DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.3150 [§82-1b] (non-rodent); Subchronic Oral Toxicity in Dogs

Work Assignment No. 4-01-128 D (MRID 46808223)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer
David A. McEwen, B.S.

Signature: David A. McEwen
Date: 12/19/06

Secondary Reviewer
Stephanie E. Foster, M.S.

Signature: Stephanie E. Foster
Date: 12/19/06

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 12/19/06

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 12/20/06

Disclaimer

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

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** Karlyn
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 5/31/05
EPA Secondary Reviewer: Myron Ottley, Ph.D. **Signature:** Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 5/31/07
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [feeding] – [Dog]; OPPTS 870.3150 [§82-1b] (non-rodent); OECD 409.

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

TEST MATERIAL (PURITY): XDE-570 (Florasulam; 99.3% a.i.; Lot # 940714)

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

CITATION: Stebbins, K.E. (1995) Amended report for XDE-570: Thirteen-week dietary toxicity study in Beagles. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: DR-0312-6565-021, September 13, 1995 (Amended date: November 20, 1997). MRID 46808223. Unpublished.

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

EXECUTIVE SUMMARY - In a 90-day oral toxicity study (MRID 46808223), XDE-570 (Florasulam; 99.3% a.i.; Lot # 940714) was administered to 4 Beagle dogs/sex/dose *ad libitum* in the diet at dose levels of 0, 5, 50, or 100 mg/kg/day (time-weighted average test substance intake was 0/0, 6/6, 56/55, and 104/94 mg/kg/day [M/F]) for 13 weeks.

There were no compound-related effects on mortality, clinical signs, body weight, body weight gain, food consumption, ophthalmoscopy, hematology, urinalysis, or gross pathology observed at any dose.

The target organ appeared to be the liver. At 50 mg/kg, alkaline phosphatase activity was increased ($p<0.05$) by 59-112% in the males and 91-127% in the females on Days 45 and 91, and there was a slight increase in the incidence of hepatic vacuolation (3/4 treated [very slight to slight severity] vs. 1/4 control [moderate severity] females). At 100 mg/kg, the following liver effects were noted: (i) alkaline phosphatase activity was increased ($p<0.05$) by 213-451% in both sexes on Days 45 and 91; (ii) increased incidence of very slight to slight hepatic vacuolation (4/4 treated vs. 3/4 control males and 3/4 treated vs. 1/4 control females); and (iii) increased ($p<0.05$) absolute (incr. 22-29%) and relative (to body; incr. 26-27%) liver weight in both sexes.

The LOAEL is 50 mg/kg/day, based on increased alkaline phosphatase (59-127%) activity

and increased incidence/severity of hepatic vacuolation in both sexes. The NOAEL is 5 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.3150; OECD 409 for a 90-day oral toxicity study in the dog.

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

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

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

46808223.PMRA.der
.pdf

Reviewer: Tom Morris , Date April 7, 2000.

STUDY TYPE: Sub-chronic Oral Toxicity [feeding]-[dog]; OPPTS 870.3150 (non-rodent); OECD 409.

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

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

CITATION: Stebbins, K. E. September 13, 1995 (Amended Date: November 20, 1997). **Amended report for XDE-570: Thirteen-Week dietary Toxicity Study in Beagles**. Performing Laboratory: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: DR-0312-6565-021. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a subchronic toxicity study, XDE-570 (Purity - 99.3%) was administered to 4 dogs (Beagle)/sex/dose *ad libitum* in the diet at dose levels of 0, 5, 50 or 100 mg/kg bw/d (time-weighted average test substance intake for ♂/♀ was 0/0, 6/6, 56/55 or 104/94 mg/kg bw/d) for 13 weeks. The control group animals received untreated diet throughout the study. The animals were sacrificed and necropsied on study days 93 (males) and 94 (females).

