DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

OPPTS 870.4300 [§83-5]; Combined Chronic Toxicity/Carcinogenicity Study in Rats

Work Assignment No. 4-1-128 N (MRID 46808236)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/carcinogenicity, dietary study in rats; OPPTS 870.4300 [§83-5]; OECD 453.

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

TEST MATERIAL (PURITY): XDE-570 (Florasulam; 99.3% 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: Johnson, K. H., K. T. Haut, and K. E. Stebbins (1997) XDE-570: Two year chronic toxicity/oncogenicity study in Fischer 344 rats. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI. Laboratory Project ID: 960004, November 24, 1997. MRID 46808236. Unpublished.

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

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (MRID 46808236), XDE-570 (Florasulam; 99.3% a.i.; Lot No. 940714) was administered in the diet for 104 weeks to 50 Fischer 344 rats/sex/dose at dose levels of 0/0, 10/10, 250/125, or 500/250 mg/kg bw/day nominally in males/females (actual intake was 0/0, 10/10, 254/127, and 506/254 mg/kg bw/day in males/females). An additional 10 rats/sex/dose were treated in a similar manner and killed after 52 weeks. A concurrent neuropathology group (5 rats/sex/dose) were treated similarly and killed at 52 weeks; however, only body weights and body weight gains were reported in this study.

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

In the 250 mg/kg/day females, body weight was decreased (p<=0.05) by approximately 3-8% after Week 52. Only a minor decrease of 6% was observed in body weight gain for Weeks 0-52, but overall body weight gain decreased by 14%. In the 500 mg/kg/day males, body weight was decreased (p<=0.05) by approximately 13-18% after Week 13. Body weight gain was similar to controls at Weeks 0-13, but was decreased at Weeks 0-52 by 27% and overall (Weeks 0-104) by 23%.

Slight nephrotoxicity was observed in males. At 250 and 500 mg/kg/day, increased absolute and relative kidney weights (5 and 3%, 8-9 and 22-24%, respectively), increased incidences of very slight to moderate renal collecting duct hypertrophy (82-98% treated vs 0% controls) and very slight to slight multi-focal mineralization in the papilla (28-78% treated vs 4% controls) were observed. Renal collecting duct hypertrophy was also observed at 12 months at 250 and 500 mg/kg/day (50-100% treated vs 0% controls). Additionally, at 500 mg/kg/day, the incidence of focal/multi-focal transitional cell hyperplasia in the papilla was increased (22% treated vs 0% controls) at 24 months.

It was not clear if the following findings were adverse and treatment-related. In the 250 mg/kg/day females, the incidence of cloudy cornea was increased (57% treated vs 20% controls); however histological examination did not corroborate an adverse effect. Urinary pH was decreased in the 500 mg/kg/day males (5.3-6.1 treated vs 7.0-8.1 controls).

The LOAEL is 250 mg/kg/day, based on decreased body weights (3-8%) and body weight gains (14%) in the females; slight nephrotoxicity (increased kidney weights, hypertrophy, and histopathology) in males. The NOAEL is 10 mg/kg/day in males; 125 mg/kg/day in females.

At the doses tested, there were no treatment-related increases in tumor incidence when compared to controls. Dosing was considered adequate based on decreased body weights and body weight gains in both sexes and slight nephrotoxicity in males.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

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 combined chronic/carcinogenicity study in rats. A detailed DER completed by the Canadian Pest Management Regulatory Agency (PMRA) is attached.

COMMENTS:

PMRA selected 125 mg/kg/day as the LOAEL, based on "equivocal urinary acidification, marginal to slight increase in kidney weight, and hypertrophy of the epithelial cells of the collecting ducts in females." In the 125 mg/kg/day females, the pH of the urine and the relative to body and absolute kidney weights were within the standard deviation of the control; thus, these effects were not considered adverse. A significant (p≤0.05) difference in kidney weights was not

found at 125 mg/kg/day. In short, the values in the treated group were similar to controls. An increased ($p \leq 0.05$) incidence of very slight hypertrophy of the collecting ducts was noted in the 125 mg/kg/day females (28/50 treated vs 0/50 controls). Due to the minimal severity of this lesion and the absence of corroborating evidence of toxicity, this effect was not considered adverse. Therefore, the LOAEL selected is 250 mg/kg/day, based on decreased body weights (3-8%) and body weight gains (14%) in the females; slight nephrotoxicity (increased kidney weights, hypertrophy, and histopathology) in males. The NOAEL is 10 mg/kg/day in males; 125 mg/kg/day in females.

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Reviewer: Tom Morris , Date April 27, 2000

STUDY TYPE: Combined chronic/oncogenicity [feeding]-[rat]; OPPTS 870.4300; OECD 453.

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

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

CITATION: Johnson, K. H., Haut, K. T. and Stebbins, K. E. November 24, 1997. **XDE-570; Two-Year Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats.** Performing Laboratory: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: 960004. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a combined chronic / carcinogenicity study, XDE-570 (Purity - 99.3%) was administered *ad libitum* via the diet to 50 Fischer 344 rats/sex/dose at dose levels of 0, 10, 125 (females only), 250 or 500 (males only) mg/kg bw/d for 104 weeks (time-weighted average test substance intake was 0, 10, 254 or 506 mg/kg bw/d for males and 0, 10, 127 or 254 mg/kg bw/d for females). In addition, 10 rats/sex/dose were treated likewise with XDE-570 and sacrificed after 52 weeks. A concurrent neuropathology group (5 rats/sex/dose, sacrificed at 52 weeks) was also included with this study. However, these animals were only included for body weight and body-weight gain data, all other data were reported separately (see DACO 4.5.11 - Shankar, M.R. and Johnson, K.A. Laboratory Project Study ID: DR-0312-6565-019N).

There was no treatment-related effect on mortality and no significant treatment-related clinical signs, ophthalmoscopic or gross pathological findings were observed. Body weight and body-weight gain were consistently lower in males at 500 mg/kg bw/d from approximately week 13 onwards and in females at 250 mg/kg bw/d from approximately week 52 onwards. The lower body-weight gain correlated with concomitant slightly lower food consumption for both sexes. RBC parameters (RBC count, HCT and HGB) were minimally but significantly lower (≈4-6%) in males at 500 mg/kg bw/d at 6 and 12 months, however, by 24 months they were significantly higher (≈11-15%) compared to controls. RBC morphology was normal throughout the study. These findings may be indicative of mild anaemia, however, it appeared to be reversible even with continued exposure. Similar findings were observed in males at ≥500 mg/kg bw/d in a 90-day dietary study with Fischer 344 rats; therefore, the lower RBC parameters in the high-dose males at 6 and 12 months were considered to be treatment-related. Significantly elevated serum bicarbonate levels were observed in the high-dose males at 24 months. Urinary acidification and reduced urinary specific gravity were consistently observed in the high-dose males. Urinary acidification was also observed in both sexes at 250 mg/kg bw/d and possibly in females at 125 mg/kg bw/d throughout most of the study. The high-dose males exhibited decreased proteinuria which was considered to represent less severe chronic renal disease although the decreased specific gravity suggest that dilution may have also contributed to lower values. Increased kidney weights were observed in males at 500 mg/kg bw/d, in both sexes at 250 mg/kg bw/d and in females at 125 mg/kg bw/d (marginal to slight increase). In the high-dose males, the increase in kidney weight was more marked and reflected the increased incidence and/or severity of elevated serum bicarbonate levels, urinalysis findings and hypertrophy of epithelial cells of the collecting duct observed in these animals when compared to both sexes at 250 mg/kg bw/d and to females at 125 mg/kg bw/d. In both sexes at 250 mg/kg bw/d and in females at 125 mg/kg bw/d the increased kidney weight correlated with urinalysis findings and hypertrophy of epithelial cells of the collecting duct. Treatment-related non-neoplastic findings were generally limited to the kidney and included hypertrophy of the epithelial cells of the collecting ducts in males at ≥250 mg/kg bw/d and in females at ≥125 mg/kg bw/d. The incidence and/or severity appeared to be dose-related, greater in males than females and appeared to increase over time in males and possibly in females. From histologic and ultra-structural appearance of the hypertrophied cells, the site within the collecting ducts which they were present, and from the presence of urinary

acidification, it is most likely that the affected cells were the α-type intercalated cells which are involved in acid secretion and bicarbonate resorption and thus, under normal conditions function in the regulation of acid-base balance. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability; therefore, the elevated serum bicarbonate levels, decreased urine specific gravity and urinary acidification were most likely associated with hypertrophy of the epithelial cells in the collecting duct. With the exception of the elevated serum bicarbonate levels in the high-dose males at 24 months, there were no significant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with urinalysis or histopathological findings in the kidney or to indicate any impairment of renal function. Hypertrophy of the epithelial cells in the collecting duct did not appear to significantly compromise renal function and continued ingestion of the test substance did not result in significant deterioration of renal function nor in renal tumours. The underlying mechanism for the hypertrophy of these cells is unknown. Morphologically, the lesions were similar to those observed in a 90-day dietary study with Fischer 344 rats at similar dose levels which appeared to be reversible following the 4-week recovery period. Other histopathological findings in the kidneys included a possible slight decreased incidence of age-related tubular degeneration/regeneration and a decrease severity of spontaneous geriatric renal degeneration (chronic progressive glomerularnephropathy) in males at ≥250 mg/kg bw/d, slight decreased incidence of spontaneous geriatric renal disease in females at 250 mg/kg bw/d and minimal reactive hyperplasia of the transitional epithelium and unilateral necrosis of the papilla in males at 500 mg/kg bw/d. Based on the data presented, there was no treatment-related difference in incidence of specific tumours, the total number of animals with tumours, the number of benign or malignant tumours or the time of their respective occurrence between the controls and the treated groups at 12 or 24 months; therefore, these data do not indicate any carcinogenic potential of XDE-570 in rats.

The LOAEL for chronic toxicity was 125 mg/kg bw/d based on equivocal urinary acidification (♀), marginal to slight increase in kidney weight (♀) and hypertrophy of the epithelial cells of the collecting duct (♀). The NOAEL for chronic toxicity was 10 mg/kg bw/d.

At the doses tested, there was no treatment related increase in tumour incidence when compared to controls. Dosing was considered adequate based on decreased body weight, body-weight gain and food consumption, urine acidification, increased kidney weights and hypertrophy of the epithelial cells of the collecting duct in females at 250 mg/kg bw/d, the highest dose tested in females and decreased body weight, body-weight gain and food consumption, haematological, clinical chemistry and urinalysis findings, increased kidney weights and histopathological findings in the kidneys in males at 500 mg/kg bw/d, the highest dose tested in males.

At the doses tested, there was no treatment-related increased incidence of tumours in the treatment groups when compared to controls up to and including 500 mg/kg bw/d in males and 250 mg/kg bw/d in females, the highest dose levels tested; therefore, under the conditions of the study, XDE-570 (florasulam) was not considered to be oncogenic.

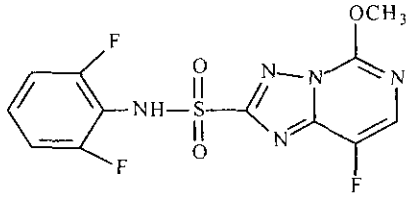
This chronic/carcinogenicity study in the rat is acceptable / guideline and satisfies the guideline requirement for a chronic/carcinogenicity study (OPPTS 870.4300); OECD 453 in rats.

