DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.4100b [§83-1b]; Chronic Toxicity in Dogs

Work Assignment No. 4-1-128 G (MRID 46808229)

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
Michael E. Viana, Ph.D., D.A.B.T.

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

Secondary Reviewer
Stephanie E. Foster, M.S.

Signature: Stephanie E. Foster
Date: 12/20/06

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:** KJ
Registration Action Branch 2, Health Effects Division (7509P) Date: 5/31/07
Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** MO
Registration Action Branch 3, Health Effects Division (7509P) Date: 5/31/07
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity - dog [feeding]; OPPTS 870.4100b [' 83-1b]; OECD 452.

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: Stebbins, K. E., and K. T. Haut (1997) XDE-570: one year dietary toxicity study in beagle. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: 960018, October 30, 1997. MRID 46808229. Unpublished.

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

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 46808229), XDE-570 (Florasulam; 99.3% a.i.; Lot No. 940714) was administered in the diet to four purebred beagle dogs/sex/dose at dose levels of 0, 0.5, 5, or 100 mg/kg/day for 52 weeks. Severe body weight loss and reduced food consumption were observed in both sexes at 100 mg/kg/day during the first three months of the study; therefore, the high dose was reduced to 50 mg/kg/day in both sexes beginning on Study Day 105 (Week 15).

No adverse treatment-related effects were observed on mortality, clinical signs, food efficiency, ophthalmoscopic examinations, hematology, urinalysis, organ weights, or gross or microscopic pathology.

At 100 mg/kg/day, both sexes exhibited loss of body weight accompanied by reduced food consumption. Following reduction of the high dose to 50 mg/kg/day, the females continued to exhibit both decreased (not significant [NS]) body weights (decr. 17% at Week 52) and food consumption, resulting in decreased (NS) overall (Week 0-52) body weight gains (decr. 68%). Male body weights and food consumption at Week 52, and overall body weight gains were similar to controls.

Additionally at 100 mg/kg/day, males and females had increased ($p<=0.05$) alkaline phosphatase (incr. 233-783%) and alanine aminotransferase (incr. 268-390%) after 3 months of dosing. Alkaline phosphatase continued to be elevated ($p<=0.05$) in both sexes through 12 months of

dosing (incr. 141-354%). No corroborating findings were observed in organ weights, or gross or microscopic pathology. Slight vacuolation of the zona reticularis and zona fasciculata was also observed in the adrenal gland of both sexes; the findings were consistent with fatty change.

The LOAEL is 100/50 mg/kg/day, based on decreased body weights (17%), body weight gains (68%), and food consumption in the females; increased liver enzymes (alanine aminotransferase and alkaline phosphatase), and slight vacuolation of the zona reticularis and zona fasciculata in the adrenal gland (consistent with fatty change) of both sexes. The NOAEL is 5 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4100b, OECD 452) for a chronic toxicity study in the dog.

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 chronic dog study. 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.

46808229.PMRA.der
.pdf

Reviewer: Tom Morris , **Date** March 10, 2000.

STUDY TYPE: Oral 1-year dog study [feeding]; OPPTS 870.4100; OECD 452.

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

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

CITATION: Stebbins, K. E. and Haut, K. T. October 30, 1997. **XDE-570: One Year Dietary Toxicity Study in Beagle**. Performing Laboratory: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: 960018. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a 1-year feeding study, XDE-570 (Purity - 99.3%) was administered to 4 purebred beagle dogs/sex/dose *ad libitum* in the diet at doses of 0, 0.5, 5.0 or 100/50 mg/kg bw/d (time-weighted average test substance intake was 0, 0.5, 4.9 or 71.4 mg/kg bw/d for males and 0, 0.5, 5.1 or 71.1 mg/kg bw/d for females) for 52 weeks. The animals were sacrificed and necropsied on study days 371 (males) and 372 (females). Due to severe body-weight loss and reduced food consumption in both sexes at 100 mg/kg bw/d during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.

There were no treatment-related effects on mortality, clinical signs, ophthalmoscopy, urinalysis, organ weights or gross pathology. At 100 mg/kg bw/d, both sexes exhibited a body weight loss during the first 15 weeks of the study, this correlated with a concomitant reduction in food consumption in both sexes. After the high-dose was reduced to 50 mg/kg bw/d (at ≈wk 15), body weight and body-weight gain were comparable to controls in males and continued to be lower throughout the study in females. At 100 mg/kg bw/d (3 months), clinical chemistry findings included increased alanine aminotransferase (ALAT) and alkaline phosphatase (AP) activity and decreased serum albumin levels in both sexes. Although not statistically identified, serum protein levels were also decreased in both sexes at 100 mg/kg bw/d, reflecting the decreased serum albumin levels. After the high-dose was reduced to 50 mg/kg bw/d, AP values diminished over time but remained significantly elevated throughout the remainder of the study in both sexes. Serum albumin and protein levels also remained lower in the high-dose males and females. There were no organ weight changes, gross pathological or histopathological findings to correlate with the increased AP activity or decreased serum albumin levels. Hypertrophy of the epithelial cells of the collecting duct was observed in males at all dose levels and in the control and high-dose females. However, the severity of the hypertrophy appeared to be increased in the high-dose males and females compared to the controls. The hypertrophied 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 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. There also appeared to be no progression in the severity of the alterations at comparable dose levels from 13 weeks to 1 year of dietary administration of the test substance. Slight vacuolization of the zona reticularis and zona fasciculata in the adrenal glands was observed in the high-dose males and females. The vacuolization was consistent with fatty changes. In the absence of any associated inflammation, necrosis or other changes, the toxicological significance of this finding was uncertain.

The LOAEL was 50 mg/kg bw/d based on lower body weight, body-weight gain and food consumption (♀), increased AP activity (♂/♀) and decreased serum albumin and protein levels (♂/♀) at 50 mg/kg bw/d and increased severity of hypertrophy of epithelial cells of the collecting ducts and slight vacuolization of the zona reticularis and zona fasciculata in the adrenal glands (♂/♀) at 100/50 mg/kg bw/d. The NOAEL was 5 mg/kg bw/d.

This study in dogs is acceptable / guideline and satisfies the guideline requirement for a 1-year oral toxicity study in dogs (OPPTS 870.4100; OECD 452).