There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, ophthalmoscopy, haematology or gross pathology. Clinical chemistry investigations revealed increased alkaline phosphatase (AP) activity in both sexes at 50 and 100 mg/kg bw/d. Increased liver weights were observed in both sexes at 100 mg/kg bw/d. The increased liver weights in both sexes at 100 mg/kg bw/d correlated with the increased AP activity and with a slight increased incidence/severity of hepatic vacuolation. A slight increased incidence/severity of hepatic vacuolation was also observed in both sexes at 50 mg/kg bw/d. In the kidney, hypertrophy of the epithelial cells of the collecting ducts was observed in 4/4 males and 3/4 females at 100 mg/kg bw/d and in 2/4 males and 1/4 females at 50 mg/kg bw/d. The hypertrophied epithelial cells were compatible with the intercalated cells which normally function 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, urinary acidification was not present and urinary specific gravity was unaffected in these animals. Kidney weights were also unaffected in these animals. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with the histopathological findings in the kidney or to indicate an impairment of renal function and there was no cellular degeneration or necrosis evident in the kidneys.

The LOAEL is 50 mg/kg bw/d based on increased alkaline phosphatase activity, slight increased incidence/severity of hepatic vacuolation and hypertrophy of the epithelial cells of the collecting ducts in both sexes. The NOAEL is 5 mg/kg bw/d.

This subchronic toxicity study in the dog is acceptable / guideline and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3150; OECD 409) in dogs.

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

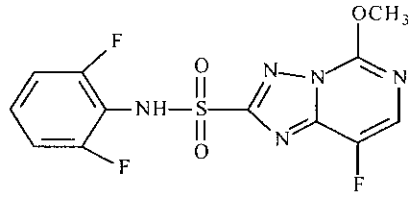
Lot/Batch #: TSN 100511 (Lot # 940714)

Purity: 99.3 % a.i. (determined by HPLC).

Compound Stability: The test substance was re-assayed after study determination and was confirmed at 99.3% (Report No. GHE-P-3714, 02/16/95)

CAS #: 145701-23-1

Structure



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

3. **Test animals:**

Species: Male and female dogs.

Strain: Beagle

Age/weight at study initiation: At study initiation, the dogs were ≈9 to 15 months of age with a body weight range of 10-13 kg for males and 8-13 kg for females.

Source: Marshall Research Laboratory, North Rose, NY.

Housing: The animals were individually housed in exercise pens.

Diet: Purina Purified Canine Chow #5007 (Purina Mills Inc., St. Louis, MO) *ad libitum*

Water: Tap water *ad libitum*

Environmental conditions:
Temperature: 65-85 °F.
Humidity: 30-70%
Air changes: 12-15 air changes/hour.
Photoperiod: ≈10 hours darkness every 24 hours.

Acclimation period: At least 14 days.

B. STUDY DESIGN:

1. **In life dates** - Start: 12/06/94. End: 13/09/94

2. **Animal assignment** - Animals were selected from a pool of 20 male and 20 female dogs on the basis of body weight, food consumption, temperament, eye examinations and clinical pathology data and randomly assigned to the study groups by weight stratification performed by computer program as summarized in Table 1. Litter mates were not included in the same dose group. The test substance was administered *ad libitum* in the diet for 13 weeks. The control group animals received untreated diet throughout the study. The animals were sacrificed and necropsied on study days 93 (males) and 94 (females).

TABLE 1: Study design.

Test Group	Dose Levels (mg/kg bw/d)	Time-Weighted Average Test Substance Intake (mg/kg bw/d)		Number of Animals	
		Males	Females	Males	Females
1	0	0	0	4	4
2	5	6	5	4	4
3	50	56	55	4	4
4	100	104	94	4	4

In an exploratory 4-week dietary toxicity study in Beagle dogs, 2 dogs/sex/dose were administered XDE-570 *ad libitum* in the diet at doses of 0, 50, 150 or 450 mg/kg bw/d (Sullivan, J.M. and Singleton, N.C., 1995., Laboratory Project ID: DR-0312-6565-018, study submitted but a full review was not done). Treatment-related findings included increased serum alkaline phosphatase (AP) activity, likely hepatic in origin, in both sexes at ≥50 mg/kg bw/d; increased aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activity in both sexes at ≥150 mg/kg bw/d; increased liver weights in males and possibly females at ≥150 mg/kg bw/d; very slight multi-focal hyperplasia of the bile ducts in males at ≥150 mg/kg bw/d and females at 450 mg/kg bw/d and biliary hyperplasia, bile stasis and hepatocellular necrosis in 1 male at 450 mg/kg bw/d which had the most significant changes in clinical chemistry parameters. A NOEL was not identified due to increased AP activity (assumed to be associated with hepatotoxicity) at ≥50 mg/kg bw/d. However, the increased AP activity at 50 mg/kg bw/d did not correlate with any histopathological findings; therefore, 50 mg/kg bw/d was considered to be a NOAEL. Based on these findings, the high dose, 100 mg/kg bw/d, was expected to produce clear evidence of treatment-related effects. The remaining dose levels were expected to provide dose-response data for any toxicity observed at the high dose and to help define a no-observed-effect dose level.

