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DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.3800 [§83-4]; Multigeneration Reproduction Study in Rats

Work Assignment No. 4-1-128 M (MRID 46808235)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - Rats; OPPTS 870.3800 [§83-4]; OECD 416

PC CODE: 129108 **DP BARCODE:** D331116
TXR#: 0054348

TEST MATERIAL (PURITY): XDE-570 (99.3% a.i.)

SYNONYMS: Florasulam; *N*-(2,6-Difluorophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo (1,5-*c*)pyrimidine-2-sulfonamide; XR-570; XRD-570; DE-570

CITATION: Liberacki, A. B., Carney, E. W. and R. J. Kociba (1997) XDE-570: two-generation dietary reproduction study in CD rats. Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: 960030, November 13, 1997. MRID 46808235. Unpublished.

SPONSOR: Dow AgroSciences Canada, Inc., 2100-450 1 St. SW, Calgary, AB, Canada

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 46808235), XDE-570 (Florasulam; 99.3% a.i.; Lot No. 940714) was administered in the diet to 30 CD (Sprague Dawley) rats/group at dose levels of 0, 10, 100, or 500 mg/kg/day. The P generation parents were dosed for at least 70 days before they were mated to produce the F1 litters. From the F1 weanlings, 30 rats/sex/dose were selected to be parents and were fed the same test diet concentrations as their parents for 70 days prior to mating to produce the F2 litters.

No adverse treatment-related effects were observed on mortality, clinical signs, or gross pathology.

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Systemic toxicity was observed at 500 mg/kg. During pre-mating, body weights ($p<=0.05$) and food consumption (not significant [NS]) generally were decreased during Weeks 3-10, resulting in decreased (NS) overall (Weeks 0-10) body weight gains in the F1 males and in the P and F1 females. During gestation, body weights ($p<=0.05$) and food consumption (NS) were decreased during gestation days (GD) 0-21, resulting in decreased ($p<=0.05$) overall (GD 0-21) body weight gains in the P and F1 females. During lactation, body weights were decreased ($p<=0.05$) during lactation days (LD) 1-14; however, food consumption and overall (LD 1-21) body weight gains were not adversely affected.

Additionally at 500 mg/kg/day, relative (to body weight) kidney weights were increased ($p<=0.05$) in the F1 males (incr. 19%) and in the P and F1 females (incr. 18-19%), and very slight multi-focal hypertrophy of the collecting ducts was observed in both sexes in both generations (70-83% treated vs. 0 controls). Although the kidney findings were not associated with histopathological findings in this study (urinalysis and clinical chemistry not measured) adverse kidney effects (increased kidney weights, hypertrophy, and histopathology) were observed in subchronic and chronic rat studies at $\geq$ 250 mg/kg/day. Therefore, these findings are considered adverse.

The LOAEL for parental toxicity is 500 mg/kg/day, based on decreased body weights, body weight gains, and food consumption, as well as increased relative kidney weights, and hypertrophy in both sexes. The NOAEL is 100 mg/kg/day.

No adverse treatment-related effects were observed on birth index, live birth index, or viability indices, clinical signs, developmental landmarks, kidney weights, or gross pathology.

Transient decreases ($p<=0.05$) in the 500 mg/kg/day pup body weights were observed on PND 4 pre-culling (F1 males) and PND 7 (F1 females and F2 males and females); however, by PND 21, all treated groups were similar to controls. These transient decreases were not considered adverse.

The LOAEL for offspring toxicity is not determined and the NOAEL is 500 mg/kg/day.

There were no effects of treatment on any reproductive parameter in either generation, including: estrous cycle length and periodicity; mating, fertility, and gestation indices; and pre-coital and gestation durations.

The LOAEL for reproductive toxicity is not determined and the NOAEL is 500 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

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

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NOTE: This DER summarizes EPA conclusions regarding effects observed in the reproduction study in rats. A detailed DER completed by the Canadian Pest Management Regulatory Agency (PMRA) is attached.

COMMENTS:

PMRA selected an offspring LOAEL of 500 mg/kg/day (NOAEL = 100 mg/kg/day) based on a decrease in pup body weight. The decreases observed were transient in nature, seen only at PND 4 pre-culling (F1 males) and PND 7 (F1 females and F2 males and females); however, by PND 21, all treated groups were similar to controls. These transient decreases were not considered adverse. Therefore, the offspring LOAEL/NOAEL is not determined.

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Reviewer: Tom Morris , Date April 27, 2000.

STUDY TYPE: Multi-generation Reproduction Study - [rat] OPPTS 870.3800; OECD 416.

TEST MATERIAL (PURITY): XDE-570 (Purity - 99.3%)

SYNONYMS: XR-570, XRD-570, DE-570, florasulam.

CITATION: Liberacki, A. B., Carney, E. W. and Kociba, R. J. . November 13, 1997. **XDE-570; Two-Generation Dietary Reproduction Study in CD Rats.** Performing Laboratory: Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: 960030. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a 2-generation study (1 litter/generation), XDE-570 (Purity - 99.3%) was administered *ad libitum* in the diet to 30 CD (Sprague-Dawley derived) rats/sex/group at dose levels of 0, 10, 100 or 500 mg/kg bw/d from about 6 weeks (P1 adults) or 3 weeks (P2 adults) of age, through to termination. The pre-mating period was at least 10 weeks for both generations. There was one 2-week mating period in each generation; animals were paired one male to one female. On day 4 postpartum, litters were culled by random selection to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible).

There were no treatment-related mortalities, adverse clinical signs or gross pathological findings. Lower body weight, body-weight gain and food consumption were observed in P2 males at 500 mg/kg bw/d throughout most of the pre-mating period. P1/P2 females at 500 mg/kg bw/d exhibited lower body weight, body-weight gain and food consumption throughout most of the pre-mating and gestation periods. Body weights remained lower throughout lactation period in the P1/P2 females at 500 mg/kg bw/d. During the last two weeks of lactation body-weight gain was significantly higher in P1/P2 females at 500 mg/kg bw/d. The higher body-weight gain in these animals was most likely a reflection of compensatory body-weight gain and not an indication of toxicity. Food consumption was slightly higher during most of the final two weeks of lactation in the P1/P2 females at 500 mg/kg bw/d, this correlated with the higher body-weight gain over the same time period in these animals. Reproductive parameters, litter parameters and sexual maturation of external sexual organs of F1 male and female weanlings were unaffected at dose levels up to and including 500 mg/kg bw/d. In the F1 pups at 500 mg/kg bw/d, body weight was significantly lower on lactation day 4 prior to cull (♂only, ↓ ≈7%) and on lactation day 7 (↓ ≈10 and 9% in ♂ and ♀, respectively). In the F2 pups at 500 mg/kg bw/d, body weight was significantly lower on lactation day 7 (↓ ≈ 15 and 12% in ♂ and ♀, respectively). By lactation day 14, body weight in these animals was comparable to controls. This transient decrease in pup body weight may have been secondary to lower food consumption of F1 and F2 dams at 500 mg/kg bw/d early in the lactation period (days 1-4 and possibly days 4-7). Relative kidney weight was significantly increased in P1 females at 500 mg/kg bw/d and in both P2 males and females at 500 mg/kg bw/d. P1 males at 500 mg/kg bw/d also exhibited increased kidney weights (≈6%), however, this was not statistically significant. Although the increased relative kidney weight may be secondary to lower terminal body weight in these animals a treatment-related effect could not be dismissed since similar findings were observed in the 13-week and 2-year dietary studies with Fischer 344 rats and the increased kidney weight correlated with histopathological findings in the kidneys, specifically with an increased incidence of hypertrophy of epithelial cells of the collecting ducts in the P1/P2 males and females at 500 mg/kg bw/d. The hypertrophied cells were compatible with the intercalated cells which are involved in the regulation of acid-base balance through the modulation of bicarbonate resorption and hydrogen ion secretion in the collecting ducts. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability, however, no urinalysis was done. Morphologically the lesions were similar to those observed in Fischer 344 rats following dietary treatment for 13-weeks and 2-years at similar dose levels where urinary acidification and decreased urinary specific gravity were observed. However, in

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the 13-week and 2-year dietary studies there were no significant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with the histopathological findings or to indicate impaired renal function and there was no increased incidence of cellular degeneration or necrosis evident in the kidneys. In the 13-week dietary study, the lesions were not apparent in either sex following the 4-week recovery period suggesting that the lesions were reversible. There were no treatment-related gross pathological findings in the F1 or F2 weanlings.

The parental LOAEL was 500 mg/kg bw/d, based on lower body weight, body-weight gain and food consumption (P2 ♂ and P1/P2 ♀), increased kidney weights (P2 ♂ and P1/P2 ♀) and hypertrophy of the epithelial cells of the collecting ducts (P1/P2 ♂/♀). The parental NOAEL was 100 mg/kg bw/d.

The LOAEL for offspring was 500 mg/kg bw/d based on a transient lower pup body weight (P1/P2 ♂/♀). The NOAEL for offspring was 100 mg/kg bw/d.

The LOAEL for reproductive effects was not determined. The NOAEL for reproductive effects was 500 mg/kg bw/d, the highest dose tested.

This study is acceptable / guideline and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800); OECD 416 in rats.

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

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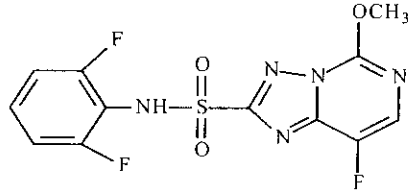
I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-570 as named in the study. Chemical Name (CA nomenclature): N-(2,6-diflurophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulphonamide

Description:	White powdery solid
Lot/Batch #:	Test Substance # 100511 / Lot # 940714
Purity:	99.3 % a.i. (determined by HPLC with ultra-violet detection).
Compound Stability:	The test substance was re-assayed after study determination and was confirmed at 99.3% (Knowles, et al., 1997, Lab Report Code GHE-P-6448)
CAS #:	145701-23-1
Structure	



2. **Vehicle and/or positive control:** Administered as a dietary admixture.

3. **Test animals:**

Species:	Male and female rats (females were nulliparous and non-pregnant)
Strain:	CD (Sprague-Dawley derived)
Age at study initiation:	(P) ≈6 weeks of age; (F_1) ≈4 weeks of age (offspring for F1 adults were selected at weaning on lactation day 21 and exposure to test substance began the following week).
Wt. at study initiation:	(P) Males: 170.4 to 171.1 g; Females: 138.6 to 140.8 g (F_1) Males: 70.4 to 200.4 g; Females: 90.2 to 156.5 g
Source:	Charles River Breeding Laboratory, Portage MI.
Housing:	During the acclimatization period and study rats were housed individually in wire mesh, stainless steel cages. From ≈day 19 of gestation, females were housed individually in plastic cages provided with ground corn cob nesting material. During the mating period females were co-housed with a single male from the same dose group until pregnancy occurred or two weeks had elapsed.
Diet:	Purina Certified Rodent Chow # 5002 (Purina Mills Inc., St. Louis. MO) *ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	**Temperature:** 22 °C **Humidity:** 40-60% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	At least 2 weeks.

