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

SEP 25 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pentachlorophenol: Review of a chronic toxicity study in dogs.

EPA Identification Numbers:

DP Barcode: D225574 P.C. Code: 063001
Submissions: S504306 MRID: 43982701

TO: Connie Welch
PM Team # 32/34
Antimicrobial Division (7510W)

FROM: Timothy F. McMahon, Ph.D. 9-25-97
Pharmacologist, RASSB
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THRU: Winston Dang, Ph.D. 9-25-97
Acting Team Leader, Team One
RASSB, Antimicrobial Division (7510W)

and

Norm Cook 09 25 97
Chief, RASSB
Antimicrobial Division (7510W)

Registrant: Pentachlorophenol Task Force

Action Requested: Review of a chronic toxicity study in dogs submitted for Pentachlorophenol.

Data Summary

In a chronic toxicity study, (MRID 43982701) pentachlorophenol (90.9% a.i.) was fed (gelatin capsules) to four beagle dogs/sex/dose at dose levels of 0, 1.5, 3.5, or 6.5 mg/kg/day for 52 weeks. At 6.5 mg/kg/day, one male and one female dog were sacrificed *in extremis* on days 247 and 305, respectively, due to significant clinical toxicity (significant weight loss, lethargy, marked dehydration, vomiting, icterus). Group mean body weight in surviving male dogs at the 6.5 mg/kg/day dose was decreased by 15% at week 13, and 21% at study termination. In females, a 19% decrease in group mean body weight was observed at week 13, and bodyweight remained significantly decreased until study termination. Decreased red cell count (16%), hemoglobin (9%), and hematocrit (8%), was observed in males at the 6.5 mg/kg/day dose at week 13. These decreases were also observed at week 26 and at necropsy. In females, significant decreases of 10-17% in these hematologic parameters were observed at 6.5 mg/kg/day from week 26 until study termination. Activities of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were significantly elevated for both sexes throughout the study at the 6.5 mg/kg/day dose. Gamma-glutamyltranspeptidase activity was increased in male dogs at week 13 by 45%. Absolute and relative liver weight in males and females was elevated by 32% and 49% over control at 6.5 mg/kg/day. Absolute and relative thyroid weight was also increased significantly in females at the 6.5 mg/kg/day dose. Gross stomach lesions consisting of multiple, raised mucosal foci were observed in increased incidence in all treated groups of male and female dogs with only one female control dog showing a similar lesion. Dark, discolored liver was also observed in increased incidence in male and female treated dogs, but a dose-response was observed only for males. Microscopically, increased incidence of lymphocytic mucosal inflammation was observed in the stomach of treated males and females. The lesion was present in all treated and control groups, but the severity of the lesion was increased, especially at the 3.5 and 6.5 mg/kg/day doses.

Although effects of toxicologic relevance were observed at the 1.5 mg/kg/day dose level (increased liver weight in female dogs, increased incidence of pigmentation of the liver, and increased incidence of lymphocytic mucosal inflammation of the stomach in both sexes), and therefore it can be concluded that a NOEL was not established for this study, the 1993 EPA Rejection Rate Document states that "in theory a NOEL would <u>not</u> be necessary if: ...4) the RfD can be determined with the addition of an extra uncertainty factor from a LOEL (lowest-observed-effect-level)." From the effects observed at the 1.5 mg/kg/day dose level, it can be observed that the liver of dogs is also a target organ of pentachlorophenol induced toxicity, similar to that observed in

other species. Thus, the endpoints in this present study can be used to support an RfD. Previously, the RfD was determined from a chronic toxicity study in rats in which pigmentation of the liver and kidneys was observed at a dose of 10 mg/kg/day. As similar effects were observed at a lower dose level in the present study, use of an extra uncertainty factor in determining the RfD would make the present study acceptable for risk characterization purposes.

The present study is considered **acceptable** and satisfies the guideline requirement (§83-1; OPPTS 870.4100) for a chronic toxicity study in non-rodents.

DATA EVALUATION RECORD

PENTACHLOROPHENOL

Study Type: 83-1b; Fifty-Two Week Repeated Dose Chronic Oral Study of Pentachlorophenol Administered via Capsule to Dogs

Work Assignment No. 2-46A (MRID 43982701)

Prepared for
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Disclaimer

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

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DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity [capsule] - dog
OPPTS Number: 870.4100 OPP Guideline Number: §83-1b

DP BARCODE: D225574 SUBMISSION CODE: S504306
P.C. CODE: 063001 TOX. CHEM. NO.: 641

TEST MATERIAL (PURITY): Pentachlorophenol (90.9% a.i.)