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-difluropheny1)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulphonamide

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

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

3. **Test animals:**

Species:	Male and female rats.	
Strain:	Fischer 344	
Age/weight at study initiation:	At study initiation, the rats were ≈8 weeks of age with a body weight range of 158.8-198.2 g for males and 93.9-138.0 g for females.	
Source:	Charles River Laboratories, Kingston, New York.	
Housing:	The animals were initially housed in pairs (same sex) in stainless steel cages. In order to allow adequate living area for each rat, it was necessary to house male rats 1/cage when they reached ≈ 12 months of age. Females continued to be housed 2/cage until termination of the study.	
Diet:	Certified Rodent Chow #5002 (Purina Mills Inc., St. Louis, MO) in meal form *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	20 - 24°C
	Humidity:	38 - 66%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 16 days.	

B. STUDY DESIGN:

1. **In life dates** - Start: February 9, 1995. End: February 12 (♂) & 13 (♀) 1996 (1-yr interim sacrifice)
February 20 1996 (sacrifice neuropathology animals)
February 10-13, 1997 (terminal sacrifice)

2. **Animal Assignment/Dose Levels:** Animals were randomly assigned to the study groups as summarized in Table 1 using a computer-generated randomization program based on body weights. The test substance was administered

ad libitum in the feed for approximately 12 (interim sacrifice) or 24 (terminal sacrifice) months. The animals scheduled for the 1-year interim sacrifice (satellite and neuropathology groups) were sacrificed and necropsied on study days 369 (♂) and 370 (♀). All surviving animals in the terminal sacrifice group were sacrificed and necropsied on study days 732-735. The control group animals received untreated diet *ad libitum* over the same time period

TABLE 1: STUDY DESIGN

Test Groups	Dose Level mg/kg bw/d	Time-Weighted Average Test Substance Intake (mg/kg bw/d)		Satellite Group (sacrifice at 52 weeks)		Neuropathology Group (a) (sacrifice at 52 weeks)		Oncogenicity Group (sacrifice at 104 weeks)	
		Male	Female	Male	Female	Male	Female	Male	Female
1	0	0	0	10	10	5	5	50	50
2	10	10	10	10	10	5	5	50	50
3	125	-	127	-	10	-	5	-	50
4	250	254	254	10	10	5	5	50	50
5	500	506	-	10	-	5	-	50	-

(a) Neuropathology group included in body weight and body-weight gain data only, for all other data collected on neuropathology group see chronic (1 year) neuropathology study. Data for neuropathology group are summarized in DACO 4.5.11 (Shankar, M.R. and Johnson, K.A. September 25, 1996. XDE-570; Chronic Neurotoxicity in Fischer 344 Rats. Laboratory Project Study ID: DR-0312-6565-019N. Unpublished).

3. Dose Selection: The dose levels were selected based on data from the acute oral, 2-week dietary and 13-week dietary (with 4-week reversibility period) studies with Fischer 344 rats. In the 2-week dietary study, 5 rats/sex/dose received XDE-570 *ad libitum* in the diet at dose levels of 0, 100, 500 or 1,000 mg/kg bw/d (Szabo, J.R. and Davis, N.L. Laboratory Project Study ID: TXT:DR-0312-6565-003, study submitted but a full review was not completed). At 1,000 mg/kg bw/d lower food consumption suggestive of minor unpalatability of the diet with subsequent lower body weights and secondary organ weight changes was observed in both sexes. At ≥500 mg/kg bw/d histopathological alterations characterized as nuclear pleomorphism of the renal proximal tubule epithelial cells were observed in both sexes. The NOAEL was 100 mg/kg bw/d. In the 13-week dietary study (with 4-week reversibility period), 10 rats/sex/dose received XDE-570 *ad libitum* in the diet at dose levels of 0, 20, 100, 500, 800 (♀ only) or 1,000 (♂ only) mg/kg bw/d (see DACO 4.3.1 - Redmond, J.M. and Johnson, K.A. Laboratory Project Study ID: DR-0312-6565-001). The LOAEL was 500 mg/kg bw/d based on lower body weight and body-weight gain (♂/♀), decreased RBC parameters (↓ RBC counts, HCT and HGB, ♂ only) indicative of anaemia (reversible after 4-week recovery period), urine acidification (♂/♀), increased kidney weights (♂/♀) and histopathological findings in the kidneys including hypertrophy of epithelial cells in collecting duct (♂/♀) and decreased degeneration/regeneration of descending portion of proximal tubules (♀). The NOAEL was 100 mg/kg bw/d. Based on these findings, the high-dose (500 mg/kg bw/d for ♂ and 250 mg/kg bw/d for ♀) was expected to produce evidence of toxicological effects. The remaining doses were expected to provide dose-response data for any treatment-related effects observed in the high-dose group and to ensure the definition of a no-observed-adverse-effect-level (NOAEL).

4. Diet preparation and analysis: Test diets were prepared by serially diluting a concentrated test substance-feed mixture (pre-mix) with ground feed. The pre-mix was mixed for an appropriate length of time to ensure a homogeneous mixture. Premixes were prepared approximately every 2-4 weeks. Diets were prepared weekly during the first 13 weeks of the study and at least once every 4 weeks for the remainder of the dosing period. Initial concentrations of the test substance in the diet were calculated from pre-study body weights and food consumption data. Subsequently, the concentrations of test substance in the diets were adjusted weekly for the first 13 weeks and monthly thereafter based on the most recent body weight and food consumption data. Stability of the test substance was established concurrent with the start of the study. Homogeneity testing of the test substance in the feed was initiated prior to the study and at two additional time points during the study. Analyses to verify the concentration of the test substance in the feed were conducted at the start and at approximately 3-month intervals thereafter. For

these analyses, aliquots of the appropriate diet concentration(s) were solvent extracted, diluted if necessary and analysed by HPLC using UV detection.

Results

Homogeneity Analysis: The analyses showed that the test material was adequately distributed in the feed for all six samples with relative standard deviations (RSD) of 2.6% for the pre-mix, 2.6% and 0.9% for the 500 mg/kg bw/d male diet and 14.3, 11.2 and 8.6% for the 10 mg/kg bw/d female diet.

Date Mixed	2/8/95	2/8/95	5/17/95	5/17/95	5/31/95	5/31/95
Dose Level (mg/kg bw/d)	10 (♀)	500 (♂)	10 (♀)	3.0% Pre-mix	10 (♂)	500 (♂)
Concentration Range (%w/w)	0.00833 - 0.0136	0.697 - 0.745	0.0157 - 0.0232	2.90 - 3.15	0.0120 - 0.0163	1.01 - 1.03
Mean Concentration (%w/w)	0.0113	0.719	0.0188	3.06	0.0148	1.02
Standard Deviation	0.00161	0.0185	0.0021	0.08	0.00127	0.0089
%RSD	14.25	2.57	11.17	2.61	8.58	0.87

Stability Analysis: Stability data was determined for the 10 mg/kg bw/d dose group (♀). Based on these findings, the test substance was found to be stable in rodent chow for at least 30 days. Since the premix and various dietary levels were mixed at least once every 4 weeks (28 days), stability data was not needed beyond 30 days.

Female - 10 mg/kg bw/d		
Days Elapsed	Observed Amount (% w/w)	% of Initial Day (day 0)
0	0.00113	-
8	0.00126	112
15	0.00123	109
30	0.00110	97

Concentration Analysis: The mean concentrations of the test substance in the diet were shown to be 97 to 102% of the targeted concentrations during the course of the study.

Dose level (mg/kg bw/d)	Range (% of target concentration)		Mean ± SD (% of target concentration)	
	Males	Females	Males	Females
10	83 - 133	88 - 115	97 ± 14	98 ± 7
125	-	91 - 105	-	99 ± 4
250	97 - 115	96 -104	101 ± 6	99 ± 2
500	98 - 115	-	101 ± 5	-
Premix	97 - 120		102 ± 7	

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

5.. **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 food consumption statistics only. Outliers, if excluded from other analyses, were excluded only for documented, scientifically sound reasons. Statistical analyses were conducted on body weight, food consumption, haematological, clinical chemistry parameters, urine specific gravity and organ weight data throughout the study. Differences in mortality patterns were tested by the Gehan-Wilcoxon procedure (Breslow, 1970) for all animals scheduled for terminal sacrifice. When a significant effect was identified for the dose groups then individual analyses were run comparing each dose to control with a Bonferroni correction to compensate for multiple comparisons with the control group. Gross pathological changes were tabulated and considered in the interpretation of final histopathological data but were not evaluated statistically. The cumulative incidence of appropriate histopathological observations on all animals scheduled for terminal sacrifice was used in statistical analysis as there were no significant differences in mortality. For organs for which all animals in all dose groups were examined as scheduled, the incidences of specific observations were first tested for deviation from linearity using ordinal spacing of the doses. If linearity was not rejected, the data were then tested for a linear curve using the Cochran-Armitage Trend test. If the trend was statistically significant, or if significant deviation from linearity was found, incidences for each dose group were compared to that of the control group using a pair-wise Chi-square test with Yate's continuity correction. For tissues which were evaluated from all control and high-dose animals, but only from selected animals in the intermediate-dose groups, statistical analysis consisted of the pair-wise comparisons of control and high dose using the pair-wise Chi-square test with Yate's continuity correction.

C. **METHODS:**

1. **Observations:** A cage-side examination was performed daily with the exception of days on which a more detailed clinical examination was performed. Detailed clinical examinations were conducted on all animals prior to the start of the study and weekly throughout the study period. Additionally, animals were observed each day during the work week and twice daily on weekends and holidays for moribundity, mortality and availability of food and water.

2. **Body weight** Animals were weighed during the pre-dosing period, weekly for approximately the first 13 weeks of the study and at approximately monthly intervals thereafter.

3. **Food consumption and compound intake:** Food consumption data were collected for all animals weekly during for the first 13 weeks and for a one week period each month thereafter by weighing the feeders at the beginning and end of a measurement cycle. From these data, food consumption (g/animal/d) was calculated. Food efficiency (g food consumed per day/g bw gain per day) was calculated for the first 13 weeks of the study to cover the period

when the animals were growing most rapidly. Food efficiency and compound intake (mg/kg bw/d) values were calculated as time-weighted averages from the food consumption and body-weight gain data.

4. Ophthalmoscopic examination The eyes were examined by indirect ophthalmoscopy pre-dosing and for all surviving animals prior to their scheduled termination. One drop of 0.5% tropicamide ophthalmoscopic solution was instilled into each eye prior to examination. At the scheduled necropsy, the eyes of each animal were evaluated by a moistened microscope slide technique.