B. PROCEDURES AND STUDY DESIGN

1. **Mating procedure:** Each female was placed with a single male from the same dose level (1:1 mating) until pregnancy occurred or two weeks had elapsed. During each breeding period, daily vaginal lavage samples were evaluated for the presence of sperm as an indication of mating. The day sperm were detected or a vaginal plug was observed *in situ* was considered day 0 of gestation. The sperm or plug positive (presumed pregnant) females were separated from the male and returned to their individual cages. If mating did not occur after two weeks, the animals were separated without further opportunity for mating. For P2 mating, co-habitation of male and female littermates was avoided.

2. **Study schedule:**

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Table 1. Study Schedule (a)

Weeks on Study	P1	F1/P2	F2
1-10	Exposure of P1 ♂/♀ prior to mating		
11-12	P1 mating period for F1 litters		
14-15		F1 born and litters culled on day 4 post-partum to 8 pups/litter	
17-18		F1 litters weaned on day 21 post-partum; offspring selected for P2 adults; one F1 pup /sex/dose/litter selected for necropsy; remaining pups euthanised.	
19-28	Necropsy of P1 adults	Exposure of P2 ♂ / ♀ prior to first mating	
29-30		*Mating period of P2 for F2 litters*	
32-33			F2 born and litters culled on day 4 post-partum to 8 pups/litter
35-36			F2 litters weaned on day 21 post-partum; 1 F2 pup / sex / dose / litter selected for necropsy; remaining pups euthanised
37			Necropsy of P2 adults

(a) Obtained from page 41 of study report.

3. Animal assignment: - For randomization procedure, the animals were weighed and ranked according to body weight and those from the extremes of the distribution were identified and removed from the population until only the number of animals required for the study remained. The animals were randomly assigned by weight to the treatment groups, as indicated in Table 2, in order to increase the probability of uniform mean weights and standard deviations at the initiation of the study.

TABLE 2 Animal Assignment.

Test Group	Dose in Diet (a) (mg/kg bw/d)	Animals/group			
		P1 Males	P1 Females	P2 Males	P2 Females
Control	0	30	30	30	30
Low	10	30	30	30	30
Mid	100	30	30	30	30
High	500	30	30	30	30

(a) Diets were administered from beginning of the study until sacrifice.

4. Dose selection rationale: The dose levels were selected based on a 13-week dietary probe study in CD rats (Liberacki, A.B. et al, September 26, 1996. Laboratory Project Study ID: DR-0312-6565-025, study submitted but a

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full review was not completed). Groups of 10 CD (Sprague-Dawley derived) rats/sex/dose were administered XDE-570 *ad libitum* in the diet at dose levels of 0, 100, 500 or 1,000 mg/kg bw/d. Significant findings at 1,000 mg/kg bw/d included increased mortality possibly due to renal papillary necrosis (3/10 ♂; 0/10 ♀) decreases in food consumption (♂/♀, 12-55%), significant decreases in body weight (41 and 24% in ♂ and ♀, respectively), significant increases in kidney weights (♂/♀, 36-73%) and histopathological findings. Histopathological observations included renal tubular degeneration / regeneration including tubular necrosis, papillary necrosis and hypertrophy of the epithelial cells of the collecting ducts in the kidneys of both sexes at 1,000 mg/kg bw/d. At 500 mg/kg bw/d, findings included slightly decreased body weight (♂ only) and slightly increased relative kidney weights (5 and 7% in ♂ and ♀, respectively) accompanied by very slight to slight hypertrophy of the epithelial cells of the collecting ducts. *The LOAEL was 500 mg/kg bw/d. The NOAEL was 100 mg/kg bw/d.* The high dose, 500 mg/kg bw/d, was selected based on anticipated parental body weight effects and histopathological alterations in the kidneys at this dose level. It was determined that the steep dose response for more severe body weight effects and renal histopathology at the higher dose (1,000 mg/kg bw/d) precluded the use of higher doses in the main 2-generation reproduction study.

5. Dosage preparation and analysis - Diets were prepared weekly by serially diluting a test substance/feed concentrate (pre-mix). The test diets were stored at room temperature. The concentration of the test substance in the diet was calculated from weekly body weight and food consumption data to maintain the targeted dose levels on a mg/kg bw/d basis. The stability of the test substance in basal rodent diet feed was determined to be at least 30 days at a concentration of approximately 0.01% w/w (Engle, K. E., 1995, Stability Analytical report 95-57, Dow Chemical Company Internal Report). As the low dose for this current study was conducted at essentially the same concentration, re-analysis to confirm stability was not conducted. Analyses of the test diets to determine test substance concentration were performed at least 3 times per generation. Homogeneity of the diets using the current mixing procedures was determined 3 times during the study. Reference samples (1/sex/dose/mixing plus pre-mix) were retained and stored at room temperature.

To avoid potential overdosing during the mating period, animals co-housed were provided with the lower of the two concentrations (female) for that dose group (low, mid or high). During gestation, females from each dose group were provided with the appropriate dietary concentration given during mating. In order to deliver constant mg/kg bw/d dose during lactation, dietary concentrations supplied during lactation were adjusted by one-third or one-half based on historical control food consumption data for lactating females. Until all litters were weaned, weanlings chosen for the P2 generation received a diet containing the same concentration of the test substance that was given to the P1 females during the third week of lactation. Dietary concentrations for the P2 generation were calculated as described for the P1 animals.

Results

Homogeneity Analysis: Analyses indicate that the distribution of the test substance in the rodent chow was homogenous.

Date Mixed	7/25/96	10/17/96	02/13/97
Dose Level (mg/kg bw/d)	10 (♀)	10 (♀)	10 (♀)
Concentration Range (%w/w)	0.00946 - 0.00975	0.0138 - 0.0144	0.0137 - 0.0140
Mean Concentration (%w/w)	0.00959	0.0141	0.0139
Standard Deviation	*0.00010*	*0.0003*	0.0001
%RSD	1.04	2.13	0.72

Stability Analysis: The stability of the test substance in basal rodent diet feed was determined to be at least 30 days at a concentration of approximately 0.01% w/w (Engle, K. E., 1995, Stability Analytical report 95-57, Dow

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Chemical Company Internal Report). As the low dose for this current study was conducted at essentially the same concentration, re-analysis to confirm stability was not conducted.

Concentration Analysis: The concentration of the test substance in the individual diets measured during the study period ranged from 88 to 104% of target with average concentrations ranging from 92-104% of target.

Dose level (mg/kg bw/d)	Range (% of target concentration)		Mean ± SD (% of target concentration)	
	Males	Females	Males	Females
10	88-95	90-96	92 ± 4	92 ± 2
100	96-99	95-98	97 ± 1	97 ± 1
500	97-101	97-101	99 ± 2	99 ± 1
10 (a)	-	98	-	98
250 (a)	-	104	-	104
500 (a)	-	102	-	102
4.5% w/w Premix	99-104		101 ± 2	

(a) Lactation diet, one half normal concentration, only one sample time.

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

C. OBSERVATIONS

1. Parental animals: - Throughout the study period, each animal on study was observed twice daily for mortality, morbidity and moribundity. Thorough clinical examinations were conducted weekly on all P1 and P2 animals. All adult animals found dead or in moribund condition were submitted for a gross pathological exam. Any pup found dead during the lactation phase was examined grossly to the extent possible and discarded. Body weight and food consumption were recorded weekly for all parental animals during the 10 week pre-mating period beginning on or before the first week of the study. Body weight for males were recorded weekly throughout the course of the study. Sperm positive females were weighed on days 0, 7, 14 and 21 days of gestation. Females that delivered litters were weighed on days 0, 4, 7, 14 and 21 of lactation. Females that failed to deliver were not weighed. Food consumption was not determined during mating due to co-housing. Following completion of mating, food consumption was determined weekly in males. During gestation, food consumption was determined at weekly intervals in sperm/plug positive females. After parturition, food consumption was determined twice during the first and second week of lactation and at 2-3 day intervals during the last week of lactation. Food consumption was not determined in non-confirmed females or in females that failed to deliver. A similar schedule was followed for the P2 generation.

2. Litter observations: - Females were observed for signs of parturition beginning on or about day 20 of gestation. In so far as possible, parturition was observed for signs of difficulty or unusual duration. The day of delivery was recorded as the first day the presence of the litter was noted and was designated as lactation day 0. All litters were examined as soon as possible after delivery. The following litter observations (X) were made (see Table 3).

TABLE 3: F_1/F_2 Litter Observations (a)

Observation	Time of observation (lactation day)						
	Day 0	Day 1	Day 4 (a)	Day 4 (b)	Day 7	Day 14	Day 21

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Number of live pups	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	X	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X	X

(a) Data extracted from pages 21-22 of study report.
(a) Before standardization (culling)
(b) After standardization (culling)

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible). Cull pups were selected using a computer generated randomization procedure. Preferential culling of runts was not performed. Litters with 8 or fewer pups were not culled. All pups which were culled were examined grossly, euthanised by deposition of Socumb euthanasia solution into the oral cavity and discarded. All litters were weaned on lactation day 21. Any weanlings not selected for the next generation adult animals or for necropsy were examined grossly, euthanised by CO_2 and discarded. All F1 weanlings selected for mating were observed daily for vaginal opening beginning on post-natal day 30 or preputial separation beginning on day 35.

3. Postmortem observations:

1) Parental animals: - A complete necropsy was conducted on all P1 and P2 adults. The scheduled necropsy was performed after the last litter of the respective generation had been weaned. Vaginal lavages of all P1 and P2 females were conducted on the day of their respective necropsy and slides were prepared to determine the stage of the estrous cycle. After being fasted overnight, the adults were weighed, anaesthetized with methoxyflurane and euthanised. The eyes were examined *in situ* by gently pressing a moistened glass slide against the cornea and observing the eyes under fluorescent light. All tissues/organs marked with an X in the following table were collected from each rat and preserved in neutral, phosphate-buffered 10% formalin, except for the testes and epididymides which were preserved in Bouin's fixative. The kidneys, testes, epididymides and ovaries were weighed prior to fixation and organ-to-body weight ratio was calculated for the P1/P2 parental animals. The lungs were infused with formalin to their approximate normal inspiratory volume. The nasal cavity was flushed with formalin via the pharyngeal duct to ensure rapid fixation of the tissue. The number of visible implantation sites present at the time of necropsy was noted for all P1 and P2 females. Moribund animals and those found dead were necropsied in a similar manner, except terminal body weight and organ weights were not recorded. Histopathological examination was done on potential target organ tissues, reproductive tissues and gross lesions (tissues marked with an XX in the following table) in the control and high-dose groups and on a single P2 adult female at 100 mg/kg bw/d which was terminated in moribund condition prior to the scheduled necropsy. Examination of tissues from the low and middle dose groups was limited to the kidney and gross lesions. Sections of urinary bladder (present as tissue adjacent to certain reproductive tissues) and skin (present as tissue adjacent to mammary gland) were not routinely subjected to histopathological examination except in those cases wherein a gross lesion had been described. Tissues were prepared for light microscopic evaluation by standard procedures, sectioned at 6 µm thickness and stained with hematoxylin and eosin.

