DATA EVALUATION RECORD-SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.4200b [§83-2b]; Carcinogenicity Study in Mice

Work Assignment No. 4-1-128 H (MRID 46808230)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity, dietary study in mice; OPPTS 870.4200b [§83-2b]; OECD 451.

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: Quast, J. F., K. T. Haut, and R. J. Kociba (1997) XDE-570: Two year oncogenicity study in B6C3F1 mice. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI. Laboratory Project ID: 960006, December 1, 1997. MRID 46808230. Unpublished.

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

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 46808230), XDE-570 (Florasulam; 99.3% a.i.; Lot No. 940714) was administered in the diet to 50 B6C3F1 mice/sex/dose at dose levels of 0, 50, 500, or 1000 mg/kg bw/day nominally (actual intake was 0/0, 50/51, 505/497, and 1009/1019 mg/kg bw/day in males/females) for 104 weeks. An additional 10 mice/sex/dose were treated in a similar manner and sacrificed after 52 weeks.

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

At 500 and 1000 mg/kg/day, mortality was increased in the females (42% each dose vs 26% controls) at Week 106; however, a dose-related effect was not observed at Week 96, and a statistically significant difference was not found at either time point. Therefore, the effect on mortality was considered equivocal. Increased ($p<=0.05$) incidences of very slight to slight renal collecting duct hypertrophy (82-96% treated vs 0% controls) and decreased slight to moderate vacuolization in the renal cortex tubule (94-96% treated vs 48% controls) were noted at 24 months in males, and similar findings were also noted at 12 months. The toxicological significance of these findings in the kidney was considered equivocal.

The LOAEL is not determined and the NOAEL is 1000 mg/kg/day.

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate because the limit dose was tested.

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

COMMENTS:

PMRA selected 500 mg/kg/day as the LOAEL, based on "decreased kidney weights in males and hypertrophy of the epithelial cells of the collecting ducts in both sexes." At 24 months, only minor decreases ($p\leq0.05$) of 5-7% were observed in the 500 and 1000 mg/kg/day males. Likewise, minor decreases of 3-10% ($p\leq0.05$; not statistically significant for the 1000 mg/kg/day relative weight) were observed at 12 months. There were no organ weight changes, clinical chemistry, gross or histopathological changes observed in the kidney. Therefore, the kidney observations are not considered adverse. The LOAEL is not determined and the NOAEL is 1000 mg/kg/day.

46808230.PMRA.der
.pdf

Reviewer: Tom Morris , Date March 20, 2000.

STUDY TYPE: Carcinogenicity - mice [feeding]; OPPTS 870.4200; OECD 451.

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

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

CITATION: Quast, J. F., Haut, K. T. and Kociba, R. J. December 1, 1997. **XDE-570: Two Year Oncogenicity Study in B6C3F1 Mice**. Performing Laboratory: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: 960006. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a carcinogenicity study, XDE-570 (Purity - 99.3%) was administered to 50 B6C3F1 mice/sex/dose *ad libitum* in the diet at dose levels of 0, 50, 500 or 1,000 mg/kg bw/d (time-weighted average test substance intake for ♂/♀ was 0/0, 50/51, 505/497 or 1,009/1,019 mg/kg bw/d) for 104 weeks. In addition, 10 B6C3F1 mice/sex/dose were treated in a similar manner and sacrificed after 52 weeks. Control animals received untreated diet *ad libitum* over the same time period.

There were no treatment-related effects on clinical signs, body weight, food consumption, ophthalmoscopy, haematology, clinical chemistry or gross pathology. The number of spontaneous deaths or animals sacrificed due to moribund conditions was slightly increased in females at 500 and 1,000 mg//kg bw/d (13/50, 14/50, 21/50 and 21/50 at 0, 50, 500 and 1,000 mg/kg bw/d, respectively), however, there were no statistically identified 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. Kidney weights were slightly but significantly decreased in males at ≥500 mg/kg bw/d at 12 and 24 months. This was considered to be treatment-related, however, the toxicological significance was uncertain. The decreased kidney weights correlate with histopathological findings, characterized as decreased cytoplasmic vacuolation of the cortical tubular epithelium cells at 12 and 24 months, however, the decreased cytoplasmic vacuolation was not considered to be toxicologically relevant since it did not adversely affect the well-being of the animals. The decreased kidney weights did not exhibit a clear dose-response relationship at either 12 or 24 months. However, the lack of a clear dose-response relationship may be associated with other histopathological findings in the kidney, specifically, the increased incidence and/or severity of hypertrophy of the epithelial cells in the collecting duct observed in males at 1,000 mg/kg bw/d compared to males at 500 mg/kg bw/d. Hypertrophy of the epithelial cells of the collecting ducts was observed in males and females at ≥500 mg/kg bw/d at 12 and 24 months. A dose-related increased severity was apparent in both sexes. In this study, there appeared to be no significant progression in the severity of the lesions over time (from 1 year to 2 years), however, when compared to the 13-week dietary study there may be a slight progression in the severity of the lesions from 13-weeks to 1 year in both sexes. The hypertrophied cells were primarily the epithelial cells lining the collecting ducts and were compatible with intercalated cells which are involved in the regulation of acid-base balance through the modulation of bicarbonate resorption and hydrogen ion secretion in the collecting ducts. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability, however, urinalysis was not performed. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with the histopathological findings in the kidneys or to indicate an impairment of renal function, there was no increased incidence of cellular degeneration or necrosis evident in the kidneys 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. In females, a decreased incidence of age-related tubular degeneration with regeneration was noted at ≥500 mg/kg bw/d at 12 months and at 1,000 mg/kg bw/d at 24 months. In males, the incidence of age-related

tubular degeneration with regeneration was comparable to controls at 12 and 24 months, however, the severity was decreased at 24 months 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 florasulam in mice.

The LOAEL for chronic toxicity was 500 mg/kg bw/d based on decreased kidney weights (♂) and hypertrophy of the epithelial cells of the collecting ducts (♂/♀). The NOAEL for chronic toxicity was 50 mg/kg bw/d.

Dosing was considered adequate based on decreased body-weight gain in both sexes at 1,000 mg/kg bw/d (≈10% ↓ compared to controls). OPPTS 870.4200 also indicates that the highest dose level tested need not exceed 1,000 mg/kg bw/d. The highest dose tested in this study was 1,000 mg/kg bw/d in both sexes.

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 1,000 mg/kg bw/d, the highest dose tested; therefore, under the conditions of the study, XDE-570 was not considered to be oncogenic.

