C12084

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 23 1996

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **p-Dichlorobenzene-** Chronic Oral Toxicity in Dogs; Section 6(a)(2)

FROM: Yung G. Yang, Ph.D. Yung G. Yang 10/21/96
Toxicology Branch II, Section II
Health Effects Division (7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 10/21/96
Section Head, Section II
Toxicology Branch II, HED (7509C)
and
Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou 10/22/96
Acting Branch Chief
Toxicology Branch II, HED (7509C)

TO: Kathleen Depukat
PM Team 51
Reregistration Division (7508W)

DP Barcode:	D226295	**Case:**	818985
Submission:	S505646	**ID No.:**	061501
Chemical:	Paradichlorobenzene	**PC No.:**	061501
Caswell No.:	632	**Registrant:**	Monsanto

ACTION REQUESTED: Review a one-year oral toxicity study in dogs (MRID# 439888-01 & -02) for Section 6(a)(2) and reregistration need.

RESPONSE: This one-year oral toxicity study in dogs has been reviewed and is found to be acceptable (§83-1).

Previously, preliminary results of this study were submitted to the Agency (MRID# 43400201) as a possibly submittable data under FIFRA Section 6(a)(2) concerning the early mortality at a dose lower than that observed in the pilot study. After reviewing the complete study (MRID# 439888-01 & -02), TB II determined that this should not be a Section 6(a)(2) issue based on the similar findings observed in the chronic study and the pilot study. No further action is required.

In addition, as indicated in the Bean sheet, this study is not required for reregistration. Nevertheless, this study is well conducted based on the guideline (§83-1); therefore, it can be used to satisfy guideline requirement as a chronic study in dogs should we need it in the future.

A Data Evaluation Record (DER) is attached and an executive summary is as follows.

EXECUTIVE SUMMARY

In a one-year oral toxicity study (MRID# 43988802), p-Dichlorobenzene (99.9% a.i.) was administered to beagle dogs (5/sex/dose) by capsule at dose levels of 0, 10, 50, or 150 mg/kg/day. Three animals (2 males and 1 female) in the highest dose and one male control animal died during the study. Due to unexpected severe toxicity at the highest dose, the initial dose of 150 mg/kg/day was adjusted to 100 mg/kg/day for males during the 3rd week, and a further decrease to 75 mg/kg/day was made for both sexes at the beginning of the 6th week of the study. Both males and females at the highest dose level were untreated during the fourth and fifth weeks to allow recovery. Lower dose level animals were administered the test material continuously at the original dose levels. All treatment-related clinical signs were primarily limited to severely affected animals at the highest dose level and to the control male which died. These clinical signs included hypoactivity, dehydration, decreased defecation, blood-like fecal color, emesis, emaciation, and/or pale (oral) mucosa.

Group mean body weight did not show a significant difference from the control in either sex. However, cumulative body weight gain at the highest dose showed a significant deficit during the first month of the study. Following reduction of the dose and adjustment of food availability, surviving dogs in this high dose group resumed a more comparable weight gain pattern for the remainder of the study.

A mild anemia (decreased RBC counts and HCT levels) in both sexes at the early stage (month 6) was observed in the high dose animals. At the end of the study, the mild anemia was resolved which correlated with microscopic findings of rib and sternal marrow erythroid hyperplasia in females and splenic excessive hematopoiesis and megakaryocyte proliferation in both sexes indicating a compensatory response to the earlier anemia.

Elevated alkaline phosphatase, ALT, AST, and GGT levels and a decreased albumin level observed in both sexes at the mid- and high-dose levels, indicated hepatic effects of the test material. These findings were supported by increases in absolute and relative liver weights (both sexes) and microscopic findings in the liver including hepatocellular hypertrophy, hepatocellular pigment deposition, bile duct/ductile hyperplasia, nodular hyperplasia, bile stasis and hepatic portal inflammation.

2

Kidney collecting duct epithelial vacuolation was present in a high-dose male and at all dose levels in females. This lesion was considered a possible effect of the test material at the mid and high doses in females where it was accompanied by increased kidney weights and grossly observed renal discoloration.

In this study, **the NOEL is 10 mg/kg/day and the LOEL is 50 mg/kg/day** based on increased liver weight, clinical chemistry data, and histological findings in the liver.