	DIGESTIVE SYSTEM		CARDIOVASC./HAEMAT.		NEUROLOGIC
X	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Peripheral .nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
XX	Stomach	X	Lymph nodes	XX	Pituitary
X	Duodenum	X	Spleen	XX	Eyes (optic n.)

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X	Jejunum	X	Thymus		**GLANDULAR**
X	Ileum			X	Adrenal gland
X	Cecum		**UROGENITAL**	X	Lacrimal / Harderian gland
X	Colon	XX	Kidneys	XX	Mammary gland
X	Rectum	XX	Urinary bladder	X	Parathyroids
XX	Liver	XX	Testes	X	Thyroids
	Gall bladder	XX	Epididymides		**OTHER**
X	Pancreas	XX	Prostate	X	Bone
XX	Mesenteric tissue	XX	Seminal vesicle	X	Skeletal muscle
	RESPIRATORY	XX	Coagulation glands	XX	Skin and subcutis
X	Trachea	XX	Ovaries	XX	All gross lesions and masses
X	Lung	XX	Uterus		
X	Nose	XX	Oviducts		
X	Pharynx	XX	vagina/cervix		
X	Larynx	XX	Preputial/clitoral gland		

X Tissue collected and preserved at necropsy
XX Tissue fixed and selected for histopathological examination

2) Offspring: - At the time of weaning, 1 pup/sex/litter from the F1 and F2 litters was randomly selected for a complete necropsy. In order to control for variation in body and organ weight, pups selected for necropsy were euthanised at the same age (postnatal day 22). The pups were anaesthetized with methoxyflurane, weighed and euthanised by decapitation. For F1 and F2 pups that were examined microscopically (1/sex/litter), the kidneys were weighed and the organ-to-body weight ratios were calculated. Gross pathological examination and preservation of tissue samples was performed as described for adults, except weanlings were not fasted overnight. Histopathological examination of weanling tissues was not performed.

D. DATA ANALYSIS

1. Statistical analyses: Descriptive statistics (means and standard deviations) were reported for food consumption. Body and organ weights were first evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, either a parametric or non-parametric ANOVA was performed. If the ANOVA was significant, a Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Gestation length, average time to mating, vaginal opening and preputial separation and litter size data were analysed using a non-parametric ANOVA. If the ANOVA was significant, the Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Statistical outliers were identified by the method of Grubs (1969) and were routinely excluded from analysis for food consumption only. Outliers for body weight, gestation length, litter size and average time to mating were only excluded from analysis for documented scientifically sound reasons. Fertility indices were analysed by the Fischer exact probability test with Bonferroni's correction. Evaluation of neonatal sex ratio was performed by the binomial distribution test. Survival indices and other incidence data among neonates were analysed using the litter as the experimental unit by the Wilcoxon test as modified by Haseman and Hoel (1974) with Bonferroni's correction. Both the Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control to keep the experiment-wise error rate at 0.05. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was much greater than the cited alpha levels would suggest. Thus the final interpretation of numerical data took into consideration statistical analyses along with other factors such as dose-response relationships and whether the results were significant in light of other biologic and pathologic findings.

2. Indices:

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

$$\text{Male Fertility Index} = \frac{\text{No. of males impregnating females}}{\text{No. of males used for mating}} \times 100$$

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$$\text{Female Fertility Index} = \frac{\text{No. of females pregnant}}{\text{No. of females paired}} \times 100$$

$$\text{Gestational Index} = \frac{\text{No. of females with live litters}}{\text{No. of females pregnant}} \times 100$$

$$\text{Live Birth Index} = \frac{\text{No. of live pups at birth}}{\text{No. of pups born}} \times 100$$

$$\text{Birth Index} = \frac{\text{total \# pups born/litter}}{\text{total \# implantations sites/litter}} \times 100$$

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

$$\text{Day 1 Survival Index} = \frac{\text{No. of live pups at day 1}}{\text{No. of live pups at day 0}} \times 100$$

$$\text{Day 4 Survival Index} = \frac{\text{No. of live pups at day 4 (precull)}}{\text{No. of live pups at day 1}} \times 100$$

$$\text{Day 7 Survival Index} = \frac{\text{No. of live pups at day 7}}{\text{No. of live pups at day 4 (postcull)}} \times 100$$

$$\text{Day 14 Survival Index} = \frac{\text{No. of live pups at day 14}}{\text{No. of live pups at day 7}} \times 100$$

$$\text{Day 21 Survival Index} = \frac{\text{No. of live pups at day 21}}{\text{No. of live pups at day 14}} \times 100$$

$$\text{Lactation Index} = \frac{\text{No. of live pups at day 21}}{\text{No. of live pups at day 4 (postcull)}} \times 100$$

$$\text{Viability index} = \frac{\text{Mean \# live pups per litter on day 4}}{\text{Mean \# pups per litter born alive}} \times 100$$

$$\text{Weaning index} = \frac{\text{Mean \# pups per litter alive on day 21}}{\text{Mean \# pups per litter kept at day 4}} \times 100$$

3. **Historical control data**: Historical data (within 5 years of study conduct) on reproduction indices, litter size and pup body weights in CD (Sprague-Dawley derived) rats were provided in the study report.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs:

Mortality: There were no treatment-related mortalities. With the exception of one P2 female at 100 mg/kg bw/d, all

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P1 and P2 adults survived to the scheduled necropsy. The P2 female at 100 mg/kg bw/d was observed to have a distended abdomen on study day 116 and was sent to necropsy in moribund condition on study day 117. Gross necropsy and subsequent histopathological examination of this animal revealed the moribund condition to be the result of severe, subacute to chronic, diffuse inflammation of the uterus. Due to the isolated nature of the occurrence in a single female at 100 mg/kg bw/d, this death was not considered to be treatment-related.

Clinical signs: There were no significant adverse treatment-related clinical observations. Soiling of the perineal area was observed in P1/P2 males and females at 500 mg/kg bw/d (Table 4). The soiling appeared to be urine dried to the fur of the perineum. Perineal soiling was considered to be a secondary or indirect effect, possibly due to lack of grooming resulting from urine acidification or the presence of excretory products of the test substance in the urine. Reddish urine was observed in one P1 male at 100 mg/kg bw/d, in two P1 males at 500 mg/kg bw/d and in one P2 male at 500 mg/kg bw/d. In one P1 male at 500 mg/kg bw/d, the reddish urine was possibly associated with inflammation of the renal papilla with resultant haemorrhagic cast in the lumen of the urinary bladder. However, in the remaining P1 and P2 animals exhibiting reddish urine there were no corroborating gross pathological or histopathological findings; therefore, the toxicological significance is uncertain. Due to the low rate of incidence of reddish urine, the absence of corroborative gross pathological or histopathological findings in most of the animals and the incidence of sporadic occurrences of reddish urine in control animals from other reproduction studies, the presence of reddish urine was considered to be an incidental finding. There were no significant treatment-related clinical observations in the P1 or P2 females during gestation or lactation.

TABLE 4: Clinical Signs (expressed as incidence/number examined). (a)

Observation		Dose Level in Diet (mg/kg bw/d)							
		P1 Generation				P2 Generation			
		0	10	100	500	0	10	100	500
Perineal soiling (urine)	Males	0/30	0/30	0/30	5/30	0/30	0/30	1/30	9/30
	Females	0/30	0/30	2/30	14/30	0/30	0/30	0/30	9/30

(a) Data extracted from page 45 of the study report for P1 adults and pages 80-82 for P2 adults.

2. Body weight and food consumption:

Pre-mating: Pre-mating body weight, body-weight gain and food consumption are summarized in Table 5 (P1/P2 males) and Table 6 (P1/P2 females).

P1 adults: Compared to controls, food consumption and body weight were lower in the high-dose P1 females throughout the pre-mating period (weeks 0-10). Food consumption was significantly lower from week 4 onwards and body weight was significantly lower from week 5 onwards. The overall pre-mating body-weight gain (weeks 0-10) was approximately 17% lower in the high-dose females compared to controls (no statistical analysis done). No treatment-related differences in body weight, body-weight gain or food consumption were noted for P1 males at any dose level or for P1 females at 10 or 100 mg/kg bw/d.

P2 adults: Compared to controls, body weight was lower in the high-dose P2 males throughout the pre-mating period, statistical significance was achieved from week 4 onwards. This was associated with a concomitant decrease in food consumption from week 3 onwards. Body weight and food consumption continued to be lower in these animals throughout the 10-week post-mating period (week 10-20). The overall pre-mating body-weight gain (weeks 0-10) in the high-dose males was approximately 10% lower compared to controls (no statistical analysis done). Compared to controls, body weight and food consumption were lower in the high-dose P2 females throughout the pre-mating period. Body weight was significantly lower from week 4 onwards. The overall pre-mating body-weight gain (weeks 0-10) in the high-dose females was approximately 16% lower compared to controls (no statistical

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analysis done). No treatment-related differences in body weight, body-weight gain or food consumption were noted for P2 males or females at 10 or 100 mg/kg bw/d.