SYNONYMS: GLAZD Penta

CITATION: Mecler, F.C. (1996) Pentachlorophenol: Fifty-Two Week Repeated Dose Chronic Oral Study of Pentachlorophenol Administered via Capsule to Dogs. TSI Mason Laboratories, 57 Union Street, Worcester, MA 01608. Laboratory Project Study ID 2-J31. March 27, 1996. MRID 43982701. Unpublished.

SPONSOR: The Pentachlorophenol Task Force, C/O SRA International, Inc., 1850 M Street, N.W., Suite 290, Washington, DC

EXECUTIVE SUMMARY:

In a chronic toxicity study, (MRID 43982701) pentachlorophenol (90.9% a.i.) was fed (gelatin capsules) to four beagle dogs/sex/dose at dose levels of 0, 1.5, 3.5, or 6.5 mg/kg/day for 52 weeks. At 6.5 mg/kg/day, one male and one female dog were sacrificed in extremis on days 247 and 305, respectively, due to significant clinical toxicity (significant weight loss, lethargy, marked dehydration, vomiting, icterus). Group mean body weight in surviving male dogs at the 6.5 mg/kg/day dose was decreased by 15% at week 13, and 21% at study termination. In females, a 19% decrease in group mean body weight was observed at week 13, and bodyweight remained significantly decreased until study termination. Decreased red cell count (16%), hemoglobin (9%), and hematocrit (8%), was observed in males at the 6.5 mg/kg/day dose at week 13. These decreases were also observed at week 26 and at necropsy. In females, significant decreases of 10-17% in these hematologic parameters were observed at 6.5 mg/kg/day from week 26 until study termination. Activities of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were significantly elevated for both sexes throughout the study at the 6.5 mg/kg/day dose. Gamma-glutamyltranspeptidase activity was increased in male dogs at week 13 by 45%. Absolute and relative liver weight in males and females was elevated by 32% and 49% over

control at 6.5 mg/kg/day. Absolute and relative thyroid weight was also increased significantly in females at the 6.5 mg/kg/day dose. Gross stomach lesions consisting of multiple, raised mucosal foci were observed in increased incidence in all treated groups of male and female dogs with only one female control dog showing a similar lesion. Dark, discolored liver was also observed in increased incidence in male and female treated dogs, but a dose-response was observed only for males. Microscopically, increased incidence of lymphocytic mucosal inflammation was observed in the stomach of treated males and females. The lesion was present in all treated and control groups, but the severity of the lesion was increased, especially at the 3.5 and 6.5 mg/kg/day doses.

Although effects of toxicologic relevance were observed at the 1.5 mg/kg/day dose level (increased liver weight in female dogs, increased incidence of pigmentation of the liver, and increased incidence of lymphocytic mucosal inflammation of the stomach in both sexes), and therefore it can be concluded that a NOEL was not established for this study, the 1993 EPA Rejection Rate Document states that "in theory a NOEL would not be necessary if: ...4) the RfD can be determined with the addition of an extra uncertainty factor from a LOEL (lowest-observed-effect-level)."

From the effects observed at the 1.5 mg/kg/day dose level, it can be observed that the liver of dogs is also a target organ of pentachlorophenol induced toxicity, similar to that observed in other species. Thus, the endpoints in this present study can be used to support an RfD. Previously, the RfD was determined from a chronic toxicity study in rats in which pigmentation of the liver and kidneys was observed at a dose of 10 mg/kg/day. As similar effects were observed at a lower dose level in the present study, use of an extra uncertainty factor in determining the RfD would make the present study acceptable for risk characterization purposes.

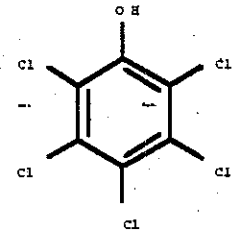
The present study is considered **acceptable** and satisfies the guideline requirement (§83-1; OPPTS 870.4100) for a chronic toxicity study in non-rodents.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Pentachlorophenol
Description: Light brown/tan flakes
Lot/Batch #: EL-064
Purity: 90.9% a.i.
Stability of compound: Not provided
CAS #: 87-86-5
Structure:



2. Vehicle and/or positive control: Gelatin capsules; Eli Lily Corp, size 000, Lot Nos. 6MM06A and 7CH16A

3. Test animals: Species: Dog
Strain: Beagle
Age and weight at study initiation: Approximately 7 months of age; male body weight range, 6.9-10.0 kg; females 6.4-8.9 kg.
Source: Hazleton Research Products, Inc., Cumberland VA.
Housing: Individually housed in stainless steel cages in two separate animal rooms, by sex.
Diet: LabDiet™, PMI Feeds, Inc., Certified Canine Diet 5007, ad libitum during a 2-hour predosing period each day. Diet was periodically supplemented with canned dog food.
Water: Filtered municipal tap water, ad libitum
Environmental conditions:
Temperature: 65-74 °F (males); 65-76 °F (females).
Humidity: 31-66% (males); 31-69% (females)
Air changes: ≥10 per hour
Photoperiod: 12-hour light/dark cycle
Acclimation period: 14-day quarantine period; 14-week pretreatment holding time

B. STUDY DESIGN:

1. In life dates - Start: 04/05/94 End: 04/07/95

2. Animal assignment

Dogs (16/sex) were randomly assigned to the test groups in Table 1 by weight using a computer generated randomization program.

Table 1: Study design

Test Group	Dose to Animal (mg/kg/day)	Animals Assigned	
		Male	Female
1 Control	0	4	4
2 Low	1.5	4	4
3 Mid	3.5	4	4
4 High	6.5	4	4

3. Dose selection rationale

The oral route was selected as the potential route of exposure to humans. The dose levels selected were based on the results of a 90-day subchronic feeding study with dogs and a subsequent discussion with EPA staff in February of 1992. At this meeting, it was agreed that (1): The 10 mg/kg/day dose level induced biologically significant systemic toxicity including fatalities; (2): The 7 mg/kg/day dose induced non-fatal systemic toxicity; and (3) The 3 mg/kg/day dose was the NOEL for systemic non-acute effects. For the chronic study the low dose was selected as the NOEL dose, the mid-dose was selected to produce minimal observable toxic effects, and the high dose was selected to elicit toxic effects but not to produce fatalities.

4. Treatment preparation and dosing

Dose-containing gelatin capsules were prepared weekly and were based on the most recently recorded body weights from weekly weighings. Capsules were prepared from powdered pentachlorophenol flakes, produced using an automated mortar, and stored in an amber bottle. Individual containers for each dog's weekly doses were prepared, labelled with the dog's ID and group numbers, and stored in racks at 22 _ 5 C. Formulation records for assembly of the gelatin capsules were provided by the registrant and showed that the doses administered were within an acceptable range of nominal for each dose group.

5. Statistics

Numerical data obtained during the course of the study were subjected to calculation of group mean values and standard deviations. The equality of means for data from the treatment groups was established using Bartlett's test for homogeneity of variances. If the variances were found to be equal ($p>0.05$), then the data were analyzed by standard one-way ANOVA followed by Dunnett's t-test to reveal differences between the control and treatment groups. If

variances proved to be unequal (heterogenous data), then the data were analyzed by the Kruskal-Wallis test followed by Dunn's summed rank test to determine differences between the control and treatment groups. A 95% confidence level was used as the criteria of statistical significance in this study for all statistical tests performed.

C. METHODS:

1. Observations

Animals were observed once daily for clinical signs of toxicity and twice daily for mortality and moribundity. Clinical observations included changes in the skin and hair, eyes and mucous membranes, respiratory system, circulatory system, central nervous system, somatomotor activity; and occurrence of behavior patterns including tremors, convulsions, salivation, diarrhea, or lethargy.

2. Body weights

Animals were weighed prior to treatment, weekly during Weeks 1-13, every fourth week thereafter until Day 291, and weekly from Day 291 through Day 361. All dogs were weighed prior to necropsy.

3. Food consumption

Food consumption for each animal was measured daily and reported weekly during Weeks 1-13 and every fourth week thereafter. Food consumption for each treatment group was reported as daily food consumption (grams) per week.

4. Ophthalmoscopic examination

Eyes were examined pretreatment, during Weeks 13 and 26, and prior to necropsy. The examinations included macroscopic and ophthalmoscopic observations of the anterior portion of the eye, the optic media, and the ocular fundus.