3. Diet preparation and analysis Diets were prepared once weekly by serially diluting a 5% test article-feed mixture with ground feed. Concentrations of the test article were calculated from pre-dose body weight and food consumption data with the intention of exposing dogs to the targeted dose levels. Test article concentration in the feed remained constant for the study duration. Actual dose was calculated using test article concentration, body weight and food consumption for each treated dog and reported at intervals corresponding with body weight and food consumption measurements. Confirmation of homogeneity and stability of the test substance were done once prior to or during the study. Concentration verification was done at the beginning, in the middle and at the end of the study. Samples of each dietary concentration, approximately 10 g (1/dose/sex and pre-mix) were retained and stored at ambient temperature.

Results - **Homogeneity Analysis:** The test substance homogeneity was considered adequate for conduct of the study.

Date Prepared	Group	Test Article Concentration (ppm)		Relative Standard Deviation (%)
		Mean Observed Concentration (a)	Standard Deviation	
12/05/94	2 - Females (5 mg/kg bw/d)	184	7	3.80
	4 - Females (100 mg/kg bw/d)	3,340	20	0.60

(a) Mean of 6 determinations.

Stability Analysis: The test substance stability was considered adequate for conduct of the study. The test substance was found to be stable in the diet for at least 13 days.

Time Point	Theoretical - 50 ppm		Theoretical - 1,943 ppm (Gr. 3 - Males)		Theoretical - 50,000 ppm (5% Pre-mix)	
	Measured (ppm)	% Initial	Measured (ppm)	% Initial	Measured (ppm)	% Initial

Initial	40.9 ± 1.7	100	1,980 ± 4	100	50,900 ± 200	100
24 hours	46.3 ± 6.1	113	-	-	-	-
48 hours	-	-	1,880 ± 10	95	49,300 ± 200	97
5 days	49.9 ± 14.5	122	-	-	-	-
6 days	-	-	1,740 ± 8	88	45,400 ± 200	89
8 days	55.2 ± 6.0	135	-	-	-	-
9 days	-	-	1,890 ± 11	95	49,800 ± 600	98
12 days	48.9 ± 7.9	117	-	-	-	-
13 days	-	-	1,890 ± 21	95	49,400 ± 200	97

Concentration Analysis: The mean concentrations of the test substance in the diet were shown to be 98 to 100% of the targeted concentrations during the course of the study.

Group	Theoretical Value (ppm)	Measured Value (ppm)		Percent of Theoretical Value	
		Range	Mean	Range	Mean
2 - Females (5 mg/kg bw/d)	178.4	169-184	177	95-103	99
3 - Females (50 mg/kg bw/d)	2,097	2020-2100	2060	96-100	98
4 - Females (100 mg/kg bw/d)	3,265	3,220-3,340	3,263	99-102	100
2 - Males (5 mg/kg bw/d)	193.3	181-198	192	94-102	99
3 - Males (50 mg/kg bw/d)	1,943	1,870-1,980	1,930	96-102	99
4 - Males (100 mg/kg bw/d)	3,311	3,250-3,360	3,307	98-101	100
Pre-mix	50,000	49,000-50,900	49,933	98-102	100