TABLE 5: Body Weight and Food Consumption - Pre-mating (P1 and P2 adult males) (a)

Observations/study week		Dose Level in Diet (mg/kg bw/d)			
		0	10	100	500
P1 Adult Males - Pre-mating (n = 30 animals/group)					
Mean body weight (g ± SD)	Week 0	170.4 ± 9.6	171.1 ± 10.9	170.5 ± 11.0	170.5 ± 10.8
	Week 1	223.8 ± 1.38	227.4 ± 12.5	226.9 ± 13.8	225.8 ± 15.1
	Week 2	273.5 ± 16.1	279.5 ± 18.0	279.3 ± 15.9	274.9 ± 19.4
	Week 3	320.6 ± 20.4	329.2 ± 19.6	328.9 ± 19.5	322.4 ± 25.8
	Week 4	353.6 ± 24.6	367.6 ± 23.4	366.0 ± 23.3	358.8 ± 31.0
	Week 5	381.6 ± 29.2	399.9 ± 26.9	397.8 ± 25.5	389.0 ± 35.4
	Week 10	478.3 ± 45.7	507.4 ± 39.0 *	506.0 ± 33.9 *	492.0 ± 53.5
Overall mean weight gain (g ± SD) (b)	Weeks 0 - 10	306.0 ± 4.4	332.4 ± 35.5	331.4 ± 37.5	320.2 ± 50.2
Mean food consumption (g/animal/d ± SD)	Week 1	23.7 ± 1.4	24.1 ± 1.3	24.1 ± 1.6	24.1 ± 1.8
	Week 3	26.0 ± 1.5	27.5 ± 2.0	27.3 ± 2.5	27.1 ± 3.0
	Week 5	26.2 ± 2.3	27.6 ± 2.0	27.7 ± 2.2	27.1 ± 3.2
	Week 10	26.4 ± 2.4	27.6 ± 2.3	28.0 ± 2.2	27.2 ± 3.6
P2 Adult Males - Pre-mating (n = 30 animals/group)					
Mean body weight (g ± SD)	Week 0	158.7 ± 20.4	156.1 ± 29.2	154.9 ± 24.1	153.9 ± 26.2
	Week 1	224.3 ± 24.1	225.7 ± 34.6	221.1 ± 27.6	219.7 ± 31.0
	Week 2	278.0 ± 27.7	282.7 ± 37.9	274.7 ± 39.0	266.1 ± 33.3
	Week 3	323.7 ± 27.7	327.4 ± 39.0	315.2 ± 29.0	303.6 ± 34.4
	Week 4	375.5 ± 30.5	382.0 ± 40.9	364.3 ± 31.1	348.8 ± 3.58 *
	Week 5	416.4 ± 32.5	426.5 ± 42.9	402.8 ± 34.3	386.7 ± 41.0 *
	Week 10	524.2 ± 40.7	546.4 ± 56.7	511.9 ± 46.5	483.5 ± 62.4 *
	Week 20	627.5 ± 47.8	652.1 ± 71.8	604.4 ± 60.7	544.0 ± 86.9 *
Overall mean weight gain (g ± SD) (b)	Weeks 0 - 10	365.5 ± 39.2	390.2 ± 40.8	357.0 ± 43.1	330.7 ± 63.1
Mean food consumption (g/animal/d ± SD)	Week 1	23.6 ± 2.1	23.7 ± 2.7	23.2 ± 2.3	23.7 ± 2.9
	Week 3	29.1 ± 2.6	29.5 ± 2.6	28.1 ± 2.3	26.6 ± 3.2
	Week 5	29.7 ± 2.5	30.4 ± 2.5	28.9 ± 2.5	29.1 ± 3.0
	Week 10	28.9 ± 2.6	29.3 ± 3.0	28.0 ± 2.2	27.6 ± 2.4
	Week 20	28.4 ± 2.5	28.2 ± 3.2	27.9 ± 1.9	25.9 ± 3.3

(a) Data extracted from pages 47-58 of the study report for P1 adults and pages 84-95 for P2 adults.
(b) Pre-mating weight gain was not provided in the study report, overall mean weight gain calculated by reviewer, no statistical analysis done.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
# Statistically different from control mean by Wilcoxon's test, $p \leq 0.05$.

TABLE 6: Body Weight and Food Consumption - Pre-mating (P1 and P2 adult females) (a)

Observations/study week	Dose Level in Diet (mg/kg bw/d)			
	0	10	100	500
P1 Adult Females - Pre-mating (n = 30 animals/group)				

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Observations/study week		Dose Level in Diet (mg/kg bw/d)			
		0	10	100	500
Mean body weight (g ± SD)	Week 0	140.7 ± 9.5	140.8 ± 8.4	138.6 ± 8.9	139.2 ± 8.5
	Week 1	164.7 ± 11.9	165.1 ± 11.8	164.9 ± 12.1	162.1 ± 11.4
	Week 2	187.5 ± 14.0	185.8 ± 14.7	187.0 ± 14.2	181.5 ± 14.1
	Week 3	205.8 ± 17.1	205.2 ± 16.0	207.0 ± 16.4	195.7 ± 17.3
	Week 4	222.3 ± 20.3	221.9 ± 18.5	223.1 ± 19.5	211.3 ± 21.7
	Week 5	236.9 ± 23.4	236.6 ± 21.5	237.2 ± 19.9	223.4 ± 21.1 *
	Week 10	282.5 ± 28.0	280.0 ± 26.7	280.7 ± 24.9	259.5 ± 24.2 *
Overall mean weight gain (g ± SD) (b)	Weeks 0 - 10	145.1 ± 27.5	139.2 ± 22.4	142.1 ± 22.7	120.2 ± 19.6
Mean food consumption (g/animal/d ± SD)	Week 1	18.0 ± 1.6	17.3 ± 1.3	17.7 ± 2.0	17.2 ± 1.5
	Week 3	18.7 ± 1.7	17.9 ± 1.7	19.0 ± 2.0	17.6 ± 1.7
	Week 5	19.8 ± 2.0	19.1 ± 1.9	19.2 ± 1.9	18.2 ± 1.7
	Week 10	19.2 ± 2.0	18.7 ± 2.3	18.6 ± 1.0	17.8 ± 1.7
P2 Adult Females - Pre-mating (n = 30 animals/group)					
Mean body weight (g ± SD)	Week 0	126.2 ± 16.3	130.1 ± 13.8	129.5 ± 14.3	125.7 ± 20.0
	Week 1	159.0 ± 15.9	163.4 ± 13.4	163.1 ± 13.2	155.1 ± 19.7
	Week 2	182.5 ± 16.6	185.8 ± 15.4	184.1 ± 14.7	173.1 ± 20.3
	Week 3	197.9 ± 19.0	203.7 ± 16.6	199.6 ± 14.9	187.6 ± 21.4
	Week 4	217.6 ± 19.2	223.4 ± 17.9	221.0 ± 17.6	202.6 ± 21.9 *
	Week 5	233.5 ± 19.8	239.8 ± 19.8	237.0 ± 18.8	216.3 ± 24.1 *
	Week 10	277.6 ± 23.3	285.4 ± 23.5	281.0 ± 28.2	252.6 ± 27.2 *
Overall mean weight gain (g ± SD) (b)	Weeks 0 - 10	151.4 ± 21.0	155.3 ± 24.0	151.5 ± 28.6	126.9 ± 22.8
Mean food consumption (g/animal/d ± SD)	Week 1	17.6 ± 1.5	17.3 ± 1.5	17.1 ± 1.3	16.6 ± 1.5
	Week 3	19.2 ± 1.8	19.0 ± 1.9	18.2 ± 1.7	17.2 ± 1.9
	Week 5	19.8 ± 1.7	19.5 ± 1.9	19.7 ± 1.8	18.5 ± 2.0
	Week 10	18.8 ± 1.8	18.5 ± 1.9	18.4 ± 1.9	17.9 ± 1.7

(a) Data extracted from pages 47-58 of the study report for P1 adults and pages 84-95 for P2 adults.
(b) Pre-mating weight gain was not provided in the study report, overall mean weight gain calculated by reviewer, no statistical analysis done.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
# Statistically different from control mean by Wilcoxon's test, $p \leq 0.05$.

Gestation: Gestation body weight, body-weight gain and food consumption are summarized in Table 7 (P1 females) and Table 8 (P2 females).

P1 females: Compared to controls, body weight remained significantly lower in the high-dose P1 females throughout gestation (≈8-11% lower). This was associated with lower food consumption throughout gestation (≈7-11% lower). In the high-dose females, the overall (gestation days 0-21) body-weight gain during gestation was significantly lower compared to controls (≈14% lower). This was attributed to a significantly lower body-weight gain during gestation days 14-21 (≈22% lower). No treatment-related differences in body weight, body-weight gain or food consumption were noted for P1 females at 10 or 100 mg/kg bw/d.

P2 females: Compared to controls, body weight remained significantly lower in the high-dose P2 females throughout gestation (≈9-14% lower). This was associated with lower food consumption throughout gestation (≈6-13% lower). Body-weight gain was lower throughout gestation in these animal. The overall (gestation days 0-21) body-weight gain during gestation was significantly lower compared to controls (≈23% lower). This was attributed to a significantly lower body-weight gain during gestation days 0-7 (≈20% lower) and gestation days 14-21 (≈29% lower). No treatment-related differences in body weight, body-weight gain or food consumption were noted for P2 females at 10 or 100 mg/kg bw/d.

Lactation: Lactation body weight, body-weight gain and food consumption are summarized in Table 7 (P1 females) and Table 8 (P2 females).

P1 females: Compared to controls, body weight in the high-dose P1 females remained lower throughout lactation

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(≈4-15% lower), this was statistically significant up to lactation day 14. However, overall body-weight gain (lactation days 1-21) was significantly higher in these animals compared to controls. This was attributed to a significantly higher body-weight gain during lactation days 7-14 and a significantly lower body-weight loss during gestation days 14-21 (all groups, including controls, exhibited a body-weight loss during lactation days 14-21). The significantly higher overall body-weight gain was considered to reflect a compensatory body-weight gain by the high-dose females and not an indication of toxicity. Food consumption in the high-dose females was lower (≈17% lower) on lactation days 1-4 and slightly higher during most of the last two weeks of lactation (≈3-12% higher). The higher food consumption during the last two weeks of lactation correlated with the body weight changes observed in these animals over the same time period. No treatment-related differences in body weight, body-weight gain or food consumption were noted for P1 females at 10 or 100 mg/kg bw/d.

P2 females Compared to controls, body weight in the high-dose P2 females remained lower throughout lactation (≈4-17% lower), this was statistically significant up to lactation day 14. However, overall body-weight gain (lactation days 1-21) was significantly higher in these animals compared to controls. This was attributed to a significantly higher body-weight gain during lactation days 7-14 and 14-21. The significantly higher overall body-weight gain was considered to reflect a compensatory body-weight gain by the high-dose females and not an indication of toxicity. Lactation food consumption in the high-dose females was lower on lactation days 1-4 (≈23% lower) and 4-7 (≈18% lower) and slightly higher during the last two weeks of lactation (≈4-14% higher). The higher food consumption during the last two weeks of lactation correlated with the higher body-weight gain over the same time period in these animals. No treatment-related differences in body weight, body-weight gain or food consumption were noted for P1 females at 10 or 100 mg/kg bw/d.

