DATA EVALUATION RECORD

3/20/2001

MESOTRIONE (ZA1296)

Study Type: §83-4a; Multigeneration Reproduction Study of Mesotrione Administered in the Diet to Sprague-Dawley Rats

Work Assignment No. 2-02-95CC (formerly 2-01-52CC) (MRID 44505033)

Prepared for

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MESOTRIONE (ZA1296) **Reproduction Study (§83-4[a])**

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DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Rat
OPPTS Number: 870.3800 OPP Guideline Number: §83-4a

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: Milburn, G.M. (1997) ZA1296: Multigeneration Study in the Rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Study No. RR0691. Report No. CTL/P/5147. December 10, 1997. MRID 44505033. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY: In a 3-generation reproduction study (MRID 44505033), mesotrione (96.8% a.i., lot # P17) was administered in the diet continuously to 3 generations of Alpk:AP$_f$SD (Sprague-Dawley) rats (26 rats/sex/dose) at dose levels of 0, 2.5, 10, 100, or 2500 ppm (equivalent to 0, 0.3/0.3, 1.1/1.2, 11.7/12.4, or 287.7/311.4 mg/kg/day [M/F] in the P and F_1 animals). The P and F_1 animals were exposed to the test substance for approximately 10 weeks prior to mating. At approximately 14 weeks after selection, the F_2 animals were subdivided into a continuous treatment group (12 animals/sex/dose) and a recovery group (14 animals/sex/dose), which received control ration only. At approximately 4 weeks after subdivision, these groups were mated to produce the F_3 generation. Excluding the recovery group, exposure of all animals to the test material was continuous throughout the study.

There was no evidence of treatment-related changes in mortality, body weight gains, food efficiency, or reproductive performance observed in the P or F_1 adults at any dose. Decreased food consumption during lactation, increased incidences of ocular opacity, cloudiness, keratitis, and increased corneal vascularization, and increased bilateral hydronephrosis were observed in the100 and 2500 ppm groups. The severity of these effects was greater than the 10 ppm groups

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in a dose-dependent manner. At 10 ppm, differences in food consumption were observed in F_1 dams (↓15-17%, LWs 3 and 4).

Ophthalmologic findings were observed during clinical observation, at necropsy, and at histological examination in the treatment groups, but not in controls, except as noted. During clinical observation, cloudy/opaque eyes were observed in the following groups: F_1 males (1/26); F_2 males before subdivision (1/26); and F_2 males in the recovery group (1/14).

At necropsy, opaque/cloudy eyes were observed in the F_1 males (1/26 treated) and F_2 recovery males (1/14 treated). At histological examination, keratitis was observed in F_1 males (5/26 treated). Corneal vascularization was observed in F_1 males (5/26 treated). Bilateral hydronephrosis at histological exam and under 2X magnification was observed in F_1 males (10/26) and F_2 continuous treatment males (2/12). No incidences were noted in the controls for any of these groups.

Increases in absolute and adjusted (to body weight) liver weights were observed. Liver weights were increased in the P, F_1, and F_2 groups as follows: In the 10, 100, and 2500 ppm P males and 100 and 2500 ppm P females, in the 10, 100, and 2500 ppm males, in the 2.5, 10, 100, and 2500 ppm F_2 continuous treatment males, and in the 2500 ppm F_2 recovery group females. Absolute and/or adjusted (to body weight) kidney weights were increased in all 2500, 100, and 10 ppm male groups, including the recovery groups. Increased kidney weights were only observed in 2500 ppm P and recovery females. The incidence of bilateral hydronephrosis at terminal necropsy as determined under 2X magnification was increased in 2500 and 100 ppm males and females and F_2 continuous treatment males and in the 10 ppm males. At histological examination, the incidence of minimal to marked bilateral hydronephrosis was increased in 2500 ppm F_1, F_2 continuous treatment, and F_2 recovery males and females; in 100 ppm F_1 males and females, F_2 continuous treatment and recovery males; and in 10 ppm F_1 males. The nephrotoxicity was apparently not reversed in the recovery groups.

Plasma tyrosine levels were significantly increased in F_2 adult males under continuous treatment at all treatment doses during the pre-mating interval and at termination (↑569 - 2478%). Levels were significantly increased in F_2 adult females under continuous treatment at 10 ppm and above during the pre-mating interval and at termination (↑289 - 285%). Animals of both sexes in the recovery groups had similar plasma tyrosine levels as the controls at all doses.

The LOAEL for systemic parental toxicity is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes) based on significantly increased plasma tyrosine levels and increased liver weights in F_2 males. No NOAEL was determined.

There was no evidence of treatment-related changes in body weights or body weight gains in the F_1 or F_2 litters at any dose.

A pattern of ocular toxicity consisting of macroscopic and microscopic ocular opacity/cloudiness was observed in all offspring generations. No opaque/cloudy eyes were observed in control animals at any time. An increase in the number of pups with cloudy eyes was observed in all 2500 groups and in the 100 ppm F_2 group (% pups[% litters]) with a range of 26-72 (36-61). An increase in the number of pups with ocular discharge was observed in the 2500 ppm F_1 (11[26] treated vs 0.4[4] controls) and F_2 litters (8[15] treated vs 0.5[5] controls), and 100 ppm F_2 litters (4[16]) but the effect was not observed in the F_3 litters.

At gross necropsy, an increase in the incidence of opaque or cloudy eyes was observed in the 2500 ppm F_1 and F_2 males and females and 100 ppm F_2 males. Closed eyelids were also observed in 2500 ppm F_1 males (18/54 treated vs 0/44 controls) and females (7/42 treated vs 1/49 controls). An increase in the incidence of minimal to marked ocular keratitis was observed at histological examination in the 2500 ppm P and F_1 males and females (18-26/26 treated vs 0/26 controls). Keratitis was also observed in 100 ppm P males and females (8-11/26 treated) and F_1 males and females (23-26/26 treated). Increased minimal to moderate corneal vascularization was observed in 2500 ppm P and F_1 males and females (16-26/26 treated vs 0/26 controls). Corneal vascularization was also observed in 100 ppm P males and females (7-8/26 treated), F_1 males and females (12-26/26 treated).

A pattern of nephrotoxicity consisting of increased kidney weights and increased macroscopic and microscopic renal hydronephrosis was observed in the pups. There was an increase in the relative kidney weights in F_2 males (↑11%) and relative and absolute kidney weights (↑15% each) in F_2 females. At gross necropsy, the incidence of bilateral renal pelvic dilatation was increased in the 100 and 2500 ppm F_3 continuous treatment males (15-18% in treated vs. 0% in controls). At histological examination, minimal to marked bilateral hydronephrosis was increased in the 100 and 2500 ppm F_1 and F_2 males and females (8-15% treated vs 1-4% controls), in the 10 ppm F_1 and F_2 males and females (5-7% treated) and in the 100 and 2500 ppm F_3 continuous treatment males and females (12-33% treated vs 2-4% controls). In the recovery animals, the incidences of bilateral hydronephrosis were low and similar to controls. The incidence of bilateral hydronephrosis at terminal necropsy, as determined under 2X magnification, was increased as follows: 100 and 2500 ppm F_1 , F_2, and F_3 continuous treatment males and females; 10 ppm F_1 males and females; and 10 ppm F_2 males.

Plasma tyrosine levels were significantly increased in F_3 male pups under continuous treatment at all treatment doses (↑979 - 2374%). Levels were significantly increased in F_3 female pups under continuous treatment at 100 and 2500 ppm (↑633 - 960%). Animals of both sexes in the recovery groups had similar plasma tyrosine levels as the controls at all doses.

The LOAEL for systemic offspring toxicity is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes) based on significantly increased plasma tyrosine levels in F_3 male pups. No NOAEL was determined.

Mean litter size was decreased 20-45% compared to the controls throughout lactation in all 2500 ppm groups, including F_3 recovery litters. Mean litter size was also decreased in the 10 ppm (↓19-23%, PND 8-29) and 100 ppm (↓20-22%, PND 5-29) F_2 litters. At 2500 ppm, the livebirth index was decreased in the F_2 (↓6%) and F_3 continuous treatment (↓12%) litters and the day 22 viability index was decreased in the F_1 and F_2 litters (↓16% each). The proportion of litters with whole litter losses was increased in the 2500 ppm F_2 litters (7/20 treated vs 1/21 controls). There was no significant difference in the F_1 and F_3 continuous treatment litters. Whole litter weights were decreased throughout lactation in the 2500 ppm F_1, F_2, and F_3 continuous treatment litters (↓19-49%, PND 1-29); at PND 1 in 2500 ppm F_3 recovery litters (↓29%); beginning at approximately the first week of lactation in the 100 ppm F_1 and F_2 litters (↓13-21); and at PND 11, 15, and 29 in the 10 ppm F_2 litters (↓18%).

The LOAEL for reproductive toxicity is 10 ppm (equivalent to 1.1/1.2 mg/kg/day [M/F]) based on decreased F_2 mean litter size. The reproductive NOAEL is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes).

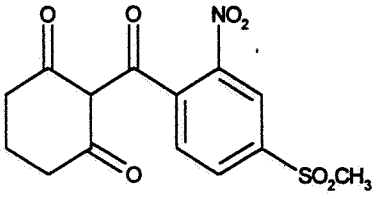
Even though no systemic NOAEL was determined for parents or offspring, the reproductive study is determined to be **acceptable/guideline (§83-4[a])** and satisfies the guideline requirement for a multigenerational reproductive toxicity study in rats as per the Hazard Identification Assessment Review 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 powder
   Lot/Batch #: P17
   Purity: 96.8% a.i.
   Storage stability: Formulations were stable at room temperature for up to 16 days.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Alpk:APfSD (Sprague-Dawley)
   Age at start of dosing: P - approx. 4 weeks; F_1 and F_2 - 29 days;
   Weight at start of dosing:
   (P, group means) Males: 87.8-89.4 g; Females: 78.1-80.5 g
   (F_1 , group means) Males: 73.2-82.0 g; Females: 68.6-76.5 g
   Source: Rodent Breeding Unit, 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 cages during gestation and lactation. The cage type used during premating and mating was not specified.
   Diet: CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 21±2°C
   Humidity: 55±15%
   Air changes: ≥15/hour
   Photoperiod: 12 h dark/12 h light
   Acclimation period: ≥5 days
   Study Duration (in life dates): start - 6/13/1995 end - 9/19/1996

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 up to 21 days. The day sperm was observed in a vaginal smear was designated gestation day (GD) 1.

2. Study schedule: The P animals (26 animals/sex/dose) were given test article diet formulations for 10 weeks prior to mating to produce the F_1 litters. The F_1 animals were reared to postnatal day (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 at least 10 weeks prior to mating to produce the F_2 litters. The F_2 animals were reared to PND 29, at which time 26 animals/sex/dose were selected to become the F_2 parents of the F_3 generation. At approximately 14 weeks after selection, the F_2 animals were subdivided into a continuous treatment group (12 animals/sex/dose) and a recovery group (14 animals/sex/dose). The recovery group received control ration only. At approximately 4 weeks after subdivision, these groups were mated to produce the F_3 generation. Adults and pups not selected for mating were sacrificed and necropsied at weaning. Excluding the recovery group, exposure of all animals to the test material was continuous throughout the study.

3. Animal assignment: The P animals were randomly assigned (stratified by body weight) to test groups as shown in Table 1. Runts or clinically abnormal pups were not used to provide F_1 or F_2 adults. Offspring used for mating were randomly chosen from the remaining pups 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	F_2 Males[c]		F_2 Females[c]	
							C	R	C	R
Control	0	0	26	26	26	26	12	14	12	14
Low-dose	2.5	0.3/0.3	26	26	26	26	12	14	12	14
Mid-dose	10	1.1/1.2	26	26	26	26	12	14	12	14
Mid-high-dose	100	11.7/12.4	26	26	26	26	12	14	12	14
High-dose	2500	287.7/311.4	26	26	26	26	12	14	12	14

a Diets were administered from the beginning of study until sacrifice, except as noted for the F_2 generation..
b Achieved dosage was calculated by the reviewers by averaging the achieved dosage during the premating period from the P and F_1 generations as shown in Table 5 of this report.
c F_2 groups were subdivided into a continuous treatment (C) and recovery (R) groups 14 weeks after selection.