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

4. Statistics - All parameters to be examined statistically were first tested for equality of variance using Bartlett's test. If the results of the Bartlett's test were significant, then the data for that parameter were subjected to a transformation to obtain equality of variance. The transformations examined included the common log, the inverse and the square root; these were applied in this order with a Bartlett's test following each transformation. When Bartlett's test was satisfied no further transformations were applied. If none of the transformations resulted in homogeneous variances, the transformation data or raw data with the lowest Bartlett's statistics were used. The selected data were then subjected to the appropriate parametric analysis. In-life body weight, haematological parameters and clinical chemistry parameters were evaluated using a three-way, repeat measure (RM) analysis of variance (ANOVA) for time (the repeated factor), sex and dose. In the three-way RM-ANOVA, the differences between the groups were detected primarily by the time-dose interaction. Parameters analysed by a three-way RM-ANOVA involved several preliminary examinations. The first examination in the three-way RM-ANOVA was of the time-sex-dose interaction. If significant, the analysis was repeated separately for each sex without examining the results of other factors. If the time-sex-dose interaction was not statistically identified, then sex-dose interaction was reviewed for significance. A significant finding was an indication that the parameter should be re-examined separately for each sex. After accounting for the influence of sex on response to treatment, the time-dose interaction was examined. If the time-dose interaction was statistically identified a linear constant was used to compare the control group to the dosed groups. If a sex-dose interaction was identified for a particular dose vs control comparison, this led to separation by sex and re-analysis. Terminal body weight, organ weight (absolute and

relative, excluding ovaries and testes) and urine specific gravity were evaluated using a 2-way ANOVA with the factors of sex and dose; differences between the groups were primarily detected by dose factor. For these parameters the first evaluation was whether the sex-dose interaction was significant; if it was, a 1-way ANOVA was performed separately for each sex. Comparisons of individual dose groups to the control group were made with ANOVA's only when a statistical difference in dose effect existed; this was subsequent to the evaluation of the sex-dose interaction. The form of the ANOVA, 1-way or 2-way, was determined by whether or not the analysis has been separated by sex or not. Results of ovaries and testes weight (absolute and relative) were analysed using a 1-way ANOVA. If significant dose effects were determined in the 1-way ANOVA then separate doses were compared to controls using separate 1-way ANOVA's. A Bonferroni's correction was used to compensate for the multiple comparisons with the control group. This was applied only when comparisons were made to the control group and were applied for the dose factor in 1-way and 2-way ANOVA's and the time-dose interaction in 3-way RM-ANOVA's. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) could be much greater than the alpha levels might suggest. The final toxicological interpretation of the data considered other factors such as dose-response relationships and whether the findings are plausible in light of other biological and toxicological findings. Descriptive statistics only (means and standard deviations) were reported for food consumption. Means only were reported for urine specific gravity. Means and standard deviation tables were generated for all other variables.

C. METHODS:

1. Observations: Animals were evaluated for morbidity, mortality, physical appearance and behaviour twice daily throughout the study. Additionally, a more thorough examination was performed once weekly at weighing.

2. Body weight: Individual body weights were recorded weekly beginning at day -14. Fasted body weights were recorded at the scheduled necropsy.

3. Food consumption and compound intake: Individual food consumption was recorded weekly beginning at day -14. From these data, food consumption (kg/animal/d) was calculated. Food efficiency was not calculated. Compound intake (mg/kg bw/d) values were also calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination: The eyes were examined by indirect and slit-lamp ophthalmoscopy pre-dosing and prior to the 13-week necropsy (examined on study day 88). A 1% tropicamide ophthalmic solution was instilled into each eye to aid visualization of the fundus.

5. Haematology & Clinical Chemistry: Blood samples were collected from the jugular vein of fasted dogs twice pre-test (approximately 1 week apart), once mid-study and prior to the scheduled necropsy for the evaluation of haematological and clinical chemistry parameters and the preparation of new methylene blue-stained blood smears. May-Grunwald Giemsa-stained bone (rib) marrow smears were prepared at the scheduled necropsy. The haematological and clinical chemistry parameters marked with an (X) in tables (a) and (b), respectively, were examined.

a. Haematology

X	Haematocrit (HCT)*	X	Leukocyte differential count*
X	Haemoglobin (HGB)*	X	Mean corpuscular haemoglobin (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular haemoglobin concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count

	Blood clotting measurements*	X	Red cell distribution (RDW)
	(Thromboplastin time)	X	Mean platelet volume (MPV)
	(Clotting time)	X	Cytologic morphology (RBC, WBC and platelets)
	(Prothrombin time)		

* Recommended for subchronic non-rodent studies based on Guideline 870.3150.