TABLE 7: Body Weight and Food Consumption - P1 Adult Females Gestation and Lactation (a)

Observations/study week		Dose Level in Diet (mg/kg bw/d)			
		0	10	100	500
P1 Adult Females - Gestation					
Mean body weight (g ± SD)		n = 24	n = 23	n = 20	n = 24
	Day 0	283.6 ± 24.8	283.8 ± 29.6	279.3 ± 19.0	257.9 ± 24.1 *
	Day 7	316.1 ± 25.9	316.9 ± 32.3	314.8 ± 21.7	288.8 ± 28.3 *
	Day 14	345.0 ± 27.3	346.5 ± 37.9	345.1 ± 20.3	317.4 ± 32.6 *
	Day 21	429.6 ± 36.2 (b)	424.8 ± 51.7	428.1 ± 23.5	383.5 ± 34.1 #
Mean weight gain (g ± SD)	Days 0-7	32.6 ± 7.5	33.2 ± 7.4	35.5 ± 9.4	30.9 ± 7.2
	Days 7-14	28.9 ± 6.4	29.6 ± 9.3	30.3 ± 8.4	28.6 ± 9.6
	Days 14-21	85.0 ± 11.6 (b)	78.3 ± 25.2	83.0 ± 13.8	66.1 ± 16.3 #
	Days 0-21	145.6 ± 19.5 (b)	141.0 ± 32.3	148.8 ± 19.8	125.6 ± 20.8 *
Mean food consumption (g/animal/d ± SD)	Days 0-7	22.5 ± 2.6	23.2 ± 2.8	22.3 ± 2.0	20.4 ± 2.3
	Days 7-14	24.4 ± 2.6	24.9 ± 3.3	24.5 ± 1.7	22.7 ± 3.7
	Days 14-21	23.5 ± 2.3	24.4 ± 3.4	23.5 ± 1.8	20.8 ± 2.8
P1 Adult Females - Lactation					
Mean body weight (g ± SD)		n = 24	n = 24 (c)	n = 22 (d)	n = 25 (e)
	Day 1	326.6 ± 26.7	329.0 ± 37.1	322.2 ± 23.9	281.8 ± 26.8 *
	Day 4	335.1 ± 27.9	334.9 ± 33.5	324.5 ± 24.0	285.2 ± 29.1 *
	Day 7	341.7 ± 26.3	342.3 ± 33.8	333.1 ± 21.5	296.9 ± 29.1 *
	Day 14	357.9 ± 24.1	357.2 ± 33.9	355.1 ± 20.6	332.4 ± 24.7 *
	Day 21	336.2 ± 22.5	340.2 ± 29.2	331.0 ± 21.7	322.4 ± 26.4
Mean weight gain (g ± SD)	Days 1-4	8.5 ± 7.9	5.9 ± 11.7	2.3 ± 12.4	3.4 ± 12.2
	Days 4-7	6.6 ± 5.1	7.4 ± 6.9	8.6 ± 14.5	11.7 ± 8.0
	Days 7-14	16.1 ± 9.1	14.9 ± 13.1	22.0 ± 10.9	35.5 ± 15.9 *
	Days 14-21	-21.7 ± 11.4	-17.0 ± 13.9	-24.1 ± 10.9	-10.0 ± 16.8 *
	Days 1-21	9.5 ± 16.2	11.2 ± 18.6	8.9 ± 16.5	40.6 ± 22.0 *

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Observations/study week		Dose Level in Diet (mg/kg bw/d)			
		0	10	100	500
Mean food consumption (g/animal/d ± SD)	Days 1-4	28.7 ± 4.5	27.1 ± 6.4	29.4 ± 7.6	23.9 ± 7.2
	Days 4-7	36.6 ± 4.6	36.6 ± 3.1	37.9 ± 4.0	35.7 ± 7.0
	Days 7-11	48.5 ± 4.1	48.3 ± 3.8	50.1 ± 3.5	50.9 ± 4.9
	Days 11-14	56.0 ± 3.5	55.2 ± 8.0	57.6 ± 4.7	60.7 ± 4.7
	Days 14-17	58.0 ± 5.7	57.2 ± 9.6	59.8 ± 5.5	65.1 ± 7.3
	Days 17-19	63.5 ± 10.4	60.2 ± 7.7	61.8 ± 6.4	65.7 ± 9.4
	Days 19-21	76.1 ± 13.1	73.3 ± 11.0	69.5 ± 7.3	74.7 ± 8.4

(a) Data extracted from pages 47-58 of the study report. Animals that were non-pregnant were excluded from analysis.
(b) Decrease in n value (n = 23) due to missing one dam that began delivering her litter prior to taking day 21 weight.
(c) Increase in n value due to inclusion of 2 dams which were never sperm positive but delivered litters and excluded 1 sperm positive dam which delivered a single dead pup.
(d) Increase in n value due to inclusion of 2 dams which were never sperm positive but delivered litters.
(e) Increase in n value due to inclusion of 1 dam which was never sperm positive but delivered a litter.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
# Statistically different from control mean by Wilcoxon's test, $p \leq 0.05$.

TABLE 8: Body Weight and Food Consumption - P2 Adult Females Gestation and Lactation (a)

Observations/study week		Dose Level in Diet (mg/kg bw/d)			
		0	10	100	500
P2 Adult Females - Gestation					
Mean body weight (g ± SD)		n = 23	n = 27	n = 25	n = 26
	Day 0	273.6 ± 26.1	286.2 ± 22.0	271.3 ± 25.0	249.5 ± 29.4 *
	Day 7	305.9 ± 26.1	316.6 ± 27.9	302.1 ± 26.7	275.3 ± 26.1 *
	Day 14	336.6 ± 27.3	343.5 ± 27.1	330.5 ± 26.7	303.5 ± 31.4 *
	Day 21	418.2 ± 32.0 (b)	419.2 ± 33.6	404.4 ± 27.7	360.9 ± 33.9 *
Mean weight gain (g ± SD)	Days 0-7	32.3 ± 7.8	30.5 ± 9.9	30.8 ± 8.0	25.8 ± 9.3 *
	Days 7-14	30.7 ± 6.6	26.9 ± 9.1	28.4 ± 9.6	28.1 ± 9.1
	Days 14-21	81.2 ± 14.8 (b)	75.7 ± 19.5	73.9 ± 1.3.3	57.4 ± 16.3 *
	Days 0-21	144.3 ± 14.8 (b)	133.0 ± 21.8	133.1 ± 16.3	111.4 ± 22.5 *
Mean food consumption (g/animal/day ± SD)	Days 0-7	21.1 ± 2.4	21.1 ± 3.0	19.6 ± 1.4	18.3 ± 2.0
	Days 7-14	23.7 ± 2.2	23.0 ± 2.9	22.9 ± 2.1	22.2 ± 2.3
	Days 14-21	22.2 ± 2.5	22.5 ± 2.2	21.4 ± 3.0	19.4 ± 3.3
P2 Adult Females - Lactation					
Mean body weight (g ± SD)		n = 24 (c)	n = 27	n = 25	n = 27 (d)
	Day 1	314.9 ± 29.6	320.9 ± 21.7	305.6 ± 21.0	266.6 ± 28.1 *
	Day 4	323.4 ± 26.1	327.5 ± 23.7	313.4 ± 23.2	267.2 ± 30.4 *
	Day 7	322.6 ± 23.7	336.8 ± 23.8	316.9 ± 22.1	271.1 ± 30.7 *
	Day 14	341.9 ± 21.7	346.9 ± 23.8	336.0 ± 25.2	313.4 ± 27.3 *
	Day 21	330.0 ± 23.3	338.0 ± 18.5	320.7 ± 21.1	316.2 ± 22.8
Mean weight gain (g ± SD)	Days 1-4	8.5 ± 11.0	6.6 ± 12.0	7.8 ± 9.4	0.6 ± 12.8 *
	Days 4-7	-0.8 ± 9.0	9.3 ± 10.2 *	3.5 ± 8.5	4.5 ± 9.8
	Days 7-14	19.3 ± 10.3	10.0 ± 14.2	19.1 ± 10.6	41.7 ± 17.9 *
	Days 14-21	-11.9 ± 13.0	-8.9 ± 14.5	-15.3 ± 16.5	2.8 ± 15.4 *
	Days 1-21	15.1 ± 14.4	17.1 ± 15.1	15.1 ± 14.9	49.6 ± 22.0 *
Mean food consumption (g/animal/d ± SD)	Days 1-4	29.5 ± 9.7	25.9 ± 5.8	29.3 ± 5.0	22.6 ± 7.0
	Days 4-7	37.7 ± 2/2	37.9 ± 5.9	38.0 ± 5.9	31.0 ± 7.6
	Days 7-11	46.9 ± 5.4	47.6 ± 5.5	48.6 ± 4.9	49.0 ± 6.0
	Days 11-14	55.8 ± 4.0	54.6 ± 5.8	56.7 ± 6.0	59.9 ± 6.1
	Days 14-17	58.3 ± 7.0	61.7 ± 5.1	60.3 ± 5.4	63.6 ± 5.7
	Days 17-19	61.5 ± 9.8	62.1 ± 9.5	64.3 ± 8.8	70.4 ± 7.9
	Days 19-21	67.7 ± 10.8	71.5 ± 7.8	72.1 ± 8.3	76.4 ± 8.2

(a) Data extracted from pages 84-95 of the study report. Animals that were non-pregnant were excluded from analysis.
(b) Decrease in n value (n = 22) due to missing one dam that began delivering her litter prior to taking day 21 weight.

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(c) Increase in n value due to inclusion of 1 dam which was never sperm positive but delivered a litter.
(d) Increase in n value due to inclusion of 1 dam which was never sperm positive but delivered a litter.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
# Statistically different from control mean by Wilcoxon's test, $p \leq 0.05$.

3. Test Substance Intake: Based on food consumption, body weight, and dietary analyses results, the doses expressed as mean daily mg test substance/kg bw/d during the 10-week pre-mating period are presented in Table 9. The concentration of the test substance in the diets was calculated weekly based on body weights and food consumption data to maintain the targeted dose levels on a mg/kg bw/d basis. The concentration of the test substance in the individual diets measured during the study period ranged from 88 to 104% of target with average concentrations ranging from 92-104% of target.

TABLE 9: Mean test substance intake during pre-mating (mg/kg bw/d) (a)

	Male			Female		
	LDT	MDT	HDT	LDT	MDT	HDT
P1	10	100	500	10	100	500
P2	10	100	500	10	100	500

(a) Data extracted from pages 19 and 43 of the study report.

4. Reproductive function:

a. Estrous cycle length and periodicity: Estrous cycle length and periodicity were not affected by treatment (based on vaginal lavage on day of necropsy). Reproductive performance and indices were unaffected by treatment and there were no treatment-related gross pathological or histopathological findings in the reproductive organs examined.

b. Sperm measures: Specific assessment of sperm was not carried out. However, reproductive performance and indices were unaffected by treatment and there were no treatment-related gross pathological or histopathological findings in the reproductive organs examined.

c. Sexual maturation (F_1): Maturation of external sexual organs in F1 males and females was unaffected by treatment up to and including 500 mg/kg bw/d (Table 10). A statistically significant decrease in the number of days to vaginal opening was noted for F1 females at 10 mg/kg bw/d. However, in the absence of a dose-related response this was not considered to be treatment-related.

