

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §82-1b, 90-Day Oral Toxicity Study in Dogs

Work Assignment No. 1-01-35 G (MRID 45710206)

Prepared for
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DATA EVALUATION RECORD**TXR#:** 0052493**STUDY TYPE:** Subchronic Oral Toxicity in Dogs (diet); OPPTS 870.3150 [§82-1b]; OECD 409.**PC CODE:** 081901**DP BARCODE:** 301496**SUBMISSION NO.:** None**TEST MATERIAL (PURITY):** Chlorothalonil technical (99.18% a.i.)**SYNONYMS:** 2,4,5,6-tetrachloro-1,3-benzo-dicarbonitrile**CITATION:** Spencer-Briggs, D.J. (1994) Toxicity to dogs by dietary administration for 13 weeks. Huntingdon Life Sciences Ltd., Huntingdon, England. Laboratory Study No.: VCM 12/920413, November 4, 1994. MRID 45710206. Unpublished.**SPONSOR:** Vischim S.r.l., Via Friuli, 20031 Cesano Maderno, Milan, Italy**EXECUTIVE SUMMARY** - In a subchronic oral study (MRID 45710206), Chlorothalonil (99.18% a.i., Lot/batch #: 71) was administered to 4 beagle dogs/sex/dose in the diet at nominal doses of 0, 160, 1600, or 16,000 ppm (equivalent to 0/0, 5.6/6.1, 56.5/61.0, and 597/570 mg/kg/day) for 13 weeks. There were no treatment-related effects on survival, hematology, ophthalmoscopy, or gross pathology.

At 16,000 ppm, body weights were decreased throughout treatment in both sexes, resulting in a decrease of 167-188% ($p \leq 0.01$) in overall body weight gains. Weekly food consumption was decreased by 3-29% in the females throughout treatment, resulting in decreased mean overall food consumption of 14%.

At ≥ 1600 ppm, in the liver, minimal foci of necrotic hepatocytes with inflammatory cell infiltration was observed (vs 0 controls) in the males (1 to 2 of 4 dogs). Also, in the liver at ≥ 1600 ppm, minimal parenchymal foci of inflammatory cells was noted in both sexes (1 to 3 of 4 dogs each treated). Adjusted liver weights were increased by 23-25% ($p \leq 0.05$) in the females at these doses. Albumin was decreased ($p \leq 0.05$) by 5% in both sexes at 1600 ppm at Week 13 and additionally by 13-21% at 16,000 ppm at Weeks 6 and 13.

Alanine aminotransferase (ALT) was severely decreased (decr. 68-90%; $p \leq 0.01$) in all treated groups of both sexes at Week 13. ALT was also decreased at Week 6 in the 16,000 ppm males

(<1 mU/mL treated vs 3 mU/mL controls; $p \leq 0.01$) and in the ≥ 1600 ppm females (decr. $\geq 90\%$; $p \leq 0.05$). Note that even the controls were decreased at Week 6 (3-10 mU/mL) compared to prior to treatment (Week -2). It was stated that normal ALT levels were attained when a pre-incubation stage was incorporated into the assay using pyridoxal-5'-phosphate (IFCC reagent) and that the initial decreases were attributed to a decrease in pyridoxal-5'-phosphate, a cofactor necessary for the full expression of ALT activity in addition to the metabolism of cysteine conjugates of β -lyase. The reduction in ALT activity was therefore considered not to be an indication of toxicity but a consequence of pyridoxal-5'-phosphate depletion by β -lyase during the metabolism of Chlorothalonil. However, no data were provided for the levels measured from the assay incorporating this pre-incubation stage with pyridoxal-5'-phosphate. Furthermore, no explanation was given for the reduction in ALT in the control animals at Week 6. Additionally at 16,000 ppm, non-protein thiol concentration was increased ($p \leq 0.05$) compared to controls in the liver in the females (incr. 280%). It was stated that this finding may have been due to the conjugation of the test substance with glutathione. Additionally at this dose at Weeks 6 and 13, total protein was decreased (decr. 7-13%; $p \leq 0.05$) in both sexes, and cholesterol was increased (incr. 32-56%; $p \leq 0.05$) in the males.