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 (TBIL)
X	Alkaline phosphatase (AP)	X	Total serum protein (PROT)*
	Cholinesterase (ChE)	X	Triglycerides (TRIG)
	Creatine phosphokinase (CPK)		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumen / globulin ration (A/G)
X	Serum alanine amino-transferase (ALAT) (also SGPT)*		
X	Serum aspartate amino-transferase (ASAT) (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GDH)		

* Recommended for subchronic non-rodent studies based on Guideline 870.3150.

6. Urinalysis * Urine samples were collected from the urinary bladder using a syringe and needle during the scheduled necropsy. The animals were fasted prior to the scheduled necropsy. The urinalysis parameters marked *with an (X) in the following table were examined.*

X	Appearance *	X	Glucose (GLUC) *
	Volume *	X	Ketones (KETO)
X	Specific gravity (SpGr) / Osmolality *	X	Bilirubin (BILI)
X	pH *	X	Blood / Blood cells *
X	Sediment (microscopic)		Nitrate
X	Protein (PROT) *	X	Urobilinogen (UROBIL)
X	Turbidity (TURB)		

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

7. Sacrifice and Pathology All animals were necropsied. Animals were fasted prior to the scheduled necropsy, sedated with acepromazine intramuscularly or subcutaneously (approximately 2.5-5.0 mg/dog), anaesthetised with sodium pentobarbital intravenously to effect (usually 35-70 mg/kg) and exsanguinated. The organs/tissues (with the exception of the eyes, testes and epididymides), in whole or in part, marked with an (X) in the following table were fixed in 10% buffered formalin. Organs/tissues marked with an (XX) in the following table were weighed prior to fixation. The eyes were collected and fixed in Davidson's fixative. The testes and epididymides were collected and fixed in Bouin's fixative. Representative samples of the fixed, collected tissues from all dogs were processed by standard procedures and examined microscopically. Special stains were not done.

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

X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland^{T}
X	Colon*	XX	Kidneys*+	X	Mammary gland*
X	Rectum*	X	Urinary bladder*	XX	Parathyroid*+
XX	Liver*+	XX	Testes*+	XX	Thyroid*+
X	Gall bladder*+		Epididymides*+		**OTHER**
X	Pancreas*	X	Prostate*	X	Bone
	RESPIRATORY	XX	Ovaries*+	X	Skeletal muscle
X	Trachea*	XX	Uterus / cervix*+	X	Skin
X	Lung*	X	Vagina	X	All gross lesions and masses*
X	Nose*			X	Tonsils
X	Pharynx*				
X	Larynx*				

* Recommended for subchronic non-rodent studies based on Guideline 870.3150
+ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ
X Organ fixed.
XX Organ weighed prior to fixation.

II. RESULTS

A. Observations :

1. Clinical signs of toxicity - There were no significant treatment-related clinical observations. Diarrhea and vomiting occurred occasionally in the treated animals but the incidence was not dose-related. Diarrhea occurred sporadically in one male at 100 mg/kg bw/d and once each in two females at 5 mg/kg bw/d. Vomiting occurred once each in two males at 100 mg/kg bw/d and once in a female at 5 mg/kg bw/d.

2. Mortality - There were no mortalities.

B. Body weight and weight gain: There were no significant treatment-related effects on body weight or body-weight gain in either sex. However, there was a non-dose related decrease in the overall body-weight gain (weeks 0 to 13) in the treated males compared to controls (Table 2). One male at 5 mg/kg bw/d (-0.006 kg), two control females (-0.115 and -0.283 kg), one female at 50 mg/kg bw/d (-0.265 kg) and one female at 100 mg/kg bw/d (-0.296 kg) exhibited an overall loss in body weight. None of these animals exhibited any incidence of vomiting or diarrhea and food consumption in these animals was comparable to controls throughout the study.

