DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §83-4; Multigeneration Reproduction Study in Rats

Work Assignment No. 2-01-35 E; formerly 1-01-35 E (MRID 45710209)

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DATA EVALUATION RECORD

TXR#: 0052493

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

PC CODE: 081901

DP BARCODES: D301496
SUBMISSION NO.: None

TEST MATERIAL (PURITY): Chlorothalonil (99.16% a.i.)

SYNONYMS: 2,4,5,6-tetrachloro-1,3 benzodicarbonitrile

CITATION: Myers, D. (1995) Chlorothalonil: a study of the effect on reproductive function of two generations in the rat. Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project Id: VCM 21/942505, March 24, 1995. MRID 45710209. Unpublished.

SPONSOR: Vischim S.r.L., Cesano Maderno, Milan, Italy.

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 45710209), Chlorothalonil (Batch # NF 28/01; 99.16% a.i.) was administered continuously in the diet to Crl: CD®BR VF/Plus rats (32 animals/sex/dose) at dose levels of 0, 400, 1200, or 3000 ppm (equivalent to 0/0, 30.8/34.3, 92.5/106.0, and 247.5/270.0 mg/kg bw/day [M/F]). The P and F_1 parents were dosed for 10 weeks and 12 weeks, respectively, before they were mated to produce the F_1 and F_2 litters. The F_1 pups were weaned on postnatal day (PND) 21, and 28 pups/sex/group (1 pup/sex/litter as nearly as possible) were randomly selected as parents of the F_2 generation.

In the parental animals, no treatment-related effects were observed on survival, or food or water consumption.

In the 3000 ppm P animals, body weight gains during Weeks 0-1 were decreased, and food consumption for Week 1 was decreased. Body weight gains were decreased during Week 1-10 in the males in this generation, but cumulative food consumption was comparable for Weeks 2-10 in both sexes in this generation. In the 3000 ppm F_1 males, body weights were decreased throughout treatment (Weeks 4-26), resulting in decreased body weight gains during pre-mating

(Weeks 4-16), that continued to termination (Weeks 17-26). Similarly in the 3000 ppm F_1 females, body weights were decreased during premating (Weeks 4-16), resulting in decreased body weight gains during this period. In the males, food consumption was not affected, while food consumption was slightly decreased during Week 1 in the females.

Treatment-related effects were observed on the kidney at 1200 and 3000 ppm. Adjusted (to terminal body weight) kidney weights were increased in both sexes in both generations. Enlarged kidney was observed in the P and F_1 males compared to controls, and the following microscopic pathological findings were noted: i) trace to moderate dilated/basophilic cortical tubules containing colloidal material in the P males compared to controls; ii) trace to moderate hyperplasia/hypertrophy of the inner cortical tubules in both sexes in both generations compared to controls; and iii) trace to minimal dystrophic mineralization at the corticomedullary junction in the P females compared to controls. Bright yellow/orange urinary staining and wetter than normal feces on the cage tray paper under the cages of the P and F_1 parents; and cage sawdust yellow stained and wet during lactation phase in the P and F_1 females was observed.

In the 400 ppm and above P and F_1 animals, the forestomach was observed to be thickened and roughened, compared to controls, with depressions in the epithelial aspect compared to controls. At 1200 ppm and above, white areas in the forestomach were noted in the P animals, and the limiting ridge of the forestomach was prominent in the F_1 animals both compared to controls. In the 400 ppm and above P and F_1 males and females, trace to moderate hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach was observed compared to controls. At 3000 ppm, minimal focal ulceration of the non-glandular epithelium of the stomach was observed in the P females, and trace to moderate erosion of the non-glandular epithelium of the stomach was noted in the F_1 females, both compared to controls. Increases were observed in relative kidney weights (↑9-13%) in the 400 ppm P and F_1 males. The kidney was enlarged in 3 males in the P generation and in 7 males in the F_1 generation. Microscopically, there was an increased incidence of trace to minimal dystrophic mineralization at the corticomedullary junction of the kidneys in the P females (34%) when compared to controls (13%).

The LOAEL for parental toxicity is 400 ppm (equivalent to 30.8/34.3 mg/kg bw/day male/female), based on macroscopic and microscopic pathological findings in the stomach-including: thickened, roughened, and white areas in forestomach with depressions in the epithelial aspect, and hyperplasia and hyperkeratosis of non-glandular epithelium in the stomach, and kidney effects, i.e., enlargement, relative weight increases and dystrophic mineralization at the corticomedullary junction. The NOAEL was not established.

In the offspring, no treatment-related effects were observed on post-implantation survival, live birth, viability, or lactation indices, on the sex ratio, clinical signs, gross pathology, or microscopic pathology.

At 3000 ppm, pup body weights were decreased during PND 12-21 in the F_1 and F_2 pups, resulting in decreased overall (PND 0-21) pup body weight gains. Litter weights were decreased in the F_1 pups during PND 16-21, and in the F_2 pups during PND 8-21, resulting in decreased

overall litter weight gains. Additionally, balano-preputial skinfold cleavage was delayed and vaginal opening was delayed compared to controls. However, since no differences were observed in the subsequent mating and reproductive performance of these animals, these effects were considered equivocal and were attributed to the reduction in body weight gain observed during lactation. This assertion is supported by the comparable body weight at attainment of criterion in all treatment groups.

At ≥400 ppm, a dose-dependent increase in the incidence of thickening and/or roughening of the forestomach was observed in both generations, and increased incidence and severity of trace to moderate hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach and trace to marked subepithelial edema in the non-glandular region of the stomach were observed compared to controls. Additionally, in the F_1 group, trace to moderate inflammatory cells in the non-glandular region of the stomach were noted compared to controls.

The LOAEL for offspring toxicity is 400 ppm (equivalent to 30.8/34.3 mg/kg bw/day male/female), based on thickening and/or roughening of the forestomach with depressions in the epithelial aspect, and hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach. The NOAEL was not established.

No treatment-related effects were observed on estrous cycle length or periodicity, pre-coital interval, duration of gestation, or on mating, gestation, or parturition indices.

The LOAEL for reproductive performance was not observed. The NOAEL for reproductive performance is 3000 ppm (equivalent to 247.5/270.0 mg/kg bw/day males/females).

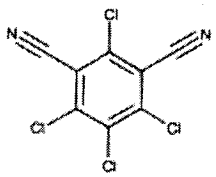
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chlorothalonil

Description:	White powder
Batch #:	NF 28/01
Purity:	99.16% a.i.
Compound Stability:	Stable in the diet for up to 4 days at room temperature, or up to 7 days, with 4 days storage at 4°C followed by 3 days at room temperature
CAS # of TGAI:	1897-45-6
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Crl: CD®BR VF/Plus
Age at study initiation:	Approximately 6 weeks old
Group mean weights on Study Day 1:	220-222 g males; 166-169 g females
Source:	Charles River UK Ltd., Manston Road, Margate, Kent, England
Housing:	Males and females during acclimation and pre-mating, and males post-mating, 4/sex/cage in Biotech® suspended stainless steel cages with solid sides and wire mesh front, back, top and floor; Week 1 of the F_1 generation, pairs during mating, and individual females during gestation and lactation in RM-2 type plastic cages.
Diet:	Biosure Laboratory Animal Diet No. 2 (Manufacturer information not provided), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature** 21±2°C **Humidity** 40-65% **Air changes** Not reported **Light cycle** 12 hrs light/12 hrs dark
Acclimation period:	12 days

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: Males and females from the same treatment group were paired on a one-to-one basis for 20 days with the following exceptions: two 3000 ppm P males were each housed with two females; and one 1200 ppm F_1 male was found dead on Day 4 of mating, but the female was not paired with another male because positive evidence of mating was observed on Day 2. Sibling matings were avoided. Vaginal smears were taken daily for seven days prior to mating and during the entire mating period from all females.