5. Blood

Following an overnight fat, blood samples (*ca* 4 ml) were collected from all surviving animals, by venipuncture, for hematology and clinical analysis at pretreatment, during Weeks 13, 26, and 39, and prior to necropsy. 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
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(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	Albumin/Globulin ratio A/G
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also		
X	SGOT)*		
	Gamma glutamyl transferase (GGT)		
X	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis

Fasting urine samples were collected from all dogs pretreatment, during Weeks 13, 26, and 39, and prior to autopsy. Samples were obtained via cage pan runoff, cystocentesis, or catheterization. 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)*	X	Nitrites
X	Leukocytes	X	Urobilinoge
X	Protein*		

* 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		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow	X	Spinal cord
X	Stomach*		(femur)*		(3 levels)*
X	Duodenum*	X	Lymph nodes*	X	Pituitary*
X	Jejunum*	X	Spleen*	X	Eyes (optic n.)*
X	Ileum*	X	Thymus*		
X	Cecum*				GLANDULAR
X	Colon*		UROGENITAL		Adrenal gland*
X	Rectum*	XX	Kidneys*+	XX	Lacrimal gland
XX	Liver*+	X	Urinary bladder*		Mammary gland*
X	Gall bladder*	XX	Testes*+	X	Parathyroids*++
X	Pancreas*	X	Epididymides	XX	Thyroids*++
		X	Prostate	XX	Tonsils
	RESPIRATORY		Seminal vesicle	X	
X	Trachea*	XX	Ovaries*+		OTHER
X	Lung*	X	Uterus*		Bone (sternum)*
	Nose	X	Oviducts	X	Skeletal muscle*
X	Pharynx	X	Cervix/Vagina	X	Skin*
X	Larynx			X	All gross lesions
				X	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. Clinical signs of toxicity - No differences in clinical signs were observed between the 1.5 or 3.5 mg/kg/day dosage groups and the controls. Some dogs in the 6.5 mg/kg/day treatment group exhibited increased incidences of lethargy (2M, 2F), inappetence (2M, 2F), emaciation, and dehydration compared to other test groups. With time, this group (6.5 mg/kg/day males and females) showed progressive signs of physical degeneration (pale mucous membranes) and of acute localized effect (gastrointestinal irritation, bleeding, and secondary sequelae).

2. Mortality - One male and one female from the 6.5 mg/kg/day treatment group was euthanized *in extremis* on days 247 and 305, respectively. Both dogs stopped eating, lost a significant amount of weight, and generally exhibited a

precipitous physical decline prior to euthanasia. The unscheduled necropsies of the euthanized animals revealed similar gross and microscopic abnormalities. Both had mottled, discolored livers corresponding to numerous morphologic alterations such as multifocal, moderate hepatocellular swelling and degeneration; fibrosis, and bile duct hyperplasia; and foci of hepatocellular hypertrophy and hyperplasia which were compatible with cirrhosis. Chronic recurrent inflammation, hepatocellular cytoplasmic pigment consistent with lipofuscin and canalicular cholestasis were also observed. Other lesions common to both dogs were significant amounts of abdominal fluid at necropsy, lymphoid depletion in spleen and lymph nodes (microscopically), mucosal hemorrhage involving the intestines and lymph nodes, increased pigment in renal proximal convoluted tubules, and increased extramedullary hematopoiesis. Morbidity was most likely due to hepatic insufficiency indicative of an exaggerated hepatic response to pentachlorophenol at the high dose (6.5 mg/kg/day).

B. Body weights and weight gains

Mean body weights and body weight gains at selected intervals during the 52 week treatment period are shown in Table 2. In males, absolute body weight was decreased at the high dose at week 13, and remained decreased until study termination. Body weight was decreased by 15% at week 13 and was decreased by 21% at study termination. No decrease in absolute body weight was observed at lower doses. In females, a 9% decrease in absolute body weight was observed at the high dose on day 25 of the study, and the decrease in absolute body weight progressed to a 19% decrease by day 95(approximately week 13). From day 95 onward, absolute body weight was significantly decreased at most measured time points in relation to control. In contrast to males, the decrease in absolute body weight in treated females appeared dose-related. At the 3.5 mg/kg/day dose level, absolute body weight in females was decreased between 8-14% vs control, and at the 1.5 mg/kg/day dose, absolute body weight in females was decreased by 4-9% over the duration of the study.

Weight gains in the first 13 weeks of the study and from initiation to 51 weeks are presented in Table 2. The rather small gains in the control groups may be associated with the maturity of the dogs at initiation (7 months of age).

