DATA EVALUATION RECORD – SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.3100 [§82-1a]; Subchronic (90-day) Oral Toxicity Study in Rats

Work Assignment No. 4-1-128 B (MRID 46808219)

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This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

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DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding]-[rat]; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

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

TEST MATERIAL (PURITY): XDE-570 (Florasulam; 99.2% a.i.)

SYNONYMS: *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: Redmond, J. M., and K. A. Johnson (1996) XDE-570: 13-week dietary toxicity and 4-week recovery in F344 rats. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: DR-0312-6565-011, January 31, 1996. MRID 46808219. Unpublished.

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

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 46808219), XDE-570 (Florasulam; 99.2% a.i.; Lot No. 930910) was administered in the diet to ten Fischer 344 rats/sex/dose at dose levels of 0, 20, 100, 500, or 1000/800 (males/females) mg/kg/day (time-weighted intake was 0/0, 22/21, 112/106, 550/528, and 1111/843 mg/kg/day [males/females]) for 13 weeks. An additional ten rats/sex/dose were fed test diets containing 0 or 1000/800 (males/females) mg/kg/day for 13 weeks, followed by a 4-week recovery period, during which time all rats were fed control diet.

No adverse treatment-related effects were observed on mortality, clinical signs, food consumption, food efficiency, ophthalmoscopic examinations, hematology, clinical chemistry, or gross pathology.

At 500 mg/kg/day, body weights were decreased ($p<=0.05$) in the females by 5-8% during Weeks 6-13, contributing to a 21% decrease ($p<=0.05$) in overall (Weeks 0-13) body weight gains. At 1000 mg/kg/day, body weights were decreased ($p<=0.05$) in both sexes by 7-17% throughout treatment, resulting in decreased ($p<=0.05$) overall body weights gains (decr. 23-30%). Body weights and body weight gains remained decreased ($p<=0.05$) in the 1000 mg/kg/day males

following recovery (decr. 11% and 17% at Week 17, respectively).
Slight nephrotoxicity was observed at 500 mg/kg/day and above. Absolute and relative (to body weight) kidney weights were increased ($p<=0.05$) by 9-37% in both sexes. Urinary pH was decreased in both the males (5.90-6.85 vs. 7.55 in controls) and females (6.65-7.10 vs. 8.20 in controls). Very slight to slight hypertrophy of the epithelial cells of the collecting ducts were observed in the males (10/10 at each dose vs. 0/10 controls) and females (8-9/10 vs. 0/10 controls); and degeneration/regeneration and inflammation (with or without necrosis) of the descending portion of the proximal tubules was noted in the females (3/10 at each dose vs. 0/10 controls). Additionally, the specific gravity of the urine was decreased ($p<=0.05$) in the 1000 mg/kg/day males (1.035 vs. 1.051 in controls), and very slight multifocal mineralization of the kidney papilla was observed in the 800 mg/kg/day females (9/10 vs. 0/10 controls). Following recovery, both very slight mineralization of the tubules of the papilla (9/10 vs. 0/10 controls) and very slight degeneration/regeneration of the cortical tubules (5/10 vs. 0.10 controls) were noted in the kidney of the 800 mg/kg/day females.

The LOAEL is 500 mg/kg/day, based on decreased body weights (5-8%) and body weight gains (21%) in the females, and evidence of slight nephrotoxicity (increased kidney weights, hypertrophy, and degeneration/regeneration and inflammation of the descending portion of proximal tubules) in both sexes. The NOAEL is 100 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100; OECD 408) for a subchronic oral toxicity study in the rat.

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

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

COMMENTS: EPA concurs with the PMRA toxicology evaluation, no conclusions have been changed.

43808219.PMRA.der
.pdf

Reviewer: Tom Morris , Date February 23, 2000

STUDY TYPE: Subchronic Oral Toxicity [feeding]-[rat]; OPPTS 870.3100 (rodent); OECD 408.

TEST MATERIAL (PURITY): XDE-570 (Purity - 99.2%)

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

CITATION: Redmond, J. M. and Johnson, K. A. January 31, 1996. **XDE-570: 13-Week Dietary Toxicity and 4-Week recovery in F344 Rats**. Performing Laboratory: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: DR-0312-6565-011. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a subchronic toxicity study, XDE-570 (Purity - 99.2%) was administered to 10 Fischer 344 rats/sex/dose *ad libitum* in the diet at dose levels of 0, 20, 100, 500, 800 (females) or 1,000 (males) mg/kg bw/d (time-weighted average test substance intake was 0, 22, 112, 550 or 1,111 mg/kg bw/d for ♂ and 0, 21, 106, 528 or 843 mg/kg bw/d for ♀) for 13 weeks. The control group animals received untreated diet (*ad libitum*) throughout the study. In addition, groups of 10 Fischer 344 rats/sex/dose were fed a control or high-dose diet for 13-weeks and then all animals were fed a control diet for an additional 4 weeks to determine the reversibility of any treatment-related effects.

There were no mortalities and no toxicologically relevant treatment-related clinical observations, clinical chemistry, ophthalmoscopic or gross pathological findings. Body weights were significantly lower in males at 1,000 mg/kg bw/d and in females at 500 and 800 mg/kg bw/d. This correlated with reduced food consumption in both sexes at the high dose and appeared to be partially reversible. Red blood cell parameters (RBC counts, HCT and HGB) were slightly but significantly reduced in males at ≥500 mg/kg bw/d. Erythrocyte morphology and RBC indices (MCV, MCH and MCHC) were unaffected by treatment. These marginal haematological findings may be indicative of anaemia although it appears to be reversible. A slight decrease in extramedullary haematopoiesis in the spleen was also observed in the high-dose males although this was considered likely to be a secondary effect. Urinalysis findings included urinary acidification (♂/♀ at ≥500 mg/kg bw/d) and decreased urinary specific gravity (♂ at 1,000 mg/kg bw/d). Following the recovery period, urinary specific gravity continued to be significantly lower. Following treatment, kidney weights were significantly increased in both sexes at ≥500 mg/kg bw/d and continued to be significantly increased in the high-dose animals following the recovery period. The increased kidney weights correlated with the histopathological findings in the kidney, specifically with hypertrophy of epithelial cells of the collecting ducts in both sexes at ≥500 mg/kg bw/d. Hypertrophy of the epithelial cells of the collecting duct was not observed following the recovery period suggesting that it was reversible. The hypertrophied cells were compatible with the intercalated cells which are normally 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 impaired concentrating ability; therefore, the urinalysis findings likely correlate with hypertrophy of the epithelial cells of the collecting ducts. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with urinalysis or histopathological findings in the kidney or to indicate an impairment of renal function. The severity of the hypertrophy of the epithelial cells of the collecting duct appeared to be dose-related and males may be slightly more sensitive than females. In the high-dose males, the increased severity of the hypertrophy of the epithelial cells likely correlated with the more pronounced urinalysis findings observed in these animals. Other significant findings in the kidney were limited to females and included degeneration / regeneration of descending portion of the proximal tubules (at ≥500 mg/kg bw/d) which was considered to be typical of acute necrosis with regeneration rather than a 13-week old lesion and multi-focal mineralization in the papilla (800 mg/kg bw/d) which were still apparent after the

4-week recovery period.

The LOAEL is 500 mg/kg bw/d based on lower body weight and body-weight gain (♀), marginal haematological findings (♂), urinalysis findings (♂/♀), increased kidney weights (♂/♀) and histopathological findings in the kidney (♂/♀). The NOAEL is 100 mg/kg bw/d.