This carcinogenicity study in the mouse is acceptable / guideline and satisfies guideline requirement for a carcinogenicity study (OPPTS 870.4200); OECD 451 in mice.

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

Description: White powdery solid

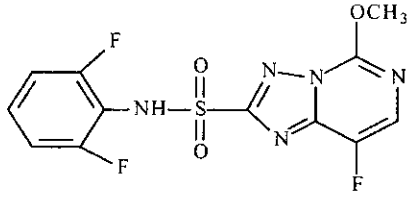
Lot/Batch #: Test Substance # 100511 / Lot # 940714

Purity: 99.3 % a.i. (determined by HPLC with ultra-violet detection using two methods).

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 mice	
Strain:	B6C3F1	
Age/weight at study initiation:	At study initiation, the mice were ≈59 days of age with a body weight range of 19.0-26.8 g for males and 17.4-22.0 g for females.	
Source:	Charles River Laboratories, Portage, Michigan.	
Housing:	The animals were individually housed in stainless steel cages..	
Diet:	Certified Rodent Chow #5002 (Purina Mills Inc., St. Louis, MO) in meal form *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	21 - 23 °C
	Humidity:	40-70%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 17 days.	

B. STUDY DESIGN:

1. **In life dates** - Start: December 2, 1994. End: December 5, 1995 (1-year interim sacrifice)
December 9, 11, 12 and 13 1996 (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 52 (satellite group) or 104 (oncogenicity group) weeks. Animals assigned to the satellite group (interim sacrifice) were sacrificed and necropsied on study day 369. All surviving animals assigned to the oncogenicity groups (terminal sacrifice) were sacrificed and necropsied on study days 739, 741, 742 or 743. The control group animals received untreated diet (*ad libitum*) for either 52 (satellite group) or 104 weeks (oncogenicity group).

TABLE 1: STUDY DESIGN

Test Groups	Dose Level mg/kg bw/d	Time-Weighted Average Test Substance Intake (mg/kg bw/d)		Satellite Group (interim sacrifice at 52 weeks)		Oncogenicity Group (terminal sacrifice at 104 weeks)	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	10	10	50	50
Low-dose	50	50	51	10	10	50	50
Mid-dose	500	505	497	10	10	50	50
High-dose	1,000	1,009	1,019	10	10	50	50

3. Dose Selection: In a 2-week dietary study, 5 B6C3F1 mice/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 20, 1992. Laboratory Project Study ID: DR-0312-6565-002, study submitted but a full review was not completed). At 1,000 mg/kg bw/d, females exhibited lower food consumption with subsequent decreases in body weights and secondary organ weight changes suggestive of a slight degree of unpalatability of the diet. There were no treatment-related findings in males up to and including 1,000 mg/kg bw/d. The NOAEL for male and female B6C3F1 mice was 1,000 mg/kg bw/d. In a 13-week dietary study, 10 B6C3F1 mice/sex/dose were administered XDE-570 *ad libitum* in the diet at dose levels of 0, 20, 100, 500 or 1,000 mg/kg bw/d (see DACO 4.3.1 - Redmond, J.M. and Johnson, K.A., January 30, 1996. Laboratory Project Study ID: DR-0312-6565-010). There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, ophthalmoscopy, haematology, clinical chemistry, organ weights or gross pathology. Histopathological examination, revealed a very slight, multi-focal bilateral hypertrophy of the epithelial cells of the collecting ducts of the kidney in 10/10 males at 500 and 1,000 mg/kg bw/d and in 8/10 females at 1,000 mg/kg bw/d. The NOAEL for male and female B6C3F1 mice was 100 mg/kg bw/d. The highest dose level of 1,000 mg/kg bw/d represents the limit dose and was expected to produce evidence of toxicological effects. The remaining dose levels were expected to provide dose-response data for any treatment-related effects observed in the high-dose group and to determine a NOEL.

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 concentration of test substance in the diets was 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 compound in rodent chow was established previously during the rat 13-week dietary study and was found to be stable for at least 30 days. Stability of the test compound was also established concurrent with this study. Stability data presented in the study report were conducted during a concurrent rat chronic toxicity / oncogenicity study (no further analysis on stability was done for the mouse oncogenicity study). Homogeneity of the test substance in the feed was initiated prior to the start of the study and at four 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. Aliquots of each diet concentration 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 6.9% for the female 50 mg/kg bw/d diet (mean of 4 time points), 1.7% for the male 50 mg/kg bw/d diet (1 time point) and 3.5% for the male 1,000 mg/kg bw/d diet (1 time point).

Date Mixed	12/01/94	03/02/95	06/01/95	10/17/95	10/17/95	01/08/96
Dose Level (mg/kg bw/d)	50 (♀)	50 (♀)	50 (♀)	50 (♀)	50 (♂)	1,000 (♂)
Concentration Range (%w/w)	0.0208-0.0265	0.0142-0.0217	0/0197-0.0206	0.0291-0.0306	0.0239-0.0284	0.697-0.782
Mean Concentration (%w/w)	0.0234	0.0179	0.0202	0.0299	0.0250	0.723
Standard Deviation	0.0018	0.0023	0.0003	0.005	0.0014	0.025
%RSD	7.69	12.85	1.49	1.67	5.60	3.46

Stability Analysis: Adequate stability data was determined in the rat chronic toxicity/oncogenicity study for the 10 mg/kg bw/d dose group (females). Stability data presented in the following table were conducted during a concurrent rat chronic toxicity / oncogenicity study, no further analysis on stability was done for the mouse oncogenicity study. 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
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 92 to 100% 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
50	84-114	85-102	92 ± 8	93 ± 6
500	95-101	95-103	97 ± 2	99 ± 2
1,000	96-102	95-99	98 ± 2	98 ± 1
Premix	97-104		100 ± 2	

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. Gross pathological observations were tabulated and considered in interpretation of final histopathological data, but were not evaluated statistically. The

cumulative incidence of appropriate histopathological observations on all animals assigned to the two-year portion of the study were used in statistical analysis. For tissues where all animals in all dose groups were microscopically 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 trend 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 those 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. Differences in mortality pattern were tested by the Gehan-Wilcoxon procedure for all animals for terminal sacrifice. If a significant effect was identified for all dose groups, then individual analyses were run comparing each dose to control and were Bonferroni corrected to compensate for multiple comparisons with the control group. If substantial differences in median survival time among the treatment groups were detected, mortality adjusted analyses for tumours such as those described by Peto (1974) or Gart et. al. (1979) were used to supplement cumulative tests, where deemed informative and valid. Data collected near the termination of the two-year study were confounded by a spectrum of geriatric changes, the presence of spontaneous tumours, secondary effects from tumours and terminal changes prior to death. As a result of these changes, statistical tests were of questionable value and biological plausibility was applied when interpreting statistical results.