Table 2. Average body weights and body weight gains during 52 weeks if treatment with pentachlorophenol.[a]

Dose rate (mg/kg/day)	Body Weights (kg)					Total Weight Gain (kg)	
	Day 0[b]	Day 95	Day 172	Day 284	Day 361	Weeks 0-13	Weeks 0-51
Male							
0	8.8	9.8	9.9	10.2	10.1	1.0	1.3
1.5	8.3	9.7	9.5	9.6	9.7	1.4	1.4
3.5	8.6	9.3	9.3	9.5	9.5	0.7	0.9
6.5	8.5	8.5	8.5	8.5	8.3	0	-0.2
Female							
0	7.6	9.1	8.8	9.4	9.6	1.5	2.0
1.5	7.5	8.5	8.5	8.7	8.7	1.0	1.2
3.5	7.5	8.1	7.9	8.5	8.4	0.5	0.8
6.5	7.4	7.4*	7.3	7.2*	7.7*	0.2	0.5

a Data obtained from pages 65-72 in the study report.
b Prior to treatment.
* Significantly different ($p<0.05$) from the controls.

C. Feed consumption

In males, food consumption at the high dose was increased in relation to control, between 5-20% over the course of the study. In females, increased food consumption was also observed at the high dose beginning at approximately week 41. Prior to week 41, food consumption in the high dose females was decreased vs control. The sudden increase in food consumption for high dose females is not readily explained. The mention of feed supplementation during the study may be relevant, if this is when supplementation began for female dogs. In any case, the data suggest that food efficiency could have been affected in high dose females.

D. Ophthalmoscopic examination

No ocular changes were observed during the treatment period.

E. Blood work

1. Hematology - At week 13, decreased red cell count (16%), hemoglobin (9%), and hematocrit (8%) were observed in male dogs at the 6.5 mg/kg/day dose level. Decreases in these parameters appeared dose-related for male dogs at all time points measured. At week 26, a 26% decrease in red cell count, 21% decrease in hemoglobin, and a 17% decrease in hematocrit were observed in high dose male dogs. At week 26, decreases in red cell count were statistically significant for the mid and high dose males, while the decrease in hemoglobin was significant for high dose males only. At necropsy, statistically significant decreases in red cell count were still observed for male dogs at the 3.5 and 6.5 mg/kg/day dose levels (15% and 22% respectively) as well as for hemoglobin in high dose males (17%).

In females, there were no significant decreases in red cell count, hemoglobin, or hematocrit at week 13 for any treatment level. Elevated lymphocyte count, was, however, observed at week 13 (46% increase). From week 26 to study termination, significant decreases in red cell count, hemoglobin, and hematocrit were observed in female dogs at the high dose. The decrease ranged from 10-17% for all these parameters. Elevation of lymphocytes was also observed at week 26 but was not statistically significant due to variability among dogs.

In contrast to males, there did not appear to be a dose-related pattern to hematologic effects. For both sexes, the 1.5 mg/kg/day dose level had no effect on hematology. Other statistically significant differences between control and 6.5 mg/kg/day females were decreased relative polymorphonuclear neutrophil counts and elevated lymphocyte counts at Week 13 as well as statistically different decreases in relative polymorphonuclear neutrophil counts and increased lymphocyte counts at Week 39 for 3.5 mg/kg/day females.

Table 3. Red blood cell, hemoglobin, and hematocrit levels in male dogs at intervals during 52 weeks of dosing with pentachlorophenol.[a]

Weeks of Dosing	Dose level (mg/kg/day)			
	0	1.5	3.5	6.5
Red Blood Cells (x 10^6/mm^3)				
0[b]	7.11	7.02	6.91	6.63
13	7.41	7.15	6.97	6.28
26	7.76	6.90	6.62*	5.92*
39	7.68	6.93	6.66	6.13
52	7.50	7.27	6.39*	5.91*
Hemoglobin (g/dL)				
0	15.6	15.8	15.6	15.1
13	16.5	16.8	16.6	15.1
26	17.6	16.1	15.7	14.0*
39	17.0	15.6	15.5	14.7
52	16.8	16.9	15.0	14.1*
Hematocrit (%)				
0	46.5	46.7	45.9	45.1
13	48.2	49.0	47.9	44.4
26	50.8	47.4	46.2	42.2
39	51.9	47.6	47.6	45.4
52	50.7	51.3	46.0	44.5

a Data obtained from Table 4, pages 79-88, in the study report.
b Prior to treatment.
* Statistically different ($p<0.05$) from the controls.