2. Study schedule: Rats were exposed to the test substance continuously in the diet throughout the study. The P animals were dosed for 10 weeks prior to mating; thus, the P animals were approximately 16 weeks old at mating. F_1 pups were selected on postnatal day (PND) 21; 28 pups/sex/group (1 pup/sex/litter, as nearly as possible) were selected (based on closest pup weight to median weight/sex for the litter) from up to 28 litters (litters selected weaned as close to the median weaning date as possible for the group), to be parents of the F_2 generation. When less than 28 litters in a group were within a reasonable range of weaning dates, extra pups were selected from appropriate litter(s). The F_1 parents had access to the same diet as the P parents throughout lactation; however, dosing of the F_1 generation was considered to formally start at Week 4. F_1 parents were then dosed for 12 weeks before they were mated; therefore, the F_1 parents were approximately 16 weeks old when mated. The F_1 pups not selected to be parents of the F_2 generation and all F_2 pups were killed shortly after weaning.

3. Animal assignment: On receipt, rats were identified by litters. Prior to treatment, all animals were weighed and animals in the weight ranges of 128-192 g for males and 107-157 g for females were randomly assigned (stratified by body weight) to the test groups shown in Table 1, while avoiding assigning litter mates to the same group.

TABLE 1. Animal assignment[a]

Test Group	Dose[b] (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	32	32	28	28
Low	400	32	32	28	28
Mid	1200	32	32	28	28
High	3000	32	32	28	28

a Data were obtained from page 16 of the study report.
b Exposure to the test substance was continuous throughout the study.

4. Dose-selection rationale: It was stated that the dose levels summarized in Table 1 were chosen in collaboration with the Sponsor, based on the results of a previous range-finding reproductive toxicity study conducted by the performing laboratory (HRC Report No. VCM 30/930559). In this study, 3000 ppm, and to a lesser extent 1500 ppm, Chlorothalonil was associated with maternal toxicity, manifested as decreased body weight gains, swollen pink rimmed eyes, wet feces, and slightly increased kidney weight. Additionally, F_1 offspring displayed retarded growth from PND 8 and slightly increased kidney and liver weight. Microscopic pathology revealed alterations in the non-glandular region of the stomach in the P females and F_1 pups at doses ≥60 ppm. Based on these findings, 3000 ppm was chosen as the highest dose and was expected to cause an appropriate degree of parental toxicity, while 400 ppm was chosen as the NOAEL (discounting pathological changes of the forestomach). No other information was provided.

5. Dosage preparation and analysis: Fresh diet was generally prepared weekly and divided

into two batches. The first batch was fed for no more than four days from the day of preparation; the second batch was stored at 4°C for no more than four days, and then was fed for no more than three days. A premix was prepared by combining the appropriate amount of test substance with diet, and final dietary formulations were prepared by direct dilution of the premix with appropriate amounts of feed. Homogeneity (top, middle, and bottom) and stability of the test substance in the diet were verified in 10 and 3000 ppm dietary formulations prior to start of the study. Concentration analyses were performed on samples of each dose level prepared for use on Weeks 1, 11, 15, 18, 30, and 34.

Results

Homogeneity (% CV): 1.70-7.44%

Stability (range of % difference from Time 0)

4 days (room temperature): 93.2-99.6%
7 days (4 days at 4°C, 3 days room temperature: 96.9-102.2%
10 days (room temperature): 91.0-104.5%

Concentration (range of % of nominal)

400 ppm = 94.5-99.7%
1200 ppm = 93.3-98.3%
3000 ppm = 93.3-100.0%

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

6. Dosage administration: All doses were administered continuously in the diet throughout the study.

C. OBSERVATIONS

1. Parental animals: All animals were handled daily. Clinical signs of toxicity were recorded daily for the first two weeks, and then weekly for the remainder of the study. Body weights were measured prior to study initiation, at the start of treatment for each generation, weekly throughout the study, and at termination. Females were weighed daily during mating and gestation (weights are reported weekly during mating and for gestation day [GD] 0, 7, 14, 17, and 20), and on lactation days (LD) 0, 7, 14, and 21. Food consumption (g/rat/week) was recorded weekly during pre-mating. Food conversion ratio was calculated as the food consumption divided by the body weight gain. Compound intake (mg/kg/day) was calculated weekly during pre-mating from the body weight and food consumption data and the nominal dietary concentration. Water consumption was measured, by weight, daily during the week prior to study initiation and on pre-mating Weeks 1, 2, 9, and 10. Vaginal smears were examined to detect any anomalies of the estrous cycle, to determine if pregnancy had occurred and continued uninterrupted after mating, and to determine the median pre-coital time and duration of pregnancy for littering females. Sperm enumeration, motility, and morphology were not evaluated.

2. Litter observations: The following litter parameters (X) were observed (Table 2).

TABLE 2. F_1/F_2 litter observations[a]

Observation	Postnatal Day					
	Day 0	Day 4	Day 8	Day 12	Day 16	Day 21
Number of live pups	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations	X	X	X	X	X	X
Clinical signs	X	X	X	X	X	X
Sex of each pup (M/F)	X					X

a Data were obtained from page 22 of the study report.

Litters were not standardized. F_1 pups selected to be parents of the F_2 generation were weighed on PND 28, but these data were not provided. Also in these pups, preputial separation was evaluated in the males daily beginning on PND 35 to 100% success; vaginal patency was evaluated in the females daily beginning on PND 28 to 100% success. Body weights were recorded when criteria were reached.

3. Postmortem observations

1) Parental animals: All animals, including those killed for humane reasons or found dead, were subjected to a complete macroscopic examination. The uteri of all females were visually inspected and the number of implantation sites per uterus was recorded. Uteri of apparently non-pregnant females were examined with a modification of the method of Salewski. All animals were killed at approximately 26 weeks of age, after the F_1 and F_2 litters had been weaned. Estrous cycle stages were determined in all adult females at termination. The following tissues were collected for histological examination (X) and fixed in 10% buffered formalin (except for the eyes, which were fixed in Davidson's fluid, and the testes, which were fixed in Bouin's fluid). Additionally, the (XX) tissues were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic	XX	Brain (multiple sections)
X	Salivary glands	XX	Heart	X	Peripheral nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal cord
X	Stomach	X	Lymph nodes	XX	Pituitary gland
X	Duodenum	X	Spleen	X	Eyes (optic nerve)
X	Jejunum	XX	Thymus		
X	Ileum				GLANDULAR
X	Cecum		UROGENITAL	XX	Adrenal gland[b]
X	Colon	XX	Kidneys[b]		Harderian/lacrimal gland
X	Rectum	X	Urinary bladder	X	Parathyroids
XX	Liver	XX	Testes	X	Thyroids
X	Pancreas	XX	Epididymides		
		XX	Prostate[a]		
	RESPIRATORY	XX	Seminal vesicle[a]		OTHER
X	Trachea	XX	Ovaries[b]	X	Bone (femur)
XX	Lung	X	Uterus	X	Skeletal muscle
		X	Mammary gland	X	Skin
		XX	Coagulating gland[a]	X	All gross lesions and masses
		X	Cervix	X	Femurotibial joint
		X	Vagina	X	Cranial vault

a Prostate was weighed with seminal vesicles and coagulating glands
b Weighed as a pair

Microscopic examination was performed on the epididymides, ovaries, pituitary, prostate, seminal vesicles (with coagulating gland), testes, uterus (with cervix), and vagina from the control and 3000 ppm groups. The above tissues from any apparently infertile animals were also examined in the 400 and 1200 ppm groups. The kidneys and stomach were examined at all doses in both parental generations. The testes were weighed individually, identified as right or left, and maximum length, width, and thickness were recorded prior to fixation.