C. **METHODS:**

1. Observations: A detailed clinical examination was conducted on all animals prior to the start of the study and at weekly intervals throughout the study period. An additional observation for moribundity, mortality and availability of food and water was made each day during the work week and twice daily on weekends.

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 start and end of a measurement cycle. From these data, food consumption (g/animal/d) was calculated. Food efficiency (g food consumed per day/kg bw gain per day) was calculated for the first 13 weeks of the study to cover the period when the animals are 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 of all animals were examined with a penlight prior to placement on study. The eyes were also routinely examined during the in-life phase of the study as part of the general clinical examination. At necropsy, the eyes were evaluated by gentle application of a moistened microscope slide to the cornea of each eye using fluorescent illumination according to established procedures.

5. Haematology & Clinical Chemistry: Blood samples for haematology and clinical chemistry were collected from 10 animals/sex/dose when necropsied following approximately 12 months of dosing. Blood samples were subsequently obtained from the first 10 and 20 surviving animals/sex/dose level from the oncogenicity group following 18 (for haematology only) and 24 (haematology and clinical chemistry) months of dosing, respectively. Animals were anaesthetized by inhalation of methoxyflurane vapours and blood samples were collected via puncture of the orbital sinus. It was not indicated in the study report whether blood samples were obtained from fasted or non-fasted animals, although at necropsy, non-fasted body weights were determined. 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 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)		

* Minimum required for carcinogenicity studies (Control and HDT unless effects are observed) based on Guideline 870.4200 & OECD 451
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 (PROT)
	Cholinesterase (ChE)	X	Triglycerides (TRIG)
	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)		

* Not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451
X Examined

6. Urinalysis - No urine was collected. Not required for carcinogenicity studies based on Guideline 870.4200 and OECD 451.

7. Sacrifice and Pathology Animals submitted for necropsy were 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 non-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. 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. 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. Paraffin embedded tissues/organs were sectioned at approximately 6 µm, stained with hematoxylin and eosin and examined using a light microscope. 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*
X	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	Cervix		
X	Pharynx*	X	Oviducts		
X	Larynx*	X	Vagina		

* Required for carcinogenicity studies based on Guideline 870.4200.
+Organ weight required in 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 treatment-related clinical observations.

2. Mortality - The number of spontaneous deaths or animals sacrificed due to moribund conditions was increased slightly in females at 500 and 1,000 mg//kg bw/d. This increase did not show a clear dose-response effect and a similar finding was not observed in the males. At the end of the study there were no statistically identified differences (Gehan-Wilcoxon Procedure) 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. Cumulative mortality (spontaneous deaths + animals sacrificed in moribund conditions) are summarized in Table 2.

Table 2: Cumulative Mortality (expressed as number of mortalities/total number of animals). (a)

Sex	Males				Females			
Dose Level (mg/kg bw/d)	0 (n = 50)	50 (n = 50)	500 (n = 50)	1,000 (b) (n = 49)	0 (n = 50)	50 (n = 50)	500 (n = 50)	1,000 (n = 50)
Weeks 0 - 8	0/50	0/50	0/50	0/49	0/50	0/50	0/50	1/50
Weeks 0 - 12	0/50	0/50	0/50	0/49	0/50	1/50	0/50	1/50

Sex	Males				Females			
Dose Level (mg/kg bw/d)	0 (n = 50)	50 (n = 50)	500 (n = 50)	1,000 (b) (n = 49)	0 (n = 50)	50 (n = 50)	500 (n = 50)	1,000 (n = 50)
Weeks 0 - 20	0/50	0/50	0/50	1/49	0/50	1/50	0/50	2/50
Weeks 0 - 24	0/50	0/50	0/50	1/49	1/50	1/50	0/50	2/50
Weeks 0 - 28	0/50	0/50	0/50	1/49	1/50	1/50	1/50	2/50
Weeks 0 - 48	1/50	1/50	0/50	1/49	1/50	2/50	1/50	3/50
Weeks 0 - 56	1/50	1/50	1/50	1/49	2/50	2/50	1/50	4/50
Weeks 0 - 64	1/50	1/50	2/50	1/49	2/50	3/50	4/50	6/50
Weeks 0 - 72	1/50	2/50	2/50	1/49	3/50	5/50	5/50	8/50
Weeks 0 - 80	2/50	2/50	3/50	1/49	6/50	7/50	5/50	8/50
Weeks 0 - 88	5/50	2/50	6/50	5/49	8/50	10/50	11/50	10/50
Weeks 0 - 96	8/50	3/50	7/50	5/49	9/50	12/50	15/50	13/50
Weeks 0 - 104	10/50	3/50	8/50	5/49	12/50	13/50	18/50	20/50
Weeks 0 - 106	10/50	3/50	8/50	6/49	13/5	14/50	21/50	21/50
Total Number of Deaths (%)	**10/50 (20)**	**3/50 (6)**	**8/50 (16)**	**6/49 (12)**	**13/50 (26)**	**14/50 (28)**	**21/50 (42)**	**21/50 (42)**

(a) Mortality data obtained from pages 78-79 in the study report.
(b) Number of males at 1,000 mg/kg bw/d excludes one animal that escaped; therefore, n = 49.

B. Body weight Mean body weight for both sexes at 1,000 mg/kg bw/d was marginally lower than control values throughout most of the study. A marginal but statistically significant difference was observed for the majority of the first 13 weeks of the study in both sexes. By week 100, body weight was approximately 3% lower in the high-dose males and females compared to controls. At the termination of the study (week 104), body weight was approximately 3 and 5% lower in males and females, respectively, compared to controls. In the high-dose animals, mean body-weight gain was consistently lower compared to controls throughout the study for both sexes. By week 100, body-weight gain was approximately 10% lower in the high-dose males and females. Overall body-weight gain (weeks 0-104) was approximately 11 and 13% lower in the high-dose males and females, respectively, compared to controls. Food consumption was comparable between the high-dose animals and the controls throughout the study. Although body-weight gain was lower in the high-dose animals, body weight was not adversely affected; therefore, these findings were not considered to be toxicologically relevant. Body weight and body-weight gain were unaffected by treatment in both sexes at 50 and 500 mg/kg bw/d. Body weight and body-weight gain data are summarized in Table 3 (males) and Table 4 (females).