This subchronic toxicity study in the rat is acceptable / guideline and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS:

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

Description: White powdery solid

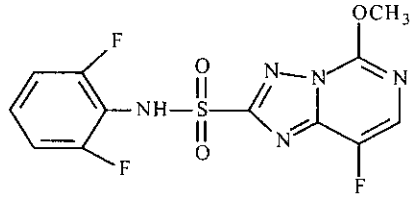
Lot/Batch #: TSN 100298 (Lot # 930910)

Purity: 99.2 % a.i. (determined by HPLC with ultra-violet detection, Certificate of Analysis, GHE-3395, Analysis Reference 93030/DA, February 1994, S. Boothroyd).

Compound Stability: The test substance was determined to be stable in the feed for at least 35 days. The compound was stable for the duration of the study (until Feb 1995)

CAS #: 145701-23-1

Structure



2. **Vehicle and/or positive control:** Dietary admixture.

3. **Test animals:**

Species: Male and female rats.

Strain: Fischer 344

Age/weight at study initiation: At study initiation, the rats were ≈7 weeks of age with a body weight range of 135.5 to 142.1 g for males and 110.8 to 112.3 g for females (animals were born on January 24, 1994).

Source: Charles River Laboratories, Kingston, New York.

Housing: The animals were individually housed.

Diet: Certified Rodent Chow #5002 (Purina Mills Inc., St. Louis, MO) in meal form *ad libitum*

Water: Tap water *ad libitum*

Environmental conditions:

Temperature:	22 ± 1 °C
Humidity:	40-70%
Air changes:	10-12 changes/hr
Photoperiod:	12 hrs dark/12 hrs light

Acclimation period: At least 7 days.

B. STUDY DESIGN:

1. **In life dates** - Start: March 15, 1994. End: Main group - June 16/17, 1994 (test days 92/93)
Recovery Group - July 15, 1994 (test day 123)

2. **Animal assignment**: Animals were randomly assigned to the study groups using a computer-generated randomization program based on body weights as summarized in Table 1. The test substance was administered (*ad libitum*) in the feed for 13 weeks (91 days). For the recovery group animals (control and high-dose groups only), the 13-week treatment period was followed by a 4-week recovery period on untreated diet (control and high-dose groups only). The main group animals were sacrificed and necropsied on study days 92 (males) and 93 (females). The recovery group animals were sacrificed and necropsied on study day 123. The control group animals received untreated diet throughout the study.

TABLE 1: Study design.

Test Group	Dose Levels (mg/kg bw/d)	Time-Weighted Average Test Substance Intake (mg/kg bw/d)		Main Group 13-weeks treatment		Recovery Group 13-weeks treatment + 4-weeks recovery	
		Males	Females	# Males	# Females	# Males	# Females
1	0	0	0	10	10	10	10
2	20	22	21	10	10	-	-
3	100	112	106	10	10	-	-
4	500	550	528	10	10	-	-
5	1,000/800 (♂/♀)	1,111	843	10	10	10	10

In a two-week dietary study, 5 Fischer 344 rats/sex/dose were administered XDE-570 *ad libitum* in the diet at doses of 0, 100, 500 or 1,000 mg/kg bw/d (Szabo, J.R. and Davis, N.L., February 19, 1993. Laboratory Project Study ID: DR-0312-6565-003, study submitted but a full review was not completed). At 1,000 mg/kg bw/d, both sexes exhibited lower food consumption with subsequent statistically significant decreased body weights and secondary organ weight changes possibly due to unpalatability of the diet. Histopathological lesions consisting of degeneration / regeneration of renal proximal tubule epithelium were observed in females at 500 mg/kg bw/d and in both sexes at 1,000 mg/kg bw/d. Nuclear pleomorphism of renal proximal tubule epithelial cells was present in both sexes at 500 and 1,000 mg/kg bw/d. Multi-focal necrosis of proximal tubule epithelial cells was observed in 1/5 male and 4/5 females at 1,000 mg/kg bw/d. The *NOAEL for male and female Fischer 344 rats was 100 mg/kg bw/d. Based on* these findings, the following dose levels were used: 1) in males the high dose (1,000 mg/kg bw/d) represented the limit dose based on acceptable guidelines (OPPTS 870.3100 and OECD 408); 2) in females, the high dose (800 mg/kg bw/d) was expected to produce lower body weights and histopathological effects in the kidneys as observed in previously conducted studies and 3) the lower dose levels were expected to provide dose-response data for any toxicity observed in the high-dose group animals and to ensure the definition of a no-observed-effect level (NOEL) *for the test substance.*

3. **Diet preparation and analysis** Diets were prepared by serially diluting a concentrated test substance-feed mixture (premix) with ground feed. Premixes were prepared approximately every two weeks. The test diets were prepared weekly throughout the dosing period based upon the most recent body weight and feed consumption data. Initial targeted concentrations of the test substance in the diet were calculated from pre-study body weights and historical feed consumption data. The diets were stored at ambient temperature. The stability and homogeneity of the test substance in the diet were determined concurrent with the conduct of the study. Analysis by HPLC of the test substance-feed mixtures to verify the concentration of the test substance in the diet was conducted prior to the beginning of the study (test day 1) and at weeks 7 and 13 of the dosing period. Reference samples (1/dose/sex) from each pre-mix and diet mix were retained and stored at ambient room temperatures.

Results - **Homogeneity Analysis:** The diet with a target concentration of 0.023% (w/w) from the female 20 mg/kg bw/d dose group was shown to be homogenous, with a standard deviation (SD) of 0.00144 and percent relative standard deviation (%RSD) of 6.23% (mean observed concentration 0.0231% w/w).

Stability Analysis: XDE-570 was shown to be stable in the diet for at least 35 days at a concentration of approximately 0.023% (w/w). The concentration was equivalent to the concentration of the female 20 mg/kg bw/d XDE-570 diet. The percent of day zero concentration varied from 91-98% over the 35 day period, but not in a time-dependent manner. The variation was considered within the error of analytical method.

Concentration Analysis: Results indicated an acceptable agreement between actual and target levels, with mean percent of target for all diets and premixes ranging from 95 to 107%.

Dose level (mg/kg bw/d)	Range (% of target concentration)		Mean ± SD (% of target concentration)	
	Males	Females	Males	Females
20	84-106	88-102	97 ± 11	96 ± 7
100	95-103	96-99	100 ± 4	98 ± 2
500	93-99	94-97	97 ± 3	95 ± 2
1,000/800 (♂/♀)	94-105	94-112	100 ± 6	103 ± 9
Premix	101-108		107 ± 10	

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

4. Statistics - Descriptive statistics only (means and standard deviations) were reported for food consumption, food efficiency, white blood cell differential counts and red blood cell indices. Body weights, organ weights, clinical chemistry data, appropriate haematological data and urine specific gravity were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by parametric or non-parametric analysis of variance (ANOVA) followed respectively by Dunnett's test or Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons. Statistical outliers were identified by a sequential test, but routinely excluded from feed consumption statistics only. Because numerous measurements were compared statistically on the same group of animals, the frequency of false positive (Type I) error was unknown, but was considered to be much greater than the nominal alpha.

C. METHODS:

1. Observations: Clinical examinations were conducted on all animals prior to the start of the study and at weekly intervals throughout the duration of the study. A daily cageside examination was made each day of the work week, except on the days when a clinical examination was performed, since the clinical examination was more thorough. An additional observation for moribundity, mortality and the availability of feed and water was made each day of the work week as well as twice daily on weekends and holidays.

2. Body weight: All animals were weighed prior to the start of the study and at weekly intervals throughout the dosing and recovery periods.