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

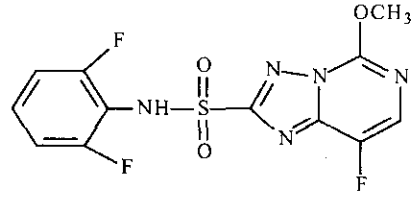
Lot/Batch #: Lot # 940714 / Test Substance # 100511

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

Compound Stability: The test substance was re-assayed after study determination and was confirmed at 99.3% (Knowles, et al., 1997, Lab Report Code GHE-P-6448)

CAS #: 145701-23-1

Structure



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

3. **Test animals:**

Species:	Male and female dogs.
Strain:	Beagle
Age/weight at study initiation:	At study initiation, the dogs were ≈8-9 months of age with a body weight range of 12-13 kg for males and 10-11 kg for females.
Source:	Marshall Research Laboratory, North Rose, NY.
Housing:	The animals were individually housed.
Diet:	Purina Purified Canine Chow #5007 (Purina Mills Inc., St. Louis, MO) *ad libitum*
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 18-26 °C Humidity: 26-78% Air changes: Not provided Photoperiod: 12 hour light / 12 hour dark
Acclimation period:	At least 14 days.

B. STUDY DESIGN:

1. **In life dates** - Start: May 28, 1996. End: male and females sacrificed on June 2/3, 1996, respectively (study days 371 and 372).

2. **Animal assignment** - 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 diet for 52 weeks. The control group animals received untreated diet throughout the study. The animals were sacrificed and necropsied on study days 371 (males) and 372 (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	0.5	0.5	0.5	4	4
3	5	4.9	5.1	4	4
4	100/50 *	71.4	71.1	4	4

* Due to significant body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.

3. Dose selection rationale: In a 4-week dietary study, 2 dogs (Beagle)/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. July 6, 1995. Laboratory Project Study ID: DR-0312-6565-018, study submitted but a full review was not completed). Food consumption was significantly lower in both sexes at 450 mg/kg bw/d with a subsequent decreased body weight. Significant treatment-related effects were confined to the liver and included biliary hyperplasia, bile stasis and hepatocellular necrosis in one male at 450 mg/kg bw/d, increased alkaline phosphatase (AP) activity (≥50 mg/kg bw/d, both sexes), increased alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activity (≥150 mg/kg bw/d, both sexes), hyperplasia of bile ducts (≥150 mg/kg bw/d, both sexes) and increased liver weights (males at 150 mg/kg bw/d). In a 13-week dietary study, 4 dogs (Beagle)/sex/dose were administered XDE-570 *ad libitum* in the diet at doses of 0, 5, 50 or 100 mg/kg bw/d (DACO 4.3.8 - Stebbins, K.E. September 13, 1995. Laboratory Project Study ID: DR-0312-6565-021). Treatment-related effects included increased AP activity (≥50 mg/kg bw/d, both sexes), increased liver weights (100 mg/kg bw/d, both sexes), possible slight increased incidence/severity of hepatic vacuolation (≥50 mg/kg bw/d, both sexes) and slight hypertrophy of the epithelial cells of the collecting ducts (≥50 mg/kg bw/d, both sexes). The LOAEL was 50 mg/kg bw/d. The NOAEL was 10 mg/kg bw/d. Based on these findings, the high-dose (100 mg/kg bw/d) was expected to produce clear evidence of toxicological effects. However, due to body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and lower feed consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare. The remaining dose levels were expected to provide dose-response data for any treatment-related effects observed in the high-dose group and to ensure definition of a no-observed-effect-level (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 4 weeks. Diets were prepared weekly during the first 13 weeks of the study and at least once every 4 weeks for the remainder of the dosing period. Initial concentrations of the test substance in the diet were calculated from pre-study body weights and food consumption data. Subsequently, the concentrations of test substance in the diets were adjusted weekly for the first 13 weeks and monthly thereafter based on the most recent body weight and food consumption data. Stability of the test substance in the feed was established concurrent with the start of the study. Homogeneity testing of test substance in the feed was initiated prior to the start of the study and validated analytically. 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**: Three homogeneity analyses were performed during the study and the test substance was shown to be homogeneous in the diet at all three time points.

Female - 0.5 mg/kg bw/d			
Date Mixed	6/17/96	8/26/96	9/9/96
Concentration Range (%w/w)	0.00164 - 0.00194	0.00183 - 0.00211	0.00176 - 0.00193
Mean Concentration (%w/w)	0.00176	0.00201	0.00184
Standard Deviation	0.00009	0.00010	0.00005
%RSD	5.11	4.98	2.72

Stability Analysis: The test substance was found to be stable in the diet for at least 44 days.

Female - 0.5 mg/kg bw/d		
Days Elapsed	Observed Amount (% w/w)	% of Initial Day
0	0.00136	-
10	0.00144	106
16	0.00123	90
22	0.00119	88
44	0.00134	99

Concentration Analysis: The mean concentrations of the test substance in the diet were shown to be 98 to 101% 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
0.5	99 - 111	97 -110	98 ± 8	101 ± 6
5	94 - 106	94 - 101	100 ± 4	100 ± 3
100/50	99 - 104	97 - 103	101 ± 2	100 ± 2
Premix	99 - 102		101 ± 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 - All parameters examined statistically were first tested for equality of variance using the Bartlett's test. If results of the Bartlett's test were significant, then the data for the parameter were subjected to transformation to obtain equality of the variances. The transformations examined included the common log, the inverse and the square root, in that order. When Bartlett's test was satisfied, that form of the data was used. If none of the transformations or the raw data resulted in homogeneous variances, the data was reviewed and an appropriate form of the data was selected. The selected form of the data was then subjected to the appropriate parametric analysis. In-life body weight, haematological parameters (excluding differential WBC, nucleated RBC and RBC indices) and clinical chemistry parameters were evaluated using a three-way, repeat measure (RM) analysis of variance (ANOVA) for time (repeated factor), sex and dose. In the three-way RM-ANOVA, the differences between the groups were detected primarily by the time-dose interaction. 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. The time-dose interaction was examined next. If the time-dose interaction was statistically identified, linear contrasts tested the time-dose interaction for the comparisons of each dose group and the control group. A Bonferroni correction was applied to control the experiment-wise error rate by compensating for the