This one-year oral toxicity study in the dog is classified as **Acceptable (§83-1).**

EPA Reviewer: Yung G. Yang, Ph.D. Yung G. Yang, Date 10/18/96
Review Section II, Toxicology Branch II (7509C)
EPA Secondary Reviewer: K Clark Swentzel K. Clark Swentzel Date 10/18/96
Review Section II, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity (Capsule)- Dogs; OPPTS 870.4100 [§83-1]

DP BARCODE: D226295
P.C. CODE: 061501
CASE: 818985

SUBMISSION CODE: S505646
TOX. CHEM. NO.: 632

TEST MATERIAL (PURITY): p-Dichlorobenzene (99.9% a.i.)

SYNONYMS: p-DCB

CITATION: Naylor, M.W. and Stout, L.D. (1996). One Year Study of p-Dichlorobenzene Administered Orally Via Capsule to Beagle Dogs. Environmental Health Laboratory, Monsanto Company, St. Louis, MO. Study No. ML-94-210, March 25, 1996. MRID# 43988802. Unpublished.

Harrington, R.M. and Thake, D.C. (1995). Four Week Range-Finding Study of Para-Dichlorobenzene Administered by Capsule to Beagle Dogs. Environmental Health Laboratory, Monsanto Company, St. Louis, MO. Study No. ML-94-090. January 5, 1995. MRID# 43988801. Unpublished.

EXECUTIVE SUMMARY

In a one-year oral toxicity study (MRID# 43988802), p-Dichlorobenzene (99.9% a.i.) was administered to beagle dogs (5/sex/dose) by capsule at dose levels of 0, 10, 50, or 150 mg/kg/day. Three animals (2 males and 1 female) in the highest dose and one male control animal died during the study. Due to unexpected severe toxicity at the highest dose, the initial dose of 150 mg/kg/day was adjusted to 100 mg/kg/day for males during the 3rd week, and a further decrease to 75 mg/kg/day was made for both sexes at the beginning of the 6th week of the study. Both males and females at the highest dose level were untreated during the fourth and fifth weeks to allow recovery. Lower dose level animals were administered the test material continuously at the original dose levels. Treatment-related clinical signs were primarily limited to severely affected animals at the highest dose level and to the control male which died. These clinical signs included hypoactivity, dehydration, decreased defecation, blood-like fecal color, emesis, emaciation, and/or pale (oral) mucosa.

Group mean body weight did not show a significant difference from the control in either sex. However, cumulative body weight gain at the highest dose showed a significant deficit during the first month of the study. Following reduction of the dose and adjustment of food availability, surviving dogs in this high dose group resumed a more comparable weight gain pattern for the remainder of the study.

A mild anemia (decreased RBC counts and HCT levels) in both sexes at the early stage (month 6) was observed in the high dose animals. At the end of the study, the mild anemia was resolved which correlated with microscopic findings of rib and sternal marrow erythroid hyperplasia in females and splenic excessive hematopoiesis and megakaryocyte proliferation in both sexes indicating a compensatory response to the earlier anemia.

Elevated alkaline phosphatase, ALT, AST, and GGT levels and a decreased albumin level, observed in both sexes at the mid- and high-dose levels, indicated hepatic effects of the test material. These findings were supported by increases in absolute and relative liver weights (both sexes) and microscopic findings in the liver including hepatocellular hypertrophy, hepatocellular pigment deposition, bile duct/ductile hyperplasia, nodular hyperplasia, bile stasis and hepatic portal inflammation.

Kidney collecting duct epithelial vacuolation was present in a high-dose male and at all dose levels in females. This lesion was considered a possible effect of the test material at the mid and high doses in females where it was accompanied by increased kidney weights and grossly observed renal discoloration.

In this study, **the NOEL is 10 mg/kg/day and the LOEL is 50 mg/kg/day** based on increased liver weight, clinical chemistry data, and histological findings in the liver.

This one year oral toxicity study in the dog is classified as **Acceptable (§83-1).**

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

5

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: p-Dichlorobenzene (p-DCB)
   Description: White solid
   Lot/Batch #: KD-5092
   Purity: 99.9% a.i.
   Stability of compound: Stable through the study.