Table 2. Average body weights and body-weight gains during 13 weeks of treatment (a)

Dose Level (mg/kg bw/d)	Body Weights (kg ± SD)				Total Weight Gain (Weeks 0 to 13)	
	Week 0	Week 1	Week 7	Week 13	kg ± SD	% of control
Male (4 animals/group)						
0	11.18 ± 0.83	11.28 ± 0.84	11.46 ± 1.14	11.85 ± 1.21	0.67 ± 0.41	100
5	11.15 ± 0.90	11.07 ± 0.90	11.30 ± 0.84	11.55 ± 0.45	0.40 ± 0.48	60

Dose Level (mg/kg bw/d)	Body Weights (kg ± SD)				Total Weight Gain (Weeks 0 to 13)	
	Week 0	Week 1	Week 7	Week 13	kg ± SD	% of control
50	11.19 ± 0.62	11.26 ± 0.77	11.44 ± 0.78	11.63 ± 0.84	0.44 ± 0.26	66
100	11.51 ± 1.07	11.33 ± 1.19	11.46 ± 1.29	11.99 ± 1.52	0.47 ± 0.46	70
Female (4 animals/group)						
0	10.07 ± 1.83	10.09 ± 1.97	10.12 ± 2.17	10.23 ± 2.16	0.16 ± 0.42	100
5	9.06 ± 0.88	8.99 ± 0.99	9.27 ± 0.81	9.46 ± 1.13	0.40 ± 0.25	250
50	9.46 ± 0.54	9.47 ± 0.54	9.62 ± 0.52	9.72 ± 0.58	0.14 ± 0.46	88
100	9.53 ± 0.64	9.40 ± 0.61	9.43 ± 0.78	9.68 ± 0.88	0.15 ± 0.33	94

(a) Data obtained from pages 32-33 in the study report for body weight and pages 133-144 for overall body-weight gain (weeks 0-13).

C. Food consumption and compound intake:

1. Food consumption - There were no treatment-related effects on food consumption in either sex. Food consumption was comparable between the controls and the treated animals at all dose levels in both sexes.

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

3. Food efficiency - Food efficiency data was not provided in the study report. However, there was no treatment-related effect on body weight, body-weight gain or food consumption which suggests that food efficiency was not affected by treatment.

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

E. Blood analyses

1. Haematology - There were no treatment-related haematological findings.

2. Clinical Chemistry - *Clinical chemistry investigations revealed a significant treatment-related increase in alkaline phosphatase (AP) activity in both sexes at 50 and 100 mg/kg bw/d (Table 3). The origin of this increase was not determined, however, increased liver weights in both sexes at 100 mg/kg bw/d and a slight increased incidence/severity in hepatic vacuolation in both sexes at 50 and 100 mg/kg bw/d may suggest a hepatic origin.*

Table 3. Clinical chemistry findings. (a)

	Dose Level (mg/kg bw/d)	Males (n = 4 animals/dose)	Females (n = 4 animals/dose)
		Alkaline Phosphatase (U/L)	Alkaline Phosphatase (U/L)
Pre-study - Day -7	0	62 ± 5.0	77 ± 27

	Dose Level (mg/kg bw/d)	Males (n = 4 animals/dose)	Females (n = 4 animals/dose)
		Alkaline Phosphatase (U/L)	Alkaline Phosphatase (U/L)
	5	73 ± 17	68 ± 29
	50	45 ± 18	49 ± 23
	100	64 ± 17	47 ± 32
Day 45	0	56 ± 16	67 ± 25
	5	72 ± 27	62 ± 12
	50	**89 ± 29 ***	**128 ± 79 ***
	100	**241 ± 49 ***	**210 ± 76 ***
Day 91	0	43 ± 6	62 ± 20
	5	56 ± 22	54 ± 13
	50	**91 ± 47 ***	**141 ± 73 ***
	100	**237 ± 79 ***	**212 ± 120 ***

(a) Data obtained from pages 143-146 in the study report for males and pages 147-150 for females.
* Statistically significant compared to control, $p \leq 0.05$.

F. Urinalysis - There were no treatment-related effects in urine parameters in either sex.

G. Sacrifice and Pathology:

1. Organ weight - Absolute and relative liver weights were significantly increased in males and females at 100 mg/kg bw/d (Table 4). The increased liver weights correlate with increased AP activity and a slight increased incidence/severity of hepatic vacuolation in both sexes at 100 mg/kg bw/d. Absolute and relative testes weights were also significantly increased in the high dose males, however, there was no clear dose-response relationship, a similar finding was not observed in the 1-year dietary study with dogs (see DACO 4.3.2- Stebbins, K.E. and Haut, K.T., October 30, 1997. Laboratory Project ID: 960018) and there were no correlating gross pathological or histopathological findings to indicate any biological or toxicological significance.