2) **Offspring:** F_1 pups not selected to become parents and all F_2 pups were killed shortly after weaning, necropsied, and sexed. Pups found dead were necropsied where possible. Additionally where possible, one pup/sex/litter was randomly selected from all F_1 and F_2 litters for preservation of liver, kidney, and stomach, and histopathological examination was performed on the forestomach. Organs were not weighed.

D. DATA ANALYSIS

1. Statistical analyses: Body weight, body weight gains, absolute and cumulative food and water consumption, organ weights, litter data, sex ratios, sexual maturation, microscopic stomach changes in weanlings, and principal microscopic changes in the stomach and kidneys of adults were analyzed as follows:

- Depending on the heterogeneity of variances between treatment groups, parametric tests (ANOVA) followed by Williams' test, or non-parametric tests (Kruskal-Wallis) followed by

Shirley's test were used to analyze these data as appropriate. For litter data and sex ratios, the basic sample unit was the litter, and due to the preponderance of non-normal distributions, non-parametric analyses were employed.

- Where appropriate, ANCOVA was used in place of ANOVA. For sexual maturation, the covariate was body weight on the day of attainment. For organ weight analysis, terminal body weight was the covariate. ANCOVA was performed when the within-group relationship between the analyzed parameter and body weight was significant ($p \leq 0.10$).
- Where 75% or more of the values for a given variable were the same, a Fisher's exact test was used.

Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. The statistics were considered appropriate.

2. Indices

Reproductive indices: The following reproductive/viability index was calculated by the performing laboratory from breeding and parturition records of animals in the study:

Post-implantation loss (%) = (# of implantation sites - total # of young born)/# of implantation sites x 100

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

Pup loss on completion of parturition (%) = (total # of young at birth - # of live young)/total # of young at birth x 100

Cumulative pup loss (%) = (total # of young at birth - # of live young at Day x)/total # of young at birth x 100

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

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: All deaths were considered unlikely to be due to the test compound because of the minimal incidence. In the P generation parents, two 3000 ppm males were killed for humane reasons due to poor physical condition associated with hemorrhage. One male was killed during Week 6 after being found lethargic and bleeding from the region of the right eye. Necropsy revealed pale kidneys, thickened, roughened, and white forestomach, and dark contents in the stomach and small intestines (stated to be suggestive of ingestion of blood). The other male was killed during Week 8 after being found with perinasal and perioral bleeding, labored respiration, lethargy, pallor, and cold to the touch. Necropsy revealed blood in the oral cavity and esophagus, pale liver and spleen, minimally roughened forestomach, and dark contents in the stomach and small intestines. Neither animal demonstrated skin lacerations. Also in the P

animals, one 1200 ppm male was found dead during Week 19. Two days prior to death, this animal was found with both hindlimbs swollen and caught in the cage flooring; one day prior to death, piloerection, red periorbital discharge, and labored respiration were noted. Necropsy revealed red/brown staining on the right periorbital region, enlarged cervical lymph nodes, pale kidneys with blood in the pelvis, severely congested urinary bladder, severely hemorrhagic seminal vesicles and prostate, and minimally thickened forestomach. In the F_1 parents, one 3000 ppm female was killed for humane reasons during Week 23 having successfully reared a litter to weaning. This animal was observed to have hunched posture, reduced body tone, lethargy, discharge around the eyes, and enlarged and hard mammary glands. Necropsy revealed severely thickened mammary glands with swellings in the cervical, inguinal, pectoral, and pelvic regions, a punctate cyst in the pituitary, enlarged cervical and mediastinal lymph nodes, swollen liver with accentuated lobular markings, pale kidneys, minimal adipose tissue, and a thickened and roughened forestomach with multiple crater-like depressions on the epithelial aspect. Also in the F_1 parents, one 1200 ppm male was found dead during Week 17. This animal did not demonstrate any clinical signs of toxicity prior to death, and necropsy failed to reveal the cause of death, although enlarged liver and kidneys and congested lungs with a few punctate subpleural foci were observed.

Clinical signs of toxicity are presented in Table 3. Yellow skin/fur was observed in all of the ≥1200 ppm P females (32/32), and in all of the 3000 ppm P males (30/30) and F_1 males (28/28) and females (27/27), beginning in Week 6 of the P group and Week 2 of the F_1 group. Hair loss was noted in the ≥1200 ppm P females (16-31/32) and in the 3000 ppm F_1 females (23/27). Additionally, the following were observed at 3000 ppm: i) brown staining on the forelimbs in the P (23/32) and F_1 (13/27) females; ii) flaky, swollen, and pink areas around the eyes in the P (13/32) females; iii) flaky and yellow skin around the mouth in all of the F_1 males (28/28) and females (27/27); iv) dry, crust, and yellow-rimmed eyes in all of the F_1 males (28/28) and females (27/27); v) soiled anogenital region in the F_1 males (8/28); vi) bright yellow/orange urinary staining and wetter than normal feces on the cage tray paper under all the cages of the P (8/8) and F_1 (7/7) parents; and vii) cage sawdust yellow stained and wet during lactation phase in the P (31/32) and F_1 (24/27) females. All other clinical signs were unrelated to treatment.

TABLE 3. Clinical signs of toxicity (# of rats with observation) in rats treated with Chlorothalonil - P and F_1 generations[a]

Observation	Dose Group (ppm)							
	Males				Females			
	0	400	1200	3000	0	400	1200	3000
P Generation								
# of animals surviving to termination	32	32	31	30	32	32	32	32
Yellow skin/fur	0	0	0	30	0	0	32	32
Hair loss	4	4	4	6	6	9	16	31
Brown staining on forelimbs	0	0	0	0	0	0	2	23
Flaky, swollen and pink areas around eyes	0	0	0	0	0	0	0	13
Bright yellow urinary staining/wetter than normal feces on cage tray paper (# cages)	0	0	0	8	0	0	0	8
Cage sawdust yellow stained and wet during lactation (# cages)	N/A	N/A	N/A	N/A	0	0	0	31
F_1 Generation								
# of animals surviving to termination	28	28	27	28	28	28	28	27
Yellow skin/fur	0	0	0	28	0	0	0	27
Hair loss	12	15	5	15	12	15	19	23
Brown staining on forelimbs	0	0	0	0	0	0	4	13
Flaky and yellow skin around mouth	0	0	0	28	0	0	0	27
Dry, crust, and yellow rimmed eyes	0	0	0	28	0	0	0	27
Soiled anogenital region	0	0	0	8	0	0	0	0
Bright yellow urinary staining/wetter than normal feces on cage tray paper (# cages)	0	0	0	7	0	0	0	7
Cage sawdust yellow stained and wet during lactation (# cages)	N/A	N/A	N/A	N/A	0	0	0	24

a Data were obtained from Tables 2:0 and 2:1 on pages 54-55 of the study report.
N/A Not applicable

2. <u>Body weight and food consumption</u>: Body weight and food consumption data are presented in Tables 4a, b, c, and d. Body weight gains were decreased ($p \leq 0.01$) in the 1200 ppm P females by 17% during Weeks 0-1, but this finding was considered incidental. In the 3000 ppm P animals, body weight gains during Weeks 0-1 were decreased ($p \leq 0.01$) by 34-41%, and food consumption for Week 1 was decreased ($p \leq 0.05$) by 7-9%. Body weight gains were decreased ($p \leq 0.05$) during Week 1-10 by 8% in the males in this generation, but cumulative food consumption was comparable for Weeks 2-10 in both sexes in this generation.