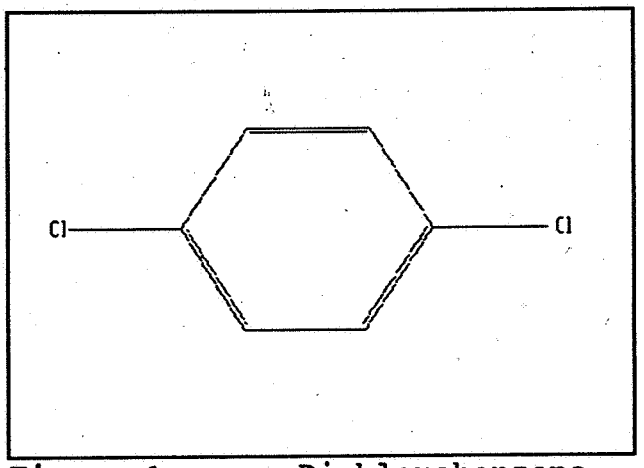

Figure 1 para-Dichlorobenzene

2. Vehicle: Gelatin Capsule

3. Test animals: Species: Dogs
   Strain: Beagle
   Age and weight at study initiation: 7 months, Males: 6.8-11.4 kg. Females: 6.9-11.1 kg
   Source: White Eagle Laboratories, Inc. Doylestown, PA
   Housing: Individual
   Diet: Certified Canine Diet#5007, 300 g/2 hours/day
   Water: Tap water, ad libitum
   Environmental conditions: Not provided; however, the report stated that animal housing and husbandry were in accordance with the provisions of "Guide to the Care and Use of Laboratory Animals", USPHS-NIH Publication No. 86-23.
   Acclimation period: Not provided

B. STUDY DESIGN:

1. In life dates - start: 8/22/94 end: 9/1/95

2. Animal assignment

Animals were assigned by computerized randomization by weight to the test groups in Table 1.

Table 1. Animal Assignment

Test Group	Dose (mg/kg/day)	Animals No. (M/F)
Control	0	5/5
Low (LDT)	10	5/5
Mid (MDT)	50	5/5
High (HDT)	75/100/150*	5/5

* Dose level was adjusted from the initial dose of 150 mg/kg/day to 100 and then 75 mg/kg/day due to severe toxicity.

3. Dose selection rationale: Dose selection was based on a range finding study (MRID# 43988801). In this study, groups of beagle dogs (2/sex/group) were administered p-DCB orally in gelatin capsules, 5 days a week at doses of 25, 75, 150, or 300 mg/kg/day for 4 weeks. Both males from the 300 mg/kg/day group died during the study. Decreased body weight gains were seen at 150 and 300 mg/kg/day groups. Elevated clinical chemistry parameters were observed in the 150 mg/kg/day males and 75, 150, and 300 mg/kg/day females. At necropsy, absolute and relative liver weights were increased in the 75 and 150 mg/kg/day males and 75, 150, and 300 mg/kg/day females. In addition, irritation of the gastrointestinal tract was noted in both males and females. It was concluded that the NOEL was 25 mg/kg/day in both sexes. The LOEL was 75 mg/kg/day based increased liver weight in males and the increase in alkaline phosphatase and liver weights, and irritation to the GI tract in females.

4. Diet preparation and analysis: The test substance was administered via gelatin capsules.

5. Statistics - The following procedures were used to detect statistically significant differences between treated animals and their respective controls:
(1) Dunnett's Multiple Comparison Test (two-tailed) was used for analysis of body weight, body weight gain, food consumption, urine specific gravity, pH and volume. (2) Decision-tree analysis (two-tailed) tests normality and homogeneity of variances, utilizing either parametric Dunnett's test and Linear Regression or nonparametric Kruskal-Wallis, Jonckheere's and/or Mann-Whitney Test, were used to detect differences and analyze for trend in hematology and blood chemistry data, body weights, absolute organ weights and organ/body weight ratios.

7

Fisher's Exact Test (one-tailed) was used for analysis of the incidence of microscopic lesions. Grubb's Test was used to detect outliers in the organ weight data.

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality.

2. Body weight

Animals were weighed prior to randomization and once weekly, thereafter.

3. Food consumption:

Food consumption was determined five days/week; this measurement was then linearly extrapolated to weekly consumption.

4. Ophthalmoscopic examination

Eyes were examined prior to the study start and just prior to study completion.

5. Blood was collected (fasted) at pretest, at month 6, and at the completion (month 12) of the study for hematology and clinical chemistry analysis from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology

x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*	x	Reticulocyte count
x	Blood clotting measurements* (Activated Partial Thromboplastin Time, APTT) (Clotting time) (Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

	ELECTROLYTES		OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected by catheterization from fasted animals at pretest, month 6 and at the completion of the study. The CHECKED (X) parameters 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

* Required for chronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

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

* Required for chronic studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.

II. RESULTS:

A. Observations

1. Toxicity - Treatment-related clinical signs were primarily limited to severely affected animals at the highest dose level and to the control male which died. These clinical signs included hypoactivity, dehydration, decreased defecation, blood-like fecal color, emesis, emaciation, and/or pale (oral) mucosa.