TABLE 3: Mean body weights and body-weight gains in males (a)

Dose Level (mg/kg bw/d)	0	50	500	1,000
Body Weight (g ± SD)				

Dose Level (mg/kg bw/d)	0	50	500	1,000
Week 0	23.6 ± 0.9 (n = 57)	23.2 ± 1.3 (n = 60)	23.4 ± 1.1 (n = 57)	23.7 ± 1.5 (n = 59)
Week 3	26.2 ± 1.4 (n = 60)	25.6 ± 1.3 *	25.7 ± 1.6 (n = 60)	25.5 ± 1.4 * (n = 60)
Week 6	27.1 ± 1.3	26.6 ± 1.5	26.9 ± 1.2	26.8 ± 1.3 (n = 59)
Week 13	29.1 ± 1.4	28.6 ± 1.6 *	28.8 ± 1.4	28.3 ± 1.4 *
Week 28	32.2 ± 2.4	31.8 ± 3.0	32.2 ± 2.2	31.5 ± 2.4 (n = 58)
Week 52	35.2 ± 2.9 (n = 59)	34.9 ± 4.0	35.0 ± 3.2	33.9 ± 3.2
Week 80	36.3 ± 3.8 (n = 48)	36.8 ± 4.8 (n = 48)	36.4 ± 3.9 (n = 44)	34.8 ± 3.6 (n = 48)
Week 100	35.5 ± 2.9 (n = 40)	35.3 ± 4.9 (n = 47)	34.8 ± 4.1 (n = 42)	34.3 ± 3.3 (n = 44)
Week 104	35.1 ± 3.2	34.9 ± 4.4	34.6 ± 3.8	33.9 ± 3.3
Body-Weight Gain (g ± SD) (b)				
Weeks 0-3	2.7 ± 1.4	2.4 ± 1.0	2.3 ± 1.6	1.8 ± 1.2
Weeks 0-6	3.5 ± 1.4	3.4 ± 1.0	3.5 ± 1.2	3.0 ± 1.1
Weeks 0-13	5.8 ± 1.3	5.3 ± 1.1	5.4 ± 1.3	4.5 ± 1.2
Weeks 0-28	8.7 ± 2.4	8.5 ± 2.4	8.8 ± 2.1	7.1 ± 1.9
Weeks 0-52	11.6 ± 2.9	11.7 ± 3.5	11.6 ± 2.9	10.1 ± 2.8
Weeks 0-80	12.6 ± 3.6	13.6 ± 4.4	13.1 ± 3.6	11.0 ± 3.4
Weeks 0-100	11.8 ± 2.8	12.0 ± 4.4	11.7 ± 3.8	10.6 ± 3.1
Weeks 0-104	11.5 ± 3.1	11.6 ± 4.0	11.5 ± 3.6	10.2 ± 2.9

(a) Data obtained from pages 88-91 in the study report for body weight and pages 92-99 for body-weight gain.
(b) There were no statistical comparison of means for body-weight gain.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.

TABLE 4: Mean body weights (BW) and body-weight gains (BWG) in females (a)

Dose Level (mg/kg bw/d)	0	50	500	1,000
Body Weight (g ± SD)				
Week 0	19.4 ± 1.0 (n = 60)	19.6 ± 0.7 (n = 60)	19.8 ± 0.8 * (n = 60)	19.5 ± 0.9 (n = 60)
Week 3	22.7 ± 1.1	22.7 ± 1.0	22.6 ± 0.9	22.2 ± 0.9 *
Week 6	23.9 ± 0.9	24.2 ± 1.2	23.5 ± 1.2	22.7 ± 1.0 * (n = 59)
Week 13	25.5 ± 1.1	25.5 ± 1.2	25.5 ± 1.4	25.1 ± 1.5
Week 28	27.9 ± 1.7 (n = 59)	28.2 ± 2.5 (n = 58)	27.9 ± 2.1 (n = 59)	27.4 ± 1.7 (n = 58)
Week 52	29.3 ± 2.3 (n = 58)	29.8 ± 3.3 (n = 57)	29.9 ± 3.1	29.0 ± 2.6 (n = 57)
Week 80	31.4 ± 3.4 (n = 44)	32.5 ± 4.6 (n = 43)	31.0 ± 3.0 (n = 45)	30.8 ± 3.1 (n = 42)
Week 100	31.7 ± 3.9 (n = 40)	31.7 ± 3.6 (n = 38)	31.1 ± 3.9 (n = 33)	30.6 ± 3.5 (n = 34)
Week 104	32.1 ± 4.1 (n = 38)	31.4 ± 3.1 (n = 36)	30.7 ± 3.0 (n = 30)	30.4 ± 3.5 (n = 30)
Body-Weight Gain (g ± SD) (b)				
Weeks 0-3	3.3 ± 0.8	3.1 ± 0.5	2.8 ± 0.7	2.7 ± 0.5
Weeks 0-6	4.5 ± 0.8	4.6 ± 0.9	3.6 ± 1.0	3.2 ± 0.7
Weeks 0-13	6.0 ± 0.8	5.8 ± 0.8	5.7 ± 1.2	5.6 ± 1.2
Weeks 0-28	8.5 ± 1.4	8.6 ± 2.1	8.1 ± 1.9	7.9 ± 1.4
Weeks 0-52	9.9 ± 2.0	10.1 ± 3.0	10.1 ± 3.0	9.5 ± 2.3
Weeks 0-80	12.0 ± 3.0	12.8 ± 4.3	11.2 ± 2.7	11.2 ± 2.6
Weeks 0-100	12.3 ± 3.6	12.0 ± 3.2	11.3 ± 3.7	11.1 ± 3.2
Weeks 0-104	12.7 ± 3.9	11.7 ± 2.8	11.0 ± 2.8	11.0 ± 3.1

(a) Data obtained from pages 100-103 in the study report for body weight and pages 104-111 for body-weight gain.
(b) There were no statistical comparison of means for body-weight gain.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.

C. **Food consumption and compound intake**

1. Food consumption - There were no definitive differences in mean food consumption values between the treatment groups and controls in either sex up to and including 1,000 mg/kg bw/d (limit dose) to indicate a treatment-related effect on food consumption.

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

3. Food efficiency During the first 13 weeks, no definitive differences in food efficiency were noted between the treatment groups and controls in either sex up to and including 1,000 mg/kg bw/d (limit dose) due to the wide variability of feed efficiency data in both sexes at all dose levels. Food efficiency was not measured after week 13, however, there was a slight decrease in body-weight gain in the high-dose animals (both sexes) with no concomitant change in food consumption.

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

E. **Blood analyses**

1. **Haematology** - There were no treatment-related changes in haematological parameters in males or females up to and including 1,000 mg/kg bw/d (limit dose).