4. Dose selection rationale: It was stated that dose levels were based on the results of a preliminary reproduction study in the rat, 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 for no more than 40 days and kept at room temperature for no more than 7 days. Concentrations for all five dosages were analyzed ten times after the start of the study. Homogeneity (duplicate samples from top, middle, and bottom) was determined on the low- and high-dose formulations. Stability was determined in previous studies (CTL studies PR1001 and PM0983) on 1 and 7000 ppm diet mixes maintained at room temperature for up to 16 days or at -20°C for up to 57 days.

   Results - Concentration analyses (range as % of nominal): 88.0-114.0%. Excessive concentration was noted in the first batch at 2.5 ppm (141.2% of nominal) and at 10 ppm (125.0% of nominal). Subsequent batches were within acceptable limits and no adverse effects were noted in the animals in these groups. Therefore, these excessive concentrations were considered not to have affected the acceptability of the study.

   Homogeneity analyses (range as % of nominal ± % standard deviation [as calculated by reviewers]): 100-129% ± 2.7-5.4%

   Stability analyses (% of day 0) - room temperature for 7 days: 86.3-92.7%; -20°C for 40-42 days: 90.5-114.6%

   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, except as noted in the F_2 recovery group.

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, 15, and 22, and on LDs 1, 5, 8, 11, 15, 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. The day of preputial separation or vaginal opening was determined in F_2 males and females selected for reproduction and in two F_3 males/litter/dose.

2. Litter observations: Litters were examined daily for clinical signs, morbidity, and mortality. Pups were weighed on PND 1, 5, 8, 11, 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 (PND)							
	Day 1	Day 5	Day 8	Day 11	Day 15	Day 16	Day 22	Day 29
Number of live pups [b]	X	X	X	X		X	X	X
Pup weight	X	X	X	X	X		X	X
External alterations	X	X	X	X		X	X	X
Number of dead pups [b]	X	X	X	X		X	X	X
Sex of each pup	X	X	X	X		X	X	X

a Data extracted from the study report, page 30.
b Pups were examined daily for morbidity, mortality and clinical signs.

3. Non-sexual developmental landmarks: Pups were not examined for developmental landmarks.

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

5. Ophthalmoscopic examinations: Ten males and ten females/dose from the P, F_1, and F_2 generations were subjected to an ophthalmoscopic exam. P animals were examined prior to termination; F_1 and F_2 animals were examined one week after selection and prior to termination. In addition, 10 F_2 animals/dose/subgroup were examined prior to allocation to the continuous treatment or recovery groups and prior to mating. One male and one female from each of the F_3 litters were examined prior to termination.

6. Postmortem observations:

   a) Parental animals: All surviving P, F_1, and F_2 adult males and females were sacrificed after littering (males) or weaning (females) of their litters, except for F_1 dams, which were sacrificed in estrus to allow oocyte quantification. All parental animals were subjected to a complete external and internal postmortem examination. Blood samples were collected from selected adults, but the analyses performed were not specified in this study report.

b) Offspring: Pups that died or were sacrificed prematurely received a gross necropsy. Five F_1 pups/sex/dose and 10 F_2 pups/sex/dose were randomly selected for a full necropsy. An additional 2 pups/sex/litter, all F_3 pups not selected for observation, and all abnormal pups received a gross necropsy only. Blood samples were collected from selected pups, but the analyses performed were not specified in this study report.

The following indicated organs or tissues 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. In addition, the noted organs (XX) were also weighed from an additional 5 F_1 pups/dose. In F_1 and F_2 pups receiving a gross exam only, macroscopic abnormalities only were preserved for future examination. In F_3 pups receiving a gross exam only, macroscopic abnormalities and kidneys were preserved for future examination.

	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[a]	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.gland		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

a Only the kidneys were fixed for the F_2 adults.

Slides prepared from these tissues were examined for control and high-dose adults, F_1 pups, and F_2 pups. Slides prepared from the eyes, kidneys, reproductive organs from infertile adults, and abnormal tissues were examined in the 2.5, 10, and 100 ppm adults and pups. Slides prepared from kidneys were examined for the F_3 pups. Hydronephrosis was assessed in P, F_1, and F_2 adults and pups using a 2x magnifying glass.

7. Plasma tyrosine levels: Plasma tyrosine levels were measured in the F_2 adults during the pre-mating interval (at approximately week 18 after selection) and at termination (Tables a and b) and at termination (day 29) in the F_3 pups (Table c). Data for F_2 adult recovery groups (received control diet after withdrawal of treatment at 4 weeks prior to mating) and F_3 pup recovery groups were also submitted. The data were not evaluated for statistical significance.

D. DATA ANALYSIS

1. Statistical analyses: F_1 and F_2 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 sexual maturation times 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 22 and >22 days, F_2 and F_3 adults with specific sexual maturation times in each treated group, 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. Analyses of pup survival and litter size were performed with and without litters with total litter loss.

2. Indices:

   Reproductive indices: The following reproductive indices as presented in the study report were calculated for the P, F_1, 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, F_2, and F_3 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%

day X viability index (%) [calculated by the reviewers] = # of live pups at day X/ # of pups born alive x 100%

3. Historical control data: Historical control data on bilateral hydronephrosis were provided for F1 males and females only. No other historical control data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: There was an increase in the incidence of cloudy/opaque eyes (Table 3) in 2500 ppm P males (16/26 treated vs 0/26 controls) and females (10/26 treated vs 0/26 controls), 2500 ppm F_1 males (26/26 treated vs 0/26 controls) and females (26/26 treated vs 0/26 controls), 2500 ppm F_2 males (26/26 treated vs 0/26 controls) and females (24/26 treated vs 0/26 controls) before subdivision, 2500 ppm F_2 males (12/12 treated vs 0/12 controls) and females (10/12 treated vs 0/12 controls) on continuous treatment after subdivision, and 2500 ppm F_2 males (14/14 treated vs 0/14 controls) and females (10/14 treated vs 0/14 controls) in the recovery group after subdivision. The incidence of cloudy/opaque eyes was also increased in the 100 ppm P males (9/26 treated vs 0/26 controls), 100 ppm F_1 males (26/26 treated vs 0/26 controls) and females (13/26 treated vs 0/26 controls), 100 ppm F_2 males (21/26 treated vs 0/26 controls) and females (11/26 treated vs 0/26 controls) before subdivision, 100 ppm F_2 males (10/12 treated vs 0/12 controls) and females (5/12 treated vs 0/12 controls) on continuous treatment after subdivision, and 100 ppm F_2 males (9/14 treated vs 0/14 controls) and females (1/14 treated vs 0/14 controls) in the recovery group after subdivision. Cloudy/opaque eyes were also observed in a single animal from each of the following groups: 2.5 ppm P males, 10 ppm F_1 males, 10 ppm F_2 males before subdivision, and 10 ppm F_2 males in the recovery group. The incidence in the 2.5 ppm P males was not dose-dependent and therefore considered not treatment-related. The following clinical signs were also observed, but were considered not of toxicological concern because they did not occur in all generations and/or were not dose-dependent:

dry or wet urine-stained fur - 2500, 100, and 10 ppm P females (10/26, 4/26, and 2/26 treated, respectively, vs 2/26 controls), 2500 ppm F_1 females (2/26 treated vs 0/26 controls);

ocular discharge - 2500 and 2.5 ppm P males (1/26 and 3/26 treated, respectively, vs 0/26 controls), 2500, 10, and 2.5 ppm P females (1/26, 1/26, and 2/26 treated, respectively, vs 0/26 controls), 2500 and 100 ppm F_1 males (3/26 each treated vs 1/26 controls), 2500 ppm F_1 females (4/26 treated vs 1/26 controls), 2500 ppm F_2 males before subdivision (1/26 treated vs 0/26 controls), 2500 ppm F_2 males on continuous treatment after subdivision (1/12 treated vs 0/12 controls), 10 ppm F_2 females on continuous treatment after subdivision (2/12 treated vs 0/12 controls), 10 ppm F_2

males in the recovery group (2/14 treated vs 0/14 controls), and 2500 ppm F_2 females in the recovery group (2/14 treated vs 1/14 controls).

There were no treatment-related mortalities observed in the P, F_1, or F_2 adults. Animals terminated for humane reasons were as follows: two 2.5 ppm P males, one 2500 ppm and one 100 ppm F_1 male, one control and one 2500 ppm F_1 females, one control and one 10 ppm F_2 males in the recovery group, and one control F_2 female in the recovery group. One control F_1 female was terminated due to difficult parturition. No P females and no animals in the F_2 continuous treatment group died prematurely.

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

Observation	Dose Group (ppm)									
	0	2.5	10	100	2500	0	2.5	10	100	2500
	P males					P females				
# Animals examined	26	26	26	26	26	26	26	26	25	26
Cloudy eyes	0	1	0	9	16	0	0	0	0	8
Opaque eyes	0	0	0	0	0	0	0	0	0	2
	F_1 males					F_1 females				
# Animals examined	26	26	26	26	26	26	26	26	26	26
Cloudy eyes	0	0	1	26	25	0	0	0	13	26
Opaque eyes	0	0	0	0	1	0	0	0	0	3
	F_2 males/pre-division					F_2 females/pre-division				
# Animals examined	26	26	26	26	26	26	26	26	26	26
Cloudy eyes	0	0	1	21	26	0	0	0	11	24
Opaque eyes	0	0	0	0	1	0	0	0	0	0
	F_2 males/continuous treatment					F_2 females/continuous treatment				
# Animals examined	12	12	12	12	12	12	12	12	12	12
Cloudy eyes	0	0	0	10	12	0	0	0	5	10
	F_2 males/recovery					F_2 females/recovery				
# Animals examined	14	14	14	14	14	14	14	14	14	14
Cloudy eyes	0	0	0	9	14	0	0	0	1	10
Opaque eyes	0	0	1	0	2	0	0	0	0	0

a Data extracted from the study report Tables 6 through 10, pages 123 through 151.

2. Body weight, body weight gains, food consumption, and food efficiency: Adjusted (to week or day 1) body weights (Tables 4a through d) during premating, gestation, and lactation were only slightly and/or sporadically different ($p \leq 0.05$ or 0.01) from concurrent controls (↓11-↑15%) in all treated groups.

Body weight gains and overall average food consumption, as calculated by the reviewers, did not appear to show any differences of toxicological concern for any generation during premating or gestation. Differences ($p \leq 0.05$ or 0.01) of toxicological concern in food consumption during lactation were observed as follows: 2500 ppm P, F_1, and F_2 continuous treatment dams - ↓16-37% throughout lactation; 100 ppm P dams - ↓11-17% throughout lactation; 100 ppm F_1 dams - ↓13-20% for lactation weeks (LWs) 2, 3, and 4; 10 ppm F_1 dams - ↓15-17% for LWs 3 and 4. No differences in food consumption were observed in the F_2 recovery groups.

Table 4a. Body weight (g), body weight gain (g), and food consumption (g/animal/day) in P and F_1 animals - pre-mating.[a]

Observations/study week	Dose Group				
	0	2.5	10	100	2500
P Generation Males - Pre-mating					
Adjusted mean body weight - Week 11	432.9	428.8	427.2	426.4	419.9
Mean weight gain - Weeks 1-11[b]	344.6	338.4	338.9	337.7	332.5
Mean food consumption - Weeks 1-10[b]	29.3	29.2	29.9	30.4	29.1
P Generation Females - Pre-mating					
Adjusted mean body weight - Week 11	252.0	249.0	249.5	246.3	239.1**(↓5)
Mean weight gain - Weeks 1-11[b]	172.9	169.5	171	167.2	159.9
Mean food consumption - Weeks 0-11[b]	21.8	21.6	22.0	21.9	21.2
F_1 Generation Males - Pre-mating					
Adjusted mean body weight - Week 11	449.6	447.3	444.7	422.7**(↓6)	415.6**(↓8)
Mean weight gain - Weeks 1-11[b]	373.6	373.4	370.0	339.2	330.8
Mean food consumption - Weeks 1-10[b]	30.1	30.1	30.3	28.9	28.4
F_1 Generation Females - Pre-mating					
Adjusted mean body weight - Week 11	253.8	256.4	253.9	260.8	258.5
Mean weight gain - Weeks 1-11[b]	180.8	184.5	181.8	187.2	184.3
Mean food consumption - Weeks 0-11[b]	22.1	22.0	22.0	22.1	22.2

a Body weight data were extracted from the study report, Tables 11 and 12, pages 155, 157, 159, and 161. Percent differences from control are presented parenthetically.

b Body weight gain data and average food consumption were calculated by the reviewers from data contained in the study report, Tables 11, 12, 14, and 15, pages 154 through 161 and 170 through 173.