Table 4. Red blood cell, hemoglobin, and hematocrit levels in female dogs at intervals during 52 weeks of dosing with pentachlorophenol.[a]

Weeks of Dosing	Dose level (mg/kg/day)			
	0	1.5	3.5	6.5
Red Blood Cells (x 10^6/mm^3)				
0[b]	6.84	7.03	7.51	7.16
13	6.90	7.22	6.81	6.64
26	6.98	7.10	7.10	6.15*
39	6.95	7.25	6.68	5.76*
52	7.09	6.83	6.98	6.97
Hemoglobin (g/dL)				
0	15.6	16.1	17.2	16.5
13	16.5	17.1	16.3	15.7
26	16.8	17.0	16.9	14.7*
39	16.4	16.9	15.5	13.7*
52	16.7	16.2	16.5	16.3
Hematocrit (%)				
0	46.4	47.4	50.6	48.3
13	47.8	50.0	47.6	46.6
26	48.6	49.3	49.5	43.8*
39	49.7	51.8	48.3	41.9*
52	51.1	49.8	51.6	51.4

a Data obtained from Table 4, pages 89-98, in the study report.
b Prior to treatment.
* Statistically different ($p<0.05$) from the controls.

2. Clinical chemistry - Male dogs exhibited elevated serum levels of alkaline phosphatase (ALK) at all sampling intervals for every treatment group, as compared to controls, and in a dose-related manner (Table 5). At week 13, increases of 48%, 68%, and 184% in the activity of ALK were observed for males. Alanine aminotransferase (ALT) levels were also elevated at the 3.5 and 6.5 mg/kg/day dose levels at week 13 (32% and 58%, respectively). GGT was observed to be elevated at the 6.5 mg/kg/day dose level at week 13 (75% increase). At subsequent sampling intervals, increases in ALK, ALT, and AST were still observed in male dogs at the high dose.

Similar patterns of enzyme changes were observed for ALT, AST, and ALK in female dogs. For example, ALT at 1.5 mg/kg/day was elevated by 700% vs control, while AST was elevated by 75% at the high dose. ALK was increased by 84% at week 13 at the high dose. At week 26, the response of female dogs to treatment was similar as for week 13. It is noted that the activity of ALT in females pre-treatment was elevated in the high dose group vs control. Other statistically significant values, but of questionable biological significance, were increased BUN for 6.5 mg/kg/day females at Week 26; decreased 6.5 mg/kg/day albumin and 3.5 and 6.5 mg/kg/day potassium at Week 39; and increased chloride at Week 39.

Table 5. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALK) levels in male dogs at intervals during 52 weeks of dosing with pentachlorophenol.[a]

Weeks of Dosing	Dose level (mg/kg/day)			
	0	1.5	3.5	6.5
ALT (U/L)				
0[b]	39	46	37	42
13	38	37	129	224
26	37	42	162	335
39	40	45	168	212
52	42	55	117	164
AST (U/L)				
0	43	33	37	40
13	31	29	41	49
26	36	32	50	63
39	39	57	67	55
52	36	32	43	45
ALK (U/L)				
0	90	95	90	93
13	45	67	76	128
26	37	81	84	232
39	40	101	87	145
52	40	74	90	197

a Data obtained from Table 5, pages 99-108, in the study report.
b Prior to treatment.

Table 6. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALK) levels in female dogs at intervals during 52 weeks of dosing with pentachlorophenol.[a]

Weeks of Dosing	Dose level (mg/kg/day)			
	0	1.5	3.5	6.5
ALT (U/L)				
0[b]	44	32	38	75
13	38	31	60	304
26	44	37	118	312
39	40	28	121	430
52	37	31	114	325
AST (U/L)				
0	45	35	39	49
13	32	35	36	56*
26	43	38	46	59
39	31	30	33	79
52	39	35	41	65*
ALK (U/L)				
0	98	77	75	89
13	51	55	65	94
26	38	56	60	118*
39	42	64	64	147*
52	34	63	90	232*

a Data obtained from Table 5, pages 109-118 in the study report.
b Prior to treatment.
* Statistically different ($p<0.05$) from the controls.

F. Urinalysis - No treatment-related differences were noted in any urinary parameter measured.

G. Sacrifice and Pathology

1. Organ weights - Liver weights in treated males were elevated vs control by 10%, 31%, and 32%, respectively, at the 1.5, 3.5, and 6.5 mg/kg/day dose levels. Relative liver weights were also increased. (Table 7). Thyroid weight in males was increased by 16% and 9% at the 1.5 and 3.5 mg/kg/day dose, but not at the 6.5 mg/kg/day dose. Relative thyroid weights were increased at all treatment levels when compared to controls, but the differences were not statistically significant. Males in the 6.5 mg/kg/day treatment group also exhibited significantly increased relative adrenal weights compared to controls.