multiple comparisons with the control group. Terminal body weight, organ weight (absolute and relative, excluding ovaries and testes) and urine specific gravity were evaluated using the two-way ANOVA with the factors of sex and dose; differences between the groups were primarily detected by the dose factor. For these parameters the first examination was whether the sex-dose interaction was significant; if it was, a one-way ANOVA was dose separately for each sex. Comparisons of individual dose groups to the control were made with the Dunnett's test only when a statistically significant dose effect existed. Dunnett's test corrected for multiple comparisons to the control and reported the experiment-wise error rate. Results of the ovaries and testes weight (absolute and relative) were analysed using a one-way ANOVA. If significant dose effects were determined then separate doses were compared to controls using the Dunnett's test. Food consumption data 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 ANOVA and if significant was followed, respectively, by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons to control. Statistical outliers were identified by a sequential test, but were routinely excluded from food consumption statistics only. Descriptive statistics only (means and standard deviations) were reported for food consumption, food efficiency, white blood cell differential counts and red blood cell indices. Because numerous measurements were compared statistically on the same group of animals, the frequency of false positive (Type I) error was unknown, but was considered to be much greater than the nominal alpha. The final toxicological interpretation of the data considered other factors such as dose-response relationships, biological plausibility and consistency and historical control data.

C. METHODS:

1. Observations: A thorough clinical examination was conducted on all animals prior to the start of the study and at weekly intervals throughout the study period. A cage-side examination was made each day of the work week, except on the days that a clinical examination was performed. 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 during the first 29 weeks of the study and at approximately monthly intervals thereafter.

3. Food consumption and compound intake Food consumption data were collected from all animals weekly during for the first 31 weeks and approximately monthly 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/kg bw gain/d) and compound intake (mg/kg bw/d) values were also calculated as time-weighted averages from the food consumption and body weight gain data.

4. *Ophthalmoscopic examination* The eyes of all animals were examined by indirect ophthalmoscopy pre-dosing, pre-terminal (study day 368), and at necropsy. One drop of 0.5% tropicamide ophthalmic solution was instilled into each eye to produce mydriasis prior to examination.

5. Haematology and Clinical Chemistry: Following an overnight fast blood samples were collected from all animals pre-study, and at 3, 6 and 12 (≈1 week prior to necropsy) months post-treatment via venipuncture of the jugular vein. An additional evaluation of haematological and clinical chemistry parameters was conducted on both sexes at the high-dose following 4-months of dosing to monitor the progression of alterations (in RBC, liver enzymes and albumin concentrations) that were noted in some high-dose animals at 3-months. 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 allowed to clot in serum tubes and the serum was collected following centrifugation. 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)*	XX	Mean corpuscular Haemoglobin (MCH)
X	Leukocyte count (WBC)*	XX	Mean corpuscular Haemoglobin Concentration (MCHC)

X	Erythrocyte count (RBC)*	XX	Mean corpuscular volume (MCV)
X	Platelet count (PLT)*		Reticulocyte count (RETIC)
	Blood clotting measurements*	X	Erythrocyte Morphology
	(Partial Thromboplastin time)	X	Leukocyte Morphology
	(Thrombin Clotting time)	X	Platelet Morphology
	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100.
X Examined
XX Examined (MCH, MCHC and MCV) at 3 months only on all animals and at 4 months only on all high-dose males and females to further characterize the treatment-related alterations in RBC parameters (RBC, HCT and HGB) which were observed in the high-dose animals at 3 months.

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 (PROTEIN)*
	Cholinesterase (ChE)	X	Triglycerides (TRIG)
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (ALAT) (also SGPT)*		
X	Serum aspartate amino-transferase(ASAT) (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GDH)		

* Recommended for chronic studies based on Guideline 870.4100.
X Examined

6. Urinalysis Urine samples were collected from fasted animals at 6 (via catheterization) and 12 months (urinary bladder aspiration during the scheduled necropsy). The urinalysis parameters marked with an (X) in the following table were examined.

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

* Recommended for chronic studies based on Guideline 870.4100.
X examined

7. Sacrifice and Pathology The animals were anaesthetized (subcutaneous injection of acepromazine at 0.1-0.3 mg/kg bw), sacrificed by intravenous overdose of sodium pentobarbital and exsanguinated. A complete necropsy was conducted on all animals. 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. Bone marrow smears were prepared from the proximal femur. All preserved tissues/organs, (with exception of the joint) were processed by conventional techniques from all animals. Tissues/organs were sectioned at approximately 6 µm, stained with hematoxylin and eosin and examined using a light microscope. A complete histopathological

examination was conducted on all animals.

	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*	XX	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Mammary gland*
X	Rectum*	X	Urinary bladder*	XX	Parathyroids*
XX	Liver*+	XX	Testes*+	XX	Thyroids*
X	Gall bladder*	X	Epididymides*+		OTHER
X	Pancreas*	X	Prostate*	X	Bone
	RESPIRATORY	XX	Ovaries*+	X	Skeletal muscle
X	Trachea*	X	Uterus*+	X	Skin*
X	Lung*++	X	Cervix	X	All gross lesions and masses*
X	Nose*	X	Oviducts		
X	Pharynx*	X	Vagina		
X	Larynx*				

* Required for chronic studies based on Guideline 870.4100.
+Organ weight required in chronic 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 significant treatment-related clinical signs. Thin appearance was noted in one male (from study days 91-350) and one female (from study days 105 to 119) at 100 mg/kg bw/d, beginning at approximately 3 months. This correlated with a treatment-related decreased body weight in these animals.

2. Mortality - There were no mortalities.

B. Body weight and weight gain: Males and females at 100 mg/kg bw/d exhibited a mean body-weight loss during the first 15 weeks of the study due to a body-weight loss in 2 males and 3 females at this dose level. By week 15, the mean body weight in the high-dose males and females was approximately 10 and 15% lower, respectively, compared to controls. Due to the body-weight loss in 2 high-dose males (-1.205 kg and -0.858 kg) and 3 high-dose females (-0.790 kg, -0.577 kg and -2.005 kg) by week 15, the high-dose level was reduced to 50 mg/kg bw/d. After the high-dose was reduced to 50 mg/kg bw/d, body-weight gain in the high-dose males was higher compared to controls (≈66 and 113% higher during wks 13-26 and 26-52, respectively). By week 52 body weight in the high-dose males was comparable to controls. The overall (wks 0-52) body-weight gain in the high-dose males was comparable to controls. After the high-dose was reduced to 50 mg/kg bw/d, the high-dose females exhibited a gain in body weight, however, body weight (≈27 and 17% lower at wks 26 and 52, respectively) and body-weight gain (≈57 and 16% lower during wks 13-26 and 26-52, respectively) continued to be lower compared to controls. By week 52 body weight was approximately 17% lower in the high-dose females compared to controls. The overall (wks 0-52) body-weight gain in the high-dose females was approximately 68% lower compared to controls. The body weight loss in the high-dose males and females during the first 15 weeks of treatment correlated with decreased food consumption in males during most of the 15 weeks and in females throughout the 15 weeks. Females at 0.5 mg/kg bw/d also exhibited lower body weights throughout the study (≈16% at wk 52) and lower overall body-weight gain (≈65%), however, this was not dose-related. Body weight and body-weight gain are summarized in Table 2 (males) and Table 3 (females).