TABLE 10: Developmental Milestones in F1 Weanlings. (a)

Dose Level in Diet (mg/kg bw/d)	Mean Age at Preputial Separation (days)	Mean Age at Vaginal Opening (days)
0 (n = 30)	46.5 ± 4.1	33.2 ± 1.9
10 (n = 30)	45.7 ± 4.9	31.9 ± 1.6 #
100 (n = 30)	45.0 ± 3.5	32.9 ± 1.8
500 (n = 30)	46.9 ± 5.1	33.0 ± 1.6

(a) Data extracted from pages 64 and 65 of study report.
Statistically different from control mean by Wilcoxon's test, $p \leq 0.05$.

5. Reproductive performance: - Reproductive performance was unaffected by treatment up to and including 500 mg/kg bw/d in the P1 and P2 adult animals (Table 11). A statistically significant increase in gestation length was

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observed in the P2 females at 10 mg/kg bw/d. However, this was within the historical control range for animals of this strain and age from this laboratory (Range: 21.5 - 22.0 days) and in the absence of a dose-response relationship this was considered to be an incidental finding.

TABLE 11: Reproductive Performance (a).

Observation	Dose Level in Diet (mg/kg bw/d)			
	0	10	100	500
P1 Adults				
Number of males/females co-housed	30/30	30/30	30/30	30/30
Time to mating (days)	2.9 ± 2.2	2.5 ± 2.1	3.2 ± 3.2	2.6 ± 2.5
Females not pregnant (not sperm/plug positive or never delivered)	6	5	8	5
Females pregnant (sperm/plug positive or delivered)	24	25	22	25
Male Fertility Index	80 (24/30)	83.3 (25/30)	73.3 (22/30)	83.3 (25/30)
Female Fertility Index	80 (24/30)	83.3 (25/30)	73.3 (22/30)	83.3 (25/30)
Females delivering	24	25	22	25
Females with liveborn pups	24	24	22	25
Females with stillborn pups	3	6	3	6
Females with no liveborn pups	0	1	0	0
Females with no pups delivered	0	0	0	0
Gestation Index	100	96	100	100
Mean Gestation Length (days)	21.6 ± 0.5	22.0 ± 0.9	21.9 ± 0.4	21.7 ± 0.5
P2 Adults				
Number of males/females co-housed	30/30	30/30	30/30	30/30
Time to mating (days)	2.9 ± 2.5	3.5 ± 3.2	3.0 ± 3.1	3.0 ± 1.4
Females not pregnant (not sperm/plug positive or never delivered)	6	3	5	3
Females pregnant (sperm/plug positive or delivered)	24	27	25	27
Male Fertility Index	80 (24/30)	90 (27/30)	83.3 (25/30)	90 (27/30)
Female Fertility Index	80 (24/30)	90 (27/30)	83.3 (25/30)	90 (27/30)
Females delivering	24	27	25	27
Females with liveborn pups	24	27	25	27
Females with stillborn pups	3	7	6	3
Females with no liveborn pups	0	0	0	0
Females with no pups delivered	0	0	0	0
Gestation Index	100	100	100	100
Mean Gestation Length (days)	21.5 ± 0.5	21.9 ± 0.3 #	21.8 ± 0.4	21.8 ± 0.5

(a) Data extracted from pages 59-60, 239-242 and 247-250 of study report for P1 adults and pages 96-97, 1170-1173 and 1178-1181 for P2 adults.
Statistically different from control mean by Wilcoxon's test, $p \leq 0.05$.

6. Parental postmortem results

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a) Organ weights: Terminal body weights and organ weights are summarized in Table 12 (P1/P2 males) and Table 13 (P1/P2 females). Relative kidney weights were significantly increased in P1 females and P2 males and females at 500 mg/kg bw/d. Absolute kidney weights were generally unaffected. P1 males at 500 mg/kg bw/d also exhibited increased kidney weights (≈6% for both absolute and relative kidney weights), however, this was not statistically significant. The increased relative kidney weights may be secondary to the decreased terminal body weight in these animals, however, a treatment-related effect can not be dismissed since similar findings were observed in the 13-week and 2-year dietary studies with Fischer 344 rats and the increased kidney weights correlate with histopathological findings in the kidney, specifically with an increased incidence of hypertrophy of the epithelial cells of the collecting duct observed in the P1/P2 males and females at 500 mg/kg bw/d.

Other significant findings included increased relative epididymal and testicular weights in the P2 males at 500 mg/kg bw/d. In the absence of any significant change in absolute epididymal and testicular weights or any corroborating gross pathological or histopathological changes, these findings were most likely secondary to the decreased terminal body weight in these animals. Ovary weights were unaffected by treatment at all dose levels in both P1 and P2 females. In the P2 generation, there were no significant differences identified for males at any dose level or for females at 10 or 100 mg/kg bw/d.

TABLE 12: Terminal Body Weight and Organ Weights - P1/P2 Adult Males. (a)

Dose Level in Diet (mg/kg bw/d)		0	10	100	500
P1 Adult Males (30 animals/dose)					
Terminal Body Weight (g ± SD)		529.3 ± 54.3	556.3 ± 53.0	553.3 ± 50.5	532.7 ± 72.4
Epididymides	Absolute (g ± SD)	1.539 ± 0.153	1.498 ± 0.220	1.491 ± 0.175	1.490 ± 0.159
	Relative (g/100 g bw ± SD)	0.292 ± 0.031	0.271 ± 0.044	0.271 ± 0.038	0.283 ± 0.036
Kidneys	Absolute (g ± SD)	3.520 ± 0.376	3.651 ± 0.752	3.604 ± 0.305	3.727 ± 0.477
	Relative (g/100 g bw ± SD)	0.668 ± 0.073	0.661 ± 0.151	0.653 ± 0.039	0.710 ± 0.131
Testes	Absolute (g ± SD)	3.561 ± 0.290	3.498 ± 0.463	3.502 ± 0.437	3.497 ± 0.294
	Relative (g/100 g bw ± SD)	0.677 ± 0.065	0.631 ± 0.073	0.637 ± 0.091	0.668 ± 0.110
P2 Adult Males (30 animals/dose)					
Terminal Body Weight (g ± SD)		594.1 ± 44.3	617.1 ± 68.6	570.5 ± 56.0	514.2 ± 84.9 #
Epididymides	Absolute (g ± SD)	2.205 ± 0.275	2.176 ± 0.233	2.155 ± 0.214	2.062 ± 0.262

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Dose Level in Diet (mg/kg bw/d)		0	10	100	500
	Relative (g/100 g bw ± SD)	0.372 ± 0.045	0.357 ± 0.055	0.380 ± 0.043	0.409 ± 0.062 *
Kidneys	Absolute (g ± SD)	3.992 ± 0.551	4.202 ± 0.670	3.974 ± 0.393	3.998 ± 0.458
	Relative (g/100 g bw ± SD)	0.672 ± 0.074	0.683 ± 0.096	0.698 ± 0.045	0.800 ± 0.175 #
Testes	Absolute (g ± SD)	3.777 ± 0.537	3.807 ± 0.463	3.923 ± 0.296	3.822 ± 0.254
	Relative (g/100 g bw ± SD)	0.638 ± 0.099	0.622 ± 0.084	0.693 ± 0.072	0.768 ± 0.161 #

(a) Data extracted from pages 66-67 of the study report for P1 adults and pages 101-102 of study report for P2 adults.
* Statistically different from control mean by Dunnett's test, p≤ 0.05
\# Statistically different from control mean by Wilcoxon test, p≤ 0.05

TABLE 13: Terminal Body Weight and Organ Weights - P1/P2 Adult Females. (a)

Dose Level in Diet (mg/kg bw/d)		0	10	100	500
P1 Adult Females (30 animals/dose)					
Terminal Body Weight (g ± SD)		300.6 ± 26.2	298.9 ± 28.0	297.1 ± 26.2	268.1 ± 23.8 *
Kidneys	Absolute (g ± SD)	2.236 ± 0.190	2.202 ± 0.249	2.211 ± 0.187	2.376 ± 0.354
	Relative (g/100 g bw ± SD)	0.746 ± 0.059	0.738 ± 0.069	0.747 ± 0.056	0.889 ± 0.131 #
Ovaries	*Absolute (g ± SD)*	*0.103 ± 0.019*	*0.104 ± 0.017*	*0.101 ± 0.021*	0.100 ± 0.017
	Relative (g/100 g bw ± SD)	0.034 ± 0.005	0.035 ± 0.005	0.034 ± 0.008	0.037 ± 0.006
P2 Adult Females (30 animals/dose)					
Terminal Body Weight (g ± SD)		301.5 ± 24.8	308.3 ± 20.6	295.2 ± 34.4	263.2 ± 2.63 *
Kidneys	Absolute (g ± SD)	2.285 ± 0.164	2.319 ± 0.309	2.216 ± 0.403	2.355 ± 0.311
	Relative (g/100 g bw ± SD)	0.761 ± 0.060	0.754 ± 0.106	0.723 ± 0.139	0.897 ± 0.100 #
Ovaries	Absolute (g ± SD)	0.191 ± 0.033	0.264 ± 0.332	0.186 ± 0.043	0.180 ± 0.049
	Relative (g/100 g bw ± SD)	0.063 ± 0.011	0.086 ± 0.110	0.063 ± 0.014	0.069 ± 0.019

(a) Data extracted from pages 66-67 of the study report for P1 adults and pages 101-102 of study report for P2 adults.
* Statistically different from control mean by Dunnett's test, p≤ 0.05
\# Statistically different from control mean by Wilcoxon test, p≤ 0.05

b) Pathology

1) Macroscopic examination: There were no treatment-related gross pathological findings in P1 or P2 adults males or females at any dose level. Presence of bloody urine within the lumen of the urinary bladder was observed in 1/30 P1 males and in 2/30 P2 males at 500 mg/kg bw/d. In the P1 male at 500 mg/kg bw/d, the presence of bloody urine in the lumen of the urinary bladder was possibly associated with inflammation of the renal papilla with resultant haemorrhagic cast in the lumen of the urinary bladder. However, in the remaining P1 and P2 animals there were no corroborating histopathological findings; therefore, the toxicological significance is uncertain. Due to the low rate of incidence, the absence of corroborative histopathological findings in most of the animals, the presence of bloody urine in the lumen of the urinary bladder was considered to be an incidental finding.