At ≥ 1600 ppm, trace to minimal brown pigment was observed (vs 0 controls) in the epithelium of the cortical tubules of the kidneys in both sexes (1 to 3 of 4 dogs). Thiol concentration was decreased prior to alkali hydrolysis in the males at this dose (decr. 64-100%; $p \leq 0.05$). Additionally at 16,000 ppm, urinalysis showed increased protein in the females (incr. 91%; $p \leq 0.01$), and thioether concentration (per μ mole creatinine) was increased in the males for the 0-8 hour urine sample (incr. 69%; $p \leq 0.01$).

In the adrenals at 16,000 ppm, increased width of the zona glomerulosa was noted in the females (3 of 4 dogs vs 0 controls), and absolute adrenal weights were increased (incr. 21%; $p \leq 0.05$) in the males.

The LOAEL for this study is 1600 ppm (equivalent to 56.5/61.0 mg/kg/day in M/F) based on minimal foci of necrotic hepatocytes with inflammatory cell infiltration in males, and minimal parenchymal foci of inflammatory cells in both sexes. The NOAEL is 160 ppm (equivalent to 5.6/6.1 mg/kg/day).

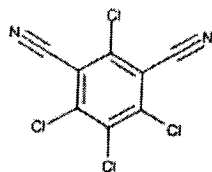
This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3150; OECD 409) for a 90-day oral toxicity study in the dog.

COMPLIANCE - Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided. A Flagging statement was provided but was not signed or dated.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Chlorothalonil Technical
- Description:** White powder
- Lot/Batch #:** 71
- Purity (w/w):** 99.18% a.i.
- Stability of compound:** Stable in the diet for up to 10 days at room temperature
- CAS #:** 1897-45-6
- Structure:**



2. Vehicle - Diet

3. Test animals

- Species:** Dog
- Strain:** Beagle
- Age/mean weight at initiation of treatment:** 31-33 weeks/ 9.5-13.3 kg for males and 9.7-12.3 kg for females
- Source:** Interfauna UK Limited, Abbots Ripton Road, Wyton, Huntingdon, UK
- Housing:** In stainless steel cages with sawdust bedding; in pairs from 5 p.m. to 9 a.m. and separated individually by a partition from 9 a.m. to 5 p.m.
- Diet:** Standard ground dry Diet A (Special Diet Services Ltd); 400 g/day presented in the morning and any uneaten food removed at 5 p.m. and recorded.
- Water:** Tap water, *ad libitum* except during urine collection in which water was withheld beginning 5 hours prior to overnight (16-hour) collection
- Environmental conditions**
- Temperature:** 15-24°C
- Humidity:** Not reported
- Air changes:** Approximately 12/hr
- Photoperiod:** 12 hrs light/12 hrs dark
- Acclimation period:** At least 4 weeks

B. STUDY DESIGN

1. **In life dates** - Start: 10/16/91 End: 01/16/92
2. **Animal assignment** - The dogs were randomly assigned, stratified by body weight, to the test groups shown in Table 1. The inclusion of litter mates in the same treatment group was avoided, when possible.

Table 1. Study design ^a

Test Group	Dose (ppm)	Achieved dose (mg/kg/day) in M/F	# of Animals (M/F)
Control	0	0/0	4/4
Low	160	5.6/6.1	4/4
Mid	1600	56.5/61.0	4/4
High	16,000	597/570	4/4

^a Data were obtained from pages 17 and 44 of the study report.

3. **Dose selection rationale** - It was stated that treatment levels were chosen following a 4-week preliminary study performed at Huntingdon Research Center in England (HRC Report # VCM 12/911435). No further data were provided.

4. **Dose preparation and administration** - Each week, the appropriate amount of test substance was mixed with diet to form a pre-mix, and a second pre-mix was made by mixing a portion of the first pre-mix with diet. The required dietary concentrations were prepared by dilution of one of these two pre-mixes with additional diet. Dietary formulations were stored at room temperature until use. Homogeneity (top, middle, bottom) and stability of the test substance in the diet for up to 10 days at room temperature were confirmed at 10, 2500, and 16,000 ppm. Homogeneity was also determined at 160 ppm. Concentration of the test substance in the diet was verified for each dose level during Weeks 1, 6, and 13 of the study.