TABLE 2: Mean body weights and body-weight gains - males (a)

Dose Level (mg/kg bw/d) (4 animals/group)		0	0.5	5.0	100/50 (b)
Body Weight (kg ± SD)	Week 0	13.2 ± 0.6	13.1 ± 1.4	12.7 ± 0.8	13.0 ± 0.8
	Week 3	13.6 ± 0.6	14.0 ± 1.7	12.9 ± 0.8	13.4 ± 0.8
	Week 6	14.0 ± 0.4	14.0 ± 1.8	13.0 ± 1.1	13.4 ± 0.7
	Week 13	14.5 ± 0.4	14.5 ± 2.7	13.7 ± 1.3	12.9 ± 1.4
	Week 15	14.5 ± 0.6	14.6 ± 2.8	13.9 ± 1.4	13.0 ± 1.7

Dose Level (mg/kg bw/d) (4 animals/group)		0	0.5	5.0	100/50 (b)
	Week 26	15.0 ± 0.4	14.9 ± 2.8	14.7 ± 2.0	13.9 ± 1.9
	Week 52	16.0 ± 1.0	16.1 ± 3.7	15.9 ± 2.4	16.1 ± 2.4
Body-Weight Gain (kg ± SD)	Weeks 0 to 3	0.46 ± 0.49	0.97 ± 0.50	0.10 ± 0.21	0.35 ± 0.18
	Weeks 3 to 6	0.42 ± 0.14	-0.05 ± 0.55	0.20 ± 0.29	-0.04 ± 0.11
	Weeks 6 to 13	0.42 ± 0.45	0.47 ± 0.98	0.66 ± 0.30	-0.51 ± 0.73
	Weeks 13 to 26	0.59 ± 0.23	0.41 ± 0.28	0.99 ± 0.83	0.98 ± 0.59
	Weeks 26 to 52	1.03 ± 0.70	1.23 ± 0.99	1.25 ± 0.92	2.19 ± 1.02
	Weeks 0 to 52 (% control)	2.90 ± 0.46	3.03 ± 2.31 (104)	3.20 ± 1.96 (110)	3.05 ± 1.61 (105)
	Weeks 0 to 15	1.30 ± 0.41	1.51 ± 1.44	1.14 ± 0.82	-0.03 ± 1.17

(a) Data obtained from pages 53-55 in the study report, body-weight gain calculated by reviewer form individual body weight data obtained from pages 184-186 in the study report.
(b) Due to significant body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.

	Animal #	Body Weight Week 0	Body Weight Week 15	Body Weight Loss
Males	2877	12.013 kg	10.808 kg	-1.205 kg (↓ ≈10%)
	2880	13.473 kg	12.615 kg	-0.858 kg (↓ ≈6.4%)
Females	2893	10.505 kg	9.715 kg	-0.790 kg (↓ ≈7.5%)
	2895	11.085 kg	10.508 kg	-0.577 kg (↓ ≈5.2%)
	2896	11.111 kg	9.106 kg	-2.005 kg (↓ ≈18%)

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

Dose Level (mg/kg bw/d) (4 animals/group)		0	0.5	5.0	100/50 (b)
Body Weight	Week 0	10.8 ± 1.4	10.8 ± 1.0	11.3 ± 1.1	10.7 ± 0.5
	Week 3	11.2 ± 1.4	10.8 ± 1.4	11.3 ± 1.1	10.5 ± 0.7
	Week 6	11.5 ± 1.4	11.0 ± 1.4	11.3 ± 1.2	10.8 ± 0.7
	Week 13	11.7 ± 0.7	11.0 ± 1.4	11.5 ± 1.1	10.2 ± 0.4

Dose Level (mg/kg bw/d) (4 animals/group)		0	0.5	5.0	100/50 (b)
	Week 15	11.9 ± 0.5	11.0 ± 1.5	11.7 ± 1.2	10.1 ± 0.9
	Week 26	12.9 ± 1.0	11.5 ± 1.7	12.4 ± 1.0	10.7 ± 0.6
	Week 52	14.1 ± 0.8	11.9 ± 2.0	13.7 ± 0.5	11.7 ± 1.0
Body-Weight Gain	Weeks 0 to 3	0.45 ± 0.76	0.04 ± 0.63	0.04 ± 0.39	-0.19 ± 0.23
	Weeks 3 to 6	0.29 ± 0.19	0.26 ± 0.041	-0.08 ± 0.24	0.32 ± 0.57
	Weeks 6 to 13	0.19 ± 0.079	-0.08 ± 0.23	0.26 ± 0.45	-0.60 ± 0.80
	Weeks 13 to 26	1.14 ± 0.77	0.56 ± 0.32	0.88 ± 0.66	0.48 ± 0.79
	Weeks 26 to 52	1.25 ± 0.27	0.38 ± 0.47	1.29 ± 0.78	1.05 ± 0.46
	Weeks 0 to 52 (% control)	3.31 ± 0.96	1.16 ± 1.23 (35)	2.39 ± 0.62 (72)	1.06 ± 0.85 (32)
	Weeks 0 to 15	1.08 ± 0.96	0.27 ± 0.69	0.35 ± 0.35	-0.58 ± 1.26

(a) Data obtained from pages 62-64 in the study report, body-weight gain calculated by reviewer form individual body weight data obtained from pages 193-195 in the study report.
(b) Due to significant body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.