Table 4. Absolute (g ± SD) and relative (g/100 g bw ± SD) organ weights. (a)

Dose Level (mg/kg bw/d)		0	5	50	100
Males (n = 4 animals/dose)					
Liver	Absolute	298.8 ± 48.9	298.0 ± 20.5	321.0 ± 25.5	**384.5 ± 32.3 ***
	Relative	2.64 ± 0.30	2.71 ± 0.30	2.84 ± 0.23	**3.32 ± 0.23 ***
Testes	Absolute	13.37 ± 1.84	15.34 ± 0.54	12.76 ± 3.51	**18.44 ± 1.81 ***
	Relative	0.119 ± 0.020	0.139 ± 0.009	0.112 ± 0.026	**0.159 ± 0.007 ***
Females (n = 4 animals/dose)					
Liver	Absolute	221.2 ± 14.6	238.0 ± 30.0	239.0 ± 14.2	**269.5 ± 27.8 ***
	Relative	2.30 ± 0.37	2.62 ± 0.20	2.57 ± 0.11	**2.91 ± 0.22 ***

(a) Data obtained from pages 58-65 in the study report for group means and pages 81-88 for individual organ weights.
* Statistically significant compared to control, $p \leq 0.05$.

2. Gross pathology - There were no treatment-related gross pathological findings.

3. Microscopic pathology - Histopathological examination revealed hypertrophy of the epithelial cells of the

collecting ducts of the kidney in 4/4 males and 3/4 females at 100 mg/kg bw/d and in 2/4 males and 1/4 females at 50 mg/kg bw/d (Table 5). Hypertrophy was characterized by enlarged epithelial cells, singly or in short rows scattered along the basement membrane of the collecting ducts, principally within the outer zone of the medulla. The cells had a granular, pale, eosinophilic cytoplasm with numerous mitochondria. The hypertrophied epithelial cells were compatible with the intercalated cells which normally function in 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, urinary acidification was not present and urinary specific gravity was unaffected in these animals. Kidney weights were also unaffected in these animals. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with the histopathological findings in the kidney or to indicate an impairment of renal function and there was no cellular degeneration or necrosis evident in the kidneys. Morphologically, the lesions were essentially the same as those reported in Fischer 344 rats following 13-weeks of treatment (see DACO 4.3.1 - Laboratory Project Study ID - DR-0312-6565-011) where urinalysis findings indicative of an acidification defect and impaired concentrating ability were observed (urinary acidification and reduced urinary specific gravity, respectively). Similar histopathological findings (no urinalysis performed) were also observed in B6C3F1 mice following 13 weeks of treatment (see DACO 4.3.1 - Laboratory Project Study ID - DR-0312-6565-010). One male at 5 mg/kg bw/d also exhibited slight hypertrophy of the epithelial cells of the collecting ducts. However, this was attributed to the congenital absence of one kidney in this animal, which was considered to make this animal more susceptible to the renal effects of the test substance compared to other animals at this dose level with anatomically normal kidneys and no evidence of treatment-related changes in the kidney. In addition, hypertrophy of the epithelial cells of the collecting duct was not observed in either sex at 5 mg/kg bw/d in the 1-year dietary study with dogs.

In both sexes at 50 and 100 mg/kg bw/d, there may be a slight increased incidence/severity of hepatic vacuolation compared to controls. This correlated with increased liver weights in both sexes at 100 mg/kg bw/d and increased alkaline phosphatase activity in both sexes at 50 and 100 mg/kg bw/d.