Results

Homogeneity (coefficient of variation): 1.8-4.8%

Stability (% initial concentration after 10 days at room temperature): 95-103%

Concentration (% nominal): 91-99%

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

5. **Statistics** - Body weight gains, food consumption, clinical pathology, and organ weight data were analyzed as follows:

- If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analyzed by appropriate methods. Otherwise,
- Bartlett's test for homogeneity of variances was performed followed by analysis of variance (ANOVA) and Student's t-test (pre-dose data) or Williams' test (treatment period data) if variances were homogeneous or by Kruskal-Wallis test and Shirley's test for pair-wise comparison with controls if variances were heterogeneous ($p \leq 0.01$). A logarithmic transformation was attempted, if necessary, to achieve homogeneous variances.
- Additionally for organ weight data, analysis of covariance (ANCOVA) was performed using

the terminal body weight as covariate when the within-group relationship between organ weight and body weight was significant ($p \leq 0.10$).

The statistics were considered appropriate.

C. METHODS

1. **Observations**: All animals were checked for mortality and clinical signs of toxicity regularly throughout the working day from 9 a.m. to 5 p.m. On weekends and holidays, dogs were examined regularly from 9 a.m. to 12 p.m. with a final daily check at approximately 5 p.m..

2. **Body weight** - All animals were weighed (before feeding) prior to initiation of treatment, weekly throughout the study, and at termination.

3. **Food consumption, food efficiency, and test substance intake** - Food consumption was measured daily for each dog throughout the study, and group mean food consumption was reported for each week (g) and for the overall (Weeks 1-13) study. Food efficiency was not reported. Group mean test substance intake (mg/kg/day) was calculated for each week and for the overall study using the weekly food consumption and body weight data and the nominal dose.

4. **Ophthalmoscopic examination** - Ophthalmoscopic examinations were conducted on all dogs prior to initiation of treatment and during Week 13, using an indirect ophthalmoscope after dilation of the pupils with Tropicamide ophthalmic solution.

5. **Hematology and clinical chemistry** - Blood samples for hematology and clinical chemistry analyses were collected from the jugular or cephalic vein of each dog prior to initiation of treatment and during Weeks 6 and 13 of treatment. Food was removed overnight before collection of blood samples. The following CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Cell morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90 day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry

ELECTROLYTES		OTHER	
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose (fasting)*
ENZYMES		X	Total bilirubin*
X	Alkaline phosphatase (ALP)*	X	Total protein *
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyltransferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90 day oral non-rodent studies based on Guideline 870.3150

6. Urinalysis - Urine samples were collected from each dog prior to treatment and during Weeks 6 and 13 of treatment. Water was withheld beginning 5 hours prior to the start of collection, and food and water were unavailable during the overnight (16-hour) collection period. The CHECKED (X) parameters were examined. Additionally, urine samples were collected from all animals prior to treatment, on Day 1, and during Weeks 5, 9, and 13 for the measurement of urinary thiol, thioether, and creatinine concentrations. For these samples, dogs were placed in metabolism cages with access to food and water, and the collection of urine was split into two periods over 24 hours (0-8 hours and 8-24 hours).

	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity*		Bilirubin
X	pH*		Blood/blood cells*
X	Sediment (microscopic)		Nitrite
X	Protein*	X	Urobilinogen
		X	Total reducing substances
		X	Bile pigments
		X	Heme pigments

* Recommended for 90 day oral non-rodent studies based on Guideline 870.3150

7. Sacrifice and pathology - At study termination, all animals were sacrificed via exsanguination under pentobarbitone anesthesia, weighed, and subjected to a gross necropsy. Prior to necropsy, bone marrow was obtained from each dog by sternal puncture; and a smear was prepared, stained, and examined. The following CHECKED (X) tissues were collected,

routinely processed, stained with hematoxylin and eosin, and examined microscopically. Additionally, the (XX) organs were weighed.

Additional sections of liver were stained for fat with Oil Red O (ORO) or for glycogen with periodic acid Schiff reagent (PAS). Non-protein thiol (mainly glutathione) concentration was determined in fresh liver and kidney samples of animals sacrificed at study termination according to a modification of a method of Ellman (1959).

DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC	
X	Tongue	X	Aorta, thoracic*	XX	Brain*+
XX	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*	XX	Spleen*+	X	Eyes (with optic nerve)*
X	Jejunum*	XX	Thymus*+	GLANDULAR	
X	Ileum*	UROGENITAL		XX	Adrenal gland*+
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	XX	Parathyroid*+
X	Rectum*	XX	Testes*+	X	Thyroid*
XX	Liver*+	X	Epididymides*+	OTHER	
X	Gall bladder*+	XX	Prostate (with urethra)*	X	Bone (sternum and/or femur)
XX	Pancreas*	XX	Ovaries*+	X	Skeletal muscle
RESPIRATORY		XX	Uterus*+	X	Skin*
X	Trachea*	X	Mammary gland*	X	All gross lesions and masses*
XX	Lungs (with bronchii)*	X	Vagina		
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for 90 day oral non-rodent studies based on Guideline 870.3150

+ Organ weight required for non-rodent studies.

a Testes and epididymides were weighed together, although microscopic evaluation was only reported for testes.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity** - Vomiting was observed during the first few days of treatment for 3/4 males and 4/4 females at 16,000 ppm and for 3 or 4/4 females at 1600 ppm. For the remainder of the study, vomiting was only noted on a further 3 occasions in these animals. There were no other treatment-related clinical signs. Liquid feces was noted in all treatment groups but was similar in incidence to the controls and was not dose-related in frequency.

2. **Mortality** - All animals survived to scheduled sacrifice.

B. BODY WEIGHT AND WEIGHT GAIN - Body weights were decreased (statistics not performed) throughout treatment in the 16,000 ppm males (14-14%) and females (18-16%), resulting in significantly decreased (167-188%; $p \leq 0.01$) body weight gains for the overall (Weeks 0-13) study (Table 2). There were no other treatment-related differences in body weights or body weight gains.

Table 2. Selected mean body weights and cumulative body weight gains (kg) in dogs treated with Chlorothalonil in the diet for up to 13 weeks ^a

Study week	Dose (ppm)			
	0	160	1600	16,000
Males				
0	11.6	11.2	11.2	11.4
2	11.3	11.1	11.1	10.8 (14)
13	12.5	12.1	11.7	10.8 (114)
Weeks 0-13 weight gain	0.9	0.9	0.5	-0.6** (1167)
Females				
0	10.8	10.6	10.6	10.6
1	10.7	10.4	10.4	9.8 (18)
13	11.6	11.0	10.9	9.8 (116)
Weeks 0-13 weight gain	0.8	0.3	0.4	-0.7** (1188)

^a Data were obtained from Table 1 on page 42 of the study report; n = 4. Percent difference from controls, calculated by the reviewers, is included in parentheses.

** Significantly different from the controls at $p \leq 0.01$.

C. FOOD CONSUMPTION - At 16,000 ppm, weekly food consumption was decreased in the 16,000 ppm females throughout treatment (13-29%; not significant [NS]), resulting in decreased (114%; NS) mean food consumption for the overall (Weeks 1-13) study (Table 3). There were no other treatment-related differences in food consumption. In the males, minor decreases of less than 2% compared to controls were observed in all treated groups during Week 1 and in the 160 ppm males during Week 13.

Table 3. Selected mean weekly and overall food consumption (g) in dogs treated with Chlorothalonil in the diet for up to 13 weeks ^a

Study week	Dose (ppm)			
	0	160	1600	16,000
Males				
1	2800	2738 (12)	2778 (1<1)	2758 (12)
13	2800	2753 (12)	2800	2800
1-13 (Overall)	2800	2792 (1<1)	2798 (1<1)	2796 (1<1)
Females				
1	2800	2800	2800	1980 (129)
11	2800	2800	2800	2715 (13)
13	2800	2800	2800	2465 (112)
1-13 (Overall)	2800	2800	2800	2403 (114)

^a Data were obtained from Table 1 on page 43 of the study report; n = 4. Percent difference from controls, calculated by the reviewers, is included in parentheses.

D. OPHTHALMOSCOPIC EXAMINATION - No treatment-related effects were noted during the ophthalmoscopic examinations.

E. BLOOD ANALYSES

1. Hematology - There were no treatment-related differences in hematology. At Week 13, the number of monocytes were increased in the 16,000 ppm females (210/mm³) compared to 0 controls. However, this finding was considered unrelated to treatment because there were no differences in the total number of leukocytes in this group, and because a comparable increase was noted in the 16,000 ppm males prior to treatment. All other hematological parameters were comparable to controls. Furthermore, bone marrow smears showed no abnormalities in cellularity, distribution, or morphology.