	Animal #	Body Weight Week 0	Body Weight Week 15	Body Weight Loss
Males	2877	12.013 kg	10.808 kg	-1.205 kg (↓ ≈10%)
	2880	13.473 kg	12.615 kg	-0.858 kg (↓ ≈6.4%)
Females	2893	10.505 kg	9.715 kg	-0.790 kg (↓ ≈7.5%)
	2895	11.085 kg	10.508 kg	-0.577 kg (↓ ≈5.2%)
	2896	11.111 kg	9.106 kg	-2.005 kg (↓ ≈18%)

C. Food consumption and compound intake:

1. Food consumption - In the high-dose males, food consumption was consistently lower than controls during most of the first 4 months of the study, averaging approximately 17% lower than controls (Table 4). Statistical significance was achieved during weeks 11, 12 and 13. During the remainder of the study (high dose ↓ to 50 mg/kg bw/d), no consistent trend was apparent with food consumption in the high-dose males. The lower food consumption in the high-dose males during the first 4 months of the study correlated with lower body weight and body-weight gain in these animals. In the high-dose females, food consumption was consistently lower compared to controls throughout the study with statistical significance being achieved on weeks 7, 8, 9 and 20. During the first 4 months, food consumption in the high-dose females was approximately 25% lower compared to controls. Food consumption in the high-dose females continued to be lower after the dose was reduced to 50 mg/kg bw/d. The trend in food consumption in the high-dose females correlated with the lower body weight and body-weight gain observed in these animals throughout the study. The lower food consumption in both sexes at 100 mg/kg bw/d may possibly indicate that the test diet at this dose level may have been unpalatable. There were no treatment-related effects on food consumption in either sex at 0.5 and 5.0 mg/kg bw/d.

TABLE 4: Mean food consumption (kg/animal/d ± SD) (a)

Dose Level (mg/kg bw/d)		0	0.5	5.0	100/50 (b)
Males	Week 1	0.292 ± 0.003 (n = 2)	0.363 ± 0.093 (n = 3)	0.350 ± 0.089 (n = 2)	0.310 ± 0.039 (n = 3)
	Week 3	0.353 ± 0.066 (n = 4)	0.377 ± 0.081 (n = 3)	0.395 ± 0.026 (n = 3)	0.315 ± 0.034 (n = 3)
	Week 6	0.353 ± 0.092 (n = 4)	0.373 ± 0.57 (n = 4)	0.446 ± 0.053 (n = 4)	0.299 ± 0.060 (n = 3)

Dose Level (mg/kg bw/d)		0	0.5	5.0	100/50 (b)
	Week 9	0.303 ± 0.063 (n = 4)	0.386 ± 0.041 (n = 2)	0.362 ± 0.103 (n = 2)	0.304 ± 0.031 (n = 3)
	Week 13	0.362 ± 0.056 (n = 4)	0.412 ± 0.038 (n = 3)	0.416 ± 0.041 (n = 3)	0.249 ± 0.005 * (n = 2)
	Week 26	0.321 ± 0.028 (n = 4)	0.361 ± 0.024 (n = 4)	0.424 ± 0.030 * (n = 3)	0.273 ± 0.072 (n = 3)
	Week 50	0.289 ± 0.042 (n = 4)	0.365 ± 0.103 (n = 3)	0.342 ± 0.051 (n = 3)	0.293 ± 0.055 (n = 3)
Females	Week 1	0.295 ± 0.047 (n = 2)	0.295 ± 0.038 (n = 3)	0.328 ± 0.099 (n = 2)	0.251 ± 0.034 (n = 4)
	Week 3	0.284 ± 0.060 (n = 4)	0.247 ± 0.152 (n = 2)	0.271 ± 0.059 (n =4)	0.188 ± 0.056 (n = 4)
	Week 6	0.296 ± 0.026 (n = 4)	0.278 ± 0.053 (n = 4)	0.242 ± 0.029 (n = 4)	0.247 ± 0.046 (n = 4)
	Week 9	0.309 ± 0.042 (n = 4)	0.319 ± 0.040 (n = 4)	0.293 ± 0.013 (n = 4)	0.208 ± 0.028 * (n = 4)
	Week 13	0.266 ± 0.113 (n = 4)	0.321 ± 0.045 (n = 3)	0.321 ± 0.054 (n = 3)	0.243 ± 0.035 (n = 4)
	Week 26	0.335 ± 0.081 (n = 4)	0.309 ± 0.067 (n = 4)	0.299 ± 0.036 (n = 3)	0.277 ± 0.055 (n = 4)
	Week 50	0.299 ± 0.098 (n = 4)	0.263 ± 0.097 (n = 4)	0.302 ± 0.098 (n = 3)	0.227 ± 0.021 (n = 4)

(a) Data obtained from pages 72-74 (males) and 75-77 (females) in the study report.
(b) Due to significant body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.
* Significantly different from control mean by Dunnett's test, $p \leq 0.05$.

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

3. Food efficiency There were no consistent trends apparent with food efficiency values to indicate a treatment-related effect. However, lower body-weight gains in the high-dose males and females correlated with reduced food consumption in these animals.

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

E. Blood analyses:

1. Haematology - There were no statistically significant haematological findings. In males at 100 mg/kg bw/d (at 3 months), red blood cell (RBC) parameters (RBC counts, HCT and HGB) were lower (≈14%) compared to controls (Table 5). RBC indices (MCV, MCH and MCHC) were unaffected by treatment at 100 mg/kg bw/d (not provided at 6 or 12 months). RBC morphology appeared to be unaffected by treatment throughout the study. No reticulocyte counts were provided. After the high-dose was reduced to 50 mg/kg bw/d (at ≈15 wks), RBC parameters continued to be lower in the high-dose males at 6 (≈12-14%) and 12 (≈6-9%) months, although an improvement was noted at 12 months. At 3 and 6 months, RBC parameters were below the normal range of the historical control values for animals of this age and strain and within the normal range at 12 months. These findings are most likely not toxicologically significant since the pre-study evaluation indicates that RBC parameters were also lower (≈10-12%) in the high-dose males compared to controls and the pre-study values for the RBC parameters were comparable to values at 3, 6 and 12 months post-treatment in the high-dose males.