In the 3000 ppm F_1 males, body weights were decreased ($p \leq 0.01$) throughout treatment (Weeks 4-26; ↓12-16%), resulting in decreased ($p \leq 0.01$) body weight gains during pre-mating (Weeks 4-16; ↓13%), that continued to termination (Weeks 17-26; ↓13%; NS). Similarly in the 3000 ppm

F_1 females, body weights were decreased ($p \leq 0.01$) during premating (Weeks 4-16; ↓7-13%), resulting in decreased ($p \leq 0.05$) body weight gains (↓8%) during this period. In the males, food consumption was not affected, while food consumption was slightly decreased ($p \leq 0.01$) during Week 1 (↓8%) in the females.

No effects of treatment were noted on body weights or body weight gains in the females of either generation during gestation or lactation. In the 3000 ppm females, overall body weight gains during lactation (LD 0-21) were increased (↑99-215%; $p \leq 0.05$), but this finding was not considered adverse. Food efficiency was not affected by treatment in either generation.

TABLE 4a. Selected mean body weights, body weight gains (g), and food consumption (g/rat/week) - P and F_1 generation males[a]

Observation/study day		Dose Group (ppm)			
		0	400	1200	3000
P Generation					
Body weight	Week 0	220	222	222	221
Body weight	Week 1	283	286	279	263
Body weight	Week 10	585	582	570	543
Body weight	Week 11	580	579	567	544
Body weight	Week 20	700	699	682	653
Body weight gain	Week 0-1	62	63	58	41** (↓34)
Body weight gain	Week 1-10	303	297	291	279* (↓8)
Body weight gain	Week 11-20	120	120	114	109
Food consumption	Week 1	188	193	192	171** (↓9)
Food consumption[b]	Week 2-10	1854	1902	1856	1855
F_1 Generation					
Body weight	Week 4	93	96	97	81** (↓13)
Body weight	Week 5	147	151	153	124** (↓16)
Body weight	Week 16	562	573	572	489** (↓13)
Body weight	Week 17	561	568	562	487** (↓13)
Body weight	Week 18	569	579	574	502** (↓12)
Body weight	Week 26	664	687	672	579** (↓13)
Body weight gain	Week 4-16	469	477	475	408** (↓13)
Body weight gain	Week 17-26	104	119	111	91 (↓13)
Food consumption	Week 5	120	124	127	114
Food consumption[b]	Week 6-16	2170	2227	2221	2097

a Data were obtained from Tables 3:0, 3:1, 5:0 and 5:1 on pages 56-57 and 60-61 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
b Cumulative intake (g/rat)
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

TABLE 4b. Selected mean body weights, body weight gains (g), and food consumption (g/rat/week) - P and F_1 generation pre-mating females[a]

Observation/study day		Dose Group (ppm)			
		0	400	1200	3000
P Generation					
Body weight	Week 0	166	166	167	169
Body weight	Week 1	195	193	190	185
Body weight	Week 10	323	325	322	311
Body weight gain	Week 0-1	29	27	24** (↓17)	17** (↓41)
Body weight gain	Week 1-10	128	132	131	126
Food consumption	Week 1	152	143	146	142* (↓7)
Food consumption[b]	Week 2-10	1345	1318	1349	1318
F_1 Generation					
Body weight	Week 4	85	89	90	75** (↓12)
Body weight	Week 5	129	133	133	112** (↓13)
Body weight	Week 7	197	202	203	183** (↓7)
Body weight	Week 16	320	331	323	291** (↓9)
Body weight gain	Week 4-16	235	242	232	216* (↓8)
Food consumption	Week 5	111	110	116	102** (↓8)
Food consumption[b]	Week 6-16	1635	1637	1664	1547

a Data were obtained from Tables 3:0, 3:1, 5:0, and 5:1 on pages 56-57 and 60-61 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
b Cumulative intake (g/rat)
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

TABLE 4c. Mean body weights and body weight gains (g) - P and F_1 generation females during gestation[a]

Observation/gestation day		Dose Group (ppm)			
		0	400	1200	3000
P Generation					
Body weight	GD 0	323.5	328.0	321.1	312.3
Body weight	GD 7	351.3	352.1	346.2	338.1
Body weight	GD 14	385.7	386.2	379.6	372.2
Body weight	GD 17	418.5	417.5	409.4	403.6
Body weight	GD 20	468.6	467.2	454.5	452.4
Body weight gain	GD 0-20	145.2	139.2	133.4	140.1
F_1 Generation					
Body weight	GD 0	319.3	321.2	322.2	291.1
Body weight	GD 7	347.1	350.8	350.1	321.4
Body weight	GD 14	378.5	383.0	383.6	357.2
Body weight	GD 17	411.3	414.6	416.6	389.1
Body weight	GD 20	461.6	464.3	468.9	437.9
Body weight gain	GD 0-20	142.3	143.2	146.6	146.8

a Data were obtained from Tables 4:0 and 4:1 on pages 58-59 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

TABLE 4d. Mean body weights and body weight gains (g) - P and F_1 generation females during lactation[a]

Observation/lactation day		Dose Group (ppm)			
		0	400	1200	3000
P Generation					
Body weight	LD 0	372.1	372.9	364.9	356.5
Body weight	LD 7	377.6	381.7	372.9	366.5
Body weight	LD 14	384.6	385.3	377.8	375.5
Body weight	LD 21	365.6	365.0	358.8	364.0
Body weight gain	LD 0-21	-6.5	-7.9	-6.1	7.5* (1215)
F_1 Generation					
Body weight	LD 0	371.9	378.8	370.4	338.6
Body weight	LD 7	374.7	377.4	375.2	349.9
Body weight	LD 14	365.8	374.9	372.2	338.4
Body weight	LD 21	349.1	356.8	355.2	338.3
Body weight gain	LD 0-21	-22.8	-22.0	-15.3	-.02** (199)

a Data were obtained from Tables 4:0 and 4:1 on pages 58-59 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

3. **Test substance intake:** The mean achieved doses of the test compound (mg/kg bw/day) in the P and F_1 generations during the pre-mating period (based on the nominal dietary concentrations, body weight data, and food consumption) are presented in Table 5. These values are considered to be representative of the test substance intake for the entire study.

TABLE 5. Mean test substance intake (mg/kg bw/day) during premating - P and F_1 generations[a]

Generation	Dose Group (ppm)		
	400	1200	3000
Males			
P	28.6	86	220
F_1	32.9	99	275
Mean[b]	30.8	92.5	247.5
Females			
P	32.5	101	251
F_1	36.0	111	289
Mean[b]	34.3	106.0	270.0

a Data were obtained from Tables 7:0 and 7:1 on pages 64-65 of the study report.
b Calculated by reviewers from data presented in this table.

4. **Water consumption:** Water consumption data are presented in Tables 6a and b. At 3000 ppm, cumulative water consumption was increased ($p \leq 0.05$) during Weeks 1-2 in the P generation (↑15-16%) and during Weeks 5-6 in the F_1 generation (↑10-15%). Water consumption was also increased in the 1200 ppm F_1 females during Weeks 5-6 (↑12%). However, water consumption returned to control levels in all of these animals by Weeks 9-10 (P) or 15-16 (F_1). Therefore, these increases were considered to be transient and not adverse. No effects of treatment were observed at 400 ppm.

TABLE 6a. Mean cumulative water consumption (g/rat) - P and F_1 generation males[a]

Study week	Dose Group (ppm)			
	0	400	1200	3000
P Generation				
Week 1-2	489	484	508	567** (116)
Week 9-10	581	552	560	580
F_1 Generation				
Week 5-6	338	335	357	388** (115)
Week 15-16	505	511	532	510

a Data were obtained from Tables 8:0 and 8:1 on pages 66-67 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
** Significantly different from controls; $p \leq 0.01$

TABLE 6b. Mean cumulative water consumption (g/rat) - P and F_1 generation pre-mating females[a]

Study week	Dose Group (ppm)			
	0	400	1200	3000
P Generation				
Week 1-2	374	382	380	429** (115)
Week 9-10	400	422	409	423
F_1 Generation				
Week 5-6	304	295	334* (112)	335* (110)
Week 15-16	403	390	397	402

a Data were obtained from Tables 8:0 and 8:1 on pages 66-67 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

5. Reproductive function

a. Estrous cycle length and periodicity: Summary data were not provided.

b. Sperm measures: Sperm measures were not performed.