2. Clinical chemistry - Selected clinical chemistry findings are presented in Table 4. Total protein was decreased (17-13%; $p \leq 0.05$) at Weeks 6 and 13 in both sexes at 16,000 ppm. Albumin was decreased in both sexes at 1600 ppm at Week 13 (17%; $p \leq 0.05$) and additionally at 16,000 ppm at Weeks 6 and 13 (113-21%; $p \leq 0.05$). Cholesterol was increased (132-56%; $p \leq 0.05$) in the 16,000 ppm males at Weeks 6 and 13. Alanine aminotransferase (ALT) was severely decreased (168-90%; $p \leq 0.01$) all treated groups of both sexes at Week 13. ALT was also decreased at Week 6 in the 16,000 ppm males (<1 mU/mL treated vs 3 mU/mL controls; $p \leq 0.01$) and in the ≥ 1600 ppm females (1290%; $p \leq 0.05$). There were no other treatment-related effects on clinical chemistry.

Table 4. Selected mean clinical chemistry parameters in male dogs treated with Chlorothalonil in the diet for up to 13 weeks ^a

Data were obtained for up to 15 weeks					
Parameter	Week	Dose (ppm)			
		0	160	1600	16,000
Males					
Total protein (g/dL)	-2	5.2	5.1	5.3	5.2
	6	5.0	5.1	5.0	4.6* (18)
	13	5.5	5.3	5.1	4.8** (113)
Albumin (g/dL)	-2	2.7	2.6	2.8	2.4
	6	2.7	2.8	2.7	2.3* (115)
	13	2.9	2.9	2.7* (17)	2.3** (121)
Alanine aminotransferase (mU/mL)	-2	18	20	28	21
	6	3	4	2	<1**
	13	19	6** (168)	3** (184)	2** (189)
Cholesterol (mg/dL)	-2	140	150	141	147
	6	121	134	152	160* (132)
	13	121	132	152	189** (156)
Females					
Total protein (g/dL)	-2	5.3	5.2	5.2	5.3
	6	5.2	5.3	5.1	4.7** (110)
	13	5.5	5.3	5.2	5.1* (17)
Albumin (g/dL)	-2	2.8	2.8	2.7	2.8
	6	2.8	3.0	2.8	2.4** (114)
	13	3.0	3.0	2.8* (17)	2.6** (113)
Alanine aminotransferase (mU/mL)	-2	22	22	26	21
	6	10	3	1* (190)	<1** (1>90)
	13	21	4** (181)	3** (186)	2** (190)

^a Data were obtained from Table 6a and 6b on pages 49-50 of the study report; n = 4. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Significantly different from the controls at $p \leq 0.05$.

** Significantly different from the controls at $p \leq 0.01$.

F. URINALYSIS - At Week 13, protein was increased (191%; $p \leq 0.01$) in the 16,000 ppm females (Table 5a). Thiol concentration was decreased (164-100%; $p \leq 0.05$) prior to alkali hydrolysis in the ≥ 1600 ppm males at Day 1 and Week 13 (Table 5b). Additionally in the 16,000 ppm males, thioether concentration (per μ mole creatinine) was increased for the 0-8 hour urine sample (169%; $p \leq 0.01$). There were no other findings in urinalysis which could be attributed to treatment.

Table 5a. Protein (mg/dL) in the urine of female dogs treated with Chlorothalonil in the diet for up to 13 weeks (food and water withheld) ^a

Week	Dose (ppm)			
	0	160	1600	16,000
-3	28	20	30	20
6	28	25	37	38
13	23	27	29	44** (191)

^a Data were obtained from Table 7b on page 53 of the study report; n = 4. Percent difference from controls, calculated by the reviewers, is included in parentheses.

** Significantly different from the controls at $p \leq 0.01$.

Table 5b. Selected mean 24-hour urinalysis parameters from male dogs treated with Chlorothalonil in the diet after 13 weeks (with access to food and water) ^a

Parameter	Time (hours)	Dose (ppm)			
		0	160	1600	16,000
Thiol concentration Pre-alkali hydrolysis (nmoles Nac cys equivs/mL urine)	0-8	24.5	40.8	18.3	1.7* (193)
	8-24	36.6	38.9	13.2* (164)	0** (1100)
Thioether concentration (nmoles thiol/ μ mol creatinine)	0-8	44.9	61.6	56.7	76.1** (169)
	8-24	NA	NA	NA	NA

^a Data were obtained from Summary Table 1 in Addendum 2 on page 172 of the study report; n = 4. Percent difference from controls, calculated by the reviewers, is included in parentheses.

NA Not applicable

* Significantly different from the controls at $p \leq 0.05$.