2. ***Clinical Chemistry*** - There were no toxicologically relevant treatment-related clinical chemistry findings in either sex up to and including 1,000 mg/kg bw/d at 12 or 24 months. At 12 months, significant findings included decreased serum cholesterol and triglyceride levels in males at 1,000 mg/kg bw/d (Table 5). These findings were considered to correlate with marginally but significantly lower body weights, decreased absolute liver weights (with no change in relative liver weights) and decreased glycogen within hepatocytes of the centrilobular region (as indicated by the altered tinctorial properties of the centrilobular hepatocytes) observed in these animals. At 24 months serum cholesterol and triglyceride levels in these animals were unaffected by treatment. Food consumption was also unaffected by treatment in these animals. The lower serum cholesterol and triglyceride levels were considered to be a secondary effect and not an indication of hepatotoxicity. At 24 months, significant findings were limited to decreased serum globulin levels in males at 500 and 1,000 mg/kg bw/d at 24 months. The decreased serum globulin level was not dose-related and total protein and levels were not identified as being different from controls; therefore, in the absence of any other corroborating clinical chemistry, gross pathological, histopathological or other findings this was considered to be an incidental finding and not toxicologically relevant. There were no relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with the histopathological findings in the kidneys or to indicate an impairment of renal function. There were no significant clinical chemistry findings at 12 or 24 months in males at 50 and 500 mg/kg bw/d or in females at any dose level.

TABLE 5: Clinical chemistry findings (a)

Dose Level (mg/kg bw/d)	0	50	500	1,000
Males- Satellite Group - 12 months (10 animals/group) (b)				
Cholesterol (mg/dL)	119 ± 8	119 ± 27	120 ± 11	**98 ± 9 ***
Triglycerides (mg/dL)	225 ± 58	189 ± 68	273 ± 63	***141 ± 27 #***
Males -Oncogenicity Group - 24 months (19-20 animals/group)				
Globulin (g/dL)	2.0 ± 1.0	1.9 ± 0.5	**1.5 ± 0.2 #**	**1.6 ± 0.7 #**

(a) Data obtained from pages 154 to 161 in the study report.
(b) The high-dose males sacrificed at 12 months, exhibited lower body weights and body-weight gains compared to controls (body weight at week 52 - 35.8 ± 1.7, 34.9 ± 3.3, 35.3 ± 3.0 and 32.4 ± 2.1 g at 0, 50, 500 and 1,000 mg/kg bw/d, respectively; body-weight gain for weeks 0 to 52 - 12.1 ± 2.5, 11.8 ± 2.8, 11.1 ± 2.6 and 9.2 ± 1.5 g at 0, 50, 500 and 1,000 mg/kg bw/d, respectively).
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
\# Statistically different from control mean by Wilcoxon's test, $p \leq 0.05$.

F. Urinalysis - Not determined.

G. Sacrifice and Pathology:

1. **Organ weight** - Absolute kidney weights were significantly decreased in males at ≥500 mg/kg bw/d at 12 and 24 months (Tables 6 and 7, respectively). Relative kidney weights were significantly decreased in males at 500 mg/kg bw/d at 12 and 24 months and in males at 1,000 mg/kg bw/d at 24 months. The decreased kidney weights were considered to be treatment-related, however, the toxicological significance was uncertain. The decreased kidney weights correlate with histopathological findings, characterized as decreased cytoplasmic vacuolation of the cortical tubular epithelium cells at 12 and 24 months, however, the decreased cytoplasmic vacuolation was not considered to be toxicologically relevant since it did not adversely affect the well-being of the animals. The decrease in kidney weights did not exhibit a clear dose-response relationship. However, the lack of a clear dose-response relationship may be associated with other histopathological findings in the kidney, specifically, the increased incidence and/or severity of hypertrophy of the epithelial cells in the collecting duct observed in males at 1,000 mg/kg bw/d compared to males at 500 mg/kg bw/d. Kidney weights were not identified as being different from controls in females at 500 or 1,000 mg/kg bw/d at either 12 or 24 months.

Other significant findings in males at 12 months included increased relative adrenal and brain weights and decreased absolute liver and heart weights at 1,000 mg/kg bw/d and decreased absolute heart weights at 500 mg/kg bw/d. Other significant findings in males at 24 months were limited to decreased absolute heart weights at 1,000 mg/kg bw/d. Absolute adrenal and brain weights were not identified as being different from controls and in the absence of any gross pathological or histopathological corroborative findings, these changes in relative adrenal and brain weights were considered to be secondary to decreased body weight in these animals and not reflective of organ-specific toxicity. Relative heart weights were not identified as being different from controls and there were no gross pathological or histopathological findings in the heart in males at 500 or 1,000 mg/kg bw/d, thus the decreased absolute heart weights were not considered to be toxicologically significant. The decreased absolute liver weights in males at 1,000 mg/kg bw/d at 12 months correlate with marginally but significantly lower body weights, decreased serum cholesterol and triglyceride levels and decreased glycogen within hepatocytes of the centrilobular region and was interpreted to be a secondary effect and not an indication of hepatotoxicity. Relative liver weights were not identified as being different from controls further suggesting that the difference in absolute liver weight was probably secondary to marginally but significantly lower body weights.

Significant findings in female mice were confined to decreased absolute and relative ovary weights in the 50 mg/kg bw/d dose group at the 12-month interim sacrifice. This decrease was not dose-related thus was not considered to be treatment-related. There were no other significant findings in females at any dose level at 12 or 24 months.

TABLE 6: Organ and organ/body weights - 12 months (a)