** Statistically different from control, $p \leq 0.01$.

Table 4b. Body weight (g), body weight gain (g), and food consumption (g/animal/day) in F_2 animals - pre-mating.[a]

Observations/study week	Dose Group				
	Control	2.5	10	100	2500
F_2 Generation Males - Continuous Treatment					
Adjusted mean body weight - Week 10	449.6	438.8	445.4	406.6**(↓10)	408.5**(↓9)
Mean weight gain - Weeks 1-10[b]	363.6	353.6	361.4	321.5	322.8
Mean food consumption - Weeks 1-10[b]	31.1	30.2	32.5	30.1	30.0
F_2 Generation Females - Continuous Treatment					
Adjusted mean body weight - Week 10	253.1	254.4	252.7	255.0	253.7
Mean weight gain - Weeks 1-10[b]	173.4	174.9	171.4	175.5	173.8
Mean food consumption - Weeks 1-10[b]	22.1	22.1	22.5	21.8	22.5
F_2 Generation Males - Recovery					
Adjusted mean body weight - Week 10	436.2	435.9	426.4	406.3*(↓7)	405.38(↓7)
Mean weight gain - Weeks 1-10[b]	351.7	351.3	343.9	321.9	321.5
Mean food consumption - Weeks 1-10[b]	30.8	30.1	30.9	29.7	29.1
F_2 Generation Females - Recovery					
Adjusted mean body weight - Week 10	246.2	242.6	247.6	247.7	258.1*(↑5)
Mean weight gain - Weeks 1-10[b]	168.2	163.9	169.2	170.0	180.3
Mean food consumption - Weeks 1-10[b]	21.7	21.3	21.9	21.4	21.9

a Body weight data were extracted from the study report, Table 13, pages 163, 165, 167, and 169. Percent differences from control are presented parenthetically.

b Body weight gain data and average food consumption were calculated by the reviewers from data contained in the study report, Tables 13 and 16, pages 162 through 169 and 174 through 177.

* or ** Statistically different from control, $p \leq 0.05$ or 0.01.

Table 4c. Body weight (g), body weight gain (g), and food consumption (g/animal/day) in P and F_1 dams - gestation and lactation.[a]

Observations/study week	Dose Group				
	0	2.5	10	100	2500
P Generation Females - Gestation					
Adjusted mean body weight - Day 22	386.7	387.9	383.8	382.8	378.1*(↓2)
Mean weight gain - Days 1-22 [b]	133.6	134.5	131.1	131.4	125.1
Mean food consumption - Weeks 1-3 [b]	28.2	27.9	28.2	28.0	27.1
P Generation Females - Lactation					
Adjusted mean body weight - Day 29	324.7	326.2	321.6	318.5	311.9**(↓4)
Mean weight gain - Days 1-29 [b]	28.0	30.4	20.6	22.5	21.0
Mean food consumption - Week 1	43.5	43.0	41.2	38.9**(↓11)	36.0**(↓17)
Mean food consumption - Week 2	66.6	64.8	60.1	55.4*(↓17)	48.7**(↓27)
Mean food consumption - Week 4	135.8	139.0	128.3	120.2*(↓11)	98.4**(↓28)
Mean food consumption - Weeks 1-4 [b]	82.0	82.8	76.9	71.2	60.7
F_1 Generation Females - Gestation					
Adjusted mean body weight - Day 22	400.1	394.8	392.8	395.4	391.4
Mean weight gain - Days 1-22 [b]	133.3	126.9	125.3	128.1	123.8
Mean food consumption - Weeks 1-3 [b]	29.0	28.3	29.1	28.3	28.9
F_1 Generation Females - Lactation					
Adjusted mean body weight - Day 29	334.3	327.5	325.1*(↓3)	326.7	326.9
Mean weight gain - Days 1-29 [b]	23.7	13.8	11.2	16.4	17.3
Mean food consumption - Week 1	36.8	36.8	34.8	34.1	30.9*(↓16)
Mean food consumption - Week 2	61.4	56.6	53.4	53.5*(↓13)	47.2**(↓23)
Mean food consumption - Week 3	83.4	73.5	70.6*(↓15)	70.4**(↓16)	60.5**(↓27)
Mean food consumption - Week 4	138	119.4	115*(↓17)	110.5**(↓20)	87.6**(↓37)
Mean food consumption - Weeks 1-4 [b]	79.9	71.6	68.5	67.1	56.6

a Body weight and food consumption data were extracted from the study report, Tables 20, 21, 23, 24, 26, 27, 29, and 30, pages 182, 183, 186, 187, 190, 191, 194, and 195.

b Body weight gain data and average food consumption were calculated by the reviewers from data contained in the study report, Tables 20, 21, 23, 24, 26, 27, 29, and 30, pages 182, 183, 186, 187, 190, 191, 194, and 195. Percent differences from control are presented parenthetically.

* or ** Statistically different from controls at p≤0.05 or 0.01.

Table 4d. Body weight (g), body weight gain (g), and food consumption (g/animal/day) in F_2 dams - gestation and lactation.[a]

Observations/study week	Dose Group				
	Control	2.5	10	100	2500
F_2 Generation Females/Continuous Treatment - Gestation					
Adjusted mean body weight - Day 22	417.8	413.4	419.6	423.6	414.0
Mean weight gain - Days 1-22 [b]	131.5	127.4	132.7	135.6	125.6
Mean food consumption - Weeks 1-3 [b]	28.4	28.1	29.0	27.6	28.8
F_2 Generation Females/Continuous Treatment - Lactation					
Adjusted mean body weight - Day 29	337.8	332.7	342.3	330.2	334.5
Mean weight gain - Days 1-29 [b]	10.3	-0.4	6.9	2.8	1.6
Mean food consumption - Week 1	38.3	37.8	34.6	33.5	30.9*(↓19)
Mean food consumption - Week 4	120.5	134.4	112	118.2	79.5*(↓34)
Mean food consumption - Weeks 1-4 [b]	74.3	79.6	67.8	68.9	50.4
F_2 Generation Females/Recovery - Gestation					
Adjusted mean body weight - Day 22	423.2	412.2	417.2	416.5	410.0
Mean weight gain - Days 1-22 [b]	138.4	127.6	132.8	129.7	123.1
Mean food consumption - Weeks 1-3 [b]	28.6	27.7	28.3	28.6	28.2
F_2 Generation Females/Recovery - Lactation					
Adjusted mean body weight - Day 29	331.8	332.1	328.6	331.5	329.7
Mean weight gain - Days 1-29 [b]	5.3	5.9	1.4	4.1	0.5
Mean food consumption - Weeks 1-4 [b]	82.5	79.1	70.7	73.3	69.3

a Body weight and food consumption data were extracted from the study report, Tables 22, 25, 28, and 31, pages 184, 185, 188, 189, 192, 193, 196, and 197.

b Body weight gain data and average food consumption were extracted from the study report or were calculated by the reviewers from data contained in the study report, Tables 22, 25, 28, and 31, pages 184, 185, 188, 189, 192, 193, 196, and 197. Percent differences from control are presented parenthetically.

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

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 11 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.

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

Male				Female			
2.5	10	100	2500	2.5	10	100	2500
P Generation							
0.3	1.1	11.6	278.1	0.3	1.2	12.4	306.8
F_1 Generation							
0.3	1.1	11.7	297.2	0.3	1.2	12.3	316.0
Average							
0.3	1.1	11.7	287.7	0.3	1.2	12.4	311.4

a Data extracted from the study report, Appendix G, pages 376 and 379.

4. Food efficiency: Mean food efficiencies were reported as food [g]/100 g body weight gain; however, the data appear to indicate that food efficiencies were reported as body weight gain [g]/100 g food consumption, as was reported in previous studies by this laboratory. Differences ($p \leq 0.05$ or 0.01) occurred in all treatment groups (↓34-↑23%), but the differences were minor, sporadic, and/or not dose-dependent. The overall food efficiencies for the premating period are reported in Table 6.

Table 6. Overall food efficiency (body weight gain [g]/100 g food consumption) during the premating period.[a]

Male					Female				
0	2.5	10	100	2500	0	2.5	10	100	2500
P Generation									
16.78	16.63	16.18* (↓4)	15.91** (↓5)	16.51	11.34	11.22	11.10	10.91* (↓4)	10.77** (↓5)
F_1 Generation									
17.74	17.74	17.42	16.76** (↓6)	16.70** (↓6)	11.74	11.97	11.82	12.10	11.87
F_2 Generation - Continuous Treatment									
18.72	18.66	17.74* (↓5)	17.12** (↓9)	17.20** (↓8)	12.46	12.56	12.21	12.73	12.29
F_2 Generation - Recovery									
18.34	18.63	17.74	17.45	17.78	12.33	12.25	12.26	12.63	13.04

a Data extracted from the study report, Tables 17, 18, and 19, pages 178 through 181. Percent differences from controls are presented parenthetically.
* or ** Statistically different from control, $p \leq 0.05$ or 0.01.

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 was delayed ($p \leq 0.05$ or 0.01) in 2.5, 10, 100, and 2500 ppm F_2 males (↑3, 3, 6, and 6%, respectively) and in the 10 ppm F_3 continuous treatment male pups selected for observation (↑5%). These differences were minor and not dose-dependent. Preputial separation was not affected in the F_3 recovery male pups selected for observation. F_1 pups were not observed for preputial separation. Vaginal opening in the F_2 females was not affected by treatment.

6. Reproductive performance: There were no differences of toxicological concern observed in the reproductive performance of the P or F_1 adults (Table 7). Differences ($p \leq 0.05$ or 0.01) were observed in the distribution of the precoital intervals in the P dams (↓19-↑22%), but no statistically significant differences were observed in the mean precoital interval. The gestation period was increased 2-3% in F_1 dams (2500 ppm - 22.9 days, 100 ppm - 22.8 days vs 22.3 days in controls), but these increases were considered minor and were not seen in the P or F_2 generations.

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

Observation	Dose Group (ppm)				
	0	2.5	10	100	2500
P Dams - F_1 Litter					
Number mating	26	26	26	26	26
Female Parturition Index-%	88.5	80.8	80.8	76.9	88.5
Mean days to mating	3.00	3.15	2.62	2.42	3.65
Gestation Length (days)	22.7	22.4*(↓1)	22.6	22.7	22.9
Number of Litters	23	21	21	20	23
F_1 Dams - F_2 Litter					
Number mating	25	26	26	26	26
Female Parturition Index-%	84.0	73.1	88.5	96.2	76.9
Mean days to mating	3.20	2.76	2.48	3.80	2.73
Gestation Length (days)	22.3	22.3	22.4	22.8**(↑2)	22.9**(↑3)
Number of Litters	21	19	23	25	20
F_2 Dams - Continuous Treatment					
Number mating	12	12	12	12	12
Female Parturition Index-%	75.0	83.3	91.7	91.7	83.3
Mean days to mating	2.36	3.50	2.08	2.75	3.00
Gestation Length (days)	23.0	22.9	22.5*(↓2)	22.9	23.1
Number of Litters	9	10	11	11	10
F_2 Dams - Recovery					
Number mating	13	14	14	14	14
Female Parturition Index-%	53.8	92.9	100*(↑86)	92.9	92.9
Mean days to mating	2.31	2.93	1.79	3.14	2.50
Gestation Length (days)	22.4	22.4	22.7	22.8	22.7
Number of Litters	7	13	14*	13	13

a Data extracted from the study report, Tables 36 through 38, pages 212 through 217. Percent differences from control are presented parenthetically.

* or ** Statistically different from control, $p \leq 0.05$ or 0.01.