In females at 100 mg/kg bw/d (3 months), RBC parameters (RBC counts, HCT and HGB) were decreased slightly compared to controls and to pre-study values (Table 5). This was primarily due to decreased values in one female that exhibited slight anisocytosis, slight macrocytosis and moderate hypochromasia. A second high-dose female exhibited slight macrocytosis (at 3 and 6 months). At 3 months, RBC parameters were decreased to a greater extent in these 2 females than in the other 2 high-dose females. RBC indices (MCV, MCH and MCHC) were unaffected by treatment at 100 mg/kg bw/d (not provided at 6 or 12 months). No reticulocyte counts were provided. At 6 and 12 months, after the high-dose was reduced to 50 mg/kg bw/d (at ≈15 wks), RBC parameters and morphology in the high-dose females were comparable to controls. These findings are most likely not toxicologically significant since

the difference between the values observed in the controls and in the high-dose females at 100 mg/kg bw/d (at 3 months) appeared to be minimal (≈1-5% lower).

Other findings in the high-dose females at 3 months included decreased mean percent neutrophils with a concomitant increase in mean percent lymphocytes. However, this change in differential WBC counts was attributed to one animal (% neutrophils - 24; % lymphocytes - 59%). At 6 and 12 months, after the high-dose was reduced, WBC differential counts in the high-dose females were comparable to controls.

TABLE 5. Haematological findings. (a)

Dose Level (mg/kg bw/d)	RBC (x 10^6/mm^3)	HGB (g/dL)	HCT (%)	RBC (x 10^6/mm^3)	HGB (g/dL)	HCT (%)
	Males (n = 4 animals/dose)			Females (n = 4 animals/dose)		
Pre-study						
0	7.49 ± 0.38	17.7 ± 0.6	53.7 ± 1.8	7.26 ± 0.45	17.0 ± 1.5	51.6 ± 3.6

154

Dose Level (mg/kg bw/d)	RBC (x 10^6/mm^3)	HGB (g/dL)	HCT (%)	RBC (x 10^6/mm^3)	HGB (g/dL)	HCT (%)
	Males (n = 4 animals/dose)			Females (n = 4 animals/dose)		
0.5	7.18 ± 0.49	16.7 ± 1.7	50.5 ± 5.0	7.09 ± 0.31	16.1 ± 0.9	49.8 ± 3.0
5	7.15 ± 0.66	16.8 ± 1.9	50.7 ± 5.4	6.78 ± 0.41	15.8 ± 1.1	48.2 ± 2.6
100 (b)	6.72 ± 0.29	15.5 ± 1.0	47.2 ± 2.9	7.24 ± 0.20	17.3 ± 0.5	52.8 ± 1.3
3 months						
0	7.96 ± 0.68	18.8 ± 1.4	55.8 ± 3.9	7.37 ± 0.23	17.2 ± 0.3	51.0 ± 0.8
0.5	7.61 ± 0.18	17.7 ± 0.7	51.9 ± 1.5	7.57 ± 0.81	17.4 ± 1.8	52.2 ± 5.7
5	7.75 ± 0.21	18.1 ± 0.8	53.6 ± 2.6	7.39 ± 0.43	17.3 ± 0.8	51.7 ± 2.2
100 (b)	6.88 ± 0.40	16.1 ± 1.2	48.1 ± 3.3	6.97 ± 0.53	16.3 ± 1.8	50.6 ± 3.3

Differential WBC count - Females:
Mean % neutrophils - 60.1 ± 6.2, 62.7 ± 7.3, 56.4 ± 7.6 and 51.5 ± 18.0% at 0, 0.5, 5 and 100 mg/kg bw/d, respectively.
Mean % lymphocytes - 27.4 ± 6.2, 27.6 ± 8.6, 32.8 ± 5.3 and 37.9 ± 14.5% at 0, 0.5, 5 and 100 mg/kg bw/d, respectively.
RBC morphology - Females: At 3 months, 1 female at 100 mg/kg bw/d exhibited slight anisocytosis, slight macrocytosis and moderate hypochromasia, a 2nd female at 100 mg/kg bw/d exhibited slight macrocytosis.

4 months (50 mg/kg bw/d group only)						
50 (b)	6.91 ± 0.49	16.3 ± 1.6	48.3 ± 4.2	6.54 ± 1.01	15.5 ± 2.7	47.3 ± 6.6
6 months						
0	7.58 ± 0.57	18.4 ± 0.9	55.4 ± 3.0	7.13 ± 0.53	16.9 ± 0.9	50.7 ± 2.8
0.5	7.23 ± 0.24	17.4 ± 0.2	51.3 ± 1.2	7.54 ± 0.07	17.7 ± 0.8	53.2 ± 1.8
5	7.38 ± 0.49	17.5 ± 1.6	52.5 ± 4.6	6.67 ± 0.78	15.9 ± 1.7	47.9 ± 5.0
50 (b)	6.66 ± 0.31	15.8 ± 0.8	47.9 ± 2.4	7.32 ± 0.35	17.8 ± 0.7	54.3 ± 2.6
12 months						
0	8.02 ± 0.75	19.6 ± 1.2	56.1 ± 3.8	7.27 ± 0.75	17.2 ± 1.3	49.4 ± 3.9
0.5	7.80 ± 0.49	18.4 ± 0.9	53.5 ± 3.2	7.51 ± 0.64	17.7 ± 1.9	51.0 ± 5.3
5	8.12 ± 0.38	19.3 ± 1.1	55.8 ± 3.7	7.06 ± 0.66	17.0 ± 1.2	48.6 ± 4.1
50 (b)	7.30 ± 0.52	17.8 ± 1.2	51.1 ± 3.4	7.74 ± 0.84	18.9 ± 2.0	55.6 ± 5.5

(a) Data obtained from pages 82-111 in the study report.
(b) Due to significant body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.

2. Clinical Chemistry - At 100 mg/kg bw/d (3 months), significant findings included increased alanine aminotransferase (ALAT) and alkaline phosphatase (AP) activity and decreased serum albumin levels in both sexes (Table 6). Although not statistically identified, serum protein (reflecting ↓ serum albumin levels) levels were also decreased in both sexes at 100 mg/kg bw/d at 3 months. After reducing the high-dose to 50 mg/kg bw/d (at ≈ 15 wks), AP values diminished over time but remained significantly elevated throughout the remainder of the study in

both sexes while ALAT activity was comparable to controls throughout the remainder of the study in both sexes. Serum albumin levels remained lower in the high-dose males and females after the high dose was reduced, this was statistically significant in the high-dose males. Serum protein levels also remained lower throughout the study in both sexes at the high-dose, this was statistically significant in males at 6 months. There were no organ weight changes, gross pathological or histopathological findings to correlate with the increased AP activity or decreased serum albumin levels. The decreased serum albumin and total protein levels may be related to the decreased food consumption observed at the high-dose. There were no treatment-related effects on clinical chemistry parameters in either sex at 0.5 or 5 mg/kg bw/d.