2) Microscopic examination: - Treatment-related histopathological findings were limited to an increased incidence of hypertrophy of epithelial cells of the collecting ducts in P1/P2 males and females at 500 mg/kg bw/d (Table 14). The lesions were characterized by enlarged individual cells lining the collecting ducts and was restricted to the inner stripe of the outer zone of the renal medulla. The hypertrophied cells were compatible with the intercalated cells with increased cytoplasmic volume and numerous mitochondria. Intercalated cells are involved in the regulation of acid-base balance through the modulation of bicarbonate resorption and hydrogen ion secretion in the collecting ducts. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired

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concentrating ability, however, no urinalysis was done. The underlying mechanism for the hypertrophy is not fully understood. Morphologically the lesions were similar to those observed in Fischer 344 rats following dietary treatment for 13-weeks and 2-years at similar dose levels (see DACO 4.3.1 - Laboratory Project Study ID - DR-0312-6565-011 and DACO 4.4.4 - Laboratory Project Study ID 960004, respectively) where urinary acidification and decreased urinary specific gravity were observed. However, in the 13-week and 2-year dietary studies there were no significant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with the histopathological findings or to indicate impaired renal function and there was no increased incidence of cellular degeneration or necrosis evident in the kidneys. In the 13-week dietary study, the lesions and urinalysis findings appeared to be reversed after a 4-week recovery period.

Other renal findings in the P1 adults at 500 mg/kg bw/d included necrosis of the renal papilla with accompanying inflammation (1 ♂/1 ♀) and inflammation of the renal papilla with resultant haemorrhagic casts in the lumen of the urinary bladder (1 ♂). Other renal findings in the P2 adults at 500 mg/kg bw/d included necrosis of the renal papilla with accompanying inflamation (4 ♂), inflammation of the renal papilla with resultant haemorrhagic casts in the lumen of the urinary bladder (2 ♂) and inflammation of the renal papilla with no haemorrhagic casts in the lumen of the urinary bladder (1 ♂). However, due to the low rate of incidence of necrosis and/or inflammation of the renal papilla with/without haemorrhagic casts in the urinary bladder, these findings were considered to be incidental findings. There were no treatment-related histopathological findings in P1 or P2 males or females at 10 or 100 mg/kg bw/d.

TABLE 14: Histopathological findings in P1/P2 adults (values expressed as incidence/total number examined). (a)

Observation			Dose Level in Diet (mg/kg bw/d)				
			Sex	0	10	100	500
P1 Adults	Kidney	- hypertrophy; collecting duct; multi-focal; very slight	♂	0/30	0/30	0/30	25/30
		- hypertrophy; collecting duct; multi-focal; very slight	♀	0/30	0/30	0/30	21/30
P2 Adults	Kidney	- hypertrophy; collecting duct; multi-focal; very slight	♂	0/30	0/30	0/30	24/30
		- hypertrophy; collecting duct; multi-focal; very slight	♀	0/30	0/30	0/30	22/30

(a) Data extracted from pages 72-76 of the study report for P1 adults and from pages 112-116 of study report for P2 adults.

B. OFFSPRING

1. **Viability and clinical signs:** There were no toxicologically relevant changes in the litter parameters examined in either the F1 or F2 pups at any dose level (Table 15). However, the number of live-born pups/litter on the day of parturition and on lactation day 1 were significantly lower than controls in the F2 generation at 500 mg/kg bw/d. This was not considered to be toxicologically relevant since the number of live-born was within the range of the historical control values for animals of this age and strain from this laboratory (mean: 14.15; range: 11.5-15.6) and a similar decrease was not observed in the F1 generation at 500 mg/kg bw/d.

TABLE 15: Mean litter parameters for F_1 and F_2 litters. (a)

Observation	Dose Level in Diet (mg/kg bw/d)			
	0	10	100	500
	F_1 Generation			
Number of litters	24	25 (b)	22	25
Mean Implantation Sites	14.3 ± 2.2	12.8 ± 4.3	13.9 ± 2.0	13.0 ± 1.6

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Observation	Dose Level in Diet (mg/kg bw/d)			
	0	10	100	500
Birth Index	95.1	95.3	98.1	98.5
Number of pups born - total (mean #/litter) - live (mean #/litter) - dead (mean #/litter)	 327 (13.6 ± 2.3) 324 (13.5 ± 2.3) 3 (0.1 ± 0.3)	 304 (12.2 ± 4.4) 296 (11.8 ± 4.4) 8 (0.3 ± 0.6)	 298 (13.5 ± 2.2) 291 (13.2 ± 2.0) 7 (0.3 ± 0.9)	 319 (12.8 ± 1.6) 310 (12.4 ± 1.7) 9 (0.4 ± 0.7)
Live birth index	99.1	97.4	97.7	97.2
Mean litter size Day 1	13.4 ± 2.3	12.2 ± 3.7	13.1 ± 2.0	12.4 ± 1.7
Day 4 (c)	13.2 ± 2.2	12.0 ± 3.8	13.1 ± 2.0	12.3 ± 1.7
Day 4 (d)	8.0 ± 0.0	7.5 ± 1.5	8.0 ± 0.2	8.0 ± 0.0
Day 7	8.0 ± 0.0	7.5 ± 1.5	8.0 ± 0.2	8.0 ± 0.0
Day 14	8.0 ± 0.0	7.5 ± 1.5	8.0 ± 0.2	8.0 ± 0.0
Day 21	8.0 ± 0.0	7.5 ± 1.5	7.9 ± 0.3	8.0 ± 0.0
Sex Ratio (males/females)	55/45	54/46	52/48	54/46
F_2 Generation				
Number of litters	24	27	25	27
Mean Implantation Sites	14.3 ± 2.5	12.4 ± 3.0	13.8 ± 1.9	12.0 ± 2.2
Birth Index	94.4	96.8	97.1	97.5
Number of pups born - total (mean #/litter) - live (mean #/litter) - dead (mean #/litter)	 330 (13.8 ± 2.3) 324 (13.5 ± 2.3) 6 (0.3 ± 0.8)	 332 (12.3 ± 3.5) 323 (12.0 ± 3.5) 9 (0.3 ± 0.7)	 342 (13.7 ± 0.4) 336 (13.4 ± 2.2) 6 (0.2 ± 0.4)	 322 (11.9 ± 2.3) 317 (11.7 ± 2.4) * 5 (0.2 ± 0.6)
Live birth index	98.2	97.3	98.2	98.4
Mean litter size Day 1	13.2 ± 2.5	11.9 ± 3.5	13.4 ± 2.2	11.6 ± 2.4 *
Day 4 (c)	12.8 ± 3.3	11.7 ± 3.5	13.2 ± 2.1	11.6 ± 2.4
Day 4 (d)	7.6 ± 1.3	7.6 ± 1.3	8.0 ± 0.0	7.9 ± 0.4
Day 7	7.6 ± 1.3	7.6 ± 1.3	8.0 ± 0.0	7.9 ± 0.4
Day 14	7.6 ± 1.3	7.6 ± 1.3	8.0 ± 0.0	7.9 ± 0.4
Day 21	7.6 ± 1.3	7.6 ± 1.3	8.0 ± 0.0	7.9 ± 0.4
Sex Ratio (males/females)	49/51	48/52	54/46	52/48

(a) Data extracted from pages 59-60, and 247-250 of study report for P1 adults and pages 96-97, and 1178-1181 for P2 adults. Mean implantations sites calculated by reviewer from individual gross pathology data obtained from pages 402-521 for P1 adult females and pages 1264-1339, 1405-1472, 1541-1603 and 1691-1777 for P2 adult females.
(b) One dam delivered a single dead pup, values were excluded from analysis.
(c) Before standardization (culling)
(d) After standardization (culling)
* Statistically different from control mean by Wilcoxon test, $p \leq 0.05$.

There were no treatment-related effects on the number of deaths between lactation days 0-4 or between lactation day 4 (after cull) and lactation day 21 in the F1 or F2 generation (Table 16). The birth, live birth, survival (on lactation days 1, 4, 7, 14 and 21), viability, lactation and weaning indices were unaffected by treatment in the F1 and F2 generation.

TABLE 16: Mean Litter Indices (a)

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Observation	Dose Level in Diet (mg/kg bw/d)							
	F_1 Generation				F_2 Generation			
	0	10	100	500	0	10	100	500
# Deaths Days 0-4 (%)	8 (2.5)	9 (3.0)	3 (1.0)	2 (0.6)	17 (5.2)	6 (1.9)	5 (1.5)	4 (1.3)
# Deaths Days 4-21 (%)	0 (0)	1 (0.6)	1 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Birth Index	95.1	95.3	98.1	98.5	94.4	96.8	97.1	97.5
Live birth index	99.1	97.4	97.7	97.2	98.2	97.3	98.2	98.4
Day 1 Survival index	99.4	98.6	99.0	99.7	97.8	99.1	99.4	99.1
Day 4 Survival index	97.5	97.0	99.0	99.4	94.8	98.1	98.5	98.7
Day 7 Survival index	100	100	100	100	100	100	100	99.5
Day 14 Survival index	100	100	100	100	100	100	100	99.5
Day 21 Survival index	100	99.4	99.4	100	100	100	100	99.5
Viability index	97.8	97.6	99.2	99.2	94.8	97.5	98.5	99.1
Lactation index	100	99.4	99.4	100	100	100	99.5	100
Weaning Index	100	100	98.8	100	100	100	100	100

(a) Data extracted from pages 59-60, and 247-250 of study report for F1 generation and pages 96-97, and 1178-1181 for F2 generation.

There were no treatment-related clinical observations or physical alterations observed in the F1 or F2 pups at any dose level during the lactation period.

2. Body weight: In the F1 pups at 500 mg/kg bw/d, body weight was significantly lower on lactation day 4 prior to cull (♂ only, ↓ ≈7%) and on lactation day 7 (↓ ≈10 and 9% in ♂ and ♀, respectively). In the F2 pups at 500 mg/kg bw/d, body weight was significantly lower on lactation day 7 (↓ ≈ 15 and 12% in ♂ and ♀, respectively). On lactation days 14 and 21, body weight of F1/F2 pups at 500 mg/kg bw/d were comparable to controls for both sexes. This transient decrease in pup body weight may have been secondary to decreased food consumption of F1 and F2 dams at 500 mg/kg bw/d early in the lactation period (days 1-4 and possibly days 4-7). There were no significant differences identified in F1 pups at 10 mg/kg bw/d or in F1 and F2 pups at 100 mg/kg bw/d. Significant increases in pup body weight were identified in F2 male and female pups at 10 mg/kg bw/d on lactation days 1, 4 and 21. A similar finding was not observed in the F1 pups at 10 mg/kg bw/d and in the absence of a dose-response relationship, this was not considered to be treatment-related. Pups body weight are summarized in Table 17.