5. Haematology & Clinical Chemistry: Blood samples for haematology and clinical chemistry determinations were obtained from 10 animals/sex/dose in the interim sacrifice group following approximately 6 and 12 months of dosing. Blood samples were subsequently obtained from the first 10 and 20 surviving animals/sex/dose level from the terminal sacrifice group following approximately 18 and 24 months of dosing, respectively. The animals were fasted overnight, anaesthetized with methoxyflurane and blood samples were collected via puncture of the orbital sinus. Blood samples for haematological determinations were mixed with EDTA and blood smears were prepared and stained with Wrights stain. Blood samples for clinical chemistry parameters were collected and the serum separated 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)*		Mean corpuscular Haemoglobin (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular Haemoglobin Concentration (MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
X	Platelet count (PLT)*		Reticulocyte count (RETIC)
	Blood clotting measurements*	X	Erythrocyte Morphology
	(Activated Partial Thromboplastin time)	X	Leukocyte Morphology
	(Thrombin Clotting time)	X	Platelet Morphology
	(Prothrombin time)		

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.
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* (PHOS)	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 (PROT)*
	Cholinesterase (ChE)	X	Triglycerides (TRIG)
X	Creatine phosphokinase (CK)		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Serum Bicarbonate (frozen sample from 24 months only)
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 combined chronic/carcinogenicity studies based on Guideline 870.4300.
X Examined

6. Urinalysis: Urine samples were collected from 10 non-fasted animals/sex/dose level at approximately 6, 12 and 18 months (1-2 weeks prior to collection of haematology and clinical chemistry samples); these were the 10 pre-designated interim necropsy animals for the 6- and 12-months samples and the first 10 surviving animals at 18 months. The first 20 animals/sex/dose level were used for urine collection prior to the 24-month necropsy. Samples were collected from animals 1 to 2 weeks prior to collection of haematology and clinical chemistry samples at each time point. Samples were obtained by external manual compression of the bladder. If an insufficient quantity was collected, a second sampling was attempted as soon as possible after the first attempt. No urine samples were obtained from animals which died or were sacrificed in a moribund condition prior to the termination of the study. 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

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.
X Examined.

7. Sacrifice and Pathology Animals submitted for necropsy were fasted overnight, anaesthetized by inhalation of methoxyflurane vapours, samples of blood/serum were obtained from the orbital sinus, their tracheas were exposed and clamped and the animals were sacrificed by decapitation. Terminal fasted body weights were determined. A complete necropsy was conducted on all animals. Similar necropsy procedures were followed for animals found dead or moribund, except that body weights, organ weights and blood samples were not obtained. The eyes were examined *in situ* utilizing fluorescent illumination and gentle application of a moistened microscope slide to each cornea. 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 nasal cavity was flushed via the pharyngeal duct and the lungs were distended to an approximately normal inspiratory volume with neutral phosphate-buffered 10% formalin. A portion of the kidney from the first 3 animals/sex from the control and high-dose groups from the 24-month scheduled necropsy was retained in 2% glutaraldehyde/2% para-formaldehyde for potential electron microscopic examination. All preserved tissues/organs (and their standard number of sections) were processed by conventional techniques from all control and high-dose animals and from animals in the low and middle dose levels that died or were sacrificed moribund prior to scheduled necropsy. Paraffin embedded tissues/organs were sectioned at approximately 6 µm, stained with hematoxylin and eosin and examined using a light microscope. The following tissues/organs from animals in the low and middle dose that survived to the terminal necropsy were also processed and examined microscopically: lungs, liver, kidneys and appropriate gross lesions with a likely histopathological correlate. To further characterize the primary effect of treatment in the collecting duct cells, sections of kidney from a control and a high-dose male were processed for electron microscopic examination by standard procedures, embedded in epoxy resin, thin sectioned, stained with

uranyl acetate and lead citrate and examined by electron microscopy. Photographs were taken of appropriate areas.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain (multiple sections)*+
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 (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Mammary gland*
X	Rectum*	X	Urinary bladder*	X	Parathyroids*
XX	Liver*+	XX	Testes*+	X	Thyroids*
	Gall bladder*	X	Epididymides*+		OTHER
X	Pancreas*	X	Prostate*	X	Bone
	RESPIRATORY	X	Seminal vesicle*	X	Skeletal muscle
X	Trachea*	XX	Ovaries*+	X	Skin*
X	Lung*++	X	Uterus*+	X	All gross lesions and masses*
X	Nose*				
X	Pharynx*				
X	Larynx*				

* Required for combined chronic/carcinogenicity studies based on Guideline 870.4300.
+Organ weight required in combined chronic/carcinogenicity studies.
++Organ weight required if inhalation route.
X Organ fixed.
XX Organ weighed prior to fixation.

II. RESULTS

A. Observations

1. Clinical signs of toxicity - There were no toxicologically relevant treatment-related clinical signs of toxicity. However, soiling of the perineal area was noted in males at 250 and 500 mg/kg bw/d and in females at 125 and 250 mg/kg bw/d throughout the study (Table 2). Perineal soiling was noted only sporadically during the weekly examinations in the control and low-dose animals. The soiling appeared to be urine dried to the fur of the perineum. Perineal soiling was considered to be a secondary or indirect effect, due to lack of grooming possibly related to acidity of the urine or to the presence of excretory products of the test substance in the urine. During the second year of the study there was a dose-related slight increased incidence of thin females. This observation was considered to be consistent with the significantly reduced body weight observed in these animals during the second year of the study. However, it was considered non-specific in nature and possibly a manifestation of other geriatric conditions.

Table 2: Clinical observations (expressed as number of animals with specified observation at least once during the weekly clinical examination/# animals examined). (a)

Sex		Males				Females			
Dose Level (mg/kg bw/d)		0	10	250	500	0	10	125	250
0 - 12 months	- perineal soiling	2/60	11/60	53/60	58/60	6/60	7/60	46/60	55/60
12 - 24 months	- perineal soiling	6/50	9/50	46/50	49/50	14/50	18/50	45/50	50/50

Sex	Males				Females			
Dose Level (mg/kg bw/d)	0	10	250	500	0	10	125	250
- thin	6/50	12/50	9/50	6/50	4/50	6/50	8/50	10/50

(a) Data obtained from pages 86-91 in the study report.

2. Mortality - At the termination of the study there were no statistically identified (by Gehan-Wilcoxon Procedure) differences in mortality pattern between the treatment groups and the controls and there was no apparent association for a cause of death with ingestion of the test substance for either sex. There was very little mortality for the first 18 months of the study in any dose group, after which time mortality rose at an increasing rate for all dose groups. Cumulative mortality (spontaneous deaths + animals sacrificed in moribund conditions) is summarized in Table 3.

Table 3: Cumulative Mortality (expressed as number of mortalities/total number of animals). (a)

Sex	Males				Females			
Dose Level (mg/kg bw/d)	0	10	250	500	0	10	125	250
Weeks 0 - 36	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50
Weeks 0 - 44	0/50	0.50	1/50	1/50	0/50	0/50	0/50	0/50
Weeks 0 - 52	0/50	0/50	2/50	1/50	0/50	0/50	0/50	0/50
Weeks 0 - 60	1/50	0/50	2/50	1/50	0/50	0/50	0/50	0/50
Weeks 0 - 64	1/50	0/50	2/50	1/50	0/50	0/50	0/50	1/50
Weeks 0 - 68	2/50	0/50	2/50	1/50	0/50	0/50	0/50	1/50
Weeks 0 - 72	2/50	0/50	2/50	1/50	0/50	2/50	0/50	1/50
Weeks 0 - 76	2/50	0/50	2/50	2/50	0/50	4/50	1/50	1/50
Weeks 0 - 80	2/50	0/50	4/50	3/50	0/50	4/50	1/50	3/50
Weeks 0 - 84	5/50	1/50	5/50	5/50	0/50	5/50	1/50	3/50
Weeks 0 - 88	5/50	1/50	5/50	6/50	2/50	7/50	2/50	3/50
Weeks 0 - 96	8/50	11/50	8/50	10/50	4/50	9/50	5/50	7/50
Weeks 0 - 104	12/50	17/50	14/50	14/50	10/50	10/50	11/50	15/50
Weeks 0 - 105	13/50	17/50	16/50	16/50	10/50	12/50	11/50	15/50
Total Number of Deaths (%)	13/50 (26)	17/50 (34)	16/50 (32)	16/50 (32)	10/50 (20)	12/50 (24)	11/50 (22)	15/50 (30)

(a) Data obtained from pages 84-85 in the study report. N values at week 0 (n = 50) includes the oncogenicity group only.

B. Body weight - Body weight in the high-dose males, 500 mg/kg bw/d, was consistently significantly lower than control values from approximately 13 weeks onwards (Table 4). After week 13, body weight in the high-dose males was approximately 13 to 18% lower compared to controls. By week 100, body weight in the high-dose males was approximately 16% lower than controls. Body weight in the high-dose females, 250 mg/kg bw/d, was significantly lower compared to controls at a few time points during the first year of the study and consistently significantly lower throughout the remainder of the study (Table 5). After week 52, body weight in the high-dose females was approximately 3 to 8% lower compared to controls. By week 100, body weight in the high-dose females was approximately 8% lower than controls. Body-weight gain in the high-dose males was consistently lower from week 13 onwards, ranging from 23-29% lower compared to controls. In the high-dose females, body-weight gain was consistently lower from week 52 onwards, ranging from 6-14% lower compared to controls. By week 100, body-weight gain was approximately 28 and 14% lower than controls in the high-dose males and females, respectively. Overall (weeks 0-104) body-weight gain was approximately 23 and 14% lower than controls in the high-dose males and females, respectively. The lower body-weight gain correlated with a slight reduction in food consumption in the high-dose males from week 13 onward and in the high-dose females from approximately week 52 onwards. Body weight and body-weight gain were unaffected by treatment in males at 10 and 250 mg/kg bw/d and in females at 10 and 125 mg/kg bw/d.