TABLE 6. Clinical chemistry findings. (a)

Dose Level (mg/kg bw/d)	ALAT U/L	AP U/L	PROT g/dL	ALB g/dL	ALAT U/L	AP U/L	PROT g/dL	ALB g/dL
Pre-study	**Males (n = 4 animals/dose)**				**Females (n = 4 animals/dose)**			
0	28 ± 6	82 ± 9	5.9 ± 0.3	3.7 ± 0.1	32 ± 9	75 ± 6.	5.8 ± 0.4	3.5 ± 0.3
0.5	33 ± 5	90 ± 32	5.9 ± 0.2	3.6 ± 0.1	30 ± 5	73 ± 12	5.9 ± 0.3	3.6 ± 0.2
5	36 ± 3	82 ± 24	5.8 ± 0.4	3.7 ± 0.1	25 ± 2	81 ± 6	5.7 ± 0.4	3.4 ± 0.2
100 (b)	30 ± 10	71 ± 7	5.6 ± 0.1	3.4 ± 0.1	28 ± 4	91 ± 33	5.6 ± 0.1	3.5 ± 0.1
3 months								
0	31 ± 9	72 ± 55	6.3 ± 0.4	4.1 ± 0.1	44 ± 13	78 ± 14	6.1 ± 0.1	4.0 ± 0.1
0.5	43 ± 7	75 ± 24	6.2 ± 0.2	4.1 ± 0.2	52 ± 19	72 ± 29	6.2 ± 0.2	4.3 ± 0.2
5	37 ± 11	54 ± 8	6.2 ± 0.2	4.2 ± 0.1	37 ± 4	57 ± 6	6.2 ± 0.3	1.9 ± 0.1
100 (b)	152 ± 107 *	240 ± 217 *	5.6 ± 0.3	3.5 ± 0.3 *	162 ± 166 *	689 ± 468 *	5.5 ± 0.2	2.5 ± 0.7 *
4 months								
50 (b)	38 ± 9	208 ± 73	5.4 ± 0.5	3.3 ± 0.1	33 ± 8	215 ± 228	5.5 ± 0.4	3.0 ± 0.8
6 months								
0	33 ± 4	60 ± 16	6.6 ± 0.5	3.9 ± 0.2	49 ± 26	88 ± 15	6.3 ± 0.4	3.8 ± 0.1
0.5	42 ± 10	64 ± 18	6.1 ± 0.1	3.8 ± 0.1	40 ± 6	81 ± 24	6.5 ± 0.5	4.2 ± 0.3
5	43 ± 10	62 ± 21	6.3 ± 0.1	3.9 ± 0.2	33 ± 3	64 ± 22	6.2 ± 0.3	3.9 ± 0.2
50 (b)	37 ± 7	178 ± 59 *	5.9 ± 0.3 *	3.3 ± 0.2 *	36 ± 7	252 ± 159 *	6.1 ± 0.2	3.6 ± 0.2
12 months								
0	173 ± 209	65 ± 29	6.8 ± 0.5	4.0 ± 0.1	45 ± 19	82 ± 9	6.3 ± 0.4	3.8 ± 0.1
0.5	53 ± 12	68 ± 25	6.7 ± 0.5	4.0 ± 0.3	45 ± 14	72 ± 12	6.3 ± 0.3	4.0 ± 0.2
5	104 ± 115	56 ± 19	6.9 ± 0.4	4.0 ± 0.1	29 ± 7	47 ± 14	6.6 ± 0.5	4.0 ± 0.1
50 (b)	32 ± 7	157 ± 36 *	6.1 ± 0.2	3.4 ± 0.1 *	46 ± 18	372 ± 312 *	6.0 ± 0.2	3.5 ± 0.3

(a) Data obtained from pages 112-131 in the study report.
(b) Due to significant body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.
* Contrast statistically significant at $\alpha = 0.02$, Bonferroni corrected comparison-wise error rate.

F. Urinalysis - No treatment-related effects on urinalysis parameters were observed in either sex.

G. Sacrifice and Pathology

1. Organ weight -No treatment-related effects on organ weights were observed in either sex.

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

3. Microscopic pathology - Hypertrophy of the epithelial cells of the collecting duct was observed in males at all dose levels and in the control and high-dose females. However, the severity of the hypertrophy appeared to be increased in the high-dose males and females compared to the controls. In both sexes in the control group and in males at 0.5 and 5.0 mg/kg bw/d the severity was graded as very slight in all animals exhibiting hypertrophy of the epithelial cells of the collecting duct. At the high-dose the severity was graded as very slight in one male and one female and as slight in a second male and second female. The hypertrophied cells were present in the inner and outer stripe of the outer zone of the medulla with a granular, pale, eosinophilic cytoplasm containing numerous mitochondria. The hypertrophied 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 (DACO 4.3.1 - Laboratory Project Study ID - DR-0312-6565-011) where urinalysis findings indicative of an acidification defect and impaired concentrating were observed (urinary acidification and reduced urinary specific gravity, respectively) and to those reported in Beagle dogs following 13-weeks of treatment (DACO 4.3.8 - Laboratory Project Study ID DR-0312-6565-021) where urinary acidification was not present and urinary specific gravity was unaffected. In the dog, there also appeared to be no progression in the severity of the alterations at comparable dose levels from 13 weeks to 1 year of dietary administration of the test substance.

Slight vacuolization of the zona reticularis and zona fasciculata in the adrenal glands was observed in the high-dose males and females. The vacuolization was characterized by multiple clear cytoplasmic vacuoles in numerous clusters of the zona reticularis and inner zona fasciculata and was consistent with fatty changes. In the absence of any associated inflammation, necrosis or other changes, the toxicological significance of this finding was uncertain. There were no treatment-related histopathological findings in either sex at 0.5 or 5.0 mg/kg bw/d. Histopathological findings are summarized in Table 7.