** Significantly different from the controls at $p \leq 0.01$.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - Adjusted liver weights were increased (123-25%; $p \leq 0.05$) in the ≥ 1600 ppm females (Table 6). In the 16,000 ppm males, absolute adrenal weights were increased (121%; $p \leq 0.05$). All other organ weights in the treated groups were comparable to controls.

Table 6. Selected mean organ weights (g) in dogs treated with Chlorothalonil in the diet for 13 weeks ^a

Organ	Dose (ppm)			
	0	160	1600	16,000
Males				
Adrenals - absolute	1.51	1.57	1.65	1.82* (121)
Females				
Liver - adjusted	338.2	348.3	422.0** (125)	415.1* (123)

^a Data were obtained from Table 8a and 8b on pages 55-56 of the study report; n = 4. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Significantly different from the controls at $p \leq 0.05$.

** Significantly different from the controls at $p \leq 0.01$.

2. **Gross pathology** - There were no macroscopic findings that could be attributed to treatment.

3. **Microscopic pathology** - Selected microscopic findings are presented in Table 7 (# dogs affected/4 vs 0/4 controls). In the liver, minimal foci of necrotic hepatocytes with inflammatory cell infiltration was observed in the ≥ 1600 ppm males (1 to 2), and minimal parenchymal foci of inflammatory cells was noted in the ≥ 1600 ppm males (3 each treated) and females (1 to 3). In the kidneys, trace to minimal brown pigment was observed in the epithelium of the cortical tubules in the ≥ 1600 ppm males (2 to 3) and females (1 to 3). In the adrenals, increased width of the zona glomerulosa was noted in the 16,000 ppm females (3). The only finding at 160 ppm was minimal parenchymal foci of inflammatory cells in the liver in one male. There were no other microscopic findings that could be attributed to treatment.

Table 7. Selected microscopic findings in dogs (# affected) treated with Chlorothalonil in the diet for 13 weeks ^a

Microscopic finding			Dose (ppm)			
			0	160	1600	16,000
Males						
Liver	Foci of necrotic hepatocytes with inflammatory cell infiltration -	Minimal	0	0	1	2
	Parenchymal foci of inflammatory cells -	Minimal	0	1	3	3
Kidneys	Brown pigment in the epithelium of cortical tubules -	Total	0	0	2	3
		Trace	0	0	2	2
		Minimal	0	0	0	1
Females						
Liver	Parenchymal foci of inflammatory cells -	Minimal	0	0	1	3
Kidneys	Brown pigment in the epithelium of cortical tubules -	Trace	0	0	1	3
Adrenals	Increased width of zona glomerulosa		0	0	0	3

^a Data were obtained from Table 9 on pages 58-67 of the study report; n = 4.

3. **Non-protein thiol concentration** - Non-protein thiol concentration was increased ($p \leq 0.05$) compared to controls in the liver in the 16,000 ppm females (1280%; Table 8).

Table 8. Non-protein thiol concentration (expressed as a fraction of the control group mean) in selected organs of dogs treated with Chlorothalonil in the diet for 13 weeks ^a

Organ	Dose (ppm)		
	160	1600	16,000
Males			
Liver	1.7	1.6	2.8
Kidney	1.3	1.1	1.7
Females			
Liver	1.0	1.3	2.8*
Kidney	1.6	1.5	2.2

^a Data were obtained from Summary Table 3 in Addendum 2 on page 174 of the study report; n = 4.

* Significantly different from the controls at $p \leq 0.05$.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - It was concluded that the LOAEL was 16,000 ppm based on decreased body weight, body weight gains, and food consumption and on microscopic pathology in the adrenals. This level was considered too high for long term administration (i.e., in the chronic study). At 1600 ppm, adrenal pathology was noted but was not accompanied by decreased body weights or food consumption. There were no effects of treatment at 160 ppm.

B. REVIEWER COMMENTS - There were no treatment-related effects on survival, hematology, ophthalmoscopy, or gross pathology.

Vomiting was observed during the first few days of treatment for 3/4 males and 4/4 females at 16,000 ppm and for 3 or 4/4 females at 1600 ppm. For the remainder of the study, vomiting was only noted on a further 3 occasions in these animals. Although dose-related, the fact that this finding was transient suggests that it was probably due to initial problems with palatability of the test substance in the diet instead of a systemic toxic response.

At 16,000 ppm, body weights were decreased throughout treatment in both sexes, resulting in significantly decreased ($\downarrow 167$ -188%; $p \leq 0.01$) overall body weight gains. Weekly food consumption was decreased in the females throughout treatment ($\downarrow 13$ -29%), resulting in decreased mean overall food consumption ($\downarrow 14$ %).