3. Food consumption and compound intake: Data were collected from all animals and calculations were made weekly during the dosing and recovery periods by weighing the feeders at the start and end of a measurement cycle. Animals for which food wastage was noted on cage side exams were excluded from the food consumption calculation. From these data, food consumption (g/animal/d) was calculated. Food efficiency (g food consumed/g bw gain/d) and compound intake (mg/kg bw/d) values were also calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination: The eyes were examined on all animals prior to the start of the study using indirect ophthalmoscopy. One drop of 0.5% tropicamide ophthalmic solution was instilled into each eye to produce mydriasis prior to each examination. At scheduled necropsy, ophthalmological examinations were conducted on all animals using a moistened slide/fluorescent light technique.

5. Haematology & Clinical Chemistry: Approximately one week prior to the scheduled necropsy, all main and recovery group animals (10 animals/sex/dose) were fasted and then lightly anaesthetized with methoxyflurane after which blood samples were collected by puncture of the orbital sinus. Haematology samples were mixed with EDTA and blood smears were prepared and stained with Wrights stain. Clinical chemistry samples were collected and serum separated from cells as soon as possible following blood collection. The haematological and clinical

chemistry parameters marked with an (X) in tables (a) and (b), respectively, were examined.

a. Haematology

X	Haematocrit (HCT)*	X	Leukocyte differential count*
X	Haemoglobin (HGB)*	X	Mean corpuscular Haemoglobin (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular Haemoglobin Concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count (PLT)*		Reticulocyte count (RETIC)
	Blood clotting measurements* (a)	X	Erythrocyte Morphology
	(Partial Thromboplastin time)	X	Leukocyte Morphology
	(Thrombin Clotting time)	X	Platelet Morphology
	(Prothrombin time)		

* Recommended for subchronic rodent studies based on Guideline 870.3100
X Examined

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium* (Ca)	X	Albumin* (ALB)
X	Chloride* (Cl)	X	Blood creatinine* (CREAT)
	Magnesium (Mg)	X	Blood urea nitrogen* (UREA)
X	Phosphorus* (P)	X	Total Cholesterol (CHOL)
X	Potassium* (K)	X	Globulins (GLOB)
X	Sodium* (Na)	X	Glucose* (GLUC)
	ENZYMES	X	Total bilirubin (TBILI)
X	Alkaline phosphatase (AP)	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides (TRIG)
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (ALAT) (also SGPT)*		
X	Serum aspartate amino-transferase(ASAT) (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GDH)		

* Recommended for subchronic rodent studies based on Guideline 870.3100
X Examined

6. Urinalysis * - Urine samples were collected from all non-fasted main group and recovery group animals (10 animals/sex/dose) approximately one week prior to their scheduled necropsy by gentle external compression of the bladder. The urinalysis parameters marked with an (X) in the following table were examined.

X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Urinalysis not required for subchronic dietary studies in rodents according to OECD Guideline 408.
X Examined

7. Sacrifice and Pathology - At the scheduled necropsy, following an overnight fast, each animal was weighed, anaesthetized with methoxyflurane, the trachea was exposed and clamped to prevent artifactual aspiration of blood, and the animal was humanely sacrificed via decapitation. The animals were examined externally and then systematically dissected. The necropsy included *in situ* examination of the eyes by a glass slide technique using

fluorescent light illumination. Internal organs were first viewed *in situ* and then removed, incised as appropriate and examined. The organs/tissues, in whole or in part, marked with an (X) in the following table were fixed in neutral, phosphate-buffered 10% formalin. Organs/tissues marked with an (XX) in the following table were weighed prior to fixation. The lungs were infused with buffered formalin to their approximately normal inspiratory volume and the nasal cavity was flushed with formalin delivered via the pharyngeal duct to ensure rapid fixation. A complete histopathological examination of the organs/tissues marked with an (X) in the following table was conducted on all animals from the control and high-dose groups. Histopathological examination of the organs/tissues from animals in the low- and mid-dose groups was limited to the liver, kidneys, lungs and grossly-observed lesions. Histopathological examination of organs/tissues from animals in the recovery groups was limited to the kidneys, mesenteric tissues including mesenteric lymph nodes, spleen and grossly observed lesions. Tissue examined histopathologically were processed by conventional techniques, sectioned at approximately 6 µm and stained with hematoxylin and eosin.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*+		GLANDULAR
X	Ileum*			X	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland[T]
X	Colon*	XX	Kidneys*+	X	Mammary gland*
X	Rectum*	X	Urinary bladder*	X	Parathyroid*
XX	Liver*+	XX	Testes*+	X	Thyroid*
	Gall bladder*	X	Epididymides*+		OTHER
X	Pancreas*	X	Prostate*	X	Bone
	RESPIRATORY	X	Seminal vesicles*	X	Skeletal muscle
X	Trachea*	X	Ovaries*+	X	Skin
X	Lung*	X	Uterus*+	X	All gross lesions and masses*
X	Nose*	X	Oviducts		
X	Pharynx*	X	Cervix		
X	Larynx*	X	Vagina		

* Recommended for subchronic rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
T = required only when toxicity or target organ
X Organ fixed.
XX Organ weighed prior to fixation.

II. RESULTS

A. Observations :

1. Clinical signs of toxicity - There were no treatment-related clinical observations. Males and females in the high-dose groups exhibited soiling of the perineal area throughout most of the 13-week treatment period. The soiling appeared to be urine dried to the fur of the perineum. The incidence was greater in females (up to 19/20 animals during weeks 11 and 12) compared to males (up to 10/20 animals during weeks 11 and 12) and was also observed

earlier in females (week 2) compared to males (week 5). Soiling of the perineal area appeared to be completely resolved during the recovery period (by week 14 in males and by week 17 in females). Perineal soiling was considered to be a secondary or indirect effect of treatment and was considered to be due to lack of grooming possibly due to urine acidification or the presence of excretory products of the test substance in the urine. Perineal soiling was noted only sporadically in the mid-dose animals; again it was more frequently observed in females than in males (1/10 animals during week 11-12 and up to 5/10 females during week 11).

2. Mortality - All animals survived until the respective scheduled sacrifice.

B. Body weight and weight gain: Compared to controls, body weight and body-weight gain were significantly lower in the high-dose males and females (1,000 and 800 mg/kg bw/d, respectively) throughout the treatment period and in females at 500 mg/kg bw/d from week 6 onward (Tables 2 and 3 for males and females, respectively). Compared to controls, the overall (weeks 0-13) body-weight gain was approximately 30 and 23%, lower in the high-dose males and females, respectively, and approximately 21% lower in females at 500 mg/kg bw/d. The treatment-related effects on body weight and body-weight gain in the high-dose males and females appeared to be partially reversible over the 4-week recovery period. During the 4-week recovery period (weeks 13-17), the high-dose males and females exhibited a higher body-weight gain compared to the controls. However, body weight and overall body-weight gain (weeks 0-17) for the high-dose males (≈10 and 17% lower, respectively) and females (≈4 and 9% lower, respectively) continued to be lower compared to the controls at the end of the 4-week recovery period. In the high-dose males this was statistically significant. The treatment-related effect on body weight and body-weight gain in the high-dose males and females and in females at 500 mg/kg bw/d were considered to be toxicologically significant. In the high-dose animals, the treatment-related effect on body weight and body-weight gain was accompanied by a reduction in food consumption (↓ up to 9 and 8% in males and females, respectively).