TABLE 4: Mean body weights and body-weight gains in males. (a)

Dose Level (mg/kg bw/d)		0	10	250	500
Body Weight (g ± SD)	Week 0	178.6 ± 7.6 (n = 65)	178.1 ± 8.1 (n = 65)	177.5 ± 7.3 (n = 65)	177.4 ± 8.7 (n = 65)
	Week 3	243.1 ± 11.3	242.3 ± 11.7	239.7 ± 10.8	241.6 ± 11.3
	Week 6	278.3 ± 13.5	278.0 ± 14.0	275.2 ± 14.3	274.4 ± 13.8
	Week 13	324.1 ± 16.1	326.0 ± 16.2	321.3 ± 15.9	317.3 ± 15.8 *
	Week 28	379.5 ± 18.7	379.4 ± 19.6	372.2 ± 18.3	332.0 ± 18.1 *
	Week 52	417.7 ± 24.1	420.7 ± 23.0	412.0 ± 20.4 (n = 64)	351.0 ± 19.2 * (n = 63)
	Week 80	418.6 ± 24.6 (n = 47)	423.2 ± 27.6 (n = 50)	409.4 ± 22.2 (n = 46)	346.2 ± 27.1 * (n = 47)
	Week 100	406.3 ± 23.9 (n = 40)	402.4 ± 38.0 (n = 36)	391.2 ± 32.6 (n = 39)	340.6 ± 21.1 # (n = 37)
	Week 104	390.0 ± 33.1 (n = 38)	385.2 ± 41.0 (n = 33)	380.7 ± 37.6 (n = 35)	339.6 ± 25.6 * (n = 35)
Body-Weight Gain (g ± SD) (b)	Weeks 0-3	64.6 ± 7.0	64.2 ± 7.4	62.2 ± 7.0	64.2 ± 6.2
	Weeks 0-6	99.7 ± 9.1	99.9 ± 9.6	97.7 ± 10.2	97.0 ± 8.6
	Weeks 0-13	145.4 ± 12.4	147.9 ± 12.8	143.8 ± 12.5	139.9 ± 11.6
	Weeks 0-28	200.9 ± 15.8	201.3 ± 16.2	194.7 ± 15.0	154.6 ± 14.4
	Weeks 0-52	239.1 ± 21.3	242.7 ± 20.3	234.6 ± 17.3	173.6 ± 15.3
	Weeks 0-80	238.6 ± 23.9	244.5 ± 26.7	231.6 ± 19.9	169.5 ± 24.1
	Weeks 0-100	226.2 ± 23.7	224.7 ± 35.8	213.7 ± 29.9	163.3 ± 17.5
	Weeks 0-104	209.9 ± 33.8	207.5 ± 38.9	203.1 ± 34.9	162.4 ± 20.7

(a) Data obtained from pages 94 to 97 in the study report for body weight and pages 98 to 105 for body-weight gain. N values at week 0 (n = 65) includes satellite group (n = 10), neuropathology group (n = 5) and the oncogenicity group (n = 50).
(b) There were no statistical comparison of means for body-weight gain included in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

TABLE 5: Mean body weights and body-weight gains in females. (a)

Dose Level (mg/kg bw/d)		0	10	125	250
Body Weight (g ± SD)		119.2 ± 6.0 (n = 65)	119.6 ± 7.3 (n = 65)	120.2 ± 6.3 (n = 65)	119.9 ± 6.3 (n = 65)
	Week 3	146.3 ± 6.4	147.8 ± 7.7	149.3 ± 7.8	147.3 ± 7.5
	Week 6	161.8 ± 8.1	162.4 ± 8.3	162.7 ± 8.8	159.6 ± 8.8

Dose Level (mg/kg bw/d)		0	10	125	250
	Week 13	181.5 ± 7.9	182.4 ± 9.2	182.9 ± 9.5	178.3 ± 9.5
	Week 28	202.6 ± 9.2	203.5 ± 10.6	202.5 ± 9.5	197.7 ± 11.6 *
	Week 52	219.6 ± 12.1	220.4 ± 13.9	217.9 ± 12.5	214.1 ± 15.3
	Week 80	266.8 ± 21.1 (n = 49)	267.7 ± 20.3 (n = 46)	262.1 ± 20.5 (n = 49)	250.7 ± 25.0 * (n = 47)
	Week 100	282.3 ± 27.2 (n = 43)	288.0 ± 20.9 (n = 40)	278.5 ± 27.3 (n = 43)	259.3 ± 36.6 # (n = 38)
	Week 104	281.0 ± 23.7 (n = 40)	281.9 ± 24.8 (n = 39)	280.5 ± 25.2 (n = 39)	257.4 ± 28.4 * (n = 35)
Body-Weight Gain (g ± SD) (b)	Weeks 0-3	27.1 ± 4.2	28.3 ± 5.8	29.1 ± 4.9	27.4 ± 4.3
	Weeks 0-6	42.7 ± 6.7	42.8 ± 6.3	42.5 ± 6.4	39.7 ± 6.1
	Weeks 0-13	62.3 ± 7.1	62.8 ± 7.5	62.8 ± 7.6	58.5 ± 7.4
	Weeks 0-28	83.5 ± 8.2	83.9 ± 8.5	82.3 ± 8.4	77.8 ± 9.6
	Weeks 0-52	100.5 ± 10.9	100.9 ± 12.0	97.7 ± 11.1	94.2 ± 13.8
	Weeks 0-80	147.6 ± 19.4	148.1 ± 17.9	141.9 ± 19.7	131.3 ± 23.1
	Weeks 0-100	163.0 ± 24.6	168.4 ± 19.2	158.2 ± 25.9	140.7 ± 34.3
	Weeks 0-104	161.3 ± 21.2	162.2 ± 22.9	159.8 ± 25.4	138.5 ± 27.3

(a) Data obtained from pages 106 to 109 in the study report for body weight and pages 110 to 117 for body-weight gain. N values at week 0 (n = 65) includes satellite group (n = 10), neuropathology group (n = 5) and the oncogenicity group (n = 50).
(b) There were no statistical comparison of means for body-weight gain included in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
\# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

C. Food consumption and compound intake

1. Food consumption - Throughout most of the first 13 weeks, food consumption was slightly greater in males at all dose levels, this was statistically significant on most occasions at both 250 and 500 mg/kg bw/d (Table 6). From week 13 onwards, food consumption was reduced slightly in the high-dose males compared to controls, with statistical significance being achieved on most occasions. The slight reduction in food consumption in the high-dose males correlated with lower body-weight gain. Food consumption in males at 10 and 250 mg/kg bw/d tended to remain slightly higher compared to controls throughout the remainder of the study (statistical significance achieved on numerous occasions at both 10 and 250 mg/kg bw/d), except for the for the last few months, during which time food consumption was slightly lower at 250 mg/kg bw/d. Food consumption in females at all dose levels tended to be comparable to or slightly higher than controls throughout the first year of the study. Females at 250 mg/kg bw/d, tended to have slightly lower food consumption than controls over the second year of the study while the other dose levels were generally comparable to controls. The slight reduction in food consumption in the high-dose females during the second year correlated with lower body-weight gain.

TABLE 6: Food consumption (g/animal/day) in males and females. (a)

Dose Level (mg/kg bw/d)		0	10	250 (♂) / 125 (♀)	500 (♂) / 250 (♀)
Males	Week 1	16.4 ± 0.6 (n = 51)	16.7 ± 0.6 (n = 65)	16.9 ± 0.5 # (n = 63)	17.3 ± 0.8 # (n = 48)
	Week 3	16.5 ± 0.5 (n = 41)	16.7 ± 0.6 (n = 55)	17.1 ± 0.8 * (n = 61)	16.8 ± 0.6 * (n = 61)

Dose Level (mg/kg bw/d)		0	10	250 (♂) / 125 (♀)	500 (♂) / 250 (♀)
	Week 6	16.4 ± 0.5 (n = 60)	16.5 ± 0.5 (n = 65)	16.6 ± 0.7 (n = 65)	16.7 ± 0.6 * (n = 61)
	Week 13	15.8 ± 0.8 (n = 65)	16.0 ± 0.5 (n = 60)	16.2 ± 0.5 # (n = 65)	16.0 ± 0.7 (n = 65)
	Week 27	16.1 ± 0.8 (n = 65)	16.9 ± 0.7 # (n = 65)	16.8 ± 0.5 # (n = 65)	15.6 ± 1.1 # (n = 53)
	Week 51	17.0 ± 0.7 (n = 65)	17.6 ± 0.7 # (n = 64)	17.6 ± 0.5 # (n = 64)	16.8 ± 1.2 (n = 59)
	Week 79	18.1 ± 1.1 (n = 46)	18.6 ± 1.3 (n = 49)	17.9 ± 1.2 (n = 47)	16.1 ± 1.7 * (n = 45)
	Week 99	18.7 ± 1.5 (n = 38)	18.8 ± 1.1 (n = 30)	18.5 ± 1.8 (n = 36)	17.4 ± 1.3 * (n = 34)
	Week 103	19.3 ± 1.5 (n = 32)	17.6 ± 2.3 * (n = 24)	18.1 ± 1.3 * (n = 27)	17.7 ± 1.7 * (n = 30)
Females	Week 1	11.5 ± 0.5 (n = 65)	11.6 ± 0.5 (n = 61)	11.8 ± 0.6 * (n = 63)	11.7 ± 0.5 (n = 62)
	Week 3	11.2 ± 0.4 (n = 61)	11.7 ± 0.5 # (n = 65)	12.2 ± 0.6 # (n = 65)	11.9 ± 0.7 # (n = 64)
	Week 6	11.7 ± 0.5 (n = 60)	11.7 ± 0.4 (n = 65)	11.7 ± 0.6 (n = 65)	11.7 ± 0.6 (n = 60)
	Week 13	11.7 ± 0.5 (n = 62)	12.1 ± 0.7 # (n = 64)	12.4 ± 0.8 # (n = 61)	11.7 ± 0.7 (n = 58)
	Week 27	11.2 ± 0.6 (n = 62)	11.4 ± 0.7 (n = 64)	11.8 ± 0.5 * (n = 65)	11.7 ± 0.7 * (n = 62)
	Week 51	11.9 ± 0.7 (n = 64)	12.1 ± 0.8 (n = 62)	12.0 ± 0.6 (n = 65)	11.9 ± 0.7 (n = 64)
	Week 79	12.5 ± 0.7 (n = 49)	12.7 ± 0.8 (n = 46)	12.6 ± 0.7 (n = 49)	12.2 ± 1.0 (n = 48)
	Weeks 99	12.7 ± 1.4 (n = 40)	13.0 ± 1.0 (n = 40)	12.9 ± 1.0 (n = 42)	12.2 ± 1.3 (n = 37)
	Week 103	12.2 ± 1.2 (n = 40)	12.3 ± 0.8 (n = 37)	12.5 ± 0.9 (n = 35)	11.8 ± 1.5 (n = 33)

(a) Data obtained from pages 118 to 121 in the study report for males and pages 122 to 125 for females. N values at week 0 (n = 65) includes satellite group (n = 10), neuropathology group (n = 5) and the oncogenicity group (n = 50).
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
\# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

2. Compound consumption (time-weighted average) - Time-weighted average test substance intakes (mg/kg bw/d) are summarized in Table 1.

3. Food efficiency - Food efficiency data for the first 13 weeks of the study showed no consistent trends which would indicate an effect on food efficiency of animals in either sex at any dose level. Food efficiency was not measured after week 13.

D. Ophthalmoscopic examination - There were no significant treatment-related ophthalmoscopic findings. However, corneal cloudiness was observed in 2/10 males at 500 mg/kg bw/d at 12 months and in 20/35 females at 250 mg/kg bw/d at 24 months (Table 7). Corneal cloudiness correlated with mineralization of the corneal basement membrane observed during the histopathological examination (see Tables 16 and 17 for non-neoplastic findings at 12 and 24 months, respectively). Mineralization of the corneal basement membrane is commonly observed in Fischer 344 and was observed at all dose levels, including the controls, at 12 and 24 months and there were no significant differences in the incidence based on the histopathological examination; therefore, the increased incidence of corneal cloudiness was considered to be an incidental finding.

Table 7: Ophthalmoscopic Observations (expressed as number of animals with specified observation/total number animals observed). (a)

Sex	Males				Females			
Dose Level (mg/kg bw/d)	0	10	250	500	0	10	125	250

0 - 12 months	- cloudy cornea	0/10	0/10	0/10	2/10	1/10	0/10	0/10	1/10
12 - 24 months	- cloudy cornea	9/38	12/33	8/36	4/36	8/40	12/39	9/39	20/35

(a) Data obtained from pages 92-93 in the study report.