TABLE 17: Mean Pup Weights. (a)

Lactation	F_1 Pups				F_2 Pups			
Day	0	10	100	500	0	10	100	500
1 ♂	6.7 ± 0.6	6.9 ± 0.5	6.9 ± 0.6	6.5 ± 0.7	6.5 ± 0.7	7.1 ± 0.7 *	6.7 ± 0.6	6.3 ± 1.0
4 (b)	9.6 ± 0.9	9.7 ± 1.0	9.8 ± 1.2	**8.9 ± 1.1** *	9.3 ± 1.1	9.5 ± 1.4 *	9.3 ± 1.0	8.5 ± 1.3

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Lactation	F_1 Pups				F_2 Pups			
Day	0	10	100	500	0	10	100	500
4 (c)	-	-	-	-	9.3 ± 1.1	10.1 ± 1.2 *	9.3 ± 1.0	8.5 ± 1.4
7	15.6 ± 1.2	15.2 ± 1.9	15.7 ± 1.6	**14.0 ± 2.2 ***	15.1 ± 1.7	15.9 ± 1.8	14.9 ± 1.5	**12.8 ± 2.1 ***
14	32.0 ± 2.2	31.0 ± 4.7	32.2 ± 2.9	31.3 ± 3.9	30.2 ± 3.3	31.6 ± 3.2	30.5 ± 2.6	28.7 ± 3.1
21	51.6 ± 4.5	50.5 ± 7.9	51.9 ± 4.1	50.9 ± 5.5	47.9 ± 5.4	51.3 ± 5.3 *	49.2 ± 4.1	47.5 ± 5.1
1 ♀	6.3 ± 0.5	6.6 ± 0.5	6.4 ± 0.6	6.2 ± 0.7	6.1 ± 0.7	6.6 ± 0.8 *	6.3 ± 0.5	6.0 ± 0.9
4 (b)	9.0 ± 0.8	9.4 ± 0.9	9.2 ± 1.1	8.5 ± 1.0	8.7 ± 1.2	9.5 ± 1.4 *	8.8 ± 0.9	8.1 ± 1.2
4 (c)	-	-	-	-	8.6 ± 1.1	9.5 ± 1.5 *	8.8 ± 0.9	8.1 ± 1.2
7	14.5 ± 1.1	14.9 ± 1.2	14.9 ± 1.4	**13.2 ± 1.7 ***	14.0 ± 1.9	14.9 ± 2.3	14.0 ± 1.4	**12.3 ± 1.9 ***
14	30.2 ± 2.2	30.8 ± 2.3	30.8 ± 2/9	30.1 ± 3.1	28.5 ± 4.1	29.8 ± 4.0	29.0 ± 2.3	27.9 ± 2.9
21	48.7 ± 4.0	49.8 ± 3.9	49.4 ± 4.0	48.6 ± 4.2	44.5 ± 6.2	48.7 ± 6.5 #	46.7 ± 3.4	45.7 ± 4.5

(a) Data extracted from page 63 of the study report for the F1 pups and page 99 for the F2 pups.
(b) Before standardization (culling)
(c) After standardization (culling)
* Statistically different from control mean by Dunnett's test, p≤ 0.05
\# Statistically different from control mean by Wilcoxon test, p≤ 0.05

3. Offspring postmortem results:

a) Organ weights: There were no treatment-related kidney weight changes (absolute of relative) noted for F1 or F2 males or female weanlings. No other organs were weighed.

b) Pathology

1) Macroscopic examination: - There were no treatment-related gross pathological observations at any dose level in F1/F2 weanlings.

III. DISCUSSION

A. Investigators' conclusions (extracted from page 12 of study report): "Dietary exposure of adult male and female rats to the high dose level of 500 mg/kg/day resulted in parental and neonatal effects. Treatment-related clinical signs included perineal soiling (P1/P2 males and females) and reddish urine (P1 males only). Other parental effects consisted of decreased food consumption and significantly lower body weights of P2 males and P1/P2 females of the 500 mg/kg/day group during most of the pre-mating, gestation and lactation periods. Body weight gains of the 500 mg/kg/day group P1 and P2 females were significantly lower than controls during gestation (day 0-21). Significant increases in relative kidney weight occurred in the 500 mg/kg/day group P1 (females only) and P2 (males and females) adults. Histologically, hypertrophy of the renal tubular collecting ducts was observed in the kidneys of most P1 and P2 rats of the 500 mg/kg/day group and was considered to represent an adaptive response rather than a pathological effect. Necrosis and/or inflammation of the renal papilla with resultant haemorrhagic casts in the lumen of the urinary bladder occurred in a few P1 and P2 rats of the 500 mg/kg/day group. No treatment-related effects were observed for P1 or P2 males or females administered 10 or 100 mg/kg/day. No treatment-related effects were observed on any reproductive parameters for P1 or P2 rats at any dose level. Minimal neonatal effects were observed at 500 mg/kg/day in the F1 and F2 litters, with transient decreases in body weight statistically identified for F1 male pups on lactation day 4 and for F1 and F2 male and female pups on lactation day seven. These body weight decrements ranged from 7 to 15% relative to controls, but by weaning on lactation day 21, pup

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body weights were similar to those of the control group. These transient decreases were attributed, in part, to the decreased feed consumption of the P1 and P2 maternal animals early in their respective lactation periods. No adverse effects were observed on pup body weights at dose levels of 10 or 100 mg/kg/day pups in either generation. Neonatal survival was not adversely affect at any dose level in either generation. In conclusion, under the conditions of this study, the parental no-observed-effect level (NOEL) for systemic effects was 100 mg/kg/day for males and females. The NOEL for reproductive effects was 500 mg/kg/day, the highest dose tested. Based upon the transient decrease in pup body weights, the NOEL for neonatal effects was conservatively determined to be 100 mg/kg/day."

B. Reviewer's discussion: There were no treatment-related mortalities, adverse clinical signs or gross pathological findings. Lower body weight, body-weight gain and food consumption were observed in P2 males at 500 mg/kg bw/d throughout most of the pre-mating period. P1/P2 females at 500 mg/kg bw/d exhibited lower body weight, body-weight gain and food consumption throughout most of the pre-mating and gestation periods. Body weights remained lower throughout lactation period in the P1/P2 females at 500 mg/kg bw/d. During the last two weeks of lactation body-weight gain was significantly higher in P1/P2 females at 500 mg/kg bw/d. The higher body-weight gain in these animals was most likely a reflection of compensatory body-weight gain and not an indication of toxicity. Food consumption was slightly higher during most of the final two weeks of lactation in the P1/P2 females at 500 mg/kg bw/d, this correlated with the higher body-weight gain over the same time period in these animals. Reproductive parameters, litter parameters and sexual maturation of external sexual organs of F1 male and female weanlings were unaffected at dose levels up to and including 500 mg/kg bw/d. In the F1 pups at 500 mg/kg bw/d, body weight was significantly lower on lactation day 4 prior to cull (♂only, ↓≈7%) and on lactation day 7 (↓ ≈10 and 9% in ♂ and ♀, respectively). In the F2 pups at 500 mg/kg bw/d, body weight was significantly lower on lactation day 7 (↓ ≈ 15 and 12% in ♂ and ♀, respectively). By lactation day 14, body weight in these animals was comparable to controls. This transient decrease in pup body weight may have been secondary to lower food consumption of F1 and F2 dams at 500 mg/kg bw/d early in the lactation period (days 1-4 and possibly days 4-7). Relative kidney weight was significantly increased in P1 females at 500 mg/kg bw/d and in both P2 males and females at 500 mg/kg bw/d. P1 males at 500 mg/kg bw/d also exhibited increased kidney weights (≈6%), however, this was not statistically significant. Although the increased relative kidney weight may be secondary to lower terminal body weight in these animals a treatment-related effect could not be dismissed since similar findings were observed in the 13-week and 2-year dietary studies with Fischer 344 rats and the increased kidney weight correlated with histopathological findings in the kidneys, specifically with an increased incidence of hypertrophy of epithelial cells of the collecting ducts in the P1/P2 males and females at 500 mg/kg bw/d. The hypertrophied cells were compatible with the intercalated cells which are involved in the regulation of acid-base balance through the modulation of bicarbonate resorption and hydrogen ion secretion in the collecting ducts. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability, however, no urinalysis was done. Morphologically the lesions were similar to those observed in Fischer 344 rats following dietary treatment for 13-weeks and 2-years at similar dose levels where urinary acidification and decreased urinary specific gravity were observed. However, in the 13-week and 2-year dietary studies there were no significant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with the histopathological findings or to indicate impaired renal function and there was no increased incidence of cellular degeneration or necrosis evident in the kidneys. In the 13-week dietary study, the lesions were not apparent in either sex following the 4-week recovery period suggesting that the lesions were reversible. There were no treatment-related gross pathological findings in the F1 or F2 weanlings.

The parental LOAEL was 500 mg/kg bw/d, based on lower body weight, body-weight gain and food consumption (P2 ♂ and P1/P2 ♀), increased kidney weights (P2 ♂ and P1/P2 ♀) and hypertrophy of the epithelial cells of the collecting ducts (P1/P2 ♂/♀). The parental NOAEL was 100 mg/kg bw/d.

The LOAEL for offspring was 500 mg/kg bw/d based on a transient lower pup body weight (P1/P2 ♂/♀). The NOAEL for offspring was 100 mg/kg bw/d.

The LOAEL for reproductive effects was not determined. The NOAEL for reproductive effects was 500 mg/kg bw/d, the highest dose tested.

C. Study deficiencies: Specific assessment of sperm was not carried out. However, reproductive performance and

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indices were unaffected by treatment and there were no significant gross pathological or histopathological findings in the reproductive organs examined; therefore, this deficiency should not significantly affect the outcome of the study. OPPTS 870.3800 indicates that the following organs weights should be determined in the P1/P2 parental animals: uterus (including cervix and oviducts), ovaries, testes, epididymides, seminal vesicles, prostate, brain, pituitary, liver, kidneys, adrenal glands and spleen. OPPTS 870.3800 also indicates that the following organ weights should be determined in at least 1 pup/litter in the F1/F2 weanlings; brain, spleen and thymus. For the P1/P2 parental animals only kidney, testes, epididymides and ovary weights were determined and for the F1/F2 pups only the kidney weights were determined. However, OECD 416 (1992) does not specifically indicate what organ weights are required for either the P1/P2 parental animals or F1/F2 pups. With the exception of the kidneys, there were no significant treatment-related gross pathological or histopathological findings in these organs; therefore, this deficiencies should not significantly affect the outcome of the study. This study is acceptable and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800); OECD 416 in rats.

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