The overall (weeks 0-13) body-weight gain in males at 500 mg/kg bw/d was significantly lower compared to controls (≈7% lower), however, body weight was not significantly different from the controls. The overall body-weight gain in males at 500 mg/kg bw/d was similar to the overall body-weight gain in males at 20 mg/kg bw/d which was not statistically significant (163.8 ± 15.9 and 163.7 ± 10.0 at 20 and 500 mg/kg bw/d). At week 13, body weight was significantly lower in males at 20 mg/kg bw/d, however, this was not dose-related. Due to the absence of a clear dose-response in body-weight gain and the absence of a significant change in body weight, the significant decrease in overall body-weight gain in males at 500 mg/kg bw/d was not considered to be toxicologically relevant. There were no treatment-related effects on body weight or body-weight gain in either sex at 20 and 100 mg/kg bw/d.

TABLE 2. Average body weights and body-weight gains in males during 13-weeks of treatment (all dose levels) plus 4-weeks of recovery (control and 1,000 mg/kg bw/d only). (a)

Dose Level (mg/kg bw/d)	0 (n = 20)	20 (n = 10)	100 (n = 10)	500 (n = 10)	1,000 (n = 20)
Body Weight (g ± SD)					
Week 0	139.5 ± 5.6	135.5 ± 3.9	141.6 ± 7.0	142.1 ± 6.9	140.4 ± 6.9
Week 3	224.4 ± 7.8	216.5 ± 10.3	224.4 ± 7.8	226.0 ± 10.0	**209.6 ± 9.7 ***
Week 6	263.3 ± 9.8	253.4 ± 12.8	261.5 ± 8.7	265.1 ± 12.7	**241.3 ± 10.5 ***

Dose Level (mg/kg bw/d)	0 (n = 20)	20 (n = 10)	100 (n = 10)	500 (n = 10)	1,000 (n = 20)
Week 9	291.2 ± 11.3	282.0 ± 13.6	291.1 ± 10.1	290.9 ± 14.6	**254.9 ± 12.5 ***
Week 13	316.0 ± 13.4	299.3 ± 17.0 *	310.1 ± 11.8	305.8 ± 14.6	**263.2 ± 15.5 ***
Week 17	335.4 ± 16.4 (n = 10)	-	-	-	**300.1 ± 14.2 *** (n = 10)
Body-Weight Gain (g ± SD)					
Weeks 0-3	84.8 ± 5.5	81.0 ± 8.7	82.9 ± 6.4	83.9 ± 6.3	**69.1 ± 4.7 ***
Weeks 0-6	123.8 ± 8.3	117.9 ± 11.1	119.9 ± 8.4	123.0 ± 8.4	**100.9 ± 6.9 ***
Weeks 0-9	151.7 ± 11.8	146.5 ± 12.5	149.5 ± 10.8	148.8 ± 11.1	**114.5 ± 9.7 ***
Weeks 0-13	176.5 ± 13.0	163.8 ± 15.9	168.5 ± 13.2	163.7 ± 10.0 *	**122.7 ± 12.9 ***
Weeks 13-17 (b)	14.4 ± 4.9	-	-	-	**31.9 ± 7.0**
Weeks 0-17	192.2 ± 17.1	-	-	-	**159.6 ± 10.8 ***

(a) Data obtained from pages 76-78 of the study report for body weight and pages 82-85 for body-weight gain.
(b) Calculated by reviewer from individual body weight data on pages 187-198 of the study report, no statistical analysis done.
* Significantly different ($p \leq 0.05$) from the control (Dunnett's test).

TABLE 3. Average body weights and body-weight gains in females during 13-weeks of treatment (all dose levels) plus 4-weeks of recovery (control and 800 mg/kg bw/d only). (a)

Dose Level (mg/kg bw/d)	0 (n = 20)	20 (n = 10)	100 (n = 10)	500 (n = 10)	800 (n = 20)
Body Weight (g ± SD)					
Week 0	110.8 ± 3.5	112.1 ± 4.0	112.3 ± 1.9	111.6 ± 3.2	111.2 ± 4.6
Week 3	143.2 ± 5.9	143.6 ± 4.7	144.8 ± 5.0	140.0 ± 6.5	**133.8 ± 7.3 ***
Week 6	160.8 ± 8.2	159.4 ± 7.5	160.5 ± 5.4	**153.3 ± 6.6 ***	**149.1 ± 8.7 ***
Week 9	171.8 ± 9.6	172.2 ± 9.1	172.4 ± 6.9	**163.0 ± 9.1 ***	**157.4 ± 8.0 ***
Week 13	180.2 ± 10.8	177.2 ± 9.7	178.5 ± 5.7	**166.4 ± 7.3 ***	**164.5 ± 9.7 ***
Week 17	189.8 ± 12.1 n = 10	-	-	-	182.5 ± 7.2 n = 10
Body-Weight Gain (g ± SD)					
Weeks 0-3	32.4 ± 4.2	31.5 ± 4.6	32.5 ± 4.8	28.3 ± 6.9	**22.6 ± 6.1 ***
Weeks 0-6	50.0 ± 6.6	47.4 ± 8.4	48.3 ± 5.6	**41.7 ± 7.3 ***	**37.9 ± 7.4 ***
Weeks 0-9	61.0 ± 7.9	60.1 ± 9.4	60.2 ± 7.1	**51.3 ± 9.9 ***	**46.1 ± 6.5 ***
Weeks 0-13	69.4 ± 8.8	65.1 ± 9.9	66.2 ± 6.3	**54.8 ± 8.4 ***	**53.3 ± 9.3 ***
Weeks 13-17 (b)	8.2 ± 3.2	-	-	-	**12.4 ± 7.1**
Weeks 0-17	79.1 ± 9.3	-	-	-	71.8 ± 6.9

(a) Data obtained from pages 79-81 of the study report for body weight and pages 86-89 for body-weight gain.
(b) Calculated by reviewer from individual body weight data on pages 199-210 of the study report, no statistical analysis done.
* Significantly different ($p \leq 0.05$) from the control (Dunnett's test).

C. Food consumption and compound intake:

1. **Food consumption** - Throughout treatment (weeks 0-13) there was a reduction in food consumption in the high-dose males and females compared to controls (↓ up to 9 and 8% in males and females, respectively). During the recovery period (weeks 13-17), food consumption continued to be slightly reduced in the high-dose males (15%) but not in the high-dose females. Food consumption in both sexes at 20, 100 and 500 mg/kg bw/d were comparable to

controls throughout the study. Food consumption values are summarized in Table 4.

TABLE 4. Average food consumption (g/animal/d ± SD) in males and females during 13-weeks of treatment (main + recovery groups) plus 4-weeks of recovery (control and 1,000/800 mg/kg bw/d only). (a)