E. Blood analyses

1. Haematology - Compared to controls, red blood cell parameters (RBC counts, HCT and HGB) were minimally but significantly decreased (≈4-6%) in the high-dose males at both the 6 and 12 month intervals (Table 8). By 18 months RBC parameters in these animals were comparable to controls and by 24 months RBC parameters were significantly higher than controls (11-15% higher). RBC morphology in these animals appeared to be normal at all time intervals. RBC indices (MCV, MCH and MCHC) and reticulocyte counts were not provided. At 6 and 12 months, RBC parameters were generally within the normal range of historical control data for animals of this age and strain from this laboratory. At 24 months, they were above the normal range of historical control data for animals of this age and strain from this laboratory. Similar findings were reported in males at 500 and 1,000 mg/kg bw/d in the 90-day dietary study with Fischer 344 rats; therefore, the decreased RBC parameters in the high-dose males at the 6 and 12 month intervals were considered to be treatment-related, although it appears to be reversible even with continued exposure. Other significant findings in the high-dose males included decreased WBC and platelet counts at the 12 and 24 month intervals, respectively. The decreased WBC and platelet counts were within the normal range of historical control data for animals of this age and strain from this laboratory, were not repeated at any other time point and differential WBC counts, WBC morphology and platelet morphology were normal at all time intervals; therefore, these findings were considered to be incidental. There were no significant treatment-related effects in females at any dose level or in males at 10 or 250 mg/kg bw/d.

TABLE 8. Haematological findings - males. (a)

Time Interval	Dose Level (mg/kg bw/d)	WBC ($x 10^3/mm^3$)	RBC ($x 10^6/mm^3$)	HGB (g/dL)	HCT (%)	PLT ($x 10^3/mm^3$)
6 months (n = 10 rats/dose)	0	8.26 ± 0.93	9.62 ± 0.21	16.0 ± 0.4	46.3 ± 1.1	600 ± 41
	10	8.58 ± 0.73	9.67 ± 0.18	15.9 ± 0.3	46.0 ± 0.8	599 ± 55

Time Interval	Dose Level (mg/kg bw/d)	WBC ($x 10^3/mm^3$)	RBC ($x 10^6/mm^3$)	HGB (g/dL)	HCT (%)	PLT ($x 10^3/mm^3$)
	250	7.99 ± 0.71	9.48 ± 0.21	15.8 ± 0.4	45.7 ± 1.0	612 ± 58
	500	7.53 ± 0.47	9.05 ± 0.19 *	15.3 ± 0.4 *	44.1 ± 0.8 *	634 ± 61
12 months (n = 10 rats/dose)	0	6.35 ± 0.66	9.40 ± 0.33	15.3 ± 0.4	45.4 ± 1.3	598 ± 41
	10	5.53 ± 0.73	9.24 ± 0.35	14.8 ± 0.6	44.2 ± 1.7	603 ± 27
	250	5.77 ± 1.26	9.11 ± 0.28	14.8 ± 0.4	44.2 ± 1.0	591 ± 49
	500	5.04 ± 0.34 #	8.87 ± 0.26 *	14.6 ± 0.5 *	43.3 ± 1.1 *	620 ± 45
18 months (n = 10 rats/dose)	0	5.50 ± 0.96	8.94 ± 1.39	15.6 ± 2.4	46.1 ± 5.7	643 ± 126
	10	5.14 ± 0.56	9.05 ± 0.44	16.0 ± 0.8	46.4 ± 2.3	697 ± 160
	250	5.84 ± 1.90	9.23 ± 0.48	15.9 ± 0.8	46.6 ± 2.4	675 ± 44
	500	5.02 ± 0.88	8.88 ± 1.07	15.3 ± 1.5	44.8 ± 3.7	682 ± 175
24 months (n = 20 rats/dose)	0	6.12 ± 1.33	8.11 ± 0.63	15.0 ± 1.0	46.2 ± 3.0	688 ± 132
	10	6.54 ± 2.41	7.09 ± 2.05	13.3 ± 3.1	42.1 ± 8.3	626 ± 211
	250	9.42 ± 15.53	7.65 ± 2.02	13.9 ± 3.5	43.6 ± 10.2	614 ± 118
	500	5.69 ± 1.71	9.32 ± 1.28 #	16.5 ± 1.9 #	51.3 ± 6.3	492 ± 98 #

(a) Data obtained from pages 132 to 167 in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

2. Clinical Chemistry - At 24 months, serum bicarbonate levels were significantly elevated in males at 500 mg/kg bw/d. Elevated serum bicarbonate levels in the high-dose males at 24 months correlate with hypertrophy of the epithelial cells in the collecting duct, specifically 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. The elevated serum bicarbonate levels in the high-dose males also reflects the increased severity of the urinalysis findings and hypertrophy of the epithelial cells of the collecting duct in these animals compared to both sexes at 250 mg/kg bw/d and females at 125 mg/kg bw/d. There were no other relevant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) in either sex to correlate with urinalysis or histopathological findings in the kidney or to indicate any impairment of renal function.

Other significant findings in the high-dose males included decreased alkaline phosphatase activity, decreased total protein, globulin, calcium and phosphate levels and increased cholesterol levels at 6 months (Table 9). At 12 months, alkaline phosphatase activity and phosphate levels continued to be significantly decreased. Cholesterol and triglyceride levels were significantly decreased at 18 and 24 months. Significant findings at 24 months also included increased albumin and bicarbonate levels and decreased globulin levels. At 250 mg/kg bw/d, significant findings were limited to decreased triglyceride levels at 18 months. Significant findings in females at 250 mg/kg bw/d included increased phosphate levels at 12 months, decreased potassium levels at 18 months and decreased albumin levels with a concomitant decrease in calcium levels at 24 months (Table 10). Although significant findings were observed in these parameters, the changes were not consistent over time, there were no corroborative gross pathological or histopathological findings to indicate toxicological significance and they were within the normal range of historical control data for animals of this age and strain from this laboratory; therefore, these findings were not considered to be toxicologically relevant. The decreased total protein, globulin, phosphate, cholesterol and triglyceride levels in the high-dose males and the decreased serum phosphate and albumin levels in the high-dose females may be secondary to decreased food consumption in these animals. The decreased calcium levels in the high-dose females at 24 months were most likely secondary to decreased albumin levels. Decreased alkaline phosphatase activity is not normally pathologically significant. In the high-dose males, the decreased alkaline phosphatase activity may be secondary to body weight and growth differences in these animals compared to the controls. There were no significant findings in either sex at 10 mg/kg bw/d or in females at 125 mg/kg bw/d.

TABLE 9. Clinical chemistry findings (males). (a)

Time Interval	Dose Level (mg/kg bw/d)	AP (mU/mL)	PROT (g/dL)	ALB (g/dL)	GLOB (g/dL)	CHOL (mg/dL)	TRIG (mg/dL)	CA (mg/dL)	PHOS (mg/dL)
6 months (n = 10 rats/dose)	0	118 ± 7	7.3 ± 0.2	5.0 ± 0.1	2.3 ± 0.2	71 ± 8	109 ± 23	10.8 ± 0.2	7.1 ± 0.4
	10	113 ± 8	7.4 ± 0.1	5.0 ± 0.1	2.4 ± 0.1	74 ± 7	119 ± 45	10.9 ± 0.1	7.2 ± 0.4
	250	113 ± 9	7.3 ± 0.2	5.0 ± 0.1	2.3 ± 0.1	79 ± 8	111 ± 41	10.8 ± 0.2	6.9 ± 0.3
	500	91 ± 7 *	7.0 ± 0.1 *	4.9 ± 0.1	2.2 ± 0.1 *	83 ± 11 *	87 ± 18	10.6 ± 0.1 *	6.5 ± 0.3 *
12 months (n = 10 rats/dose)	0	93 ± 8	7.0 ± 0.1	4.6 ± 0.2	2.4 ± 0.2	86 ± 12	101 ± 25	10.2 ± 0.3	7.0 ± 0.4
	10	87 ± 8	7.2 ± 0.2	4.5 ± 0.1	2.7 ± 0.1	79 ± 11	83 ± 25	10.1 ± 0.2	6.9 ± 0.3
	250	90 ± 9	7.0 ± 0.2	4.6 ± 0.1	2.4 ± 0.2	93 ± 17	99 ± 34	10.3 ± 0.3	6.7 ± 0.3
	500	83 ± 6 *	6.9 ± 0.3	4.5 ± 0.1	2.4 ± 0.2	80 ± 10	79 ± 19	10.2 ± 0.2	6.5 ± 0.4 *
18 months (n = 10 rats/dose)	0	101 ± 11	7.1 ± 0.2	4.7 ± 0.2	2.4 ± 0.1	142 ± 33	111 ± 28	10.9 ± 0.2	6.1 ± 0.5
	10	105 ± 29	7.2 ± 0.3	4.7 ± 0.2	2.5 ± 0.3	155 ± 30	96 ± 19	11.0 ± 0.2	6.0 ± 0.3
	250	100 ± 13	7.3 ± 0.2	4.8 ± 0.2	2.5 ± 0.2	135 ± 24	88 ± 17 *	10.9 ± 0.3	6.2 ± 0.3
	500	94 ± 17	7.0 ± 0.3	4.8 ± 0.1	2.2 ± 0.2	105 ± 28 *	54 ± 10 *	10.7 ± 0.3	6.2 ± 0.6
24 months (n = 20 rats/dose)	0	85 ± 31	6.5 ± 0.3	4.4 ± 0.3	2.2 ± 0.3	167 ± 47	123 ± 56	10.2 ± 0.3	6.0 ± 0.5
	10	90 ± 51	6.4 ± 0.5	4.3 ± 0.3	2.1 ± 0.3	186 ± 72	193 ± 157	10.3 ± 0.6	6.0 ± 0.7
	250	104 ± 72	6.4 ± 0.3	4.4 ± 0.3	2.0 ± 0.2	152 ± 54	121 ± 66	10.2 ± 0.4	6.1 ± 0.8
	500	78 ± 12	6.6 ± 0.4	4.7 ± 0.3 *	1.8 ± 0.3 *	100 ± 44 *	65 ± 23 #	10.0 ± 0.5	5.9 ± 0.7

HCO_3 at 24 months (only measured at 24 months): 21.9 ± 1.8, 21.7 ± 1.3, 22.2 ± 2.6 and 23.1 ± 1.3 mmol/L at 0, 10, 250 and 500 mg/kg bw/d, respectively. Statistically significant from control mean by Wilcoxon's test at 500 mg/kg bw/d, $p \leq 0.05$

(a) Data obtained from pages 168 to 183 in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
\# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

TABLE 10. Clinical chemistry findings (females). (a)

Time Interval	Dose Level (mg/kg bw/d)	AB (g/dL)	CA (mg/dL)	PHOS (mg/dL)	POTASSIUM (mmol/L)
6 months (n = 10 rats/dose)	0	5.1 ± 0.1	10.7 ± 0.2	6.2 ± 0.5	4.7 ± 0.2
	10	5.2 ± 0.2	10.7 ± 0.2	6.3 ± 0.6	4.9 ± 0.3
	125	5.1 ± 0.2	10.7 ± 0.3	6.5 ± 0.8	4.7 ± 0.3