2. Mortality - In the 150 mg/kg/day dose group, one male dog was sacrificed *in extremis* on day 12 of the study, a second male dog died on day 25, and one female dog died on day 24. A control male dog died on day 83. All other dogs survived to the scheduled completion of the study.

B. Body weight

Results of mean body weights are summarized in Table 2. Group mean body weight in either sex did not show statistically significant differences from respective controls. However, cumulative body weight gain at the highest dose showed a significant deficit during the first month of the study in both sexes. Following

reduction of the dose and adjustment of food availability, surviving dogs at this high dose group resumed a more comparable weight gain pattern for the remainder of the study.

Table 2. Summary of Mean Body Weight and Cumulative Body Weight Changes (kg)

Interval (day)	Dose (mg/kg/day)							
	Males				Females			
	0	10	50	75[a]	0	10	50	75[a]
Pretest	10.2	9.7	9.8	9.7	8.7	8.5	8.7	8.9
5	10.3	9.8	10.1	9.7	8.7	8.6	8.7	8.9
12	10.4	10.0	10.0	9.9	8.8	8.7	8.7	8.7
33	10.6	10.3	10.2	10.4	8.9	8.8	8.7	7.9
61	11.2	10.8	10.6	10.8	9.0	8.9	8.9	8.4
89	12.2	11.0	11.2	11.2	9.1	9.3	9.1	8.6
145	12.9	11.3	11.5	11.2	9.5	9.7	9.5	9.2
250	13.9	11.9	12.5	12.1	9.9	9.8	9.5	9.0
369	14.3	11.9	12.7	12.0	10.3	10.2	10.1	9.3
Wt Gain								
2-5	0.15	0.10	0.32	0.07	0.09	0.09	-0.04	0.00
2-12	0.26	0.25	0.26	-0.34*	0.19	0.25	0.03	-0.14
2-19	0.41	0.17	0.03	-0.56**	0.18	0.24	-0.05	-0.90**
2-26	0.42	0.48	0.26	-0.23	0.38	0.36	0.02	-0.81**
2-33	0.42	0.53	0.45	-0.17	0.22	0.31	-0.01	-0.44*
2-47	0.65	0.90	0.67	0.08	0.38	0.45	0.12	-0.04
2-180	2.84	1.75	2.07	0.76	1.18	1.35	0.92	1.29
2-369	3.89	2.19	2.95	1.43	1.67	1.69	1.39	0.99

Data extracted from Tables 2&3, pages 43-74 of the report.

a Due to unexpectedly severe toxicity, the initial dose of 150 mg/kg/day was adjusted to 100 mg/kg/day for males during the 3rd week, and a further decrement to 75 mg/kg/day was made for both sexes at the beginning of the 6th week of the study.

* Statistically significant ($p \leq 0.05$).

** Statistically significant ($p \leq 0.01$).

C. Food consumption- Food consumption by males and females at all dose levels was comparable to controls. However, the report indicated that, for humane reasons, adjustments such as moistening the food, allowing an extended period of time for consumption, additional dry dog chow were made to improve food intake by animals that were not eating sufficiently. Therefore, the results of the food consumption were questionable.

D. Ophthalmoscopic examination - There were no treatment-related ophthalmologic findings reported.

E. Blood work

1. Hematology - At month 6, increased platelet counts were noted at the mid and high doses (statistically significant in females only); RBC counts (both sexes) and HCT levels (males only) were reduced at the high-dose at

11

this interval (Table 3). At the end of the study (month 12), an increased platelet count was seen in the high dose females only. In addition, the report indicated that the number of basophils in high dose (females only) was decreased at month 6. However, the total WBC count at this dose was comparable to the control; therefore, this finding is not considered biologically significant.

Table 3. Significant Findings in Hematology Data

Parameters / Dose level (mg/kg/day)	Males		Females	
	Month 6	Month 12	Month 6	Month 12
RBC(10^6/uL)				
0	7.115	7.025	7.302	7.088
10	6.808	6.738	7.620	7.706
50	6.660	6.848	6.552	6.944
75[a]	6.363*	6.840	6.475*	6.418
HCT(%)				
0	48.375	46.300	49.960	47.160
10	46.320	45.220	51.900	51.620
50	46.140	47.280	46.460	47.600
75[a]	43.267*	45.900	46.125	44.300
PLT(10^3/mm3)				
0	257.75	231.50	275.40	266.60
10	308.40	265.20	240.80	221.80
50	373.60	327.00	411.80*	322.80
75[a]	453.67	372.00	450.50*	413.25*

Data extracted from Table 6, pages 90-96 of the report.
a Due to unexpectedly severe toxicity, the initial dose of 150 mg/kg/day was adjusted to 100 mg/kg/day for males during the 3rd week, and a further decrement to 75 mg/kg/day was made for both sexes at the beginning of the 6th week of the study.
* Statistically significant ($p \leq 0.05$).
** Statistically significant ($p \leq 0.01$).