7. Parental postmortem results

a) Organ weights: Kidney weights were increased ($p \leq 0.05$ or 0.01) in P, F_1, and F_2 groups as follows: In the P groups, absolute and adjusted (to body weight) weights were increased 9-11% in 100 and 2500 ppm males and adjusted weights were increased 12% in the 2500 ppm females; in the F_1 groups, adjusted weights were increased 7-11% in the 10, 100 and 2500 ppm groups males; in the F_2 continuous treatment groups, adjusted weights were increased 18-26% in the 10, 100 and 2500 ppm males; in the F_2 recovery groups, adjusted weights were increased 9-10% in the 10, 100, and 2500 ppm males and absolute kidney weights were increased 12% in the 2500 ppm females (Tables 8a and b). Hydronephrosis was observed in the treatment

groups, and therefore these increases in kidney weights are considered treatment-related.

Liver weights were increased ($p \leq 0.05$ or 0.01) in the P, F_1, and F_2 groups as follows: In the P groups, absolute and adjusted (to body) weights were increased 9-17% in the 10, 100, and 2500 ppm males and 8-11% in the 100 and 2500 ppm females; in the groups, adjusted liver weights were increased 11-12% in the 10, 100, and 2500 ppm males; in the F_2 continuous treatment groups, adjusted weights were increased in the 2.5, 10, 100, and 2500 ppm males; in the F_2 recovery group, absolute liver weights were increased 12% in the 2500 ppm females. These increases in liver weights were not accompanied by abnormal hepatic histopathology, and may be due to an adaptive response to treatment or related to the tyrosinemia.

Adjusted testes weights were increased 4-5% in the 10, 100, and 2500 ppm P males. Absolute and adjusted epididymides weights were decreased 7-8% in 100 and 2500 ppm males. These changes are small and not associated with abnormal histopathology, and therefore, are considered not treatment-related.

Table 8a. Selected organ weights in P and F_1 males and females.[a]

Organ	Dose Group (ppm)				
	0	2.5	10	100	2500
P Generation					
Males					
Terminal body weight	512.1	515.2	506.6	503.1	500.7
Kidney, absolute	3.49[b]	3.52	3.61	3.84**(↑10)	3.81**(↑9)
adjusted to body	3.48[b]	3.46	3.61	3.86**(↑11)	3.85**(↑11)
Liver, absolute	20.1[b]	21.5	21.9*(↑9)	22.7**(↑13)	23.3**(↑16)
adjusted to body	20.1[b]	21.2	21.9*(↑9)	22.8**(↑13)	23.6**(↑17)
Testes, absolute	3.53	3.64	3.72*(↑5)	3.70	3.70
adjusted to body	3.55	3.62	3.72*(↑5)	3.70*(↑4)	3.71*(↑5)
Females					
Terminal body weight	298.4	298.0	307.8	296.8	289.8
Kidney, absolute	2.33	2.37	2.44*(↑5)	2.32	2.37
adjusted to body	2.33	2.37	2.37	2.33	2.43*(↑12)
Liver, absolute	12.5	12.9	13.5*(↑8)	13.5*(↑8)	13.5*(↑8)
adjusted to body	12.5	12.9	12.9	13.6**(↑9)	13.9**(↑11)
F_1 Generation					
Males					
Terminal body weight	551.0	540.5	554.8	501.3	489.8
Epididymides, absolute	1.44	1.51	1.44	1.34*(↓7)	1.32*(↓8)
adjusted to body	1.44	1.51	1.45	1.34*(↓7)	1.32*(↓8)
Kidney, absolute	3.64	3.62	3.90*(↑7)	3.70	3.70
adjusted to body	3.51	3.55	3.75**(↑7)	3.86**(↑10)	3.91**(↑11)
Liver, absolute	21.6	21.6	24.3**(↑1	22.1	21.6
adjusted to body	20.8	21.2	23.3**(↑12)	23.0**(↑11)	23.0**(↑11)
Females					
Terminal body weight	299.3	306.0	310.9	306.0	297.4
Kidney, absolute	2.48	2.36	2.57	2.61	2.47
adjusted to body	2.53	2.35**(↓7)	2.51	2.60	2.52
Liver, absolute	13.4	13.4	14.1	15.8**(↑18)	13.8
adjusted to body	13.7	13.3	13.7	15.7**(↑15)	14.3

a Data extracted from the study report, Tables 56 and 57, pages 255 through 268.
b One outlier was excluded from calculations.

Table 8b. Selected organ weights in F_2 males and females.[a]

Organ	Dose Group (ppm)				
	0	2.5	10	100	2500
F_2 Generation, Continuous Treatment					
Males					
Terminal body weight	639.2	608.8	604.8	559.3	547.0
Kidney, absolute	3.91	3.84	4.38*(↑12)	3.95	4.17
adjusted to body	3.56	3.71	4.28**(↑20)	4.19**(↑18)	4.50**(↑26)
Liver, absolute	2.22	23.6	26.4**(↑19)	23.2	23.3
adjusted to body	20.0	22.8**(↑14)	25.8**(↑29)	24.8**(↑24)	25.4**(↑27)
Females					
Terminal body weight	323.6	322.3	330.5	319.6	322.3
F_2 Generation, Recovery					
Males					
Terminal body weight	609.4	600.7	622.8	599.6	597.7
Kidney, absolute	3.61	3.72	4.00*(↑11)	3.89	3.92
adjusted to body	3.59	3.75	3.91*(↑9)	3.92**(↑9)	3.96**(↑10)
Liver, absolute	20.7	20.8	22.7	20.8	21.6
adjusted to body	20.7	21.0	22.1*(↑7)	21.0	21.9*(↑6)
Females					
Terminal body weight	312.1	309.1	314.6	325.2	332.9
Kidney, absolute	2.17	2.20	2.29	2.29	2.42*(↑12)
adjusted to body	2.21	2.26	2.31	2.25	2.34
Liver, absolute	12.1	12.3	12.7	12.5	13.5**(↑12)
adjusted to body	12.4	12.8	12.9	12.2	12.8

a Data extracted from the study report, Table 58, pages 255 through 268.

b) Pathology:

1) Macroscopic examination: There was an increase in the incidence of opaque/cloudy eyes at necropsy (Table 8c) which reflected the increased incidence of these observations during clinical examination. At 2500 ppm, the incidences of opaque/cloudy eyes were as follows: P males (14/26 treated vs 0/26 controls, $p \leq 0.01$); F_1 males (25/26 treated vs 0/26 controls, $p \leq 0.01$); F_1 females (19/26 treated vs 0/26 controls, $p \leq 0.01$); F_2 continuous treatment males (8/12 treated vs 0/12 controls, $p \leq 0.01$); F_2 continuous treatment females (7/12 treated vs 0/12 controls, $p \leq 0.01$); and F_2 recovery males (5/14 treated vs 0/14 controls, $p \leq 0.05$). Opaque/cloudy eyes were also observed at 100 ppm in P males (5/26 treated, p=not significant); F_1 males (25/26 treated, $p \leq 0.01$); and F_2 continuous treatment males (7/12 treated, $p \leq 0.01$).

Table 8c. Incidence of opaque or cloudy eyes at necropsy in P, F_1, and F_2 males and females.[a]

Observation	Dose Group (ppm)									
	0	2.5	10	100	2500	0	2.5	10	100	2500
	P Males					P Females				
No. of animals examined	26	26	26	26	26	26	26	26	26	26
Opaque eyes	0	0	0	5	14**	0	0	0	0	2
	F_1 Males					F_1 Females				
No. of animals examined	26	26	26	26	26	26	26	26	26	26
Opaque eyes	0	0	0	1	1	0	0	0	0	3
Cloudy eyes	0	0	1	24**	24**	0	0	0	2	16**
Total	0	0	1	25**	25**	0	0	0	2	19**
	F_2 Continuous Treatment Males					F_2 Continuous Treatment Females				
No. of animals examined	12	12	12	12	12	12	12	12	12	12
Cloudy eyes	0	0	0	7**	8**	0	0	0	0	7**
	F_2 Recovery Males					F_2 Recovery Females				
No. of animals examined	14	14	14	14	14	13	14	14	14	14
Opaque eyes	0	0	0	0	2	0	0	0	0	0
Cloudy eyes	0	0	1	0	3	0	0	0	0	2
Total	0	0	1	0	5*	0	0	0	0	2

a Data extracted from the study report, Tables 59, 61, 63, and 64, pages 269, 270, 277, 280, 286, 287, 288, 289, and 291.

* or ** Statistically significant at $p \leq 0.05$ or 0.01 as analyzed by the reviewers using Fisher's Exact Test.

2) Microscopic examination: An increase ($p \leq 0.05$ or 0.01) in the incidence of minimal to marked ocular keratitis (Table 8d) was observed in the 2500 ppm P males (18/26 treated vs 0/26 controls), P females (25/26 treated vs 0/26 controls), F_1 males (26/26 treated vs 0/26 controls), and F_1 females (26/26 treated vs 0/26 controls). Keratitis was also observed in 100 ppm P males (11/26 treated), P females (8/26 treated), F_1 males (26/26 treated), F_1 females (23/26 treated), and 10 ppm F_1 males (5/26 treated, p=not significant). Increased ($p \leq 0.05$ or 0.01) minimal to moderate corneal vascularization was observed in 2500 ppm P males (16/26 treated vs 0/26 controls), P females (21/26 treated vs 0/26 controls), F_1 males (26/26 treated vs 0/26 controls), and F_1 females (26/26 treated vs 0/26 controls). Corneal vascularization was also observed in 100 ppm P males (7/26 treated), P females (8/26 treated), F_1 males (26/26 treated), F_1 females (12/26 treated), and 10 ppm F_1 males (5/26 treated, p=not significant).

The incidence of minimal to marked bilateral hydronephrosis (Table 8e) was increased ($p \leq 0.05$ or 0.01) as follows: (i) in 2500 ppm F_1 males (21/26 treated vs 0/26 controls), F_1 females (14/26 treated vs 4/26 controls), F_2 continuous treatment males

(8/12 treated vs 0/12 controls), and F_2 recovery males and females (5/14 each treated vs 0/13 or 14 controls); (ii) in 100 ppm F_1 males (14/26 treated), F_1 females (15/26 treated), F_2 continuous treatment males (6/12 treated), F_2 recovery males (5/14 treated); and (iii) in 10 ppm F_1 males (10/26 treated). Historical control data for bilateral hydronephrosis was requested by the Agency from the performing laboratory. The Sponsor submitted data from F_1 parents stating that these were the only historical control data collected by them and that they were considered to represent the best data for comparative purposes. The historical control ranges for bilateral hydronephrosis in F_1 males and females was 0-10% and 0-8%, respectively. The incidences of bilateral hydronephrosis in the F_1 and F_2 10, 100, and 2500 ppm males and the F_1 100 and 2500 ppm females were greater than the submitted historical control ranges.

Bilateral hydronephrosis was also seen in 10 ppm F_2 recovery males (1/14 treated), 2.5 ppm F_1 males and females (1/26 each treated) and 2.5 ppm F_2 recovery males (2/14 treated) and females (1/14 treated), but the incidences were low, less than controls values, and/or not dose-dependent and therefore were considered not treatment-related.