Time Interval	Dose Level (mg/kg bw/d)	AB (g/dL)	CA (mg/dL)	PHOS (mg/dL)	POTASSIUM (mmol/L)
	250	5.1 ± 0.2	10.7 ± 0.3	6.7 ± 0.6	4.8 ± 0.3
12 months (n = 10 rats/dose)	0	5.4 ± 0.4	10.6 ± 0.4	6.3 ± 0.5	4.8 ± 0.3
	10	5.4 ± 0.3	10.5 ± 0.3	6.2 ± 0.7	4.9 ± 0.4
	125	5.1 ± 0.5	10.3 ± 0.5	6.3 ± 0.6	4.8 ± 0.2
	250	5.1 ± 0.3	10.3 ± 0.3	7.0 ± 0.5 *	4.9 ± 0.2
18 months (n = 10 rats/dose)	0	5.2 ± 0.8	10.9 ± 0.5	5.6 ± 0.6	4.4 ± 0.3
	10	5.4 ± 0.3	11.1 ± 0.4	6.0 ± 0.7	4.4 ± 0.3
	125	5.7 ± 0.3	11.2 ± 0.3	6.1 ± 0.6	4.2 ± 0.2
	250	5.3 ± 0.3	11.0 ± 0.3	5.6 ± 0.8	4.1 ± 0.2 *
24 months (n = 20 rats/dose)	0	5.1 ± 0.3	10.6 ± 0.4	5.5 ±1.0	4.8 ± 0.3
	10	4.9 ± 0.3	10.5 ± 0.3	5.9 ± 0.6	4.8 ± 0.3
	125	4.9 ± 0.4	10.4 ± 0.4	5.7 ± 0.7	4.9 ± 0.5
	250	4.6 ± 0.7 #	10.2 ± 0.5 *	6.0 ± 1.6	4.8 ± 0.6

(a) Data obtained from pages 168 to 183 in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

F. Urinalysis - Urinary acidification and lower urinary specific gravity were consistently observed in males at 500 mg/kg bw/d (Table 11). Other findings in the high-dose males included decreased proteinuria at 6, 12, 18 and 24 months and slightly decreased ketone levels at 12 and 18 months. Urinary pH was also lower in males at 250 mg/kg bw/d 6, 18 and 24 months and slightly lower at 12 months. There may also be slightly decreased proteinuria at 6 and 12 months and slightly decreased ketone levels at 18 months in males at 250 mg/kg bw/d. In females urinalysis findings were limited to lower urinary pH at 250 mg/kg bw/d and slightly lower urinary pH at 125 mg/kg bw/d at 6, 18 and 24 months (Table 12). There were no treatment-related urinalysis findings in either sex at 10 mg/kg bw/d. It was indicated in the study report that, although urinary specific gravity was consistently lower in the high-dose males it was always above the specific gravity of renal ultra-filtrate suggesting that the kidneys in these animals were still able to concentrate urine. Urinary acidification and lower urinary specific gravity likely correlate with hypertrophy of the epithelial cells of the collecting duct, specifically the α-type 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 in the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability. The incidence and/or severity of the urinalysis findings and hypertrophy of the epithelial cells of the collecting ducts appeared to be more prominent in high-dose males. This also correlates with the elevated serum bicarbonate levels being observed only in the high-dose males. There were no other notable clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with the urinalysis or histopathological findings in the kidney or to indicate any impairment of renal function. The decreased proteinuria in the high-dose males was considered to represent less severe chronic renal disease compared to controls although the decreased specific gravity suggest that dilution may have contributed to these lower values.

TABLE 11. Urinalysis findings (males). (a)

Dose Level (mg/kg bw/d)	0	10	250	500
	Male - 6 months (10 animals/dose)			
Specific gravity	1.055 ± 0.007	1.063 ± 0.004 *	1.064 ± 0.005 *	1.035 ± 0.005 *
pH	8.1 ± 0.9	7.8 ± 0.9	7.0 ± 0.6	5.5 ± 0.4
Protein - slight	-	1/10	2/10	3/10

Dose Level (mg/kg bw/d)		0	10	250	500
	- moderate	5/10	1/10	3/10	7/10
	- severe	5/10	8/10	5/10	-
Ketones	- negative	10/10	-	-	6/10
	- trace	-	1/10	4/10	4/10
	- slight	-	9/10	6/10	-
Male - 12 months (n = 10 animals/dose)					
Specific gravity		1.055 ± 0.004	1.055 ± 0.005	1.059 ± 0.004	1.039 ± 0.007 *
pH		7.3 ± 0.3	7.0 ± 0.6	7.1 ± 0.3	6.1 ± 0.3
Protein	- slight	-	-	2/10	4/10
	- moderate	-	3/10	2/10	5/10
	- severe	10/10	7/10	6/10	1/10
Ketones	- negative	-	-	-	4/10
	- trace	8/10	7/10	9/10	6/10
	- slight	2/10	3/10	1/10	-
Male - 18 months (n = 10 animals/dose)					
Specific gravity		1.048 ± 0.006	1.049 ± 0.012	1.050 ± 0.006	1.030 ± 0.006 *
pH		7.5 ± 0.7	7.3 ± 0.4	7.0 ± 0.4	5.7 ± 0.6
Protein	- negative	1/10	-	-	1/10
	- trace	-	1/10	-	-
	- slight	-	-	-	6/10
	- moderate	-	-	-	2/10
	- severe	9/10	9/10	10/10	1/10
Ketones	- negative	2/10	4/10	5/10	10/10
	- trace	8/10	6/10	5/10	-
Male - 24 months (n = 20 animals/dose)					
Specific gravity		1.045 ± 0.008	1.041 ± 0.009	1.041 ± 0.004	1.028 ± 0.006 *
pH		7.0 ± 0.3	6.7 ± 0.4	6.1 ± 0.3	5.3 ± 0.3
Proteins	- moderate	-	-	-	6/20
	- severe	20/20	20/20	20/20	14/20
Ketones	- negative	20/20	19/20	20/20	19/20
	- trace	-	1/20	-	1/20

(a) Data obtained from pages 184 to 197 in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.

TABLE 12. Urinalysis findings (females). (a)

Dose Level (mg/kg bw/d)		0	10	125	250
pH	- 6 months (10 animals/dose)	7.9 ± 0.6	7.8 ± 0.6	7.5 ± 0.7	7.3 ± 0.4
	- 12 months (10 animals/dose)	6.6 ± 0.6	7.3 ± 0.6	7.1 ± 0.6	6.9 ± 0.7
	- 18 months (10 animals/dose)	7.4 ± 0.9	7.5 ± 0.4	6.7 ± 0.3	6.6 ± 0.6

Dose Level (mg/kg bw/d)		0	10	125	250
	- 24 months (20 animals/dose)	6.5 ± 0.6	6.0 ± 0.5	6.1 ± 0.4	5.6 ± 0.3

(a) Data obtained from pages 184 to 199 in the study report.

G. Sacrifice and Pathology

1. Organ weight - At 12 months, absolute and relative kidney weights were significantly increased (≈8 and 24%, respectively) in males at 500 mg/kg bw/d (Table 13). At 24 months kidney weight continued to be increased (≈ 9 and 22%, respectively) in males at 500 mg/kg bw/d compared to controls, however, statistical significance was achieved for relative kidney weight only (Table 14). At 12 months, there were no significant changes in organ weights in females at any dose level. At 24 months, females at 250 mg/kg bw/d exhibited a significant increase (≈ 12%) in relative kidney weight with only a marginal increase in absolute kidney weight (≈1%). A slight increase in absolute and relative kidney weight (≈ 5 and 3%, respectively) was also noted in males at 250 mg/kg bw/d at 12 months. A marginal increase in absolute and relative kidney weight (≈ 3% for both) was also noted in females at 125 mg/kg bw/d at 24 months, this correlated with an increased incidence of very slight hypertrophy of the epithelial cells of the collecting duct observed in these animals. In the high-dose males, the increase in kidney weight was more marked and reflected the increased incidence and/or severity of elevated serum bicarbonate levels, urinalysis findings and hypertrophy of epithelial cells of the collecting duct observed in these animals when compared to both sexes at 250 mg/kg bw/d and to females at 125 mg/kg bw/d. In both sexes at 250 mg/kg bw/d and in females at 125 mg/kg bw/d the increased kidney weight correlate with urinalysis findings and hypertrophy of epithelial cells of the collecting duct.

Other significant findings included increased relative brain, heart and testes weights and decreased absolute liver and heart weights in males at 500 mg/kg bw/d at 12 months and increased relative brain weights, decreased absolute heart weights and decreased absolute and relative liver weights in males at 500 mg/kg bw/d and increased relative heart weights in females at 250 mg/kg bw/d at 24 months. In the absence of any corroborating clinical chemistry, gross pathological and/or histopathological findings, these changes were considered to be secondary effects and not reflective of organ-specific toxicity. At 12 months, organ weights were unaffected by treatment in males at 10 and 250 mg/kg bw/d and in females at all dose levels and at 24 months in males at 10 and 250 mg/kg bw/d and in females at 10 mg/kg bw/d.

TABLE 13. Absolute and relative organ weights at 12 months. (a)

Dose Level (mg/kg bw/d)		0	10	250	500
12 months - Males (n = 10 animals/dose)					
Final Body Weight (g ± SD)		391.2 ± 18.1	392.1 ± 26.2	397.4 ± 22.5	338.5 ± 10.9 *
Brain	Absolute (g ± SD)	1.992 ± 0.071	2.042 ± 0.054	2.021 ± 0.047	2.017 ± 0.063

Dose Level (mg/kg bw/d)		0	10	250	500
	Relative (g/100 g bw ± SD)	0.510 ± 0.022	0.523 ± 0.030	0.510 ± 0.026	0.596 ± 0.015 *
Heart	Absolute (g ± SD)	0.976 ± 0.038	1.004 ± 0.058	1.002 ± 0.066	0.932 ± 0.024
	Relative (g/100 g bw ± SD)	0.250 ± 0.014	0.256 ± 0.012	0.253 ± 0.016	0.276 ± 0.011 *
Kidneys	Absolute (g ± SD)	2.298 ± 0.127	2.361 ± 0.180	2.411 ± 0.132	2.471 ± 0.084 *
	Relative (g/100 g bw ± SD)	0.587 ± 0.021	0.602 ± 0.027	0.607 ± 0.022	0.731 ± 0.035 *
Liver	Absolute (g ± SD)	9.022 ± 0.741	8.868 ± 0.706	9.053 ± 0.635	7.661 ± 0.404 *
	Relative (g/100 g bw ± SD)	2.304 ± 0.121	2.263 ± 0.129	2.279 ± 0.106	2.264 ± 0.109
Testes	Absolute (g ± SD)	3.102 ± 0.167	3.346 ± 0.273	3.152 ± 0.135	3.129 ± 0.336
	Relative (g/100 g bw ± SD)	0.793 ± 0.036	0.857 ± 0.091	0.796 ± 0.059	0.924 ± 0.094 *

(a) Data obtained from pages 200 to 203 in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

TABLE 14. Absolute and relative organ weights at 24 months. (a)