6. Reproductive performance: There were no effects of treatment on the precoital or gestation intervals or on reproductive performance in either generation (Table 7).

TABLE 7. Reproductive performance[a]

Observation	Dose Group (ppm)			
	0	400	1200	3000
P Generation				
Number males paired	32	32	32	30
Number females paired	32	32	32	32
Mating index (%)[b]	97	100	97	97
Females non-pregnant	2	0	2	1
Females with total litter resorption	0	2	0	0
Females with total litter loss post-partum	0	0	1	0
Pregnancy rate (%)	94	100	94	97
Median pre-coital time (days)	3.0	2.5	2.0	2.0
Duration of gestation (mean days)	21.8	21.6	21.8	21.8
F_1 Generation				
Number males paired	28	28	28	28
Number females paired	28	28	28	28
Mating index (%)[b]	100	93	96	96
Females non-pregnant	1	5	1	3
Females with total litter resorption	0	0	0	0
Females with total litter loss post-partum	2	0	2	0
Pregnancy rate (%)	96	82	96	89
Median pre-coital time (days)	2.0	3.0	2.0	3.0
Duration of gestation (mean days)	22.0	21.9	21.8	21.9

a Data were obtained from Tables 1:0, 1:1, 9:0, 9:1, 11:0 and 11:1 on pages 52-53, 68-69, and 70-73 of the study report.

b Calculated by reviewers from data presented in Appendices 7:0 and 7:1 on pages 267-275 using the following formula:
Mating index = # of females mated (females with vaginal sperm or that gave birth to a litter)/# of females paired x 100

7. Parental postmortem results

a) Organ weights: Organ weight data are presented in Tables 8a and b. Increases ($p \leq 0.01$) were observed in adjusted (to terminal body weight) kidney weights in the ≥400 ppm P (↑19-35%) and F_1 (↑13-55%) males and in the ≥1200 ppm P (↑12-24%) and F_1 (↑12-26%) females. Increases ($p \leq 0.05$) in adjusted liver weights were noted in the ≥1200 ppm P males (↑7-21%) and at 3000 ppm in the P females (↑7%) and F_1 males and females (↑8-16%); however, in the absence of corroborating microscopic pathological findings, this is considered an adaptive response. Other alterations ($p \leq 0.05$) in adjusted organ weights were observed, but were minor and/or not corroborated by pathological findings.

TABLE 8a. Selected absolute and adjusted to terminal body weight (g) organ weights - P and F_1 generation males[a]

Organ weight	Dose Group (ppm)			
	0	400	1200	3000
P Generation				
Terminal body weight	693	690	673	645
Kidney				
absolute	5.002	5.429	5.814	6.441
adjusted	4.88	5.31** (19)	5.79** (119)	6.58** (135)
Liver				
absolute	27.54	28.55	28.31	30.81
adjusted	26.67	27.82	28.47* (17)	32.36** (121)
F_1 Generation				
Terminal body weight	663	685	672	577
Kidney				
absolute	4.676	5.451	5.869	6.420
adjusted	4.536	5.140** (113)	5.634** (124)	7.015** (155)
Liver				
absolute	25.72	27.09	27.40	25.83
adjusted	25.11	25.45	26.36	29.09** (116)

a Data were obtained from Tables 16:0:1 and 16:1:1 on pages 80 and 82 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

TABLE 8b. Selected absolute and adjusted to terminal body weight (g) organ weights - P and F_1 generation females[a]

Organ weight	Dose (ppm)			
	0	400	1200	3000
P Generation				
Terminal body weight	356	362	358	345
Kidney				
absolute	2.952	3.050	3.325	3.569
adjusted	2.945	3.007	3.306** (112)	3.638** (124)
Liver				
absolute	15.55	15.37	15.79	16.28
adjusted	15.51	15.15	15.69	16.64** (17)
F_1 Generation				
Terminal body weight	366	384	367	331
Kidney				
absolute	2.912	3.078	3.273	3.463
adjusted	2.892	2.958	3.246** (112)	3.636** (126)
Liver				
absolute	15.62	15.63	16.18	15.71
adjusted	15.51	14.93	16.03	16.72* (18)

a Data were obtained from Tables 16:0:2 and 16:1:2 on pages 81 and 83 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

b) Pathology

1) Macroscopic examination: Macroscopic findings are presented in Tables 9a and b. In the ≥400 ppm P and F_1 animals, the forestomach was observed to be thickened (78-100%) and roughened (88-100%), compared to 0 controls, with depressions in the epithelial aspect (10-100%) compared to controls (0-4%). At ≥1200 ppm, white areas (19-59%) in the forestomach were noted in the P animals, and the limiting ridge of the forestomach was prominent (21-86%) in the F_1 animals, both compared to 0 controls. Enlarged kidney was noted in the ≥1200 ppm males of both generations (10-50%), both compared to 0 controls. In the ≥400 ppm P males and in the 3000 ppm P females, the caecum was distended (16-57%) compared to 0 controls; however, in the absence of corroborating microscopic pathological findings, this finding is considered equivocal. In the 3000 ppm P males, the liver was enlarged (27%) compared to controls (6%), but this was considered an adaptive response. Also, petechiae were observed in the lungs (27%) in this group compared to controls (3%), but in the absence of microscopic pathological data, this was considered incidental. No effects of treatment were observed on testicular measurements. All other macroscopic pathological findings were unrelated to dose.

TABLE 9a. Selected macroscopic pathology findings (% animals affected) - P and F_1 generation males[a]

Finding	Dose Group (ppm)			
	0	400	1200	3000
P Generation				
Forestomach				
thickened	0	78	100	100
roughened	0	88	97	97
depressions	0	13	10	13
white	0	3	19	57
Caecum				
distended	0	16	26	57
Kidney				
enlarged	0	3	10	50
Liver				
enlarged	6	13	13	27
Lungs				
petechiae	3	9	3	27
F_1 Generation				
Forestomach				
thickened	0	89	100	100
roughened	0	93	100	100
depression/s	0	54	81	96
limiting ridge prominent	0	0	26	86
Kidney				
enlarged	0	7	26	29

a Data were obtained from Tables 18:0 and 18:1 on pages 88-89 and 93 of the study report.

TABLE 9b. Selected macroscopic pathology findings (% animals affected) - P and F_1 generation females[a]

Finding	Dose Group (ppm)			
	0	400	1200	3000
P Generation				
Forestomach				
thickened	0	94	100	100
roughened	0	97	100	97
depressions	3	13	13	13
white	0	9	28	59
Caecum				
distended	0	0	9	50
F_1 Generation				
Forestomach				
thickened	0	100	100	100
roughened	0	100	100	100
depression/s	4	64	100	100
limiting ridge prominent	0	0	21	85

a Data were obtained from Tables 18:0 and 18:1 on pages 89 and 93 of the study report.

2) **Microscopic examination:** Microscopic findings are presented in Tables 10a and b. In the ≥400 ppm P and F_1 animals, trace to moderate hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach (44-100%) was observed compared to controls (0-7%). At 3000 ppm, minimal focal ulceration of the non-glandular epithelium of the stomach (19%) was observed in the P females, and trace to moderate erosion of the non-glandular epithelium of the stomach (22%) was noted in the F_1 females, both compared to 0 controls. In the kidney of the ≥1200 ppm P males, trace to moderate dilated/basophilic cortical tubules containing colloidal material (42-53%) were noted compared to controls (16%). Trace to moderate hyperplasia/hypertrophy of the inner cortical tubules of the kidney was observed in the ≥1200 ppm F_1 males (44-100%), in the 3000 ppm P males (93%), and in the 3000 ppm P and F_1 females (48-63%), all compared to 0 controls. In the ≥400 ppm P females, trace to minimal dystrophic mineralization at the corticomedullary junction (34-38%) of the kidney was noted compared to controls (13%) No other treatment-related microscopic findings were observed.