2. Clinical chemistry - Although some of the analyses did not show statistical significance, dose-related decreases in albumin levels and elevations in alkaline phosphatase, ALT, AST, and GGT were seen in the mid and/or high doses of both sexes at months 6 and/or 12 (Table 4). In addition, sporadic changes were noted in some chemistry parameters including total and direct bilirubin, glucose, creatinine and cholesterol.

Table 4. Significant Findings in Clinical Chemistry Data

Parameters / Dose level (mg/kg/day)	Males		Females	
	Month 6	Month 12	Month 6	Month 12
Albumin(g/L)				
0	3.83	3.93	3.80	3.92
10	3.62	3.68	3.90	4.00
50	3.22*	3.40	3.26	3.48
75[a]	3.13*	3.30	3.08*	3.03
Alk Phos(IU/L)				
0	151.3	162.5	175.8	173.4
10	147.6	130.8	176.0	181.8
50	1256.2*	1170.8*	1098.2**	745.8**
75[a]	1058.3	1256.3	1513.5*	1351.8*
ALT(IU/L)				
0	46.0	41.8	29.6	28.2
10	32.8	33.2	37.8	37.2*
50	90.4	106.8	57.6	38.8
75[a]	237.7	382.7	247.5	99.5*
AST(IU/L)				
0	31.75	30.50	30.40	36.20
10	35.40	34.40	34.60	38.80
50	40.20	42.00	39.80	39.00
75[a]	51.00	69.67	64.25	55.50
GGT(IU/L)				
0	5.5	4.5	4.0	4.6
10	6.0	5.0	3.8	4.4
50	7.0	6.0	5.0	6.0
75[a]	8.7	13.3	9.3*	12.0*

Data extracted from Table 7, pages 99-109 of the report.

a Due to unexpectedly severe toxicity, the initial dose of 150 mg/kg/day was adjusted to 100 mg/kg/day for males during the 3rd week, and a further decrement to 75 mg/kg/day was made for both sexes at the beginning of the 6th week of the study.

* Statistically significant ($p \leq 0.05$).

** Statistically significant ($p \leq 0.01$).

F. Urinalysis - There were no adverse effect in the urine of both sexes.

G. Sacrifice and Pathology

1. Organ weight - Increased absolute organ weights were seen in livers of males (high dose) and females (mid and high doses) and the thyroid of females (mid dose only) (Table 5). Increased relative organ/body weight ratios were seen in the adrenals (females, high dose), kidney (females, mid dose), liver (both sexes, mid and high doses) and thyroid (females, mid dose).

Table 5. Significant Findings in Organ Weights and Organ to Body Weight Ratios

Organ/ Dose (mg/kg/day)	Males		Females	
	Absolute (g)	Relative	Absolute (g)	Relative
Body wt				
0	13780		9684	
10	11290		9680	
50	11930		9338	
75[a]	11290		8907	
Adrenal				
0	1.2015	0.0089	1.2230	0.0128
10	1.3036	0.0117	1.2410	0.0129
50	1.5046	0.0127	1.6534	0.0176
75[a]	1.5637	0.0140	1.7255	0.0195*
Liver				
0	379.80	2.7738	261.80	2.7078
10	318.64	2.8821	291.42	3.0504
50	473.22	3.9663**	388.68**	4.2028**
75[a]	531.90*	4.7260**	407.40**	4.6070**
Kidney				
0	66.7650	0.4881	45.0650	0.4704
10	56.6228	0.5085	44.5988	0.4650
50	64.1948	0.5429	52.7756	0.5643*
75[a]	66.8830	0.5952	62.7873	0.7266
Thyroid				
0	1.2192	0.0090	0.8062	0.0085
10	1.0286	0.0094	0.8894	0.0093
50	1.4386	0.0120	1.1200*	0.0121*
75[a]	1.4967	0.0134	1.0625	0.0119

Data extracted from Tables 8-9, pages 111-115 of the report.

a Due to unexpectedly severe toxicity, the initial dose of 150 mg/kg/day was adjusted to 100 mg/kg/day for males during the 3rd week, and a further decrement to 75 mg/kg/day was made for both sexes at the beginning of the 6th week of the study.