Dose Level (mg/kg bw/d)		0	10	250 (♂)/125 (♀)	500 (♂)/250(♀)
24 months - Males (n = 33-40 animals/dose)					
Final Body Weight (g ± SD)		355.3 ± 30.3	352.8 ± 39.5	350.5 ± 33.8	316.5 ± 19.9 #
Brain	Absolute (g ± SD)	2.072 ± 0.070	2.082 ± 0.078	2.095 ± 0.072	2.085 ± 0.097
	Relative (g/100 g bw ± SD)	0.587 ± 0.058	0.598 ± 0.072	0.064 ± 0.076	0.661 ± 0.043 #
Heart	Absolute (g ± SD)	1.067 ± 0.099	1.064 ± 0.089	1.057 ± 0.089	1.009 ± 0.100 *
	Relative (g/100 g bw ± SD)	0.302 ± 0.038	0.305 ± 0.037	0.304 ± 0.034	0.319 ± 0.027
Kidneys	Absolute (g ± SD)	2.926 ± 0.518	2.774 ± 0.287	2.883 ± 0.318	3.211 ± 1.171
	Relative (g/100 g bw ± SD)	0.828 ± 0.165	0.795 ± 0.112	0.829 ± 0.111	1.013 ± 0.358 #
Liver	Absolute (g ± SD)	10.927 ± 1.442	11.354 ± 2.340	10.920 ± 1.855	8.489 ± 0.813 #
	Relative (g/100 g bw ± SD)	3.089 ± 0.443	3.236 ± 0.686	3.123 ± 0.602	2.680 ± 0.164 #
24 months - Females (n = 33-40 animals/dose)					
Final Body Weight (g ± SD)		261.4 ± 23.2	263.9 ± 21.3	262.0 ± 25.4	239.6 ± 28.9 *
Heart	Absolute (g ± SD)	0.764 ± 0.063	0.793 ± 0.110	0.797 ± 0.061	0.777 ± 0.084
	Relative (g/100 g bw ± SD)	0.294 ± 0.030	0.302 ± 0.044	0.307 ± 0.037	0.330 ± 0.062 #
Kidneys	Absolute (g ± SD)	1.849 ± 0.159	1.862 ± 0.160	1.897 ± 0.169	1.874 ± 0.159
	Relative (g/100 g bw ± SD)	0.711 ± 0.071	0.707 ± 0.051	0.732 ± 0.112	0.794 ± 0.129 #

(a) Data obtained from pages 200 to 203 in the study report.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.
# Significantly different from control mean by Wilcoxon's test, $p \leq 0.05$.

2. Gross pathology - There were no treatment-related gross pathological findings at 12 or 24 months in either sex.

3. Microscopic pathology -

a) Non-neoplastic - Treatment-related non-neoplastic findings were observed in the kidneys. The most notable finding was an increased incidence of hypertrophy of the epithelial cells of the collecting ducts in females at 125 mg/kg bw/d at 24 months, in both sexes at 250 mg/kg bw/d at 12 and 24 months and in males at 500 mg/kg bw/d at

12 and 24 months. The incidence and/or severity appeared to be dose-dependent in both sexes and appeared to be greater in males than females. The incidence and/or severity also appeared to increase over time in males and possibly in females. In the high-dose males, the increased incidence and/or severity of hypertrophy of the epithelial cells of the collecting duct correlate with the increased severity of the urinalysis findings, increased kidney weights and elevated serum bicarbonate levels in these animals. The hypertrophy was characterized by enlargement of individual cells rather than generalized enlargement of all cells in the collecting duct. In males, the hypertrophied cells were present in both the inner and outer stripe of the renal medulla while in females they were only prominent in the inner stripe of the outer medulla. The hypertrophied cells were compatible with intercalated cells with increased cytoplasmic volume and numerous mitochondria. Based on the histologic and ultra-structural appearance of the hypertrophied cells, the site within the collecting ducts in which they were present, and the presence of urinary acidification, the affected cells were thought to be the α-type intercalated cells which are involved in acid secretion and bicarbonate resorption and thus, under normal conditions function in the regulation of acid-base balance. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability. At 500 mg/kg bw/d (♂ only), hypertrophy of the epithelial cells was accompanied by decreased urinary specific gravity and urinary acidification and increased kidney weights at 12 and 24 months. At 250 mg/kg bw/d, hypertrophy of the epithelial cells was accompanied by slight urinary acidification in both sexes and increased kidney weights in males at 12 months and females at 24 months. In females at 125 mg/kg bw/d, hypertrophy of the epithelial cells was accompanied by slight urinary acidification. With the exception of elevated serum bicarbonate levels in the high-dose males at 24 months, there were no other notable clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with the urinalysis findings or with hypertrophy of the epithelial cells or to indicate any impairment of renal function. The hypertrophy of the epithelial cells did not appear to significantly compromise renal function and the continued ingestion of the test substance did not result in significant deterioration of renal function nor in renal tumours. The underlying mechanism for hypertrophy of these cells is unknown. Morphologically, the lesions were similar those reported in Fischer 344 rats following 13-weeks of treatment at similar dose levels (see DACO 4.3.1 - Laboratory Project Study ID - DR-0312-6565-011) where urinalysis findings indicative of impaired concentrating and an acidification defect were also observed (reduced urinary specific gravity and pH, respectively). However, in the 13-week dietary study these lesions and urinalysis findings appeared to be reversed after a 4-week recovery period.

There may also be a slight decrease in the incidence of age-related tubular degeneration with regeneration in males at 250 and 500 mg/kg bw/d at 12 months. In females at 250 mg/kg bw/d, there may be a possible slight increased incidence of age-related tubular degeneration with regeneration. This diagnosis was used at the 12-months necropsy to denote the earliest manifestations of spontaneous chronic renal disease. At 24 months, the severity of spontaneous geriatric renal degeneration (chronic progressive glomerulonephropathy) appeared to decrease in males at 250 and 500 mg/kg bw/d. At 24 months, females at 250 mg/kg bw/d exhibited a slight decreased incidence of spontaneous geriatric renal degeneration (chronic progressive glomerulonephropathy). The high-dose males also tended to have decreased proteinuria which was considered to represent less severe chronic renal disease compared to controls although the decreased specific gravity suggest that dilution may have contributed to these lower values.

At 24 months, the surface of the papilla near the formix in males at 500 mg/kg bw/d exhibited small folds with minimal reactive hyperplasia of the transitional epithelium instead of a normal smooth surface. Males at 250 and 500 mg/kg bw/d, exhibited an increased incidence of small round or linear foci of mineralisation within the papilla at 24 months. However, the severity was considered to be of minimal degree and may represent mineralisation of sloughed off epithelial cells within the Loop of Henle. At 24 months, 3 males at 500 mg/kg bw/d exhibited unilateral necrosis of the papilla. This incidence was not statistically significant, however, it was considered treatment-related as papillary necrosis is considered to be an uncommon finding in untreated Fischer 344 rats.

At 24 months, males at 500 mg/kg bw/d exhibited a significantly higher incidence of mineralisation of the blood vessels in the mesenteric tissue, however, this was considered to represent normal variability in the occurrence of a common geriatric change. Mineralization of the corneal basement membrane, a finding commonly observed in Fischer 344 rats, was observed at all dose levels, including controls, at 12 and 24 months. This finding appeared to correlate with corneal cloudiness observed during the ophthalmoscopic examination which appeared to increase in females at 250 mg/kg bw/d (see Table 7). However, there was no significant difference in the incidence of corneal mineralization based on the histopathological examination; therefore, the increased incidence of corneal cloudiness

observed during the ophthalmoscopic examination could be considered an incidental finding. All other histopathological observations were considered to be geriatric conditions unrelated to treatment and to represent normal variability in the occurrence of common geriatric changes. Non-neoplastic histopathological findings at 12 and 24 months are summarized in Table 15 and Table 16, respectively.

TABLE 15: Non-neoplastic histopathological findings in males and females at 12 months (expressed as # animals with specified observation / # animals examined). (a)

Dose Level (mg/kg bw/d)				0	10	250 (♂) 125 (♀)	500 (♂) 250 (♀)
Males							
Kidney	- hypertrophy, collecting duct	- very slight (b)		0/10	0/10	5/10	2/10
		- slight (b)		0/10	0/10	0/10	8/10
		- total		0/10	0/10	5/50	10/10
	- tubule degeneration/regeneration	- focal / multi-focal	- very slight	8/10	9/10	10/10	8/10
			- slight	2/10	1/10	0/10	0/10
			- total	10/10	10/10	10/10	8/10
Cornea	- mineralization	- unilateral		7/10	-	1/0	4/10
		- bilateral		1/10	-	0/10	4/10
Females							
Kidney	- hypertrophy, collecting ducts	- very slight (b)		0/10	0/10	0/10	5/10
	- tubule degeneration/regeneration	- focal / multi-focal	- very slight	3/10	2/10	1/10	1/10
			- slight	1/10	3/10	0/10	6/10
			- total	4/10	5/10	1/10	7/10
Cornea	- mineralization	- unilateral		4/10	-	-	6/10
		- bilateral		3/10	-	-	3/10

(a) Data obtained from pages 206 to 220 in the study report
(b) Grading system used for hypertrophy, collecting ducts:
very slight - only a few hypertrophied cells (<5) were identified in any collecting duct with many collecting ducts lacking hypertrophied cells.
slight - more hypertrophied cells in an affected collecting duct and more collecting ducts contained hypertrophied cells.

TABLE 16: Non-neoplastic histopathological findings in males and females at 24 months (expressed as # animals with specified observation / # animals examined). (a)

Dose Level (mg/kg bw/d)	0	10	250 (♂) 125 (♀)	500 (♂) 250 (♀)
Males				

Dose Level (mg/kg bw/d)			0	10	250 (♂) 125 (♀)	500 (♂) 250 (♀)
Kidney	- hypertrophy, collecting duct	- very slight (b)	0/50	0/50	29/50	11/50
		- slight (b)	0/50	0/50	12/50	20/50
		- moderate (b)	0/50	0/50	0/50	18/50
		- total	0/50	0/50	41/50 *	49/50*
	- papilla; hyperplasia; transitional epithelium; reactive; focal/multi-focal		0/50	0/50	0/50	11/50 *
	- papilla; mineralization; tubule; multi-focal; very slight to slight		2/50	5/50	14/50 *	39/50 *
	- papilla; necrosis; unilateral		0/50	0/50	0/50	3/50
	- chronic progressive glomerulonephropathy	- very slight	9/50	6/50	13/50	22/50 *
		- slight	14/50	15/50	19/50	21/50
		- moderate	15/50	16/50	16/50	3/50 *
		- severe	7/50	11/50	0/50 *	1/50 *
		- total	45/50	48/50	48/50	47/50
Mesenteric Tissue	- blood vessels; mineralization		39/50	15/23	12/17	48/50 *
Cornea	- mineralization	- unilateral	18/50	10/30	8/21	17/50
		- bilateral	8/50	7/30	4/21	9/50
Females						
Kidney	- hypertrophy, collecting ducts	- very slight (b)	0/50	0/50	28/50 *	39/50 *
	- chronic progressive glomerulonephropathy	- very slight	32/50	38/50	37/50	28/50
		- slight	7/50	2/50	7/50	6/50
		- moderate	6/50	4/50	2/50	4/50
		- severe	0/50	0/50	0/50	0/50
		- total	45/50	44/50	46/50	38/50
Cornea	- mineralization	- unilateral	14/50	13/23	12/24	19/50
		- bilateral	12/50	1/232	6/24	12/50

(a) Data obtained from pages 241 to 290 in the study report. Data at the 24 month necropsy includes all modes of death, scheduled and unscheduled deaths were combined.