TABLE 10a. Selected microscopic pathology findings (% incidence) - P and F_1 generation males[a]

Finding	Dose Group (mg/kg/day)			
	0	400	1200	3000
P Generation				
Stomach				
hyperplasia and hyperkeratosis of non-glandular epithelium (total)	0	44	94	100
trace	0	19	10	0
minimal	0	25	32	43
moderate	0	0	52	57
Kidney				
dilated/basophilic cortical tubules containing colloidal material (total)	16	16	42	53
trace	0	13	23	30
minimal	16	3	16	23
moderate	0	0	3	0
hyperplasia/hypertrophy of inner cortical tubules (total)	0	0	0	93
minimal	0	0	0	60
moderate	0	0	0	33
F_1 Generation				
Stomach				
hyperplasia and hyperkeratosis of non-glandular epithelium (total)	4	93	100	100
trace	4	32	19	0
minimal	0	39	48	64
moderate	0	21	33	36
Kidney				
hyperplasia/hypertrophy of inner cortical tubules (total)	0	0	44	100
trace	0	0	19	36
minimal	0	0	22	50
moderate	0	0	4	14

a Data were obtained from Tables 20:0 and 20:1 on pages 98-99 and 106-107 of the study report.

TABLE 10b. Selected microscopic pathology findings (% incidence) - P and F_1 generation females[a]

Finding	Dose Group (ppm)			
	0	400	1200	3000
P Generation				
Stomach				
hyperplasia and hyperkeratosis of non-glandular epithelium (total)	0	97	97	100
trace	0	25	13	0
minimal	0	63	56	53
moderate	0	9	28	47
focal ulceration of non-glandular epithelium (total)	0	3	0	19
minimal	0	3	0	19
Kidney				
dystrophic mineralization at the corticomedullary junction (total)	13	34	38	38
trace	9	25	31	22
minimal	3	9	6	16
hyperplasia/hypertrophy of inner cortical tubules (total)	0	0	0	63
minimal	0	0	0	63
F_1 Generation				
Stomach				
hyperplasia and hyperkeratosis of non-glandular epithelium (total)	7	93	100	93
trace	0	25	14	0
minimal	0	57	50	37
moderate	7	11	36	56
erosion of non-glandular epithelium (total)	0	4	4	22
trace	0	0	4	7
minimal	0	4	0	11
moderate	0	0	0	4
Kidney				
hyperplasia/hypertrophy of inner cortical tubules (total)	0	0	7	48
trace	0	0	4	33
minimal	0	0	4	15

a Data were obtained from Tables 20:0 and 20:1 on pages 98-100 and 106-107 of the study report.

B. OFFSPRING

1. Viability and clinical signs: Litter data for the F_1 and F_2 pups are presented in Table 11. There were no effects of treatment on the post-implantation survival, live birth, viability, or lactation indices or on the sex ratio in any generation. In the 3000 ppm group, the mean number of implantation sites was decreased ($p \leq 0.05$) by 5%; however, a greater non-significant decrease was observed in the 400 ppm group. Therefore, this finding was considered incidental. Clinical signs of toxicity were not reported.

TABLE 11. Litter parameters for F_1 and F_2 generations[a]

Observation		Dose Group (ppm)			
		0	400	1200	3000
F_1 Generation					
Mean implantation sites		16.7	16.5	16.1	16.8
Number born live[b]		449	450	425	477
Number born dead[b]		10	8	11	1
Mean sex ratio (% live ♂) on Day 0		51.5	48.1	49.8	52.4
# Deaths Days 1-4[b]		10	8	6	6
# Deaths Days 5-21[b]		14	19	11	21
Mean litter size	Day 0	15.3	15.3	15.0	15.4
	Day 4	14.6	14.7	14.4	15.2
	Day 8	14.4	14.4	14.2	14.9
	Day 12	14.3	14.2	14.1	14.7
	Day 16	14.2	14.1	14.1	14.5
	Day 21	14.2	14.1	14.1	14.5
Post-implantation loss (%)		9.1	7.2	7.3	8.3
Live birth index (%)[c]		97.8	98.3	97.5	99.8
Viability (Days 0-4) index (%)[d]		97.8	98.2	98.6	98.7
Lactation (Days 4-21) index (%)[e]		96.8	95.7	97.4	95.5

(table continues next page)

Observation	Dose Group (ppm)			
	0	400	1200	3000
F_2 Generation				
Mean implantation sites	16.7	15.6	16.4	15.8* (15)
Number born live[b]	385	324	384	372
Number born dead[b]	5	5	3	4
Mean sex ratio (% live ♂) on Day 0	51.2	49.8	50.4	49.1
# Deaths Days 1-4[b]	11	6	4	7
# Deaths Days 5-21[b]	12	8	16	19
Mean (±SD) litter size Day 0	15.6	14.3	15.5	15.0
Day 4	15.0	13.8	15.2	14.6
Day 8	14.7	13.7	14.9	14.0
Day 12	14.5	13.6	14.7	14.0
Day 16	14.5	13.5	14.6	13.9
Day 21	14.5	13.5	14.6	13.8
Implant loss (%)	6.4	8.2	5.2	4.7
Live birth index (%)[c]	98.7	98.5	99.2	98.9
Viability (Days 1-4) index (%)[d]	97.1	98.1	99.0	98.1
Lactation (Days 4-21) index (%)[e]	96.8	97.5	95.8	94.8

a Data were obtained from Tables 12:0, 12:1, 13:0, and 13:1 and Appendices 8:0 and 8:1 on pages 74-77 and 276-291 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
b Calculated by reviewers
c Calculated by reviewers as # pups born live/total # of pups born x 100
d Calculated by reviewers as # pups alive on PND 4/# pups born live x 100
e Calculated by reviewers as # pups alive on PND 21/# pups alive on PND 4 x 100
* Significantly different from controls; $p \leq 0.05$

2. <u>Body weight</u>: Offspring body and litter weight data are presented in Tables 12a and b. At 3000 ppm, pup body weights were decreased ($p \leq 0.05$) during PND 12-21 in the F_1 and F_2 pups (↓10-21%), resulting in decreased (NS) overall (PND 0-21) pup body weight gains (↓17-24%). Litter weights were decreased ($p \leq 0.05$) in the F_1 pups during PND 16-21 (↓7-11%), and in the F_2 pups during PND 8-21 (↓10-23%), resulting in decreased (NS) overall litter weight gains (↓14-27%). No effects of treatment were observed on body or litter weights at 400 or 1200 ppm.