TABLE 5. Histopathological findings (expressed as # of animals affected / # of animals examined). (a)

Dose Level (mg/kg bw/d)			0	5	50	100
Males						
Kidney	- hypertrophy, collecting ducts	- very slight	0/4	1/4	2/4	3/4
		- slight	0/4	0/4	0/4	1/4
		- total	0/4	1/4	2/4	4/4
Liver	- vacuolation, glycogen / hydropic changes	- very slight	2/4	1/4	1/4	1/4
		- slight	1/4	0/4	1/4	3/4
		- moderate	0/4	0/4	1/4	0/4
		- total	3/4	1/4	3/4	4/4
Females						
Kidney	- hypertrophy, collecting ducts	- very slight	0/4	0/4	1/4	3/4
		- slight	0/4	0/4	0/4	0/4
		- total	0/4	0/4	1/4	3/4
Liver	- vacuolation, glycogen / hydropic changes	- very slight	0/4	1/4	1/4	2/4
		- slight	0/4	0/4	2/4	1/4
		- moderate	1/4	0/4	0/4	0/4
		- total	1/4	1/4	3/4	3/4

(a) Data obtained from pages 67-70 in the study report for group means and pages 92-123 for individual histopathological observations.

III. DISCUSSION

A. Investigators' conclusions (extracted from page 10 of the study report): "Calculated daily consumption of XDE-570 closely approximated the targeted doses of 5, 50, or 100 mg/kg/day. Treatment-related effects included:

- Increased alkaline phosphatase activity (ALP), likely of hepatic origin, in males and females given ≥50 mg/kg/day;
- Increased liver weights in males and females given 100 mg/kg/day with no histopathologic correlates; and
- Slight hypertrophy of individual epithelial cells (consistent with intercalated cells) lining renal collecting ducts in the majority of Beagles given 50 or 100 mg/kg/day. Only one Beagle given 5 mg/kg/day (no. 24) had slight hypertrophy of individual epithelial cells lining renal collecting ducts. However, this dog had congenital absence of one kidney, which likely made it more uniquely more susceptible to the renal effects of XDE-570 as compared to other dogs given 5 mg/kg/day that had anatomically normal kidneys with no evidence of treatment-related effects. No evidence of treatment-related kidney changes were seen in any other dog given 5 mg/kg/day.

Conclusions: Based on the results of this study, the no-observed-effect-level (NOEL) in anatomically normal dogs was 5 mg/kg/day."

B. Reviewer comments: There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, ophthalmoscopy, haematology or gross pathology. Clinical chemistry investigations revealed increased alkaline phosphatase (AP) activity in both sexes at 50 and 100 mg/kg bw/d. Increased liver weights were observed in both sexes at 100 mg/kg bw/d. The increased liver weights in both sexes at 100 mg/kg bw/d correlated with the increased AP activity and with a slight increased incidence/severity of hepatic vacuolation. A slight increased incidence/severity of hepatic vacuolation was also observed in both sexes at 50 mg/kg bw/d. In the kidney, hypertrophy of the epithelial cells of the collecting ducts was observed in 4/4 males and 3/4 females at 100 mg/kg bw/d and in 2/4 males and 1/4 females at 50 mg/kg bw/d. The hypertrophied epithelial cells were compatible with the intercalated cells which normally function 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, urinary acidification was not present and urinary specific gravity was unaffected in these animals. Kidney weights were also unaffected in these animals. There were no toxicologically relevant clinical chemistry findings (serum creatinine, nitrogen or electrolyte levels) to correlate with the histopathological findings in the kidney or to indicate an impairment of renal function and there was no cellular degeneration or necrosis evident in the kidneys.

The LOAEL is 50 mg/kg bw/d based on increased alkaline phosphatase activity, slight increased incidence/severity of hepatic vacuolation and hypertrophy of the epithelial cells of the collecting ducts in both sexes. The NOAEL is 5 mg/kg bw/d.

C. Study deficiencies: Urine volume was not provided, however, according to OECD Guideline 409, urinalysis is not required on a routine basis but only when there is an indication based on expected or observed toxicity. Urinalysis is required according to EPA Guideline OPPTS 870.3150 (urinalysis should be performed prior to treatment, midway through treatment and at the end of treatment using timed urine collection). There were treatment-related histopathological findings in the kidneys, however, there were no significant treatment-related changes in serum urea nitrogen or creatinine, urinary parameters (specifically specific gravity and pH) or kidney weights to suggest any functional impairment in the kidneys and there was no cellular degeneration or necrosis evident in the kidneys. Based on these findings, this deficiency should not impact on the outcome of this study; therefore, this study is considered acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3150; OECD 409) in dogs.