Dose Level (mg/kg bw/d)		0	50	500	1,000
12 months - Satellite Group Males (10 animals/sex/dose level)					
Final Body Weight (g ± SD)		35.8 ± 1.7	34.9 ± 3.3	35.3 ± 3.0	**32.4 ± 2.1 ***
Adrenals	Absolute (g ± SD)	0.04 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.005 ± 0.001
	Relative (g/100 g bw ± SD)	0.0115 ± 0.0026	0.0106 ± 0.0026	0.0122 ± 0.0028	**0.0146 ± 0.0022 ***
Brain	Absolute (g ± SD)	0.482 ± 0.015	0.475 ± 0.027	0.471 ± 0.017	0.473 ± 0.025
	Relative (g/100 g bw ± SD)	1.349 ± 0.050	1.373 ± 0.157	1.341 ± 0.110	**1.463 ± 0.061 #**
Heart	Absolute (g ± SD)	0.191 ± 0.009	0.184 ± 0.012	**0.173 ± 0.013 ***	**0.171 ± 0.014 ***
	Relative (g/100 g bw ± SD)	0.537 ± 0.037	0.530 ± 0.042	0.494 ± 0.050	0.528 ± 0.028
Kidneys	Absolute (g ± SD)	0.736 ± 0.027	0.696 ± 0.036	**0.663 ± 0.032 ***	**0.646 ± 0.049 ***
	Relative (g/100 g bw ± SD)	2.061 ± 0.095	2.005 ± 0.156	**1.889 ± 0.162 ***	1.997 ± 0.107
Liver	Absolute (g ± SD)	1.876 ± 0.086	1.977 ± 0.578	1.866 ± 0.151	**1.689 ± 0.076 ***
	Relative (g/100 g bw ± SD)	5.255 ± 0.288	5.700 ± 1.755	5.304 ± 0.454	5.229 ± 0.254
12 months - Satellite Group Females (10 animals/sex/dose level)					
Ovaries	Absolute (g ± SD)	0.016 ± 0.006	0.010 ± 0.002 *	0.013 ± 0.005	0.012 ± 0.003
	Relative (g/100 g bw ± SD)	0.053 ± 0.018	0.035 ± 0.005 #	0.041 ± 0.014	0.039 ± 0.009

(a) Data obtained from pages 162 and 177 in the study report for males and pages 163 and 178 for females.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
# Statistically different from control mean by Wilcoxon test, $p \leq 0.05$.

TABLE 7: Organ and organ/body weights - 24 months (a)

Dose Level (mg/kg bw/d)		0 (n = 39)	50 (n = 47)	500 (n = 42)	1,000 (n = 43)
24 months - Oncogenicity Group Males					
Heart	Absolute (g ± SD)	0.187 ± 0.018	0.188 ± 0.018	0.179 ± 0.017	**0.177 ± 0.016** *
	Relative (g/100 g bw ± SD)	0.545 ± 0.54	0.549 ± 0.079	0.528 ± 0.072	0.528 ± 0.047
Kidneys	Absolute (g ± SD)	0.732 ± 0.050	0.705 ± 0.069	**0.678 ± 0.073** *	**0.681 ± 0.062** *
	Relative (g/100 g bw ± SD)	2.136 ± 0.213	2.047 ± 0.187	**1.984 ± 0.143** *	**2.030 ± 0.187** *

(a) Data obtained from pages 162 and 177 in the study report for males and pages 163 and 178 for females.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.

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

3. Microscopic pathology:

a) Non-neoplastic - Significant non-neoplastic histopathological findings are summarized in Table 7 (12 months) and Table 8 (24 months).

Hypertrophy of the epithelial cells of the collecting ducts was observed in both sexes at ≥500 mg/kg bw/d at 12 and 24 months. A dose-related increased severity was apparent in both sexes. In this study, there appeared to be no significant progression in the severity of the lesions over time (from 1 year to 2 years), however, when compared to the 13-week dietary study there may be a slight progression in the severity of the lesions from 13-weeks to 1 year in both sexes. Overall, the effect was considered to be minimal and was graded as very slight or slight based on the number of hypertrophied cells present in the tubules. The hypertrophy was characterized by enlargement of individual cells rather than generalized enlargement of all cells in the collecting duct. The hypertrophied cells exhibited a granular, pale, eosinophilic cytoplasm with increased cytoplasmic volume and numerous mitochondria. The cells were primarily the epithelial cells lining the collecting ducts, principally of the inner stripe of the outer zone of the medulla and were compatible with 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. Occasionally individual hypertrophied cells were also found in the proximal portion of the papilla. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability, however, urinalysis was not performed. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with the histopathological findings in the kidneys or to indicate an impairment of renal function and there was no increased incidence of cellular degeneration or necrosis evident in the kidneys. 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. Similar histopathological findings (no urinalysis performed) were also observed in the 13-week dietary study with B6C3F1 mice at similar dose levels (see DACO 4.3.1 - Laboratory Project Study ID - DR-0312-6565-010). Morphologically, the lesions were also similar to those reported in the 13-week and 2-year dietary studies with Fischer 344 rats at similar dose levels (see DACO 4.3.1.2 - Laboratory Project Study ID - DR-0312-6565-011 and DACO 4.4.4 - Laboratory Project Study ID - 960004, respectively) where urinalysis findings indicative of an acidification defect and impaired concentrating were observed (urinary acidification and reduced urinary specific gravity, respectively). In the 13-week dietary study the lesions and urinalysis findings appeared to be reversed after a 4-week recovery period.

At 12 and 24 months, cytoplasmic vacuolation of the cortical tubular epithelium cells was decreased in males at ≥500 mg/kg bw/d. It was indicated that these lipid appearing vacuoles are normally observed in male mice maintained on an *ad libitum* diet but not in female mice at any age. The decreased cytoplasmic vacuolation was consistent with decreased kidney weights in males at 500 and 1,000 mg/kg bw/d. This was considered to be treatment-related, however, it was not considered to be toxicologically significant since it did not adversely affect the well-being of these animals.

In females, a decreased incidence of age-related tubular degeneration with regeneration was noted at ≥500 mg/kg

bw/d at 12 months and at 1,000 mg/kg bw/d at 24 months. In males, the incidence of age-related tubular degeneration with regeneration was comparable to controls at 12 and 24 months, however, the severity was decreased at 24 months at ≥500 mg/kg bw/d. This was not considered to be toxicologically significant. Spontaneous tubular degeneration/regeneration is considered a normally occurring disease process.

At 12 months, males at 1,000 mg/kg bw/d, microscopic changes in the liver were characterized by altered tinctorial properties of the centrilobular hepatocytes. This was considered to be consistent with marginally but significantly lower body weights and decreased absolute liver weights (with no change in relative liver weights) and most likely reflected decreased glycogen within hepatocytes of the centrilobular region. This was considered to be a secondary effect and not an indication of hepatotoxicity.

At 24 months there was a slight increase in the incidence of inflammation of the lacrimal/Harderian gland in females at 1,000 mg/kg bw/d, however, this was not statistically significant.