TABLE 12a. Mean pup body weights (g)[a]

PND	Dose Group (ppm)			
	0	400	1200	3000
F_1 Pups				
0	6.3	6.3	6.4	6.5
4	9.4	9.5	10.1	9.6
8	16.1	16.1	16.9	15.2
12	24.2	24.0	24.7	21.9* (↓10)
16	31.5	31.8	31.9	28.2* (↓10)
21	46.1	46.2	46.3	39.5** (↓14)
Gain (PND 0-21)[b]	39.8	39.9	39.9	33.0 (↓17)
F_2 Pups				
0	6.3	6.5	6.3	6.3
4	9.9	10.2	9.8	9.5
8	16.2	17.2	16.1	15.1
12	23.7	24.9	23.4	21.0**(↓11)
16	30.1	32.1	29.6	25.6** (↓15)
21	44.6	47.3	43.2	35.4** (↓21)
Gain (PND 0-21)[b]	38.3	40.8	36.9	29.1 (↓24)

a Data were obtained from Tables 12:0 and 12:1 on pages 74-75 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

b Calculated by reviewers from data presented in this table

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

TABLE 12b. Mean litter weights (g)[a]

PND	Dose Group (ppm)			
	0	400	1200	3000
F_1 Pups				
0	93.4	93.8	93.2	99.4
4	133.7	138.5	142.7	143.8
8	222.9	228.6	234.2	223.9
12	330.2	333.3	340.7	316.2
16	428.6	439.6	437.1	399.1** (↓7)
21	627.4	639.1	631.9	559.1** (↓11)
Gain (PND 0-21)[b]	534.0	545.3	538.7	459.7 (↓14)
F_2 Pups				
0	96.4	90.9	96.5	93.9
4	145.6	139.2	147.9	137.1
8	233.7	230.5	238.3	211.1* (↓10)
12	336.8	331.0	340.4	291.5** (↓13)
16	426.3	424.1	429.4	351.4** (↓18)
21	629.7	621.8	623.5	484.7** (↓23)
Gain (PND 0-21)[b]	533.3	530.9	527.0	390.8 (↓27)

a Data were obtained from Tables 12:0 and 12:1 on pages 74-75 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
b Calculated by reviewers from data presented in this table
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

3. <u>Sexual maturation (F_1)</u>: Sexual maturation data are presented in Table 13. At 3000 ppm, balano-preputial skinfold cleavage was delayed ($p \leq 0.01$) by 3.2 days and vaginal opening was delayed ($p \leq 0.01$) by 3.4 days compared to controls. However, since no differences were observed in the subsequent mating and reproductive performance of these animals, these effects were considered equivocal and were attributed to the reduction in body weight gain observed during lactation. This assertion is supported by the comparable body weight at attainment of criterion in all treatment groups. No effects of treatment were observed on age or body weight at balano-preputial separation or vaginal patency in the 400 or 1200 ppm groups.

TABLE 13. Sexual maturation (F_1)[a]

Observation	Dose Group (ppm)			
	0	400	1200	3000
Balano-preputial skinfold cleavage - Males				
Mean age (days)	43.6	43.8	44.2	46.8**
Mean body weight (g) at completion	217	223	222	205
Vaginal opening - Females				
Mean age (days)	33.9	34.1	34.6	37.3**
Mean body weight (g) at completion	116	120	123	124

a Data were obtained from Tables 14:1 and 15:1 on pages 78-79 of the study report.
** Significantly different from controls; $p \leq 0.01$

4. Offspring postmortem results

a) Organ weights: Organ weights were not recorded.

b) Pathology

1) Macroscopic examination: Macroscopic findings are presented in Table 14. A dose-dependent increase in the incidence of thickening and/or roughening of the forestomach was observed in the ≥400 ppm weanlings of both generations. No other macroscopic pathology data were presented.

TABLE 14. Incidence (% pups affected [% litters affected]) of thickening and/or roughening of the forestomach of F_1 and F_2 weanlings[a]

Observation	Dose Group (ppm)			
	0	400	1200	3000
F_1 Generation				
# of pups (litters) examined	369 (30)	367 (30)	352 (29)	394 (31)
Thickening and/or roughening of forestomach	0 (0)	44 (67)	87 (97)	97 (97)
F_2 Generation				
# of pups (litters) examined	362 (25)	310 (23)	364 (25)	346 (25)
Thickening and/or roughening of forestomach	0 (0)	8 (30)	48 (64)	67 (92)

a Percent incidence calculated by reviewers from data presented in Tables 19:0 and 19:1 on pages 95-96 of the study report.

2) **Microscopic examination:** Microscopic findings are presented in Tables 15a and b. At ≥400 ppm in both generations, increased ($p \leq 0.05$) incidence and severity of trace to moderate hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach (30-100%) and trace to marked subepithelial edema in the non-glandular region of the stomach (48-100%) were observed compared to controls (0-4%). In the ≥400 ppm F_1 group, trace to moderate inflammatory cells in the non-glandular region of the stomach (13-40%) was noted compared to 0 controls. All other microscopic findings were unrelated to dose.

TABLE 15a. Microscopic findings in the stomach (% incidence) of F_1 and F_2 males[a]

Observation	Dose Group (ppm)			
	0	400	1200	3000
F_1 Generation				
Hyperplasia and hyperkeratosis of non-glandular epithelium	0	73	100	100
trace	0	57**	17*	16*
minimal	0	10	69**	58**
moderate	0	7	14	26**
Subepithelial edema in non-glandular region	0	50	79	81
trace	0	20*	45**	23*
minimal	0	23*	21*	55**
moderate	0	7	10	3
marked	0	0	3	0
Inflammatory cells in non-glandular region	0	13	14	23
trace	0	3	7	10
minimal	0	10	7	10
moderate	0	0	0	3
F_2 Generation				
Hyperplasia and hyperkeratosis of non-glandular epithelium	0	61	88	68
trace	0	43	32**	24*
minimal	0	17	40**	24*
moderate	0	0	16	20*
Subepithelial edema in non-glandular region	4	57	80	100
trace	4	43**	52**	44**
minimal	0	9	24*	48**
moderate	0	4	4	8

a Percent incidence calculated by reviewers from data presented in Tables 20:0:2 and 20:1:2 on pages 105 and 114 of the study report.

TABLE 15b. Microscopic findings in the stomach (% incidence) of F_1 and F_2 females[a]

Observation	Dose Group (ppm)			
	0	400	1200	3000
F_1 Generation				
Hyperplasia and hyperkeratosis of non-glandular epithelium	0	83	97	100
trace	0	43**	24*	13
minimal	0	37**	41**	57**
moderate	0	3	31**	30**
Subepithelial edema in non-glandular region	0	67	76	93
trace	0	37**	45**	23*
minimal	0	30**	28**	60**
moderate	0	0	3	10
Inflammatory cells in non-glandular region	0	17	28	40
trace	0	10	10	27**
minimal	0	7	17*	7
moderate	0	0	0	7
F_2 Generation				
Hyperplasia and hyperkeratosis of non-glandular epithelium	0	30	80	68
trace	0	17	40**	20*
minimal	0	13	40**	36**
moderate	0	0	0	12
Subepithelial edema in non-glandular region	0	48	80	76
trace	0	30*	56**	36*
minimal	0	13	20*	36**
moderate	0	4	4	4

a Percent incidence calculated by reviewers from data presented in Tables 20:0:2 and 20:1:2 on pages 105 and 114 of the study report.

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: It was concluded that the LOAEL for parental toxicity was 3000 ppm based on body weight gain, food consumption, water intake, and macroscopic and microscopic changes in the stomach and kidney. All stomach changes observed in adults and weanlings were considered to reflect the anticipated irritant effect of the test compound on the stomach. The NOAEL for mating and reproductive performance was 3000 ppm. Growth of the offspring during the pre-weaning period was retarded by treatment at 3000 ppm. The NOAEL for offspring toxicity was 1200 ppm.

B. REVIEWER COMMENTS

1. PARENTAL ANIMALS: In the P generation, two 3000 ppm males were killed for humane reasons due to poor physical condition associated with hemorrhage: one male was killed during Week 6; the other was killed during Week 8. Additionally in the P animals, one 1200 ppm male was found dead during Week 19. In the F_1 parents, one 3000 ppm female was killed for humane reasons during Week 23 having successfully reared a litter to weaning, and one 1200 ppm male was found dead during Week 17. All deaths were considered incidental.