Dose Level (mg/kg bw/d)	0	20	100	500	1,000/800 (b)
Males					
Days 1-4	16.6 ± 0.6 n = 20	16.5 ± 0.9 n = 10	16.5 ± 0.8 n = 10	17.7 ± 1.0 n = 9	16.8 ± 1.0 n = 18
Days 17-24	18.0 ± 0.8 n = 19	18.0 ± 0.8 n = 10	16.2 ± 0.9 n = 10	18.9 ± 0.8 n = 9	16.9 ± 0.9 n = 20
Days 39-45	17.6 ± 0.8 n = 20	17.3 ± 0.9 n = 10	18.0 ± 0.8 n = 9	17.6 ± 1.0 n = 9	16.5 ± 1.7 n = 18
Days 59-66	17.1 ± 1.2 n = 20	16.9 ± 1.2 n = 10	17.9 ± 0.9 n = 10	17.8 ± 0.9 n = 10	15.6 ± 1.7 n = 20
Days 87-93	18.6 ± 1.2 n = 20	17.7 ± 1.2 n = 10	18.9 ± 1.2 n = 10	18.6 ± 1.3 n = 10	17.0 ± 5.8 n = 18
Days 114-119	17.6 ± 0.8 n = 10	-	-	-	16.7 ± 0.9 n = 10
Females					
Days 1-4	11.3 ± 0.8 n = 20	11.4 ± 0.5 n = 10	11.6 ± 0.5 n = 10	12.8 ± 1.1 n = 9	10.1 ± 1.4 n = 16
Days 17-24	13.1 ± 1.0 n = 18	13.8 ± 1.0 n = 10	13.3 ± 1.4 n = 9	12.8 ± 1.1 n = 8	12.1 ± 0.9 n = 19
Days 39-45	12.7 ± 0.9 n = 19	13.1 ± 1.3 n = 10	13.6 ± 1.7 n = 10	12.7 ± 1.6 n = 10	11.8 ± 0.7 n = 19
Days 59-66	12.5 ± 0.9 n = 18	13.9 ± 2.5 n = 10	13.8 ± 1.6 n = 9	12.2 ± 0.7 n = 8	11.8 ± 0.8 n = 20
Days 87-93	12.0 ± 1.3 n = 20	11.0 ± 0.9 n = 10	11.3 ± 0.5 n = 10	10.6 ± 0.8 n = 10	11.0 ± 1.1 n = 17
Days 114-119	12.5 ± 0.7 n = 10	-	-	-	12.5 ± 0.8 n = 10

(a) Data obtained from pages 90-91 of the study report for males and pages 92-93 for females. Animals for which food wastage was noted on cage-side exam were excluded from feed consumption calculation; therefore, the number of animals/group may vary from one observation period to the next.
(b) High-dose levels - 1,000 mg/kg bw/d for males and 800 mg/kg bw/d for females.

2. <u>Compound consumption</u> Time-weighted average test substance intake (mg/kg bw/d) and diet concentration (ppm) are summarized in Table 5.

TABLE 5. Average test substance intake (mg/kg bw/d) and diet concentration (ppm) in males and females during 13-weeks of treatment. (a)

Dose Level (mg/kg bw/d)	0	20	100	500	1,000/800 (b)
Males					
Average test substance intake (mg/kg bw/d)	-	22	112	550	1,111

Dose Level (mg/kg bw/d)	0	20	100	500	1,000/800 (b)
Average diet concentration (ppm)	-	298	1,522	7,396	14,538
Females					
Average test substance intake (mg/kg bw/d)	-	21	106	528	843
Average diet concentration (ppm)	-	256	1,276	6,313	10,350

(a) Data obtained from pages 96 and 98 of the study report for males and pages 97 and 99 for females.
(b) High-dose levels - 1,000 mg/kg bw/d for males and 800 mg/kg bw/d for females.

3. Food efficiency There was no consistent pattern or definitive difference in food efficiency data to indicate a treatment-related effect (Table 6). The decreased body-weight gain in the high-dose males and females was accompanied by a reduction in food consumption (↓ up to 9 and 8% in males and females, respectively). In females at 500 mg/kg bw/d body-weight gain was significantly decreased with little or no change in food consumption.

TABLE 6. Average food efficiency (g/g bwg/d) in males and females during 13-weeks of treatment (main + recovery groups). (a)

Dose Level (mg/kg bw/d)	0	20	100	500	1,000/800 (b)
Males					
Days 4-10	0.157	0.118	0.162	0.124	0.150
Days 17-24	0.104	0.092	0.097	0.101	0.098
Days 39-45	0.084	0.076	0.088	0.081	0.077
Days 60-67	0.070	0.067	0.075	0.074	0.063
Days 88-91	0.069	0.065	0.074	0.073	0.064
Overall Average	0.088	0.077	0.090	0.085	0.083
Females					
Days 4-10	0.128	0.113	0.132	0.110	0.122
Days 17-24	0.115	0.111	0.116	0.103	0.104
Days 39-45	0.096	0.094	0.103	0.093	0.088
Days 60-67	0.086	0.094	0.099	0.082	0.081
Days 88-91	0.078	0.071	0.074	0.07	0.071
Overall Average	0.096	0.08	0.079	0.083	0.089

(a) Data obtained from page 94 of the study report for males and page 95 for females.
(b) High-dose levels - 1,000 mg/kg bw/d for males and 800 mg/kg bw/d for females.

D. Ophthalmoscopic examination - There were no adverse treatment-related ophthalmoscopic findings.

E. Blood analyses

1. Haematology - A dose-related statistically significant decrease in red blood cell parameters (RBC count, HCT and HGB) was observed in males at 500 and 1,000 mg/kg bw/d (Table 7). Erythrocyte morphology and RBC indices (MCH, MCHC and MCV) in these animals appeared to be normal (no reticulocyte counts provided). The high-dose males also exhibited a slight decrease in extramedullary haematopoiesis in the spleen compared to controls. After the 4-week recovery period (control and high-dose males only), HGB and HCT values were

comparable between the control and high-dose males while RBC counts continued to be significantly lower in the high-dose males. However, RBC counts in the high-dose males following the 4-week recovery period were within the normal range of historical control data for animals of this age and strain from this laboratory. These findings in males at 500 and 1,000 mg/kg bw/d were considered to be treatment-related and may be indicative of anaemia which appears to be reversible. The statistically significant decreased RBC count in males at 100 mg/kg bw/d was within the normal range of historical control data for animals of this age and strain from this laboratory and in the absence of any other significant haematological findings was not considered to be toxicologically relevant.

White blood cell counts (WBC) were significantly higher in the high-dose females compared to controls following treatment (Table 7). There were no significant differences in differential WBC count or WBC morphology between the controls and the high-dose females, the increased WBC counts did not correlate with any histopathological changes in these animals and WBC counts in the high-dose females were comparable to controls following the 4-week recovery period; therefore, the increased WBC counts were not considered to be toxicologically relevant.

TABLE 7. Haematological findings in males and females following 13-weeks of treatment (all dose levels) and 4-weeks recovery (control and *1,000/800* mg/kg bw/d *only*). (a)

Dose Level (mg/kg bw/d)	0	20	100	500	1,000/800 (b)
Male Treatment (13 weeks of treatment) (n = 10 animals/dose)					
RBC count (x 10^6/mm^3)	9.68 ± 0.15	9.50 ± 0.17	9.42 ± 0.16 *	**9.29 ± 0.22 ***	**8.83 ± 0.24 ***
HGB (g/dL)	16.2 ± 0.2	16.0 ± 0.2	15.9 ± 0.3	**15.8 ± 0.4 ***	**15.2 ± 0.4 ***
HCT (%)	46.3 ± 0.7	45.4 ± 1.0	45.4 ± 0.8	**44.8 ± 1.2 ***	**43.0 ± 1.2 ***
Male Recovery (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)					
RBC count (x 10^6/mm^3)	9.81 ± 0.24	-	-	-	9.41 ± 0.17 *
HGB (g/dL)	16.0 ± 0.2	-	-	-	15.8 ± 0.3
HCT (%)	46.3 ± 1.3	-	-	-	45.7 ± 1.0
Female Treatment (13 weeks of treatment) (n = 10 animals/dose)					
WBC count (x 10^3/mm^3)	4.90 ± 0.89	4.67 ± 0.71	5.43 ± 1.22	4.70 ± 0.76	6.30 ± 1.37 *
Female Recovery (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)					
WBC count (x 10^3/mm^3)	4.86 ± 0.48	-	-	-	4.51 ± 0.65

(a) Data obtained from pages 100-115 of the study report.
(b) High-dose levels - 1,000 mg/kg bw/d for males and 800 mg/kg bw/d for females.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.