Table 7. Mean absolute and relative weights of the livers and thyroids of rats after 52 weeks of treatment with pentachlorophenol.[a]

Dose rate (mg/kg/day)	Organ Weights			
	Absolute liver (g)	Relative liver (% body wt)	Absolute thyroid (g)	Relative thyroid (% body wt)
Male				
0[b]	267.68	2.804	0.8542	0.0089
1.5	295.76	3.210*	0.9949	0.0106
3.5	351.63	3.889*	0.9287	0.0104
6.5	354.48	4.664*	0.7856	0.0103
Female				
0[b]	232.11	2.612	0.5252	0.0058
1.5	288.50*	3.593*	0.8123	0.0100*
3.5	282.94*	3.660*	0.7297	0.0095
6.5	346.73*	5.074*	0.9285*	0.0138*

a Data obtained from Table 6, pages 119-120, in the study report.
b Prior to treatment.
* Statistically different ($p<0.05$) from the controls.

In females, absolute liver weight was increased by 24%, 21%, and 49% at the 1.5, 3.5, and 6.5 mg/kg/day dose levels. These increases were identified as statistically significant. Females also exhibited higher absolute and relative thyroid weights in all treatment groups, with increases of 55%, 38%, and 77% at the 1.5, 3.5, and 6.5 mg/kg/day dose levels, respectively. Females treated at the 6.5 mg/kg/day dose showed decreases in absolute brain weights.

2. Gross pathology - Gross lesions were found in the stomachs, livers, and kidneys of animals in the 1.5, 3.5, and 6.5 mg/kg/day treatment groups. The stomachs of most dogs treated with pentachlorophenol had multiple, raised mucosal foci which ranged from 1-3 mm in diameter. Gross stomach lesions were observed in 2/4, 3/4, and 2/3 males in the 1.5, 3.5, and 6.5 mg/kg/day treatment groups, respectively, and in 2/4, 4/4, and 2/3 females in the respective 1.5, 3.5, and 6.5 mg/kg/day treatment groups. Only one control (female) animal had similar gross lesions involving the stomach.

Male dogs exhibited dark, discolored livers in 1/4, 1/4, and 3/3 of the 1.5, 3.5, and 6.5 mg/kg/day treatment groups, respectively. Females exhibited more severe dark, discolored livers in 3/4, 3/4, and 2/3 of the respective 1.5, 3.5, and 6.5 mg/kg/day treatment groups.

3. Microscopic pathology

a) Non-neoplastic - Several different treatment-related morphologic alterations were observed in livers of males and females treated with 1.5, 3.5, or 6.5 mg/kg/day of pentachlorophenol (Table 8).

Table 8. Liver histopathology incidence and severity for terminal animals.[a]

Lesion	Females/(mg/kg/day)				Males/(mg/kg/day)			
	0	1.5	3.5	6.5	0	1.5	3.5	6.5
# examined	4	4	4	3	4	4	4	3
Pigment	0	4 (2.3)[a]	4 (2.8)	3 (3.3)	0	4 (3)	4 (3)	3 (3.3)
Cytoplasmic vacuolation	3 (1)	3 (2)	4 (2.3)	3 (3.3)	1 (3)	1 (2)	4 (2.8)	3 (3.3)
Minimum necrosis	0	0	0	2	0	0	0	1
Chronic inflammation	2 (1)	2 (1.5)	4 (1.8)	3 (1.7)	0	4 (1)	4 (1.3)	3 (1.3)

a Data obtained from Table 5; pages 45-46 in the study report.
b The values in parentheses are grades of lesion:1-minimum; 2-mild; 3-moderate; 4-marked.

In the male (1/4) and female (1/4) dogs receiving 6.5 mg/kg/day that were sacrificed moribund there were numerous histologic lesions in the livers. Multifocal, moderate hepatocellular swelling and degeneration of hepatocytes were observed as well as fibrosis, bile duct hyperplasia, foci of hepatocellular hypertrophy and hyperplasia

consistent with cirrhosis. Chronic inflammation, accumulation of pigment consistent with lipofuscin and canalicular cholestasis were also seen.

Increased amounts of a yellow/brown globular pigment were observed in the cortexes of kidneys for most dogs in the 3.5 and 6.5 mg/kg/day treatment groups.

A significant increase in lymphocytic mucosal inflammation was observed in microscopic sections from stomachs of males and female dogs treated with 1.5, 3.5, or 6.5 mg/kg/day of pentachlorophenol, compared to controls.