TABLE 7. Histopathological findings (expressed as # of animals affected / # of animals examined). (a)

Dose Level (mg/kg bw/d)			0	0.5	5.0	100/50 (b)
Males						
Kidney	- hypertrophy, collecting ducts	- very slight (b)	2/4	2/4	2/4	1/4
		- slight (b)	0/4	0/4	0/4	1/4

Dose Level (mg/kg bw/d)			0	0.5	5.0	100/50 (b)
		- total	2/4	2/4	2/4	2/4
Adrenal glands	- vacuolization of zona reticularis and zona fasciculata	- very slight	1/4	0/4	0/4	1/4
		- slight	0/4	0/4	0/4	1/4
		- total	1/4	0/4	0/4	2/4
Females						
Kidney	- hypertrophy, collecting ducts	- very slight (b)	2/4	0/4	0/4	1/4
		- slight (b)	0/4	0/4	0/4	1/4
		- total	2/4	0/4	0/4	2/4
Adrenal glands	- vacuolization of zona reticularis and zona fasciculata	- very slight	0/4	1/4	0/4	1/4
		- slight	0/4	0/4	0/4	2/4
		- total	0/4	1/4	0/4	3/4

(a) Data obtained from pages 164-172 in the study report.
(b) Due to significant body-weight loss in 2 males and 3 females at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare.

III. DISCUSSION

A. Investigators' conclusions (extracted from page 35 of the study report): "Dogs from the high-dose group were given 100 mg XDE-570/kg bw/day for the first 104 days of the study. Due to significant decreases in body weights and feed consumption in the high-dose animals, it was necessary to lower the dose level to 50 mg/kg bw/day on test day 105 for reasons of animal welfare. Dogs given 100 mg/kg bw/day also had decreases in red blood cell parameters and serum albumin, and increases in serum alanine aminotransferase and alkaline phosphatase. Following the change to 50 mg/kg bw/day, body weights, feed consumption, red blood cell parameters and alanine aminotransferase gradually returned to levels comparable to control-group values for the remainder of the study. Alkaline phosphatase remained elevated and serum albumin remained decreased in high-dose animals for the remainder of the study. Treatment-related histopathological alterations consisted of slight hypertrophy of individual cells of collecting ducts in the kidneys, and slight vacuolization of the zona reticularis and zona fasciculata of the adrenal glands, in a few male and female dogs given 100/50 mg/kg bw/day. There were no treatment-related changes in male or female dogs given 0.5 or 5 mg/kg bw/day. Under the conditions of this study, the no-observed-effect-level (NOEL) was 5 mg XDE-570/kg bw/day, for male and female Beagle dogs following one year of dietary administration."

B. Reviewer comments: There were no treatment-related effects on mortality, clinical signs, ophthalmoscopy, urinalysis, organ weights or gross pathology. At 100 mg/kg bw/d, both sexes exhibited a body weight loss during the first 15 weeks of the study, this correlated with a concomitant reduction in food consumption in both sexes. After the high-dose was reduced to 50 mg/kg bw/d (at ≈wk 15), body weight and body-weight gain were comparable to controls in males and continued to be lower throughout the study in females. At 100 mg/kg bw/d (3 months), clinical chemistry findings included increased alanine aminotransferase (ALAT) and alkaline phosphatase (AP) activity and decreased serum albumin levels in both sexes. Although not statistically identified, serum protein levels were also decreased in both sexes at 100 mg/kg bw/d, reflecting the decreased serum albumin levels. After the high-dose was reduced to 50 mg/kg bw/d, AP values diminished over time but remained significantly elevated throughout the remainder of the study in both sexes. Serum albumin and protein levels also remained lower in the high-dose males and females. There were no organ weight changes, gross pathological or histopathological findings to correlate with the increased AP activity or decreased serum albumin levels. Hypertrophy of the epithelial cells of the collecting duct was observed in males at all dose levels and in the control and high-dose females. However, the severity of the hypertrophy appeared to be increased in the high-dose males and females compared to the controls. 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 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. There also appeared to be no progression in the severity of the alterations at comparable dose levels from 13 weeks to 1 year of dietary administration of the test substance. Slight vacuolization of the zona reticularis and zona fasciculata in the adrenal glands was observed in the high-dose males and females. The vacuolization was consistent with fatty changes. In the absence of any associated inflammation, necrosis or other changes, the toxicological significance of this finding was uncertain.

The LOAEL was 50 mg/kg bw/d based on lower body weight, body-weight gain and food consumption (♀), increased AP activity (♂/♀) and decreased serum albumin and protein levels (♂/♀) at 50 mg/kg bw/d and increased severity of hypertrophy of epithelial cells of the collecting ducts and slight vacuolization of the zona reticularis and zona fasciculata in the adrenal glands (♂/♀) at 100/50 mg/kg bw/d. The NOAEL was 5 mg/kg bw/d.

C. Study deficiencies: OECD guideline 452 recommends that urine volume should be determined and that urine samples should be collected from all non-rodents at 3 months, 6 months and at 6 month intervals thereafter and at termination of the study. Urine volume was not determined and there was no urine collection at 3 months. 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 pH and specific gravity) 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. Due to a body-weight loss in 2 males (-1.205 kg and -0.858 kg) and 3 females (-0.790 kg, -0.577 kg and -2.005 kg) at 100 mg/kg bw/d and reduced food consumption in these animals during the first three months of the study (up to study day 104), the high-dose level was reduced to 50 mg/kg bw/d (beginning study day 105) for reasons of animal welfare. At week 0 mean body weight was comparable between the high-dose animals and controls for both sexes, however, by week 15, mean body weight in the high-dose males and females was approximately 10 and 15% lower, respectively, compared to controls. In the 90-day dietary study in dogs, there were no treatment-related effects on body weight, body-weight gain or food consumption in either sex at 100 mg/kg bw/d. It is questionable whether it was justifiable to decrease the high-dose from 100 mg/kg bw/d to 50 mg/kg bw/d at week 15. However, the LOAEL for the 90-day and 1-year dietary studies were identical, 50 mg/kg bw/d, based on similar treatment-related findings. In addition, a definite NOAEL, 5.0 mg/kg bw/d, was determined for this study which was also similar to that observed in the 90-day dietary study; therefore, this study is considered to be acceptable and satisfies the guideline requirement for a 1-year oral toxicity study in dogs (OPPTS 870.4100; OECD 452).