(b) Grading system used for hypertrophy, collecting ducts:

very slight - only a few hypertrophied cells (<5) were identified in any collecting duct with many collecting ducts lacking hypertrophied cells.

slight - more hypertrophied cells in an affected collecting duct and more collecting ducts contained hypertrophied cells.

moderate - involved higher number of cells and collecting ducts affected, at times the outer stripe of the outer medulla appeared to consist almost entirely of affected cells.

* Statistically different from controls by Yate's Chi-square, $p \leq 0.10$.

b) Neoplastic - There were no treatment-related increased incidences of neoplastic lesions in either sex up to the highest dose levels tested, 500 and 250 mg/kg bw/d for males and females, respectively, at 12 or 24 months. Based on the data presented, there was no treatment-related difference in incidence of specific tumours, the total number of animals with tumours, the number of benign or malignant tumours or the time of their respective occurrence between the controls and the treated groups at 12 or 24 months; therefore, these data do not indicate any carcinogenic potential of XDE-570 (florasulam) in rats.

The only statistically significant identified difference was a decreased incidence of adenomas of the pituitary gland for females at 250 mg/kg bw/d at 24 months (Table 17). At 24 months, a similar pattern was noted for males at 500 mg/kg bw/d but the decrease was not statistically significant. At 12 months, the incidence of adenomas of the pituitary gland was similar between the high-dose males and females and the respective control group. Carcinomas, malignant tumours of the pars distalis were also somewhat decreased in males at 500 mg/kg bw/d and in females at 250 mg/kg bw/d at 24 months. The incidence of hyperplasia of the pars distalis was comparable between the controls and the high-dose animals at 12 and 24 months.

TABLE 17: Neoplastic histopathological findings at 12 and 24 months (expressed as # animals with specified

observation / # animals examined). (a)

Dose Level (mg/kg bw/d)		0	10	250 (♂) 125 (♀)	500 (♂) 250 (♀)
Males - 12-month interim sacrifice					
Pituitary Gland	- Pars distalis; adenoma; benign; primary	2/10	-	-	2/10
Males - 24-month terminal sacrifice					
Pituitary Gland	- Pars distalis; adenoma; benign; primary	23/50	18/30	18/33	17/50
	- Pars distalis; carcinoma; malignant without metastasis; primary	6/50	3/30	1/33	1/50
	- Pars distalis; carcinoma; malignant with metastasis; primary	0/50	0/30	0/33	0/50
Females - 12-month interim sacrifice					
Pituitary Gland	-Pars distalis; adenoma; benign; primary	0/10	0/10	1/10	2/10
Females - 24-month terminal sacrifice					
Kidney	- Pars distalis; adenoma; benign; primary	26/50	25/33	21/31	14/50 *
	- Pars distalis; carcinoma; malignant without metastasis; primary	3/50	1/33	2/31	2/50
	- Pars distalis; carcinoma; malignant with metastasis; primary	3/50	0/33	0/31	0/50

(a) Data obtained from pages 206-220 of the study report for the 12-month interim sacrifice and pages 241 to 290 for the 24-month terminal sacrifice. Data at the 24 month necropsy includes all modes of death, scheduled and unscheduled deaths were combined.
* Statistically different from controls by Yate's Chi-square, $p \leq 0.10$.

III. DISCUSSION

A. <u>Investigators' conclusions (extracted from page 54 of the study report)</u>: "There were no tumorigenic effects ascribed to XDE-570 in any of the treatment groups. Non-neoplastic effects were present in a dose-dependent manner with the kidneys identified as the primary target organ. The major dose-related effects were changes in

some urinalysis parameters (predominantly decreased pH with less substantial or equivocally decreased urinary protein and ketone levels) and histopathologic effects characterized as hypertrophy of selected populations of cells of the collecting ducts believed to be Type A (or α)-intercalated cells. These effects were found in male rats given 250 or 500 mg XDE-570/kg bw/day and in female rats given 125 or 250 mg XDE-570/kg bw/day. A related effect, perineal urine soiling, was also noted at these dose levels. Increased kidney weights, particularly relative to body weight, was present for the high dose group of either sex. Male rats receiving 500 mg XDE-570/kg bw/day had consistently lower urine specific gravity than controls but this was not found for other treated groups. Body weights were slightly decreased for rats of both sexes given 250 mg XDE-570/kg bw/day while body weight was substantially decreased for males given 500 mg XDE-570/kg bw/day. Male rats from this high dose group also had slight decreases of red blood cell parameters; however, these decreases were minimal and were not present in the hematological parameters measured after 18 or 24 months on study. There were no effects on rats of either sex receiving 10 mg XDE-570/kg bw/day and this represents the No Observed Effect Level for this study. The only effects for female rats given 125 mg XDE-570/kg bw/day were very slight hypertrophy of collecting duct cells, equivocal urine acidification and perineal urine soiling. As these were of minimal degree, have been shown to be reversible, possibly represent a physiologic response, and there were no other effects that might be considered adverse, this is considered a No Observed Adverse Effect Level."

B. Reviewer comments: There was no treatment-related effect on mortality and no significant treatment-related clinical signs, ophthalmoscopic or gross pathological findings were observed. Body weight and body-weight gain were consistently lower in males at 500 mg/kg bw/d from approximately week 13 onwards and in females at 250 mg/kg bw/d from approximately week 52 onwards. The lower body-weight gain correlated with concomitant slightly lower food consumption for both sexes. RBC parameters (RBC count, HCT and HGB) were minimally but significantly lower (≈4-6%) in males at 500 mg/kg bw/d at 6 and 12 months, however, by 24 months they were significantly higher (≈11-15%) compared to controls. RBC morphology was normal throughout the study. These findings may be indicative of mild anaemia, however, it appeared to be reversible even with continued exposure. Similar findings were observed in males at ≥500 mg/kg bw/d in a 90-day dietary study with Fischer 344 rats; therefore, the lower RBC parameters in the high-dose males at 6 and 12 months were considered to be treatment-related. Significantly elevated serum bicarbonate levels were observed in the high-dose males at 24 months. Urinary acidification and reduced urinary specific gravity were consistently observed in the high-dose males. Urinary acidification was also observed in both sexes at 250 mg/kg bw/d and possibly in females at 125 mg/kg bw/d throughout most of the study. The high-dose males exhibited decreased proteinuria which was considered to represent less severe chronic renal disease although the decreased specific gravity suggest that dilution may have also contributed to lower values. Increased kidney weights were observed in males at 500 mg/kg bw/d, in both sexes at 250 mg/kg bw/d and in females at 125 mg/kg bw/d (marginal to slight increase). In the high-dose males, the increase in kidney weight was more marked and reflected the increased incidence and/or severity of elevated serum bicarbonate levels, urinalysis findings and hypertrophy of epithelial cells of the collecting duct observed in these animals when compared to both sexes at 250 mg/kg bw/d and to females at 125 mg/kg bw/d. In both sexes at 250 mg/kg bw/d and in females at 125 mg/kg bw/d the increased kidney weight correlated with urinalysis findings and hypertrophy of epithelial cells of the collecting duct. Treatment-related non-neoplastic findings were generally limited to the kidney and included hypertrophy of the epithelial cells of the collecting ducts in males at ≥250 mg/kg bw/d and in females at ≥125 mg/kg bw/d. The incidence and/or severity appeared to be dose-related, greater in males than females and appeared to increase over time in males and possibly in females. From histologic and ultra-structural appearance of the hypertrophied cells, the site within the collecting ducts which they were present, and from the presence of urinary acidification, it is most likely that the affected cells were the α-type intercalated cells which are involved in acid secretion and bicarbonate resorption and thus, under normal conditions function in the regulation of acid-base balance. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability; therefore, the elevated serum bicarbonate levels, decreased urine specific gravity and urinary acidification were most likely associated with hypertrophy of the epithelial cells in the collecting duct. With the exception of the elevated serum bicarbonate levels in the high-dose males at 24 months, there were no significant clinical chemistry findings (serum nitrogen, creatinine or electrolyte levels) to correlate with urinalysis or histopathological findings in the kidney or to indicate any impairment of renal function. Hypertrophy of the epithelial cells in the collecting duct did not appear to significantly compromise renal function and continued ingestion of the test substance did not result in significant deterioration of renal function nor in renal tumours. The underlying mechanism for the hypertrophy of these cells is unknown. Morphologically, the

lesions were similar to those observed in a 90-day dietary study with Fischer 344 rats at similar dose levels which appeared to be reversible following the 4-week recovery period. Other histopathological findings in the kidneys included a possible slight decreased incidence of age-related tubular degeneration/regeneration and a decrease severity of spontaneous geriatric renal degeneration (chronic progressive glomerularnephropathy) in males at ≥250 mg/kg bw/d, slight decreased incidence of spontaneous geriatric renal disease in females at 250 mg/kg bw/d and minimal reactive hyperplasia of the transitional epithelium and unilateral necrosis of the papilla in males at 500 mg/kg bw/d. Based on the data presented, there was no treatment-related difference in incidence of specific tumours, the total number of animals with tumours, the number of benign or malignant tumours or the time of their respective occurrence between the controls and the treated groups at 12 or 24 months; therefore, these data do not indicate any carcinogenic potential of XDE-570 in rats.

The LOAEL for chronic toxicity was 125 mg/kg bw/d based on equivocal urinary acidification (♀), marginal to slight increase in kidney weight (♀) and hypertrophy of the epithelial cells of the collecting duct (♀). The NOAEL for chronic toxicity was 10 mg/kg bw/d.

At the doses tested, there was no treatment-related increased incidence of tumours in the treatment groups when compared to controls up to and including 500 mg/kg bw/d in males and 250 mg/kg bw/d in females, the highest dose levels tested; therefore, under the conditions of the study, XDE-570 (florasulam) was not considered to be oncogenic.

C. Study deficiencies: Based on OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies), a haematological examination should be performed at 3 months, 6 months and at 6 month intervals thereafter and at termination of the study. The guideline also indicate that urinalysis examination should also be performed at the same time intervals as the haematological examination. In this study no haematological or urinalysis examination was performed at 3 months. Determination made during the urinalysis examination should include urine volume. In this study, urine samples were obtained from the individual animals by external manual compression of the bladder, if an insufficient quantity was collected a second sampling was attempted as soon as possible after the first attempt; therefore, due to the method of sampling, urine volume was not measured. While these deficiencies, if addressed, would supply additional data on treatment-related findings, they would most likely not significantly change the outcome of this study. OPPTS 870.4300 indicates that spleen, epididymides and uterus weights are required, however, they were not provided in the study report. Based on OECD guideline 453, these organ weights are not required for a combined chronic toxicity / oncogenicity study in rodents. There are no deficiencies which would significantly affect the outcome of the study; therefore, this study is acceptable/guideline and satisfies the guideline requirement for a chronic/carcinogenicity study (OPPTS 870.4300); OECD 453 in rats.