2. Clinical Chemistry - There were no toxicologically relevant treatment-related clinical chemistry findings. Following treatment, significant findings observed in the high-dose males included decreased alkaline phosphatase (AP) activity, decreased total protein (with no significant changes in albumen or globulin levels) and triglyceride levels and increased cholesterol and potassium levels (Table 8). Following treatment, significant findings in the high-dose females included decreased glucose levels and increased phosphate levels (Table 8). With the exception of serum potassium levels, which continued to be significantly higher in the high-dose males, there were no significant differences in these parameters between the high-dose animals (both sexes) and the controls following the 4-week recovery period. The increased potassium (males) and decreased phosphate (females) were within the normal range of historical control data for animals of this age and strain from this laboratory and thus were not considered to be toxicologically relevant. However, the increased potassium levels in the high-dose males may be associated with urinalysis findings and with histopathological findings in the kidneys. Decreased AP activity levels

are not normally pathologically significant. The decreased total protein and triglyceride levels in the high-dose males and the decreased glucose levels in the high-dose females may be secondary to reduced food consumption in these animals and in the absence of any related findings following the recovery period were not considered to be toxicologically relevant. In the absence of any corroborating gross pathological, histopathological or other findings and the absence of a significant change in serum cholesterol levels following the recovery period, the increased cholesterol levels in the high-dose males were not considered to be toxicologically relevant. There were no significant clinical chemistry findings in either sex at 20, 100 or 500 mg/kg bw/d.

TABLE 8. Significant clinical chemistry findings in males and females following 13-weeks of treatment (all dose levels) and 4-weeks recovery (control and 1,000/800 mg/kg bw/d only). (a)

Dose Level (mg/kg bw/d)	0	20	100	500	1,000/800 (b)
Male Treatment (13 weeks of treatment) (n = 10 animals/dose)					
Alkaline phosphatase (mU/mL)	91 ± 8	90 ± 7	85 ± 7	84 ± 8	71 ± 5 *
Total protein (g/dL)	7.4 ± 0.2	7.4 ± 0.2	7.3 ± 0.2	7.4 ± 0.3	7.1 ± 0.3 *
Cholesterol (mg/dL)	59 ± 4	59 ± 5	62 ± 4	59 ± 3	68 ± 9 #
Triglycerides (mg/dL)	85 ± 18	77 ± 15	80 ± 10	71 ± 8	68 ± 10 *
Potassium (mmol/L)	4.3 ± 0.2	4.5 ± 0.4	4.4 ± 0.2	4.4 ± 0.2	4.8 ± 0.3 *
Male Recovery (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)					
Alkaline phosphatase (mU/mL)	77 ± 4	-	-	-	80 ± 7
Total protein (g/dL)	7.5 ± 0.1	-	-	-	7.4 ± 0.1
Cholesterol (mg/dL)	61 ± 7	-	-	-	60 ± 3
Triglycerides (mg/dL)	80 ± 20	-	-	-	67 ± 10
Potassium (mmol/L)	4.2 ± 0.1	-	-	-	4.4 ± 0.2 *
Female Treatment (13 weeks of treatment) (n = 10 animals/dose)					
Glucose (mg/dL)	102 ± 7	102 ± 8	99 ± 6	95 ± 7	86 ± 7 *
Phosphate (mg/dL)	5.8 ± 0.5	5.7 ± 0.5	6.1 ± 0.5	6.1 ± 0.4	6.5 ± 0.4 *
Female Recovery (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)					
Glucose (mg/dL)	94 ± 6	-	-	-	89 ± 4
Phosphate (mg/dL)	5.2 ± 0.5	-	-	-	5.3 ± 0.3

(a) Data obtained from pages 128-135 of the study report.
(b) High-dose levels - 1,000 mg/kg bw/d for males and 800 mg/kg bw/d for females.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
\# Statistically different from control mean by Wilcoxon test, $p \leq 0.05$.

F. Urinalysis - Significant urinary findings following 13 weeks of treatment, included urinary acidification at 500 (both sexes), 800 (females only) and 1,000 (males only) mg/kg bw/d and significantly reduced urinary specific gravity in males at 1,000 mg/kg bw/d (Table 9). Following the 4-week recovery period, urinary pH in the high-dose males and females was comparable to controls while urinary specific gravity in the high-dose males continued to be significantly lower. The urinary acidification and decreased urinary specific gravity were considered to be treatment-related and likely correlate with hypertrophy of the epithelial cells in the collecting duct where functional abnormalities manifest primarily as an acidification defect and impaired concentrating ability. The urinalysis findings were more pronounced in the high-dose males and likely correlated with the increased severity of the histopathological findings in the collecting duct in these animals. The reduced urinary specific gravity in the high-dose males may suggest a decreased ability of these animals to concentrate urine. Animals that exhibit a loss of ability to concentrate urine generally exhibit reduced specific gravity and increased urine volume, however, urine volume was not measured. These findings may represent changes in excretory processes due to histopathological

findings in the kidneys. There were no toxicologically relevant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with urinalysis findings or to indicate an impairment of renal function. However, the increased serum potassium levels in the high-dose males may be associated with the urinalysis findings. Urinary specific gravity was significantly increased in males at 20 and 100 mg/kg bw/d and in females at 500 mg/kg bw/d, however, the increase was not dose-related and was within the normal range of historical control data for animals of this age and strain from this laboratory; therefore, this was considered to be an incidental finding at these animals.

TABLE 9. Significant urinalysis findings in males and females following 13-weeks of treatment (all dose levels) and 4-weeks recovery (control and 1,000/800 mg/kg bw/d only). (a)

Dose Level (mg/kg bw/d)	0	20	100	500	1,000/800 (b)
Male Treatment (13 weeks of treatment) (n = 10 animals/dose)					
Specific gravity	1.051 ± 0.006	1.058 ± 0.006 *	1.063 ± 0.003 *	1.055 ± 0.007	1.035 ± 0.006 *
pH	7.55 ± 0.28	7.95 ± 0.37	7.70 ± 0.48	6.85 ± 0.34	5.90 ± 0.32
Male Recovery (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)					
Specific gravity	*1.060 ± 0.004*	-	-	-	*1.052 ± 0.005 **
pH	7.80 ± 0.35	-	-	-	7.95 ± 0.50
Female Treatment (13 weeks of treatment) (n = 10 animals/dose)					
Specific gravity	1.044 ± 0.014	1.051 ± 0.010	1.053 ± 0.008	1.057 ± 0.008 *	1.051 ± 0.008
pH	8.20 ± 0.63	7.85 ± 0.63	8.05 ± 0.96	7.10 ± 0.52	6.65 ± 0.53
Female Recovery (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)					
Specific gravity	1.047 ± 0.009	-	-	-	1.042 ± 0.012
pH	8.30 ± 0.67	-	-	-	8.25 ± 0.72

(a) Data obtained from pages 116-127 of the study report.
(b) High-dose levels - 1,000 mg/kg bw/d for males and 800 mg/kg bw/d for females.
* Statistically different from control mean by Dunnett's test, $p < 0.05$.

G. Sacrifice and Pathology:

1. Organ weight - Following the 13-week treatment period, males at 500 and 1,000 mg/kg bw/d and females at 500 and 800 mg/kg bw/d exhibited significantly increased absolute and relative kidney weights (Table 10). The increased kidney weights correlate with histopathological findings in the kidney, specifically with hypertrophy of the epithelial cells of the collecting ducts, and were considered to be treatment-related and toxicologically significant. Following the 4-week recovery period, kidney weights were still slightly increased in the high-dose males (relative kidney weights only) and females (absolute and relative kidney weights) compared to controls. In the absence of any corroborating clinical chemistry, gross pathological or histopathological findings, statistically significant findings in the heart and liver were not considered to be primary treatment-related effects and were not considered to be biologically or toxicologically significant.