At ≥ 1600 ppm, in the liver, minimal foci of necrotic hepatocytes with inflammatory cell infiltration was observed (vs 0 controls) in the males (1 to 2 of 4 dogs), and minimal parenchymal foci of inflammatory cells was noted in both sexes (1 to 3 of 4 dogs each treated). It is important to note that the severity of these microscopic findings was minimal; thus, they are unlikely to be adverse. Adjusted liver weights were increased ($\uparrow 23$ -25%; $p \leq 0.05$) in the females at these doses. Albumin was decreased in both sexes at 1600 ppm at Week 13 ($\downarrow 7$ %; $p \leq 0.05$) and additionally at 16,000 ppm at Weeks 6 and 13 ($\downarrow 13$ -21%; $p \leq 0.05$).

Additionally at 16,000 ppm, non-protein thiol concentration was increased ($p \leq 0.05$) compared to controls in the liver in the females ($\uparrow 280$ %). It was stated that this finding may have been due to the conjugation of the test substance with glutathione. Additionally at this dose at Weeks 6 and 13, total protein was decreased ($\downarrow 7$ -13%; $p \leq 0.05$) in both sexes, and cholesterol was increased ($\uparrow 32$ -56%; $p \leq 0.05$) in the males.

Alanine aminotransferase (ALT) was severely decreased ($\downarrow 68$ -90%; $p \leq 0.01$) in all treated groups of both sexes at Week 13. ALT was also decreased at Week 6 in the 16,000 ppm males (< 1 mU/mL treated vs 3 mU/mL controls; $p \leq 0.01$) and in the ≥ 1600 ppm females ($\downarrow \geq 90$ %; $p \leq 0.05$). Note that even the controls were decreased at Week 6 (3-10 mU/mL) compared to prior to treatment (Week -2). It was stated that normal ALT levels were attained when a pre-incubation stage was incorporated into the assay using pyridoxal-5'-phosphate (IFCC reagent) and that the initial decreases were attributed to a decrease in pyridoxal-5'-phosphate, a cofactor necessary for the full expression of ALT activity in addition to the metabolism of cysteine conjugates by β -lyase. The reduction in ALT activity was therefore considered not to be an indication of toxicity

but a consequence of pyridoxal-5'-phosphate depletion by β -lyase during the metabolism of Chlorothalonil. However, no data were provided for the levels measured from the assay incorporating this pre-incubation stage with pyridoxal-5'-phosphate. Furthermore, no explanation was given for the reduction in ALT in the control animals at Week 6.

At ≥ 1600 ppm, trace to minimal brown pigment was observed (vs 0 controls) in the epithelium of the cortical tubules of the kidneys in both sexes (1 to 3 of 4 dogs). Thiol concentration was decreased prior to alkali hydrolysis in the males at this dose (164-100%; $p \leq 0.05$). Additionally at 16,000 ppm, urinalysis showed increased protein in the females (191%; $p \leq 0.01$), and thioether concentration (per μ mole creatinine) was increased in the males for the 0-8 hour urine sample (169%; $p \leq 0.01$).

In the adrenals at 16,000 ppm, increased width of the zona glomerulosa was noted in the females (3 of 4 dogs vs 0 controls), and absolute adrenal weights were increased (121%; $p \leq 0.05$) in the males.

The LOAEL for this study is 1600 ppm (equivalent to 56.5/61.0 mg/kg/day in M/F) based on minimal foci of necrotic hepatocytes with inflammatory cell infiltration in males, and minimal parenchymal foci of inflammatory cells in both sexes. The NOAEL is 160 ppm (equivalent to 5.6/6.1 mg/kg/day).

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

C. STUDY DEFICIENCIES - The following minor deficiencies were noted but do not alter the conclusions of this DER:

- Standard deviations were not presented with the summary data.
- No data were provided for the ALT levels measured from the assay incorporating this pre-incubation stage with pyridoxal-5'-phosphate, in which the Sponsor stated that normal levels were attained.
- The Flagging statement was not signed or dated.
- Mean corpuscular hemoglobin (MCH) and blood serum sorbitol dehydrogenase (SDH) were not measured.
- Urine appearance and the presence of occult blood in urine were not reported.
- The parathyroid glands, epididymides and gall bladder were not weighed.
- The nose, pharynx, and larynx were not examined microscopically.