Table 8d. Selected histological findings in P and F_1 males and females.[a]

Observation		Dose Group (ppm)									
		0	2.5	10	100	2500	0	2.5	10	100	2500
		Males					Females				
		P Generation									
No. of animals examined		26	24	26	26	26	26	26	26	26	26
Ocular keratitis	Minimal	0	0	0	2	2	0	0	0	5	6*
	Slight	0	0	0	3	7**	0	0	0	3	14**
	Moderate	0	0	0	4	8**	0	0	0	0	5
	Marked	0	0	0	2	1	0	0	0	0	0
	Total	0	0	0	11**	18**	0	0	0	8**	25**
Corneal vascularization	Minimal	0	0	0	0	8**	0	0	0	8**	11**
	Slight	0	0	0	5	8**	0	0	0	0	10**
	Moderate	0	0	0	2	0	0	0	0	0	0
	Total	0	0	0	7**	16**	0	0	0	8**	21**
		F_1 Generation									
No. of animals examined		26	26	26	26	26	26	26	26	26	26
Ocular keratitis	Minimal	0	0	3	2	1	0	0	0	13**	3
	Slight	0	0	2	7**	7**	0	0	0	9**	12**
	Moderate	0	0	0	16**	9**	0	0	0	1	10**
	Marked	0	0	0	1	9**	0	0	0	0	1
	Total	0	0	5	26**	26**	0	0	0	23**	26**
Corneal vascularization	Minimal	0	0	4	4	7**	0	0	0	11**	10**
	Slight	0	0	1	22**	17**	0	0	0	1	16**
	Moderate	0	0	0	0	2	0	0	0	0	0
	Total	0	0	5	26**	26**	0	0	0	12**	26**

a Data extracted from the study report, Tables 65 and 66, pages 293, 299, 302, and 303.
* or ** Statistically significant at $p \leq 0.05$ or 0.01 as analyzed by the reviewers using Fisher's Exact Test.

Table 8e. Bilateral hydronephrosis in F_1 and F_2 males and females.[a]

Observation		Dose Group (ppm)										Historical controls (%)
		0	2.5	10	100	2500	0	2.5	10	100	2500	
		Males					Females					
F_1 Generation												
No. of animals examined		26	26	26	26	26	26	26	26	26	26	
Bilateral hydronephrosis	Minimal	0	0	0	0	0	1	0	2	3	1	
	Slight	0	1	7**	3	15**	2	1	1	4	5	
	Moderate	0	0	3	11**	6*	0	0	1	8**	7**	
	Marked	0	0	0	0	0	1	0	0	0	1	
	Total	0	1	10**	14**	21**	4	1	4	15**	14**	
	% Incidence	0	4	39	54	81	15	4	15	58	54	0-10 (M) and 0-8 (F)
F_2 Generation, Continuous Treatment												
No. of animals examined		12	12	12	12	12	12	12	12	12	12	
Bilateral hydronephrosis	Minimal	0	0	0	1	0	0	0	0	1	0	
	Slight	0	0	1	2	1	0	0	0	1	1	
	Moderate	0	0	1	3	6*	0	0	0	0	0	
	Marked	0	0	0	0	1	0	0	0	0	0	
	Total	0	0	2	6*	8**	0	0	0	2	1	
	% Incidence	0	0	17	50	67	0	0	0	17	8	
F_2 Generation, Recovery												
No. of animals examined		14	14	14	14	14	13	14	14	14	14	
Bilateral hydronephrosis	Minimal	0	1	0	1	0	0	0	0	1	0	
	Slight	1	1	0	2	0	0	1	0	1	3	
	Moderate	0	0	1	2	5	0	0	0	1	2	
	Marked	0	0	0	0	0	0	0	0	0	0	
	Total	1	2	1	5*	5*	0	1	0	3	5*	
	% Incidence	7	14	7	36	36	0	7	0	21	36	

a Data extracted from the study report, Tables 66, 67 and 68, pages 299, 304, 309, 310, 312, and 313. Historical control data submitted by the Sponsor upon request by the Agency, response from Sponsor dated 9/3/2000. Historical control data for F1 males and females only.

* or ** Statistically significant at $p \leq 0.05$ or 0.01 as analyzed by the reviewers using Fisher's Exact Test.

8. Macroscopic bilateral hydronephrosis: The incidence of bilateral hydronephrosis at terminal necropsy (Table 9), as determined under 2X magnification, was increased ($p \leq 0.05$ or 0.01) at 2500 ppm as follows: F_1 males (20/25 treated vs 0/26 controls), F_1 females (12/24 treated vs 4/24 controls), and F_2 continuous treatment males (8/12 treated vs 0/12 controls). At 100 ppm, bilateral hydronephrosis was observed in the F_1 males (13/25 treated), F_1 females (15/26 treated), and F_2 continuous treatment males (6/12

treated). At 10 ppm, bilateral hydronephrosis was observed in F_1 males (10/26 treated). No bilateral hydronephrosis was observed in the P animals.

Table 9. Incidence of bilateral hydronephrosis visible at 2X magnification in F_1 and F_2 adults.[a]

	Dose Group (ppm)									
	0	2.5	10	100	2500	0	2.5	10	100	2500
	Males					Females				
Observation	F_1 Generation									
No. of animals examined	26	26	26	25	25	24	26	26	26	24
Bilateral hydronephrosis	0	1	10**	13**	20**	4	1	4	15**	12*
% Incidence	0	4	39	52	80	17	4	15	58	50
	F_2 Generation, Recovery									
No. of animals examined	13	14	13	14	14	13	14	14	14	14
Bilateral hydronephrosis	1	2	1	5	5	0	1	0	3	5*
% Incidence	8	14	8	36	36	0	7	0	21	36
	F_2 Generation, Continuous Treatment									
No. of animals examined	12	12	12	12	12	12	12	12	12	12
Bilateral hydronephrosis	0	0	2	6*	8**	0	0	0	2	1
% Incidence	0	0	17	50	67	0	0	0	17	8

a Data extracted from the study report, Table 85, page 357.
* or ** Statistically significant at $p \leq 0.05$ or 0.01 as analyzed by the reviewers using Fisher's Exact Test.

B. OFFSPRING

1. Viability and clinical signs: An increase in the number of pups with cloudy eyes (Table 10a) was observed in all 2500 groups and in the 100 ppm F_2 group (% pups[% litters]: F_1 - (38[61]) at 2500 ppm; F_2 - (72[55]) and 26[36, at 2500 and 100 ppm, respectively; F_3 continuous treatment - (41[50]) at 2500 ppm). No ocular cloudiness was observed in the recovery litters or any of the controls. An increase in the number of pups with ocular discharge was observed in the 2500 ppm F_1 (11[26] treated vs 0.4[4] controls) and F_2 litters (8[15] treated vs 0.5[5] controls), and 100 ppm F_2 litters (4[16]) but the effect was not observed in the F_3 litters.

Mean litter size (Tables 10b and c) was decreased ($p \leq 0.05$ or 0.01 or not significant) in all 2500 ppm groups throughout lactation: F_1 litters - ↓20-32%; F_2 litters - ↓27-45%, F_3 continuous treatment litters - ↓34-41%; and F_3 recovery litters - ↓30-31%. Mean litter size was also decreased in the 100 ppm F_2 litters (↓20-22%, PND 5-29, $p \leq 0.05$) and the 10 ppm F_2 litters (↓19-23%, PND 8-29, $p \leq 0.05$). Other differences occurred, but these were sporadic and not dose-dependent. The livebirth index was decreased ($p \leq 0.05$ or 0.01) in 2500 ppm F_2 (↓6%) and F_3 continuous treatment (↓12%) litters. The livebirth index was decreased in the

100 ppm F_3 recovery litters (↓14%, $p \leq 0.01$). The day 22 viability index was decreased ($p \leq 0.01$) in F_1 and F_2 litters (↓16% in each). The proportion of litters with whole litter losses was increased in the 2500 ppm F_2 litters (7/20 treated vs 1/21 controls, $p \leq 0.05$) and F_3 continuous treatment litters (3/10 vs 0/9, p = not significant). There was no difference in the F_1 litters and, as noted, the difference in the F_3 continuous treatment litters was not significant.

Table 10a. Select clinical signs (# of pups[# of litters]) in F_1, F_2 and F_3 pups.[a]

Observation	Dose Group (ppm)				
	0	2.5	10	100	2500
F_1 litters					
No. of litters	23	21	21	20	23
No. of live pups at PND 22	251	234	196	184	169
Cloudy eyes	0	0	0	1[1]	65[14]
% Incidence	0	0	0	0.5[5]	38[61]
Ocular discharge	1[1]	0	5[4]	3[1]	18[6]
% Incidence	0.4[4]	0	3[19]	2[5]	11[26]
F_2 litters					
No. of litters	21	19	23	25	20
No. of live pups at PND 22	205	160	166	188	75
Cloudy eyes	0	0	0	48[9]	54[11]
% Incidence	0	0	0	26[36]	72[55]
Ocular discharge	1[1]	0	0	8[4]	6[3]
% Incidence	0.5[5]	0	0	4[16]	8[15]
F_3 litters/continuous treatment					
No. of litters	9	10	11	11	10
No. of live pups at PND 22	86	98	80	85	41
Cloudy eyes	0	0	0	3[3]	17[5]
% Incidence	0	0	0	3.5[27]	41[50]
Ocular discharge	1[1]	4[1]	0	2[1]	0
% Incidence	1[11]	4[10]	0	2[9]	0
F_3 litters/recovery					
No. of litters	7	13	14*	13	13
No. of live pups at PND 22	78	119	116	103	100
Cloudy eyes	0	0	0	0	0

a Data extracted from the study report Tables 44 and 45 through 47, pages 230 through 232 and 234 through 239.

Table 10b. F_1 and F_2 generation mean litter size and viability.[a]

Observation	Dose Group (ppm)				
	0	2.5	10	100	2500
F_1 litters					
Mean litter size					
Day 1	11.9	12.4	11.0	10.3	9.5**(↓20)
Day 22	11.4	11.1	10.3	9.7	7.7**(↓32)
Day 29	11.4	11.1	10.3	9.7	7.7**(↓32)
Number live pups					
Day 1	261	260	209	195	209
Day 22	251	234	196	184	169
Number deaths [b]					
Days 1-22	10	26	13	11	40
Deaths/litter [b]	0.43	1.24	0.62	0.55	1.74
Viability indices (%)					
Stillborn[b]	2.9	3.9	0.9	5.1	3.1
Livebirth	97.1	96.1	99.1	94.9	96.9
Viability (Day 22)	96.6	89.9	94.2	94.4	81.1**(↓16)
Whole litter losses	1	0	2	1	1
F_2 litters					
Mean litter size					
Day 1	11.7	9.8	9.6	10.2	8.5**(↓27)
Day 22	10.3	8.4	7.9*(↓23)	8.2*(↓20)	5.8**(↓44)
Day 29	10.3	8.4	7.9*(↓23)	8.2*(↓20)	5.8**(↓44)
Number live pups					
Day 1	234	187	201	235	110
Day 22	205	160	166	188	75
Number deaths[b]					
Days 1-22	29	27	35	47	35
Deaths/litter [b]	1.38	1.42	1.52	1.88	1.75
Viability indices (%)					
Stillborn[b]	2.2	2.6	3.0	1.9	7.8
Livebirth	97.8	97.4	97.0	98.1	92.2**(↓6)
Viability (Day 22)	88.1	88.7	87.3	82.3	74.1*(↓16)
Whole litter losses	1	0	2	2	7*

a Data extracted from the study report, Table 40, 41, and 44, page 220, 222, 224, and 230. Percent differences from control are presented parenthetically. Sex ratio was not reported.

b Calculated by the reviewers from data contained in this table or in the study report, Table 40 page 220.

NR Not reported.

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

Table 10c. F_3 generation mean litter size and viability.[a]

Observation	Dose Group (ppm)				
	0	2.5	10	100	2500
F_3 litters-continuous treatment					
Mean litter size					
Day 1	10.6	10.8	8.9	9.1	7.0(↓34)
Day 22	9.6	9.8	8.0	8.5	5.9*(↓39)
Day 29	9.6	9.7	7.6	8.4	5.7*(↓41)
Number live pups					
Day 1	95	108	89	91	49
Day 22	86	98	80	85	41
Number deaths[b]					
Days 1-22	9	10	9	6	8
Deaths/litter [b]	1.00	1.00	0.82	0.55	0.80
Viability indices (%)					
Stillborn[b]	5.4	1.8	7.8	10.7	16.6
Livebirth	94.6	98.2	92.2	89.3	83.4*(↓12)
Viability (Day 22)	90.3	93.0	88.2	93.9	82.2
Whole litter losses	0	0	1	1	3
F_3 litters-recovery					
Mean litter size					
Day 1	11.7	10.5	9.6	9.7	8.2*(↓30)
Day 22	11.1	9.2	8.3	8.6	7.7*(↓31)
Day 29	11.1	9.2	8.3	8.6	7.7*(↓31)
Number live pups					
Day 1	82	136	134	116	107
Day 22	78	119	116	103	100
Number deaths[b]					
Days 1-22	4	17	18	13	7
Deaths/litter [b]	0.57	1.31	1.29	1.00	0.54
Viability indices (%)					
Stillborn[b]	2.2	1.8	10.0	15.5	4.6
Livebirth	97.8	98.2	90.0	84.5**(↓14)	95.4
Viability (Day 22)	93.3	88.7	86.6	88.6	94.4
Whole litter losses	0	0	0	1	0

a Data extracted from the study report, Table 40, 41, and 44, page 221, 226, 228, and 232. Percent differences from control are presented parenthetically.

b Calculated by the reviewers from data contained in this table or in the study report, Table 40 page 221.