* Statistically significant ($p \leq 0.05$).

** Statistically significant ($p \leq 0.01$).

2. Gross pathology - Animals that died or were sacrificed *in extremis* showed abnormal color and/or contents and/or consistency in gastrointestinal tract tissue, the liver, lungs, lymph nodes, mesentery/omentum, thyroids, and urinary bladder. Other treatment-related gross necropsy findings included abnormal kidney color in two high dose females, abnormal color and/or enlargement of the mesenteric and pancreatic lymph modes in a high dose female, splenic foci/enlargement in two/one high level females, white focus(i) in the lungs of one male at each dose level, one female at the mid level and two females at the highest level, and a liver mass/nodule in high level females.

3. Microscopic pathology -

a) Non-neoplastic - Liver lesions of mild to severe hepatocellular hypertrophy were present in all males and females at the mid and high doses and one female at the lowest dose level. Hepatocellular pigment deposition occurred in two males and one female each in the mid- and high-dose levels. Bile duct/ductule hyperplasia was observed in one male and one female in the highest dose group. Hepatic portal inflammation (periportal accumulation of neutrophils) was seen in males of the two high dose groups.

In the lung, chronic active inflammation was present only in treated males (two males each in all test groups) and females (one female each in the mid- and high-dose levels). The significance of these pulmonary lesions is uncertain.

In the kidney, one male (high dose) and four females (one each in the low and mid doses and two in high dose) exhibited excessive vacuolation of medullary ray collecting duct epithelium causing the medullary rays to be very prominent. In the two high dose females, these lesions also corresponded to grossly observed renal linear discoloration.

Several test animals in the high dose level had hyperplastic changes in hematopoietic tissues. These included splenic excessive hematopoiesis, megakaryocyte proliferation and rib and sternal bone marrow erythroid hyperplasia.

b) Neoplastic - No significant neoplastic findings were reported.

III. DISCUSSION

A. Initial administration of the highest dose level, 150 mg/kg/day, resulted in the early death of one female and two male dogs within 25 days. The dose was reduced to 100 mg/kg/day at the third week, then to 75 mg/kg/day at the 6th week and for the remainder of the study. Lower dose levels, 50 and 10 mg/kg/day, remained constant for the entire study.

Treatment-related clinical signs were primarily limited to severely affected animals at the highest dose level and to the control male which died. These clinical signs included hypoactivity, dehydration, decreased defecation, blood-like fecal color, emesis, emaciation, and/or pale (oral) mucosa.

Group mean body weight did not show a significant difference from the control in either sex. However, cumulative body weight gain at the highest dose showed a significant deficit during the first month of the study. Following reduction of the dose and adjustment of food availability, surviving dogs in this high dose group resumed a more comparable weight gain pattern for the remainder of the study.

A mild anemia (decreased RBC counts and HCT) in both sexes at the early stage was observed in the high dose animals. At the end of the study, the mild anemia was resolved which correlated with microscopic findings of rib and sternal marrow erythroid hyperplasia in females and splenic excessive hematopoiesis and megakaryocyte proliferation in both sexes, which may have been evidence of a compensatory response to the earlier mild anemia.

Elevated alkaline phosphatase, ALT, AST, and GGT levels, observed in both sexes at the mid- and high-dose levels, indicated hepatic effects. This finding was supported by increases in absolute liver weights and relative liver/body weight ratios plus the microscopic findings in the liver which included hepatocellular hypertrophy, hepatocellular pigment deposition, bile duct/ductile hyperplasia, nodular hyperplasia, bile stasis and hepatic portal inflammation.

Increases in absolute and relative adrenal and thyroid weights in both sexes at the mid and high doses were considered possible treatment-related effects; however, no histopathologic lesions were found which would explain the increases.

Kidney collecting duct epithelial vacuolation was present in a high level male and at all levels in females. This lesion was considered to be a possible effect of the test material at the mid and high doses in females where it was accompanied by increased kidney weights and grossly observed renal discoloration.

In this study. the NOEL was estimated to be 10 mg/kg/day. The LOEL was 50 mg/kg based on increased liver weight, clinical chemistry data, and histological findings in the liver.

Although this study is not required under Data Call-In or reregistration, the study is acceptable (§83-1)

B. Study deficiencies - No significant deficiencies were noted.