TABLE 7: 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	50	500	1,000
12 months - Satellite Group Males (10 animals/sex/dose level)						
Kidney	- hypertrophy, collecting ducts	- very slight (b)	0/10	0/10	10/10	0/10
		- slight (b)	0/10	0/10	0/10	10/10
		- total	0/10	0/10	**10/10**	**10/10**
	- tubule degeneration with regeneration, very slight, focal/multi-focal		7/10	8/10	7/10	6/10
	- decreased vacuolization renal tubule, cortex,	- very slight	0/10	1/10	5/10	3/10
		- slight	0/10	0/10	0/10	2/10
		- moderate	1/10	0/10	0/10	4/10
		- total	1/10	1/10	**5/10**	**9/10**
Liver	- altered tinctorial properties, centrilobular	- very slight	3/10	1/10	2/10	7/10
		- slight	0/10	1/10	0/10	3/10
		- total	3/10	2/10	2/10	**10/10**
12 months - Satellite Group Females (10 animals/sex/dose level)						
Kidney	- hypertrophy, collecting ducts	- very slight (b)	0/10	0/10	10/10	1/10
		- slight (b)	0/10	0/10	0/10	9/10
		- total	0/10	0/10	**10/10**	**10/10**
	- tubule degeneration with regeneration, very slight, focal/multi-focal		7/10	5/10	3/10	3/10

(a) Data obtained from pages 165-176 of 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 8: 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	50	500	1,000

24 months - Oncogenicity Group Males						
Kidney	- hypertrophy, collecting ducts	- very slight (b)	0/50	0/50	**41/50 ***	**27/49 ***
		- slight (b)	0/50	0/50	0/50	**20/49 ***
		- total	0/50	0/50	**41/50 ***	**47/49 ***
	- tubule degeneration *with regeneration*	*- very slight*	20/50	17/50	**33/50 ***	**32/49 ***
		- slight	30/50	31/50	**16/50 ***	**17/47 ***
		- total	50/50	48/50	49/50	49/49
	- decreased vacuolization renal tubule, cortex,	- slight	11/50	17/50	**27/50 ***	**14/49 ***
		- moderate	13/50	9/50	20/50	**33/49 ***
		- total	24/50	26/50	**47/50 ***	**47/49 ***
lacrimal / Harderian gland	- inflammation; subacute to chronic; unilateral	- very slight	4/50	0/4	0/7	4/49
		- slight	2/50	0/4	1/7	3/49
		- total	6/50	0/4	1/7	7/49
24 months - Oncogenicity Group Females						
Kidney	- hypertrophy, collecting ducts	- very slight (b)	0/50	0/50	**18/50 ***	**34/50 ***
		- slight (b)	0/50	0/50	0/50	0/50
		- total	0/50	0/50	**18/50 ***	**34/50 ***
	- tubule degeneration with regeneration	- very slight	8/50	4/50	4/50	1/50 *
		- slight	0/50	0/50	0/50	0/50
		- total	8/50	4/50	4/50	1/50 *
lacrimal / Harderian gland	- inflammation; subacute to chronic; unilateral	- very slight	1/50	1/16	0/23	0/41
		- slight	1/50	2/16	2/23	6/41
		- total	2/50	3/16	2/23	6/41

(a) Data obtained from pages 197-223 of the study report.
* Statistically different from controls by Yate's Chi-square, $p \leq 0.10$.
(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

b) Neoplastic - Neoplastic histopathological findings are summarized in Table 9 (12 months) and Table 10 (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 florasulam in mice.

At 12 and 24 months, there were no treatment-related neoplastic findings in either sex. The neoplasms observed were generally of the common types present in this strain of mice of this age and their incidence had no relationship to treatment. However, at 24 months a slight increased incidence of benign adenomas of the lacrimal/harderian gland was observed in males at 1,000 mg/kg bw/d. The incidence of these tumours in the high-dose males was not statistically different from controls. Benign adenomas of the lacrimal/harderian gland were not present in males at any dose level at 12 months. Ductal hyperplasia of the lacrimal gland was not observed in either sex at any dose level and there was no significant increase in the incidence of inflammation in the lacrimal/harderian gland (see Table 8). The incidence of benign adenomas was within the range of the historical control data for animals of this age and strain from this laboratory (incidence range: 2-18%) and the National Toxicology Program (NTP) historical control database (incidence range: 0-18%; NTP update 1998 for 2-year dietary oncogenicity study in B6C3F1 mice); therefore, the tumours were considered to be spontaneous in nature. A review of the literature also suggests that the spontaneous occurrence of benign adenomas of the lacrimal/harderian glands is not uncommon in B6C3F1 male mice.

For all neoplastic findings there was neither a dose-dependent change in total number of animals with tumours nor in the number of tumour bearing animals sacrificed or dying during treatment as compared with those sacrificed

according to schedule. Furthermore, no difference in the incidence of benign and malignant tumours in animals sacrificed or dying during treatment compared with those sacrificed after 2 years was observed. There was no dose-related effect detected on the time-dependent occurrence of tumour bearing animals.

TABLE 9: 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	50	500	1,000	0	50	500	1,000
		Males				Females			
Lacrimal/ Harderian Gland	- benign adenoma	0/10	-	-	0/10	1/10	0/1	-	0/10
Liver	- benign hepatocellular adenoma	2/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
Lungs	- benign bronchioloalveolar adenoma	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Salivary Glands	- benign hemangioma, adipose tissue	0/10	-	-	0/10	1/10	0/1	-	0/10
Spleen	- malignant hemangiosarcoma	0/10	-	-	0/10	1/10	0/1	-	0/10
Testes	- benign hemangioma	0/10	1/1	-	0/10	-	-	-	-
Uterus	- benign endometrial stromal polyp	-	-	-	-	1/10	0/10	0/8	0/9

(a) Data obtained from page 176 of the study report. No statistically identified differences from control by Yate's Chi-square, $p \leq 0.10$.

TABLE 10: 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	50	500	1,000	0	50	500	1,000
		Males				Female			
Lacrimal / Harderian Gland (b)	- benign adenoma	2/50	1/4	4/11	7/49	4/50	2/16	3/23	3/50

(a) Data obtained from pages 224-230 of the study report. No statistically identified differences from control by Yate's Chi-square, $p \leq 0.10$.
Historical control incidence of benign adenomas of the lacrimal/harderian gland in male B6C3F1 mice (2-yr dietary chronic toxicity/oncogenicity):
Performing Laboratory : Mean - 6/50 (12%) Range - 2-18% (1/50 to 9/50).
NTP database historical controls: Mean - 4.7% (64/1,355). Range - 0-18% (**Reference**: Haseman, J.K., et al (1998). Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F1 Mice in Two-year Carcinogenicity Studies: A National Toxicology Program Update. Toxicologic Pathology 26: 428-441).