NR Not reported.

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

2. Body weights, bodyweight gains, and litter weights: There were no differences of toxicological concern in body weights (Tables 11a and b). Occasional differences (↓10-↑16%, $p \leq 0.05$ or 0.01) were noted in the treatment groups, but the differences were sporadic, not dose-dependent, and/or increases. There were no apparent differences of toxicological concern in pup body weight gains (as calculated by the reviewers). Whole litter weights (Table 11c) were decreased during lactation ($p \leq 0.05$ or 0.01) in 2500 ppm F_1 (↓19-36%, PND 1-29), F_2 (↓32-43%, PND 1-29), F3 continuous treatment (↓35-49%, PND 1-29), and F3 recovery litters (↓29%, PND 1 only); in 100 ppm F_1 (↓13-19%, PND 8-29) and F_2 litters (↓17-21, PND 5-29); and 10 ppm F_2 litters (↓18%, PND 11, 15, and 29).

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Table 11a. Mean F_1 and F_2 adjusted (to PND 1) pup bodyweights and bodyweight gains (g).[a]

Postnatal Day	Dose Group (ppm)				
	0	2.5	10	100	2500
F_1 litters					
Males					
Day 1[b]	6.3	6.0	6.3	6.4	6.4
Day 11	19.2	19.3	19.9	18.2	19.0
Day 22	42.3	43.5	43.6	38.2*(↓10)	39.1
Day 29	79.6	81.7	82.7	73.7*(↓7)	74.7
Day 29 absolute[b]	78.7	79.3	82.3	75.2	76.8
Gain (0-29)[c]	72.4	73.3	76.0	68.8	70.4
Females					
Day 1[b]	5.9	5.6	6.0	6.1	5.9
Day 11	18.4	18.3	18.9	17.4	17.9
Day 22	40.5	41.0	41.9	36.5*(↓10)	37.4
Day 29	74.1	75.8	75.6	68.1*(↓8)	69.4
Day 29 absolute[b]	72.8	72.9	75.7	70.6	70.1
Gain (0-29)[c]	66.9	67.3	69.7	64.5	64.2
F_2 litters					
Males					
Day 1[b]	6.1	6.2	6.4	6.4	6.2
Day 11	19.6	19.8	20.1	19.8	21.6*(↑10)
Day 22	45.0	46.5	47.6	44.7	48.3
Day 29	84.4	85.5	86.9	81.9	85.6
Day 29 absolute[b]	82.9	84.7	89.1	82.9	84.8
Gain (0-29)[c]	76.8	78.5	82.7	76.5	78.6
Females					
Day 1[b]	5.6	5.9	6.1**(↑9)	5.9	6.0
Day 11	19.1	19.3	19.4	19.6	20.5
Day 22	43.3	44.9	44.8	43.8	44.3
Day 29	78.7	79.2	80.0	77.7	77.2
Day 29 absolute[b]	76.2	79.6	82.4	77.4	77.7
Gain (0-29)[c]	70.6	73.7	76.3	71.5	71.7

a Data extracted from the study report, Tables 48 and 49, pages 240, 241, 242, and 243. Percent difference from controls is listed parenthetically.
b Absolute body weight.
c Calculated by the reviewers from data contained in this table.
* Statistically different from controls at $p \leq 0.05$.

Table 11b. Mean F_3 continuous treatment and recovery adjusted (to PND 1) pup weights and body weight gains (g).[a]

Postnatal Day	Dose Group (ppm)				
	0	2.5	10	100	2500
F_3 continuous treatment litters					
Males					
Day 1[b]	6.5	6.4	6.3	6.6	6.1
Day 11	20.8	21.4	21.4	21.0	21.5
Day 22	47.9	50.5	49.8	45.0	48.2
Day 29	85.3	92.2	90.4	83.9	88.9
Day 29 absolute[b]	88.6	92.6	89.2	86.3	85.2
Gain (0-29)[c]	82.1	86.2	82.9	79.7	79.1
Females					
Day 1[b]	5.9	6.1	6.0	6.2	5.6
Day 11	18.7	21.0	19.7	20.1	20.2
Day 22	44.5	48.1	46.6	43.6	45.3
Day 29	78.1	85.5	83.2	78.3	79.8
Day 29 absolute[b]	77.1	87.5	83.1	80.6	76.6
Gain (0-29)[c]	71.2	81.4	77.1	74.4	71.0
F_3 recovery litters					
Males					
Day 1[b]	6.2	6.3	6.3	6.4	6.3
Day 11	20.1	21.4	20.8	22.0	23.3*(↑16)
Day 22	48.2	50.8	49.7	51.4	54.4*(↑13)
Day 29	87.5	91.4	90.3	91.9	96.5*(↑10)
Day 29 absolute[b]	85.5	91.5	90.7	92.5	96.3
Gain (0-29)[c]	79.3	85.2	84.4	86.1	90.0
Females					
Day 1[b]	5.8	5.9	5.9	6.0	6.0
Day 11	19.9	20.3	20.4	22.2	22.4*(↑13)
Day 22	45.8	48.0	47.9	50.5	51.4
Day 29	80.7	84.2	84.2	87.5	88.7
Day 29 absolute[b]	79.0	84.4	84.5	87.3	88.9
Gain (0-29)[c]	73.2	78.5	78.6	81.3	82.9

a Data extracted from the study report, Table 50, pages 244, 245, 246, and 247. Percent difference from controls is listed parenthetically.

b Absolute body weight.

c Calculated by the reviewers from data contained in this table.

Table 11c. Mean F_1, F_2, and F_3 litter weights (g).[a]

Postnatal Day	Dose Group (ppm)				
	0	2.5	10	100	2500
F_1 Litter Weights					
Day 1	70.4	72.2	65.9	63.4	57.1**(↓19)
Day 15	276.0	269.6	255.0	239.5*(↓13)	190.7**(↓31)
Day 22	452.2	440.9	416.1	364.9**(↓19)	288.1**(↓36)
Day 29	848.6	836.1	778.1	688.6**(↓19)	550.6**(↓36)
F_2 Litter Weights					
Day 1	68.7	57.7	57.6	60.8	46.8**(↓32)
Day 15	267.5	222.3	220.2*(↓18)	220.8*(↓17)	168.1**(↓37)
Day 29	805.9	684.6	658.2*(↓18)	637.5*(↓22)	463.1**(↓43)
F_3 Continuous Treatment Litter Weights					
Day 1	63.9	66.5	50.8	54.1	32.4**(↓49)
Day 15	246.3	274.2	216.5	235.2	160.5*(↓35)
Day 29	764.6	850.9	638.7	679.4	458.1*(↓40)
F_3 Recovery Litter Weights					
Day 1	69.9	64.3	58.6	56.4	49.9*(↓29)
Day 29	892.9	805.2	717.5	755.6	692.3

a Data extracted from the study report, Tables 51, 52, and 53, pages 248 through 251. Percent difference from controls is listed parenthetically.

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

3. Anogenital distance: Not determined.

4. Offspring developmental landmarks: Not determined. Sexual development results are reported in Section II.A.5.c.

5. Offspring behavioral tests: Not determined.

6. Offspring postmortem results:

   a) Organ weights: There was an increase ($p \leq 0.05$ or 0.01) in the kidney weights in F_2 males (adjusted to body weight - ↑11%) and females (adjusted and absolute - ↑15% each)(Table 12). Bilateral hydronephrosis was observed microscopically in these and the F_1 pups, although there were no differences in kidney weights in the F_1 pups. There were differences ($p \leq 0.05$ or 0.01) observed in liver (↑9-18%) and epididymides (↓22-24%) weights, but the differences were not clearly dose-dependent.

Table 12. Selected organ weights in and F_2 males and females.[a]

Organ	Dose Group (ppm)				
	0	2.5	10	100	2500
F_1 Generation					
Males					
Terminal body weight	80.9	84.5	73.6	72.3	77.1
Epididymides, absolute	0.098	0.086	0.076*	0.074*	0.081
adjusted to body	0.096	0.082	0.078	0.077	0.081
F_2 Generation					
Males					
Terminal body weight	84.2	82.8	89.4	82.4	82.1
Kidney, absolute	1.05	1.06	1.16	1.05	1.14
adjusted to body	1.05	1.07	1.10	1.07	1.17**(↑11)
Females					
Terminal body weight	75.9	84.8	80.5	80.5	76.1
Kidney, absolute	0.97	1.07	1.01	1.08	1.12*(↑15)
adjusted to body	1.02	1.01	1.01	1.07	1.17**(↑15)
Liver, absolute	4.04	4.75*(↑18)	4.29	4.65*(↑15)	4.49
adjusted to body	4.25	4.50	4.26	4.62*(↑9)	4.68*(↑10)

a Data extracted from the study report, Table 58, pages 320 through 327.

b) Pathology:

1) Macroscopic examination: There was an increase in the incidence of opaque or cloudy eyes (Table 13) in 2500 ppm F_1 males (4/54 treated vs 0/44 controls) and females (3/42 treated vs 0/49 controls), 2500 ppm F_2 males (9/13 treated vs 0/45 controls) and females (7/10 treated vs 0/46 controls), and 100 ppm F_2 males (13/50 treated). Closed eyelids were also observed in 2500 ppm F_1 males (18/54 treated vs 0/44 controls) and females (7/42 treated vs 1/49 controls), but this observation was not made in the F_2 pups, and was therefore considered of equivocal toxicological concern. The incidence of bilateral renal pelvic dilatation was increased in 100 and 2500 ppm F_3 continuous treatment males (15-18% in treated vs 0% in controls) and females (7-12% in treated vs 2% in controls), but the findings were not dose-dependent in the females.

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

Observation	Dose Group (ppm)									
	0	2.5	10	100	2500	0	2.5	10	100	2500
	Males					Females				
F_1 Pups										
No. of animals examined	44 [21]	43 [19]	40 [18]	38 [17]	54 [21]	49 [22]	46 [21]	42 [18]	43 [18]	42 [18]
Closed eyelids	0	0	0	0	18 [7]	1 [1]	0	0	0	7 [4]
Cloudy eyes	0	0	0	0	4 [4]	0	0	0	0	3 [2]
F_2 Pups										
No. of animals examined	45 [19]	35 [15]	39 [16]	50 [21]	13 [8]	46 [20]	35 [16]	37 [17]	37 [20]	10 [6]
Opaque eyes	0	0	0	0	1 [1]	0	0	0	0	3 [1]
Cloudy eyes	0	0	0	13 [5]	8 [6]	0	0	0	10 [6]	4 [3]
Total opaque/cloudy eyes	0	0	0	13 [5]	9 ND	0	0	0	10 [6]	7 ND
F_3 Pups-Continuous Treatment										
No. of animals examined	50 [13]	41 [18]	28 [12]	33 [14]	17 [7]	57 [14]	86 [21]	83 [22]	73 [20]	59 [17]
Unilateral renal pelvic dilatation	8 [4]	5 [5]	5 [4]	4 [2]	5 [3]	6 [3]	26 [10]	11 [5]	17 [8]	5 [4]
Bilateral renal pelvic dilatation	0	2 [2]	1 [1]	5 [3]	3 [2]	1 [1]	3 [3]	5 [4]	9 [3]	5 [4]
F_3 Pups-Recovery										
No. of animals examined	50 [13]	41 [18]	28 [12]	33 [14]	17 [7]	57 [14]	86 [21]	83 [22]	73 [20]	59 [17]
Unilateral renal pelvic dilatation	13 [5]	13 [9]	9 [4]	9 [7]	3 [2]	5 [4]	8 [6]	10 [7]	7 [5]	9 [3]
Bilateral renal pelvic dilatation	0	0	0	0	0	0	1	0	1	0

a Data extracted from the study report, Tables 73 through 76, pages 328 through 336.
ND Could not be determined from data provided.