TABLE 10. Absolute (g ± SD) and relative (g/100 g bw ± SD) organ weights in males and females following 13-weeks of treatment (all dose levels) and 4-weeks recovery (control and 1,000/800 mg/kg bw/d only). (a)

Dose Level (mg/kg bw/d)		Males			Females		
		Kidneys	Heart	Liver	Kidneys	Heart	Liver
Treatment Groups (13 weeks of treatment) (n = 10 animals/dose)							
0	Absolute	2.022 ± 0.085	0.813 ± 0.060	7.401 ± 0.393	1.281 ± 0.082	0.580 ± 0.036	4.630 ± 0.322

Dose Level (mg/kg bw/d)		Males			Females		
		Kidneys	Heart	Liver	Kidneys	Heart	Liver
	Relative	0.702 ± 0.031	0.282 ± 0.015	2.569 ± 0.077	0.773 ± 0.030	0.350 ± 0.021	2.794 ± 0.112
20	Absolute	2.028 ± 0.105	0.801 ± 0.056	7.531 ± 0.562	1.285 ± 0.075	0.574 ± 0.029	4.652 ± 0.311
	Relative	0.731 ± 0.036	0.289 ± 0.020	2.714 ± 0.165	0.788 ± 0.024	0.352 ± 0.007	2.795 ± 0.078
100	Absolute	2.062 ± 0.058	0.836 ± 0.054	7.432 ± 0.435	1.296 ± 0.063	0.582 ± 0.025	4.553 ± 0.186
	Relative	0.720 ± 0.024	0.292 ± 0.017	2.592 ± 0.099	0.787 ± 0.024	0.354 ± 0.021	2.764 ± 0.055
500	Absolute	**2.283 ± 0.097** *	0.849 ± 0.046	7.797 ± 0.415	**1.396 ± 0.069** *	0.571 ± 0.037	4.352 ± 0.161 *
	Relative	**0.806 ± 0.034** #	0.300 ± 0.011	2.755 ± 0.178 #	**0.909 ± 0.036** #	0.372 ± 0.024	2.835 ± 0.117
1,000/800 (b)	Absolute	**2.296 ± 0.179** *	0.748 ± 0.032 *	6.268 ± 0.749 *	**1.460 ± 0.134** *	0.542 ± 0.027 *	4.135 ± 0.190 *
	Relative	**0.961 ± 0.080** #	0.313 ± 0.016 *	2.626 ± 0.261	**1.010 ± 0.099** #	0.375 ± 0.029	2.857 ± 0.082
Recovery Groups (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)							
0	Absolute	2.124 ± 0.116	0.825 ± 0.052	7.789 ± 0.595	1.237 ± 0.069	0.559 ± 0.029	4.344 ± 0.301
	Relative	0.683 ± 0.017	0.265 ± 0.009	2.501 ± 0.084	0.726 ± 0.028	0.328 ± 0.015	2.546 ± 0.078
1,000/800 (b)	Absolute	2.102 ± 0.146	0.760 ± 0.044 *	6.956 ± 0.523 *	**1.368 ± 0.036** *	0.566 ± 0.037	4.586 ± 0.435
	Relative	**0.762 ± 0.039** *	0.276 ± 0.009 *	2.521 ± 0.118	**0.838 ± 0.028** *	0.347 ± 0.019 *	2.813 ± 0.288 #

(a) Data obtained from pages 136 to 139 of the study report.
(b) High-dose levels - 1,000 mg/kg bw/d for males and 800 mg/kg bw/d for females.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
# Statistically different from control mean by Wilcoxon test $p \leq 0.05$.

2. Gross pathology - There were no toxicologically relevant treatment-related gross pathological findings. Following the 13-week treatment period, decreased adipose tissue was observed in 8/10 high-dose females (incidence: 0/10, 0/10, 0/10, 0/10 and 8/10 at 0, 20, 100, 500 and 800 mg/kg bw/d, respectively). The decreased adipose tissue was most noticeable around the reproductive tract and was not observed after the 4-week recovery period. At the high dose, urinary soiling of the perineal area was observed in 5/10 males and 8/10 females, this correlated with clinical observations and was considered to be a secondary or indirect effect of treatment.

3. Microscopic pathology - In the kidney, hypertrophy of epithelial cells of the collecting ducts was observed in males at 500 and 1,000 mg/kg bw/d and in females at 500 and 800 mg/kg bw/d following 13 weeks of treatment. The lesions were characterized by enlarged individual cells lining the collecting ducts and were restricted to the inner stripe of the outer zone of the medulla. The cells generally exhibited a granular, pale cytoplasm. Small foci of mineralization were infrequently noted in the areas of the hypertrophied cells and were thought to be an end-stage of hypertrophied cells. The affected tubules appeared slightly dilated. The hypertrophied cells were compatible with the intercalated cells with increased cytoplasmic volume and numerous mitochondria. Intercalated cells are normally 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 impaired concentrating ability; therefore, the urinalysis findings likely correlate with the hypertrophy of the epithelial cells in the collecting ducts. This histopathological finding also correlates with the

increased kidney weights. There were no toxicologically relevant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) in either sex to correlate with the histopathological findings in the kidney or to indicate an impairment of renal function. However, the increased serum potassium levels in the high-dose males may be associated with the histopathological findings in the collecting ducts. The severity of the lesions appeared to be dose-related. In males and females at 500 mg/kg bw/d the severity was graded as very slight hypertrophy while in the high-dose males and females the severity ranged from very slight to slight. Males may be slightly more sensitive than females. In the high-dose males, the increased severity of the hypertrophy of the epithelial cells likely correlated with the more pronounced urinalysis findings observed in these animals. Following the recovery period, the lesions were not apparent in either sex at the high dose suggesting that it was reversible. Morphologically, the lesions were essentially the same as those reported in B6C3F1 mice following 13-weeks of treatment at similar dose levels (Dow Chemical Company Laboratory Project Study ID - DR-0312-6565-010).

Other histopathological findings in the kidney were limited to females and included degeneration / regeneration of proximal tubules (descending part), multi-focal mineralization in the papilla and degeneration / regeneration of cortical tubules. Degeneration / regeneration of descending portion of the proximal tubules was observed in females at 500 and 800 mg/kg bw/d. Lesions were observed on the outer stripe of the outer zone of the medulla and appeared as necrosis and/or regeneration of the tubular epithelium with a variable regenerative response. The lesions were considered to be typical of subacute necrosis with regeneration likely to be of only a few days duration rather than a 13-week old lesion. The lesions were still apparent after the 4-week recovery period. Females at 800 mg/kg bw/d also exhibited small foci of mineralized tubular debris in the tubules of the papilla. The mineralization was restricted to the medullary region and was thought to be due to tubular debris from the proximal tubules and was still present following the 4-week recovery period. Females also exhibited very slight degeneration / regeneration of the cortical tubules. The incidence was not dose-related and was considered to be a reflection of a spontaneous lesion (incidence - 1/10, 2/10, 0/10, 4/10 and 3/10 at 0, 20, 100, 500 and 800 mg/kg bw/d, respectively).

Extramedullary haematopoiesis in the spleen was observed in all animals (both sexes) but the severity may be slightly decreased in the high-dose males with 4/10 animals graded as slight in the high-dose males compared to 10/10 animals in all other dose groups (both sexes). Atrophy of adipose tissue was observed in both sexes at the high-dose. This consisted of adipocytes having a central lipid vacuole of decreased size or lacking the vacuole. This correlated with the decreased adipose tissue observed during the gross pathological examination and with the lower body weights at this dose level. The decreased extramedullary haematopoiesis in the spleen and atrophy of adipose tissue were minimal and were considered likely to be secondary effects. Histopathological findings are summarized in Tables 11 and 12 for males and females, respectively.