In the parental animals, no treatment-related effects were observed on survival, food or water consumption, estrous cycle length or periodicity, pre-coital interval, duration of gestation, or on mating, gestation, or parturition indices.

In the 3000 ppm P animals, body weight gains during Weeks 0-1 were decreased ($p \leq 0.01$) by 34-41%, and food consumption for Week 1 was decreased ($p \leq 0.05$) by 7-9%. Body weight gains were decreased ($p \leq 0.05$) during Week 1-10 by 8% in the males in this generation, but cumulative food consumption was comparable for Weeks 2-10 in both sexes in this generation. In the 3000 ppm F_1 males, body weights were decreased ($p \leq 0.01$) throughout treatment (Weeks 4-26; ↓12-16%), resulting in decreased ($p \leq 0.01$) body weight gains during pre-mating (Weeks 4-16; ↓13%), that continued to termination (Weeks 17-26; ↓13%; NS). Similarly in the 3000 ppm F_1 females, body weights were decreased ($p \leq 0.01$) during premating (Weeks 4-16; ↓7-13%), resulting in decreased ($p \leq 0.05$) body weight gains (↓8%) during this period. In the males, food consumption was not affected, while food consumption was slightly decreased ($p \leq 0.01$) during Week 1 (↓8%) in the females.

Treatment-related effects were observed on the kidney at 3000 ppm. Adjusted (to terminal body weight) kidney weights were increased ($p \leq 0.01$) in both sexes in both generations (↑24-55%). Enlarged kidney was observed in the P and F_1 males (29-50%) compared to 0 controls, and the following microscopic pathological findings were noted: i) trace to moderate dilated/basophilic cortical tubules containing colloidal material (53%) in the P males compared to controls (16%); ii) trace to moderate hyperplasia/hypertrophy of the inner cortical tubules in both sexes in both generations(48-100%) compared to 0 controls; and iii) trace to minimal dystrophic mineralization at the corticomedullary junction (38%) in the P females compared to controls (13%). Bright yellow/orange urinary staining and wetter than normal feces on the cage tray paper under the cages of the P (8/8) and F_1 (7/7) parents; and cage sawdust yellow stained and wet during lactation phase in the P (31/32) and F_1 (24/27) females was observed.

Treatment-related effects were observed on the kidney at 1200 ppm. Increases ($p \leq 0.01$) were observed in adjusted (to terminal body weight) kidney weights in the 1200 ppm P and F_1 (↑19-24%) males and P and F_1 (↑12%) females. Enlarged kidney was observed in the P and F_1 males (10-26%) compared to 0 controls, and the following microscopic pathological findings were noted: i) trace to moderate dilated/basophilic cortical tubules containing colloidal material (42%) in the P males compared to controls (16%); ii) trace to moderate hyperplasia/hypertrophy of the inner cortical tubules in F_1 males and females (7-44%) compared to 0 controls; and iii) trace to

minimal dystrophic mineralization at the corticomedullary junction (38%) in the P females compared to controls (13%). Yellow skin/fur was observed in all of the 1200 ppm P females and in 100% of the rats at 3000 ppm in both generations, beginning in Week 6 of the P group and Week 2 of the F_1 group. Hair loss was noted in the ≥1200 ppm P females (16-31/32) and in the 3000 ppm F_1 females (23/27). Additionally, the following were observed at 3000 ppm: i) brown staining on the forelimbs in the P (23/32) and F_1 (13/27) females; ii) flaky, swollen, and pink areas around the eyes in the P females (13/32); iii) flaky and yellow skin around the mouth in all of the F_1 males (28/28) and females (27/27); iv) dry, crust, and yellow-rimmed eyes in all of the F_1 males (28/28) and females (27/27); and v) soiled anogenital region in the F_1 males (8/28).

Increases were observed in relative kidney weights (↑9-13%) in the 400 ppm P and F_1 males. Macroscopically, the kidney was enlarged in 3 males in the P generation and in 7 males in the F_1 generation. Microscopically, there was an increased incidence of trace to minimal dystrophic mineralization at the corticomedullary junction (34%) of the kidneys in females when compared to controls (13%). In the ≥400 ppm P and F_1 animals, the forestomach was observed to be thickened (78-100%) and roughened (88-100%), compared to 0 controls, with depressions in the epithelial aspect (10-100%) compared to controls (0-4%). At ≥1200 ppm, white areas in the forestomach were noted in the P animals (19-59%) , and the limiting ridge of the forestomach was prominent in the F_1 animals (21-86%), both compared to 0 controls. In the ≥400 ppm P and F_1 males and females, trace to moderate hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach (44-100%) was observed compared to controls (0-7%). At 3000 ppm, minimal focal ulceration of the non-glandular epithelium of the stomach (19%) was observed in the P females, and trace to moderate erosion of the non-glandular epithelium of the stomach (22%) was noted in the F_1 females, both compared to 0 controls.

The LOAEL for parental toxicity is 400 ppm (equivalent to 30.8/34.3 mg/kg bw/day male/female), based on macroscopic and microscopic pathological findings in the stomach- including: thickened, roughened, and white areas in forestomach with depressions in the epithelial aspect, and hyperplasia and hyperkeratosis of non-glandular epithelium in the stomach, and kidney effects, i.e., enlargement, relative weight increases and dystrophic mineralization at the corticomedullary junction. The NOAEL was not established.

2. **OFFSPRING:** In the offspring, no treatment-related effects were observed on post-implantation survival, live birth, viability, or lactation indices, on the sex ratio, clinical signs, gross pathology, or microscopic pathology.

At 3000 ppm, pup body weights were decreased ($p \leq 0.05$) during PND 12-21 in the F_1 and F_2 pups (↓10-21%), resulting in decreased (NS) overall (PND 0-21) pup body weight gains (↓17-24%). Litter weights were decreased ($p \leq 0.05$) in the F_1 pups during PND 16-21 (↓7-11%), and in the F_2 pups during PND 8-21 (↓10-23%), resulting in decreased (NS) overall litter weight gains (↓14-27%). Additionally, balano-preputial skinfold cleavage was delayed ($p \leq 0.01$) by 3.2 days and vaginal opening was delayed ($p \leq 0.01$) by 3.4 days compared to controls. However, since no differences were observed in the subsequent mating and reproductive performance of these animals, these effects were considered equivocal and were attributed to the reduction in

body weight gain observed during lactation. This assertion is supported by the comparable body weight at criterion in all treatment groups.

At ≥400 ppm, a dose-dependent increase in the incidence of thickening and/or roughening of the forestomach was observed in both generations, and increased ($p \leq 0.05$) incidence and severity of trace to moderate hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach (30-100%) and trace to marked subepithelial edema in the non-glandular region of the stomach (48-100%) were observed compared to controls (0-4%). Additionally, in the F_1 group, trace to moderate inflammatory cells in the non-glandular region of the stomach (13-40%) was noted compared to 0 controls.

The LOAEL for offspring toxicity is 400 ppm (equivalent to 30.8/34.3 mg/kg bw/day male/female), based on thickening and/or roughening of the forestomach with depressions in the epithelial aspect, and hyperplasia and hyperkeratosis of the non-glandular epithelium of the stomach. The NOAEL was not established.

No treatment-related effects were observed on estrous cycle length or periodicity, pre-coital interval, duration of gestation, or on mating, gestation, or parturition indices.

The LOAEL for reproductive performance was not observed. The NOAEL for reproductive performance is 3000 ppm (equivalent to 247.5/270.0 mg/kg bw/day males/females).

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.

C. **STUDY DEFICIENCIES:** The following minor deficiencies were noted but do not change the conclusions of this review:

- Data were presented as group mean values; however, standard deviations were not provided.
- Sperm enumeration, motility, and morphology were not evaluated in the parental males.
- Pup body weight data were not presented by sex.
- Pup organ weights were not measured.