2) Microscopic examination: There was an increase in the incidence of minimal to marked ocular keratitis (Tables 14 a, b, and c) in 2500 ppm F_1 males (25% treated vs 0% controls) and females (16% treated vs 0% controls); in 2500 ppm F_2 males (13% treated vs 0% controls) and females (11% treated vs 0% controls); in 100 ppm F_1 males (4% treated) and females (4% treated); in 100 ppm F_2 males (15% treated) and females (10% treated); and in 10 ppm F_2 males (1% treated). Increases in minimal to moderate corneal vascularization was observed in 2500 ppm F_1 males (24% treated vs

0% controls) and females (16% treated vs 0% controls); in 2500 ppm F_2 males (9% treated vs 0% controls) and females (8% treated vs 0% controls); and in 100 ppm F_1 males (4% treated) and females (4% treated); and in 100 ppm F_2 males (5% treated) and females (4% treated). Slight to marked unilateral or bilateral cataractous change was observed in the 2500 ppm F_1 males (3% treated vs 0% controls) and females (2% treated vs 0% controls). No changes of this nature were reported for the F_2 or F_3 groups.

Minimal to marked bilateral hydronephrosis was increased in 2500 ppm F_1 males (13% treated vs 1% controls) and females (8% treated vs 2% controls); in 2500 ppm F_2 males (11% treated vs 4% controls) and females (13% treated vs 4% controls); in 100 ppm F_1 males (15% treated) and females (15% treated); in 100 ppm F_2 males (15% treated) and females (9% treated); in 10 ppm F_1 males (6% treated) and females (6% treated); and in 10 ppm F_2 males (7%) and females (5%). Minimal to marked bilateral hydronephrosis was also observed in F_3 continuous treatment 2500 ppm males (12% treated vs 4% controls) and females (33% treated vs 2% controls) and 100 ppm males (14% treated) and females (29% treated). In the recovery animals, the incidences of bilateral hydronephrosis were low and similar to controls.

Table 14a. Incidence of selected microscopic findings at necropsy in F_1 pups.[a]

Observation		Dose Group (ppm)									
		0	2.5	10	100	2500	0	2.5	10	100	2500
		Males					Females				
No. of animals on study		142	135	109	101	122	133	135	122	115	98
No. of animals examined		11	18	17	23	38	11	17	14	22	23
Ocular keratitis	minimal	0	0	0	0	0	0	0	0	0	1
	slight	0	0	0	0	9	0	0	0	3	3
	moderate	0	0	0	4	17	0	0	0	2	8
	marked	0	0	0	0	5	0	0	0	0	4
	total	0	0	0	4	31	0	0	0	5	16
	% Incidence	0	0	0	4	25	0	0	0	4	16
Corneal vascularization	minimal	0	0	0	3	18	0	0	0	3	7
	slight	0	0	0	1	11	0	0	0	1	9
	total	0	0	0	4	29	0	0	0	4	16
	% Incidence	0	0	0	4	24	0	0	0	3	16
Bilateral hydronephrosis	minimal	0	0	0	0	1	1	0	1	1	0
	slight	2	1	3	4	5	0	0	2	4	1
	moderate	0	2	3	9	6	1	1	3	11	7
	marked	0	0	0	2	4	0	0	1	1	0
	total	2	3	6	15	16	2	1	7	17	8
	% Incidence	1	2	6	15	13	2	1	6	15	8
Unilateral cataractous change	slight	0	0	0	0	1	0	0	0	0	0
	moderate	0	0	0	0	2	0	0	0	0	1
	total	0	0	0	0	3	0	0	0	0	1
	% Incidence	0	0	0	0	2	0	0	0	0	1
Bilateral cataractous change	moderate	0	0	0	0	1	0	0	0	0	0
	marked	0	0	0	0	0	0	0	0	0	1
	total	0	0	0	0	1	0	0	0	0	1
	% Incidence	0	0	0	0	1	0	0	0	0	1
Total uni- and bilateral cataractous change		0	0	0	0	4	0	0	0	0	2
% Incidence		0	0	0	0	3	0	0	0	0	2

a Data extracted from the study report, Table 77, pages 337 through 341. % Incidence calculated by the reviewers from data contained in this table.

Table 14b. Incidence of selected microscopic findings at necropsy in F_2 pups.[a]

Observation		Dose Group (ppm)									
		0	2.5	10	100	2500	0	2.5	10	100	2500
		Males					Females				
No. of animals on study		129	100	119	130	87	125	93	106	125	79
No. of animals examined		19	14	15	29	12	14	17	11	22	10
Ocular keratitis	minimal	0	0	1	9	1	0	0	0	7	2
	slight	0	0	0	5	3	0	0	0	2	3
	moderate	0	0	0	4	5	0	0	0	3	4
	marked	0	0	0	2	2	0	0	0	0	0
	total	0	0	1	20	11	0	0	0	12	9
	% Incidence	0	0	1	15	13	0	0	0	10	11
Corneal vascularization	minimal	0	0	0	5	5	0	0	0	2	5
	slight	0	0	0	2	2	0	0	0	3	1
	moderate	0	0	0	0	1	0	0	0	0	0
	marked	0	0	0	0	0	0	0	0	0	0
	total	0	0	0	7	8	0	0	0	5	6
	% Incidence	0	0	0	5	9	0	0	0	4	8
Bilateral hydronephrosis	minimal	0	0	0	1	0	2	0	1	5	0
	slight	2	3	4	5	2	2	2	3	1	4
	moderate	2	1	4	13	5	1	3	1	5	4
	marked	1	0	0	1	3	0	0	0	0	2
	total	5	4	8	20	10	5	5	5	11	10
	% Incidence	4	4	7	15	11	4	5	5	9	13

a Data extracted from the study report, Table 78, pages 343 through 347. % Incidence calculated by the reviewers from data contained in this table.

Table 14c. Incidence of selected microscopic findings at necropsy in F_3 pups.[a]

F_3 Generation, Continuous Treatment											
No. of animals on study		51	44	48	51	41	51	66	51	52	27
Bilateral hydronephrosis	minimal	0	0	0	0	1	0	0	0	0	0
	slight	0	0	1	3	3	0	3	3	3	2
	moderate	2	2	0	4	1	1	2	0	7	5
	marked	0	0	0	0	0	0	0	0	5	2
	total	2	2	1	7	5	1	5	3	15	9
	Incidence	4	5	2	14	12	2	8	6	29	33
F_3 Generation, Recovery											
No. of animals on study		47	77	66	68	43	37	62	82	67	68
Bilateral hydronephrosis	minimal	1	0	0	0	0	1	0	0	1	0
	slight	1	0	0	1	0	0	1	0	2	0
	moderate	0	0	0	0	0	0	1	0	0	1
	marked	0	0	0	0	0	0	0	0	0	0
	total	2	0	0	1	0	1	2	0	3	1
	Incidence	4	0	0	1	0	3	3	0	4	1

a Data extracted from the study report, Table 79 and 80, pages 349 through 351. % Incidence calculated by the reviewers from data contained in this table.

7. Macroscopic bilateral hydronephrosis: The incidence of bilateral hydronephrosis at terminal necropsy, as determined under 2X magnification (Table 15), was increased as follows: 2500 ppm F_1 males (30% treated vs 5% controls) and females (19% treated vs 4% controls); 2500 ppm F_2 males (77% treated vs 11% controls) and females (100% treated vs 11% controls); 2500 ppm F_3 continuous treatment males (63% treated vs 9% controls) and females (90% treated vs 3% controls); 100 ppm F_1 males (39% treated) and females (40% treated); 100 ppm F_2 males (40% treated) and females (30% treated); 100 ppm F_3 continuous treatment males (58% treated) and females (44% treated); 10 ppm F_1 males (15%) and females (17% treated); and 10 ppm F_2 males (21% treated).

Table 15. Incidence of bilateral hydronephrosis visible at 2X magnification in F_1, F_2, and F_3 pups.[a]

	Dose Group (ppm)									
	0	2.5	10	100	2500	0	2.5	10	100	2500
	Males					Females				
Observation	F_1 Generation									
No. of animals examined	44	43	39	38	54	49	46	42	43	42
Bilateral hydronephrosis	2	3	6	15	16	2	1	7	17	8
% Incidence	5	7	15	39	30	4	2	17	40	19
	F_2 Generation									
No. of animals examined	45	35	39	50	13	45	35	37	37	10
Bilateral hydronephrosis	5	4	8	20	10	5	5	5	11	10
% Incidence	11	11	21	40	77	11	14	14	30	100
	F_3 Generation, Recovery									
No. of animals examined	28	30	18	21	9	24	38	50	39	49
Bilateral hydronephrosis	2	0	0	1	0	1	2	0	3	1
% Incidence	7	0	0	5	0	4	5	0	8	2
	F_3 Generation, Continuous Treatment									
No. of animals examined	22	11	10	12	8	33	48	33	34	10
Bilateral hydronephrosis	2	2	1	7	5	1	5	3	15	9
% Incidence	9	18	10	58	63	3	10	9	44	90

a Data extracted from the study report, Table 86, page 358.

8. Plasma tyrosine levels: In the 2.5, 10, 100, and 2500 ppm dose groups during the pre-mating interval, a dose dependent increase in plasma tyrosine levels from controls was observed in the F_2 males (↑569, 1650, 2427, and 2478%, respectively) and females (↑79, 408, 1821, and 2536%, respectively). At termination, a similar dose dependent increase in plasma tyrosine levels was observed in the F_2 males (↑648, 1302, 1642, and 2054%, respectively) and females (↑60, 289, 1310, and 2855%, respectively). Additionally at termination in the F_3 pups, a dose dependent increase in plasma tyrosine levels was observed in the males (↑979, 1370, 1918, 2374% in the 2.5, 10, 100, and 2500 ppm dose groups, respectively) and females (↑97, 100, 633, 960% in the 2.5, 10, 100, and 2500 ppm dose groups, respectively). During the pre-mating interval, the values in the 100 and 2500 ppm males appear as if they are approaching a plateau, however, a clear dose dependent increase in plasma tyrosine levels is observed at termination in the F_2 adults and F_3 pups.

Plasma tyrosine levels in all recovery groups were similar to or approaching control levels.

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Table 16a. Plasma tyrosine levels (nmol/ml± SD) in F_2 adults during the pre-mating interval (approximately week 18 after selection).[a]

Sex	Treatment group	Dietary concentration of mesotrione (ppm)				
		0	**2.5**	**10**	**100**	**2500**
Males	Continuous treatment	129.2±19	864.8±205.7 (↑569)	2260.5±277.3 (↑1650)	3265.4±63.6 (↑2427)	3330.4±160.6 (↑2478)
Males	Recovery[b]	188±38.9	125.5±12.5 (↓33)	145.6±25.4 (↓23)	133.0±7.1 (↓29)	126.9±17.5 (↓33)
Females	Continuous treatment	89.3±20.0	159.7±42.4 (↑79)	454.0±54.2 (↑408)	1715.4±95.9 (↑1821)	2354.1±136.8 (↑2536)
Females	Recovery	99.8±22.3	84.1±8.7 (↓16)	120.4±13.8 (↑21)	119.8±30.0 (↑20)	133.9±17.6 (↑34)

a Data submitted by the Sponsor upon request by the Agency, response from Sponsor dated 9/13/2000; differences from controls are presented parenthetically. n=5

b Recovery group animals were dosed for approximately 14 weeks (4 weeks prior to mating)and then received control diet until the end of the study.