Increased extramedullary hematopoiesis (EMH) was observed in several males and females in the 3.5 and 6.5 mg/kg/day treatment groups. The increased EMH was minimal to mild in these dogs and was characterized by discrete aggregates of megakaryocytes and other cell types consistent with erythropoiesis.

b) Neoplastic - No neoplastic tissue was observed in dogs in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the NOEL, for daily administration of pentachlorophenol to beagle dogs for 52 weeks, was 3.5 mg/kg in both males and females and that administration of the test article at 6.5 mg/kg/day resulted in adverse morphologic effects to livers of both male and female dogs. The author also concluded that administration of the test article via capsules to dogs for 52 weeks was associated with gross and microscopic lesions in livers, kidneys, and stomachs of male and female dogs in the 1.5, 3.5 and 6.5 mg/kg/day treatment groups, but that adverse morphologic effects were limited only to dogs in the 6.5 mg/kg/day treatment group and were accompanied by several serum chemistry alterations indicative of hepatic disease.

Daily oral administration of pentachlorophenol was associated with lipofuchsin in liver and kidneys and cytoplasmic hepatocellular vacuolation in dogs from the 1.5, 3.5, or 6.5 mg/kg treatment groups. The report stated that lipofuschin is a known product of the metabolism of chlorinated phenols and is an expected response. Lymphocytic inflammation of the gastric mucosa, often occurring as grossly observable foci corresponding to discrete lymphoid follicles, was present in most test article-treated dogs.

B. Reviewer's Discussion

In the present study, male and female beagle dogs were administered pentachlorophenol in gelatin capsules at doses of 0, 1.5, 3.5, and 6.5 mg/kg/day for 52 weeks. Significant toxicologic effects were observed at the 6.5 mg/kg/day dose level, which consisted of decreased body weight in male and female dogs, moribund sacrifice of 1 male and 1 female dog, hematological effects (decreased red cell count, hemoglobin, and hematocrit), elevated serum enzymes associated with liver function (alkaline phosphatase, aspartate and alanine aminotransferase, and gamma-glutamyl transpeptidase), increased absolute and relative liver weight [both sexes] and thyroid weight [females only], and macroscopic and microscopic alterations of the liver (pigmentation, necrosis, vacuolation), kidney (yellow/brown pigment deposition), and stomach (lymphocytic mucosal inflammation). At the 3.5 mg/kg/day dose level, many of these effects were also evident, but no mortality occurred. It is noted that the increased alkaline phosphatase activity observed especially at the low dose could be artefactual, based on a decreased control activity of this enzyme after 13 weeks of treatment. Whether activity was elevated at the start of the study or whether some factor in the conduct of the study contributed to a decline after 13 weeks of treatment with pentachlorophenol cannot be answered. It is further noted with respect to the percentage changes in the hematologic and clinical chemistry parameters that changes were calculated on the basis of comparison to concurrent controls. This would tend to lead to a slightly exaggerated representation, as comparison of the treated groups to historical baseline values would lead to results showing less severe alterations. In either event, the basis for determination of the systemic LOEL was not based upon changes in hematology or clinical chemistry.

At the 1.5 mg/kg/day dose level, toxicologic effects (in the form of increased liver weight in female dogs and increased incidence of microscopic pathology of the stomach of males and females) were observed. These effects, of themselves, appear to constitute relevant toxicologic effects, insofar as the liver and kidney pigmentation have been previously observed in long-term studies with pentachlorophenol, and have, in fact, formed the basis for establishment of the Reference Dose. In addition, effects on the liver of other species (enzyme induction, tumor production) have also been observed. Thus, although the 1.5 mg/kg/day dose produced adverse effects and the study would not be considered to have a NOEL, the 1993 EPA Rejection Rate Document states that "in theory a NOEL would <u>not</u> be necessary if: ...4) the RfD can be determined with the addition of an extra uncertainty factor from a LOEL (lowest-observed-effect-level)." From the effects observed at the 1.5 mg/kg/day dose level, it is apparent that effects similar to that observed in other species occur in dogs at a lower dose level. Thus, if this study is used in selection of the RfD, a supplemental safety factor could be employed to determine the RfD and to satisfy the guideline requirement for a chronic toxicity study in non-

rodents for pentachlorophenol. The reviewer supports the inclusion of this study for consideration of the RfD for pentachlorophenol.

The present study is **acceptable** and satisfies the guideline requirement (§83-1; 870.4100) for a chronic toxicity study in non-rodents.

IV. STUDY DEFICIENCIES

Originally, data on the intake of test chemical was not provided in the report. A request by the reviewer for these data was fulfilled by the registrant, and the data show that the intake of test article was within acceptable limits of the reported nominal dose levels.