TABLE 11. Histopathological findings in males (values expressed as incidence/total number examined). (a)

Dose Level (mg/kg bw/d)			0	20	100	500	1000
Treatment Group (13 weeks of treatment) (n = 10 animals/dose)							
Kidney	- hypertrophy, collecting ducts	- very slight (b) - slight (c) - total	0/10 0/10 0/10	0/10 0/10 0/10	0/10 0/10 0/10	10/10 0/10 10/10	3/10 7/10 10/10
Spleen	- extramedullary haematopoiesis	- very slight - slight	0/10 10/10	0/10 10/10	0/10 10/10	0/10 10/10	6/10 4/10
Mesenteric tissue	- atrophy of adipose tissue	- very slight - slight	0/10 0/10	-	-	-	6/10 1/10
Recovery Group (13 weeks of treatment + 4 week of recovery) (n = 10 animals/dose)							

Dose Level (mg/kg bw/d)			0	20	100	500	1000
Spleen	- extramedullary haematopoiesis	- very slight	0	-	-	-	1/10
Mesenteric tissue	- atrophy of adipose tissue	- very slight	0	-	-	-	1/10

(a) Data obtained from pages 143-156 of the study report.
(b) Very slight hypertrophy - 1 to 3 enlarged cell, if any, per tubule profile were present and many tubules did not have any affected cells.
(c) Slight hypertrophy - characterized by generally greater numbers of affected cells per tubule profile and more tubules were generally affected, although normal cells and tubular profiles predominated.

TABLE 12. Histopathological findings in females (values expressed as incidence/total number examined). (a)

Dose Level (mg/kg bw/d)			0	20	100	500	800
Treatment Group (13 weeks of treatment) (n = 10 animals/dose)							
Kidney	- hypertrophy, collecting ducts	- very slight (b)	0/10	0/10	0/10	8/10	6/10
		- slight (c)	0/10	0/10	0/10	0/10	3/10
		- total	0/10	0/10	0/10	0/01	9/10
	- proximal tubules (descending part) degeneration/regeneration and inflammation with or without necrosis		0/10	0/10	0/10	3/10	3/10
	- mineralization in papilla (multi-focal, very slight)		0/10	0/10	0/10	0/10	9/10
	- cortical tubules, degeneration / regeneration (very slight)		1/10	2/10	0/10	4/10	3/10
Mesenteric tissue	- atrophy of adipose tissue	- very slight	0/10	-	0/10	-	3/10
		- slight	0/10		0/10		5/10
Recovery Group (13 weeks of treatment + 4 weeks of recovery) (n = 10 animals/dose)							
Kidney	- papilla mineralization, tubules (very slight)		0/10	-	-	-	9/10
	- cortical tubules, degeneration / regeneration (very slight)		0/10	-	-	-	5/10
Mesenteric tissue	- atrophy of adipose tissue	- very slight	0/10	-	-	-	4/10

(a) Data obtained from pages 143-156 of the study report.
(b) Very slight hypertrophy - 1 to 3 enlarged cell, if any, per tubule profile were present and many tubules did not have any affected cells.
(c) Slight hypertrophy - characterized by generally greater numbers of affected cells per tubule profile and more tubules were generally affected, although normal cells and tubular profiles predominated.

III. DISCUSSION

A. <u>Investigators' conclusions (extracted from page 40 of the study report)</u>: "The kidney was identified as a target organ at dose levels of 500, 800 (females) or 1000 (males) mg/kg bw/day XDE-570 based on histopathologic findings, organ weights and urinalysis changes. Other parameters affected by treatment with XDE-570 included lower body weights and body-weight gains, secondary organ weight changes, lower feed consumption and perineal soiling of both sexes of high-dose rats and females rats given 500 mg/kg bw/day. Minimal decreases of erythrocyte parameters were present in males rats given 500 or 1,000 mg/kg bw/day. Slight increases in serum potassium and cholesterol levels were present in the high-dose males, and a slight decrease in serum glucose level was present in high-dose females. These serum clinical chemistry changes may have been related to treatment with XDE-570, but these effects were of minor toxicological significance and were considered of no biological consequence. All effects observed during the subchronic study lessened or were absent at the end of the recovery period. Under the conditions of this study, the no-observed-effects-level (NOEL) was 100 mg/kg bw/day XDE-570 for male and female F344 rats."

B. Reviewer comments: There were no mortalities and no toxicologically relevant treatment-related clinical observations, clinical chemistry, ophthalmoscopic or gross pathological findings. Body weights were significantly lower in males at 1,000 mg/kg bw/d and in females at 500 and 800 mg/kg bw/d. This correlated with reduced food consumption in both sexes at the high dose and appeared to be partially reversible. Red blood cell parameters (RBC counts, HCT and HGB) were slightly but significantly reduced in males at ≥500 mg/kg bw/d. Erythrocyte morphology and RBC indices (MCV, MCH and MCHC) were unaffected by treatment. These marginal haematological findings may be indicative of anaemia although it appears to be reversible. A slight decrease in extramedullary haematopoiesis in the spleen was also observed in the high-dose males although this was considered likely to be a secondary effect. Urinalysis findings included urinary acidification (♂/♀ at ≥500 mg/kg bw/d) and decreased urinary specific gravity (♂ at 1,000 mg/kg bw/d). Following the recovery period, urinary specific gravity continued to be significantly lower. Following treatment, kidney weights were significantly increased in both sexes at ≥500 mg/kg bw/d and continued to be significantly increased in the high-dose animals following the recovery period. The increased kidney weights correlated with the histopathological findings in the kidney, specifically with hypertrophy of epithelial cells of the collecting ducts in both sexes at ≥500 mg/kg bw/d. Hypertrophy of the epithelial cells of the collecting duct was not observed following the recovery period suggesting that it was reversible. The hypertrophied cells were compatible with the intercalated cells which are normally 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 impaired concentrating ability; therefore, the urinalysis findings likely correlate with hypertrophy of the epithelial cells of the collecting ducts. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with urinalysis or histopathological findings in the kidney or to indicate an impairment of renal function. The severity of the hypertrophy of the epithelial cells of the collecting duct appeared to be dose-related and males may be slightly more sensitive than females. In the high-dose males, the increased severity of the hypertrophy of the epithelial cells likely correlated with the more pronounced urinalysis findings observed in these animals. Other significant findings in the kidney were limited to females and included degeneration / regeneration of descending portion of the proximal tubules (at ≥500 mg/kg bw/d) which was considered to be typical of acute necrosis with regeneration rather than a 13-week old lesion and multi-focal mineralization in the papilla (800 mg/kg bw/d) which were still apparent after the 4-week recovery period.

The LOAEL is 500 mg/kg bw/d based on lower body weight and body-weight gain (♀), marginal haematological findings (♂), urinalysis findings (♂/♀), increased kidney weights (♂/♀) and histopathological findings in the kidney (♂/♀). The NOAEL is 100 mg/kg bw/d.

C. Study deficiencies: OECD Guideline 408 (Subchronic Oral toxicity - Rodent: 90-day Study) recommend that adrenal gland weights be determined, in this study adrenal glands were not weighed although there were no significant gross pathological or histopathological findings in the adrenal glands. Urine volume was not provided, however, urinalysis is not required for subchronic dietary studies in rodents according to OECD Guideline 408. These deficiencies should not impact on the outcome of this study; therefore, this study is considered acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in rat.