Table 16b. Plasma tyrosine levels (nmol/ml± SD) in F_2 adults at termination.[a]

Sex	Treatment group	Dietary concentration of mesotrione (ppm)				
		0	**2.5**	**10**	**100**	**2500**
Males	Continuous treatment	132.7±17.0	993.1±195.2 (↑648)	1860.0±290.4 (↑1302)	2312.0±123.4 (↑1642)	2858.7±245.9 (↑2054)
Males	Recovery	98.8±8.1	114.8±11.6 (↑16)	123.6±15.8 (↑25)	133.6±19.9 (↑35)	137.8±28.3 (↑39)
Females	Continuous treatment	85.6±11.9	137.3±38.5 (↑60)	332.8±79.7 (↑289)	1207.0±339.1 (↑1310)	2529.7±119.5 (↑2855)
Females	Recovery	93.1±12.1	95.2±11.0 (↑2)	129.3±37.6 (↑39)	113.7±170.1 (↑22)	150.1±15.7 (↑61)

a Data submitted by the Sponsor upon request by the Agency, response from Sponsor dated 9/13/2000; Recovery group animals were dosed for approximately 14 weeks (4 weeks prior to mating)and then received control diet until the end of the study. n=3-5

Table 16c. Plasma tyrosine levels (nmol/ml± SD) at termination (day 29) of F_3 pups.[a]

Sex	Treatment	Dietary concentration of mesotrione (ppm)				
		0	2.5	10	100	2500
Males	Continuous treatment	124.17±8.38 n=7	1340.29±83.61 (↑979) n=8	1825.85±106.31 (↑1370) n=5	2505.63±90.17 (↑1918) n=7	3071.37±78.44 (↑2374) n=3
Males	Recovery	112.98±17.93 n=5	133.05±27.13 (↑18) n=13	120.25±33.48 (↑6) n=10	134.39±31.18 (↑19) n=10	112.27±55.78 (↓1) n=7
Females	Continuous treatment	163.0±30.78 n=8	320.95±61.57 (↑97) n=10	325.97±128.57 (↑100) n=10	1194±150.42 (↑633) n=8	1728.38±146.46 (↑960) n=7
Females	Recovery	137.32±16.37 n=7	158.18±43.1 (↑15) n=12	156.32±22.77 (↑14) n=14	77.13±40.29 (↓44) n=11	90.49±16.34 (↓34) n=13

a Data submitted by the Sponsor upon request by the Agency, response from Sponsor dated 9/13/2000. n=3-14

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: There were no adverse effects of mesotrione on mating performance, but offspring survival was reduced at 2500 ppm (F_1, F_2, and F_3 continuous treatment) and 100 ppm (F_2 only). There was no effect on pup survival in the F_3 recovery animals. There was also a reduced number of pups/litter at 2500, 100 and 10 ppm. Treatment-related effects due to tyrosinemia were seen in bodyweights, in the eyes, and in the kidneys at 2500, 100, and 10 ppm. There were no effects on the eyes or kidneys of pups in recovery groups. Effects on litter size in these groups were also less marked than in the continuous treatment group.

B. REVIEWER'S DISCUSSION:

1. Parental toxicity: There was no evidence of treatment-related changes in mortality, body weights, body weight gains, food efficiency, or reproductive performance observed in the P or F_1 adults.

A pattern of ocular toxicity consisting of macroscopic and microscopic ocular opacity/cloudiness was observed in all parental generations. No opaque/cloudy eyes were observed in control animals at any time. The findings are as follows:

At clinical examination, there was an increase in the incidence of opaque/cloudy eyes in all P, F_1, and F2 2500 ppm groups and in all 100 ppm groups except for 100 ppm P females. Opaque/cloudy eyes were also observed in single animals from each of the following groups:

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2.5 ppm P males, 10 ppm F_1 males, 10 ppm F_2 males before subdivision, and 10 ppm F_2 males in the recovery group. The incidence in the 2.5 ppm P males was not dose-dependent and therefore considered not treatment-related. At gross necropsy, there was an increase in the incidence of opaque/cloudy eyes in 2500 ppm P males, F_1 males and females, F_2 continuous treatment males and females, and F_2 recovery males. Opaque/cloudy eyes were also observed at 100 ppm in P males, F_1 males, and F_2 continuous treatment males. No opaque/cloudy eyes were observed in control animals at any time. At histological exam, an increase ($p \leq 0.05$ or 0.01) in the incidence of minimal to marked ocular keratitis and minimal to moderate corneal vascularization was observed in the 2500 and 100 ppm P and F_1 males and females. Keratitis and corneal vascularization was also observed in 10 ppm F_1 males. No keratitis or corneal vascularization were observed in control animals at any time. Eyes from F_2 animals were not examined histologically. The ocular lesions were reversible as evidenced by the reduced incidences in the recovery animals.

A pattern of nephrotoxicity consisting of increased kidney weights and increased macroscopic and microscopic renal hydronephrosis was observed in all parental generations. The findings are as follows:

Absolute and/or adjusted (to body weight) kidney weights were increased ($p \leq 0.05$ or 0.01) in all 2500, 100, and 10 ppm male groups, including the recovery groups. Increased kidney weights were only observed in 2500 ppm P and recovery females. The incidence of bilateral hydronephrosis at terminal necropsy as determined under 2X magnification was increased ($p \leq 0.05$ or 0.01) in 2500 and 100 ppm males and females and F_2 continuous treatment males and in 10 ppm males. At histological examination, the incidence of minimal to marked bilateral hydronephrosis was increased ($p \leq 0.05$ or 0.01) in 2500 ppm F_1, F_2 continuous treatment, and F_2 recovery males and females; in 100 ppm F_1 males and females, F_2 continuous treatment and recovery males; and in 10 ppm F_1 males. The nephrotoxicity was apparently not reversed in the recovery groups. The Sponsor submitted data from F_1 parents stating that these were the only historical control data collected by them and that they were considered to represent the best data for comparative purposes. The historical control ranges for bilateral hydronephrosis in F_1 males and females was 0-10% and 0-8%, respectively. The incidences of bilateral hydronephrosis in the F_1 and F_2 10, 100, and 2500 ppm males and the F_1 100 and 2500 ppm females were greater than the submitted historical control ranges.

Increases in absolute and adjusted (to body weight) liver weights were observed, but were not accompanied by abnormal hepatic histopathology, and may be due to an adaptive response to treatment or related to the tyrosinemia. Liver weights were increased ($p \leq 0.05$ or 0.01) in the P, F_1, and F_2 groups as follows: In the 10, 100, and 2500 ppm P males and 100 and 2500 ppm P females; in the 10, 100, and 2500 ppm males; in the 2.5, 10, 100, and 2500 ppm F_2 continuous treatment males; and in the 2500 ppm F_2 recovery group females.

Plasma tyrosine levels were significantly increased in F_2 adult males under continuous treatment at all treatment doses during the pre-mating interval (↑569 - 2478%) and at

termination (↑648 - 2054%). Levels were significantly increased in F_2 adult females under continuous treatment at 10 ppm and above during the pre-mating interval (↑408 - 2536%) and at termination (↑289 - 2855%). Animals of both sexes in the recovery groups had similar plasma tyrosine levels as the controls at all doses.

The LOAEL for systemic parental toxicity is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes) based on significantly increased plasma tyrosine levels and increased liver weights in F_2 males. No NOAEL was determined.

2. Offspring and Reproductive Toxicity: No treatment-related differences in pup body weights or body weight gains were observed. Toxicity was characterized by ocular and renal changes, decreased litter size and weight, and increased plasma tyrosine levels.

A pattern of ocular toxicity consisting of macroscopic and microscopic ocular opacity/cloudiness was observed in all offspring generations. No opaque/cloudy eyes were observed in control animals at any time. The findings are as follows:

At clinical examination, an increase in the number of pups with cloudy eyes was observed in all 2500 ppm groups and in the 100 ppm F_2 group. No ocular cloudiness was observed in the recovery litters or any of the controls. At gross necropsy, there was an increase in the incidence of opaque or cloudy eyes was observed in 2500 ppm F_1 and F2 males and females and 100 ppm F_2 males.

At histological examination, increases were observed in the incidence of minimal to marked ocular keratitis in 100 and 2500 ppm F_1 and F2 males and females and in 10 ppm F_2 males. Increases in minimal to moderate corneal vascularization were observed in 100 and 2500 ppm F_1 and F2 males and females. Slight to marked unilateral or bilateral cataractous change was observed in the 2500 ppm F_1 males and females, but the incidences were low (2-3% treated vs 0% controls) and no changes of this nature were reported for the F_2 or F_3 groups.

At clinical examination, an increase in the number of pups with ocular discharge was observed in the 2500 ppm F_1 and F_2 litters and 100 ppm F_2 litters. The effect was not observed in any of the F_3 litters. This finding may be related to the increased incidences of closed eyelids at gross necropsy that were observed in 2500 ppm F_1 males and females.

A pattern of nephrotoxicity consisting of increased kidney weights and increased macroscopic and microscopic renal hydronephrosis was observed in the pups. The findings are as follows:

There was an increase ($p \leq 0.05$ or 0.01) in the absolute and/or adjusted (to body weight) kidney weights in F_2 males and females. At gross necropsy, the incidence of bilateral renal pelvic dilatation was increased in 100 and 2500 ppm F_3 continuous treatment males and females, but the findings were not dose-dependent in the females. The incidence of bilateral hydronephrosis at terminal necropsy, as determined under 2X magnification, was increased as follows: 100 and 2500 ppm F_1 , F_2, and F_3 continuous treatment males and females; 10 ppm F_1 males and

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females; and 10 ppm F_2 males. At histological examination, minimal to marked bilateral hydronephrosis was increased in 10, 100, and 2500 ppm F_1 and F_2 males and females. Minimal to marked bilateral hydronephrosis was also observed in 100 and 2500 ppm F_3 continuous treatment males and females. In the recovery animals, the incidences of bilateral hydronephrosis were low and similar to controls.

There were differences ($p \leq 0.05$ or 0.01) observed in liver (↑9-18%) and epididymides (↓22-24%) weights, but the differences were not clearly dose-dependent.

Plasma tyrosine levels were significantly increased in F_3 male pups under continuous treatment at all treatment doses (↑979 - 2374%). Levels were significantly increased in F_3 female pups under continuous treatment at 100 and 2500 ppm (↑633 - 960%). Animals of both sexes in the recovery groups had similar plasma tyrosine levels as the controls at all doses.

The LOAEL for systemic offspring toxicity is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes) based on significantly increase plasma tyrosine levels in male pups. No NOAEL was determined.

Decreased mean litter sizes with a related decrease in whole litter weights were observed. Mean litter size was decreased ($p \leq 0.05$ or 0.01) throughout lactation in all 2500 ppm groups, including F_3 recovery litters. Mean litter size was also decreased in the 10 and 100 ppm F_2 litters. The livebirth index was decreased ($p \leq 0.05$ or 0.01) in 2500 ppm F_2 and F_3 continuous treatment litters and in the 100 ppm F_3 recovery litters. The day 22 viability index was decreased ($p \leq 0.01$) in F_1 and F_2 litters. The proportion of litters with whole litter losses was increased in the 2500 ppm F_2 and F_3 continuous treatment litters. There was no difference in the F_1 litters and the difference in the F_3 continuous treatment litters was not significant. Whole litter weights were decreased throughout lactation ($p \leq 0.05$ or 0.01) in the 2500 ppm F_1, F_2, and F_3 continuous treatment litters; beginning at approximately the first week of lactation in the 100 ppm F_1 and F_2 litters; and at PND 11, 15, and 29 in the 10 ppm F_2 litters. In the F_3 recovery litters, mean litter size was decreased ($p \leq 0.05$) throughout lactation in the 2500 ppm litters. The livebirth index was decreased ($p \leq 0.01$) only in the 100 ppm litters and whole litter weights were decreased ($p \leq 0.05$) in the 2500 ppm litters only on PND 1.

The LOAEL for reproductive toxicity is 10 ppm (equivalent to 1.1/1.2 mg/kg/day [M/F]) based on decreased litter size. The reproductive NOAEL is 2.5 ppm (equivalent to 0.3 mg/kg/day for both sexes).

Even though no systemic NOAEL was determined for parents or offspring, the reproductive study is determined to be **acceptable/guideline (§83-4[a])** and satisfies the guideline requirement for a multigenerational reproductive toxicity study in rats 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 data was provided for

the dose rationale. In addition, no observations were made pertaining to estrus cycle length and periodicity or sperm and male reproductive organ measures; however, these tests were not required by the guidelines in use at the time the study was performed.