III. DISCUSSION

A. Investigators' conclusions (extracted from page 47 of the study report): "Treatment-related effects were limited to middle and high dose mice of both sexes with the kidneys identified as the only target organ. Renal effects were characterized by the following: decreased absolute and relative weight (males only), intercalated cell hypertrophy (both sexes), decreased vacuolization of cortical epithelial cells (males only), and a decreased incidence of age-related tubular degeneration/regeneration (both sexes). The microscopic renal effects were not associated with any clinical chemistry or electrolyte changes indicative of altered renal function. Slightly decreased body weight and weight gain were observed in the high dose mice of each sex over the course of the study. Other incidental organ weight changes observed were unassociated with any histopathology, and were considered secondary to decreased body weights. No treatment-related effects on mortality were observed. Survival after 104 weeks of treatment was 80 - 94% in males and 58 - 74% in females. There were no tumorigenic effects in male or female mice at any of the dose levels of 50, 500, or 1000 mg XDE-570/kg bw/day. The effects on body weights, kidney weights, and kidney morphology were minor and did not adversely affect the well-being of the mice administered 500 or 1000 mg XDE-570/ kg bw/day for two years. The no-observed-effect-level (NOEL) in male and female B6C3F1 mice was 50 mg XDE-570/kg bw/day."

B. Reviewer comments: There were no treatment-related effects on clinical signs, body weight, food consumption,

ophthalmoscopy, haematology, clinical chemistry or gross pathology. The number of spontaneous deaths or animals sacrificed due to moribund conditions was slightly increased in females at 500 and 1,000 mg//kg bw/d (13/50, 14/50, 21/50 and 21/50 at 0, 50, 500 and 1,000 mg/kg bw/d, respectively). However, at the end of the study there were no statistically identified 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.

Kidney weights were slightly but significantly decreased in males at ≥500 mg/kg bw/d at 12 and 24 months. This was considered to be treatment-related, however, the toxicological significance was uncertain. The decreased kidney weights correlate with histopathological findings, characterized as decreased cytoplasmic vacuolation of the cortical tubular epithelium cells at 12 and 24 months, however, the decreased cytoplasmic vacuolation was not considered to be toxicologically relevant since it did not adversely affect the well-being of the animals. The decreased kidney weights did not exhibit a clear dose-response relationship at either 12 or 24 months. However, the lack of a clear dose-response relationship may be associated with other histopathological findings in the kidney, specifically, the increased incidence and/or severity of hypertrophy of the epithelial cells in the collecting duct observed in males at 1,000 mg/kg bw/d compared to males at 500 mg/kg bw/d.

Hypertrophy of the epithelial cells of the collecting ducts was observed in both sexes at ≥500 mg/kg bw/d at 12 and 24 months. A dose-related increased severity was apparent in both sexes. In this study, there appeared to be no significant progression in the severity of the lesions over time (from 1 year to 2 years), however, when compared to the 13-week dietary study there may be a slight progression in the severity of the lesions from 13-weeks to 1 year in both sexes. The hypertrophied cells were primarily the epithelial cells lining the collecting ducts and were compatible with intercalated cells which are involved in the regulation of acid-base balance through the modulation of bicarbonate resorption and hydrogen ion secretion in the collecting ducts. Functional abnormalities of the collecting duct manifest primarily as an acidification defect and as impaired concentrating ability, however, urinalysis was not performed. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with the histopathological findings in the kidneys or to indicate an impairment of renal function and there was no increased incidence of cellular degeneration or necrosis evident in the kidneys. 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. In females, a decreased incidence of age-related tubular degeneration with regeneration was noted at ≥500 mg/kg bw/d at 12 months and at 1,000 mg/kg bw/d at 24 months. In males, the incidence of age-related tubular degeneration with regeneration was comparable to controls at 12 and 24 months, however, the severity was decreased at 24 months at ≥500 mg/kg bw/d.

At 24 months, a slight increased incidence of benign adenomas of the lacrimal/harderian gland was observed in males at 1,000 mg/kg bw/d (2/50, 1/4, 4/11 and 7/49 at 0, 50, 500 and 1,000 mg/kg bw/d, respectively). The incidence of these tumours in the high-dose males was not statistically different from controls. Benign adenomas of the lacrimal/harderian gland were not present in males at any dose level at 12 months. Ductal hyperplasia of the lacrimal gland was not observed in either sex at any dose level and there was no significant increase in the incidence of inflammation in the lacrimal/harderian gland. The incidence of benign adenomas was within the range of the historical control data for animals of this age and strain from this laboratory (incidence range; 2-18%) and the National Toxicology Program (NTP) historical control database (incidence range: 0-18%; NTP update 1998 for 2-year dietary oncogenicity study in B6C3F1 mice) therefore, the tumours were considered to be spontaneous in nature. A review of the literature also suggests that the spontaneous occurrence of benign adenomas of the lacrimal/harderian glands is not uncommon in B6C3F1 male mice. 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 florasulam in mice.

The LOAEL for chronic toxicity was 500 mg/kg bw/d based on decreased kidney weights (♂) and hypertrophy of the epithelial cells of the collecting ducts (♂/♀). The NOAEL for chronic toxicity was 50 mg/kg bw/d.

Dosing was considered adequate based on decreased body-weight gain in both sexes at 1,000 mg/kg bw/d (≈10% ↓ compared to controls). OPPTS 870.4200 indicates that the highest dose level tested need not exceed 1,000 mg/kg bw/d. The highest dose tested in this study was 1,000 mg/kg bw/d in both sexes.

ɔses tested, there was no treatment-related increased incidence of tumours in the treatment groups .ompared to controls up to and including 1,000 mg/kg bw/d, the highest dose tested; therefore, under .onditions of the study, XDE-570 was not considered to be oncogenic.

C. **Study deficiencies:** According to OECD Guideline 451 (Carcinogenicity Studies) a blood smear should be obtained from all animals at 12 and 18 months and prior to sacrifice. No blood smears were obtained for any animals, however, blood samples were obtained at the interim sacrifice at 12 months (10 animals/sex/dose) and from the first 10 and 20 surviving animals/sex/dose level from the oncogenicity group following 18 (haematology) and 24 (haematology and clinical chemistry) months of dosing, respectively. From these blood samples RBC, WBC and platelet counts, differential WBC counts, erythrocyte, leukocyte and platelet morphology were determined. There were no significant treatment-related haematological findings; therefore, this deficiency should not impact upon the outcome of the study. OPPTS 870.4200 indicates that spleen, epididymides and uterus weights are required, however, they were not provided in the study report. Based on OECD guideline 451, these organ weights are not required for an oncogenicity study in rodents. There are no deficiencies which would significantly impact upon the outcome of the study; therefore, this study is acceptable and satisfies the guideline requirement for a carcinogenicity study (OPPTS 870.4200); OECD 451 in mice.