DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §83-1b, Chronic Toxicity Study in Dogs

Work Assignment No. 1-01-35 H (MRID 45710210)

Prepared for
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DATA EVALUATION RECORD

TXR#: 0052493

STUDY TYPE: Chronic Toxicity in Dogs (diet); OPPTS 870.4100b [§83-1b]; OECD 452.

PC CODE: 081901 **DP BARCODE:** 301496
SUBMISSION NO.: None

TEST MATERIAL (PURITY): Chlorothalonil technical (99.28% a.i.)

SYNONYMS: 2,4,5,6-tetrachloro-1,3-benzo-dicarbonitrile

CITATION: Spencer-Briggs, D.J. (1995) Toxicity to dogs by repeated dietary administration for 52 weeks. Huntingdon Life Sciences Ltd., Huntingdon, England. Laboratory Study No.: VCM 14/943124, December 21, 1995. MRID 45710210. Unpublished.

SPONSOR: Vischim S.r.l., Via Friuli, 20031 Cesano Maderno, Milan, Italy

EXECUTIVE SUMMARY - In a chronic oral toxicity study (MRID 45710210), 4 beagle dogs/sex/dose were given Chlorothalonil (99.28% a.i., Lot/Batch # NF 28/01) in the diet at nominal doses of 0, 160, 1280, or 10,240 ppm (equivalent to 0/0, 5.10/5.92, 43.26/45.30, and 374/354 mg/kg/day [M/F] for up to 52 weeks.

There were no effects of treatment on ophthalmoscopy, hematology, or urinalysis.

Vasodilatation of the gums and/or ears was noted in all of the 10,240 ppm males and females compared to 0 controls, with males affected for 33-84 days and females affected for 30-191 days. With the exception of a single female, this effect tapered off after 26 weeks.

One 10,240 ppm female (#370) was killed for humane reasons on the first day of Week 31, and the death was considered to be treatment-related. The following were noted in this animal prior to death: vasodilatation of the gums, later followed by paleness of gums; decreased body weight, food consumption, red cell indices, and albumin; reticulocytosis; and increased total globulin. Post-mortem examination revealed microscopic changes in the heart, spleen, bone marrow, stomach, liver, and kidneys.

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At 10,240 ppm, body weights were decreased (decr 4-11%; statistics not performed) in the males beginning at Week 2, resulting in significantly decreased (p<=0.05) cumulative body weight gains beginning at Week 8 (decr 36-47%) and an overall (Weeks 0-52) body weight gain decrease of 44% compared to controls. In the females at this dose, cumulative body weight gains were decreased (p<=0.01) early in treatment, with decreases of 50% compared to controls at Week 4 and decreases of 38% at Week 8. However, overall body weight gains were comparable to controls.

At 10,240 ppm, weekly food consumption was decreased during the first six months of the study in the males and generally throughout treatment in the females, with greater decreases in the females (decr <=18% compared to controls) than in the males (decr <=8%). Average food consumption for the overall study was decreased in both sexes by 2% compared to controls, with the difference attaining significance (p<=0.05) in the females.

Cholesterol was increased (incr 7-48%) in the >=1280 ppm males throughout treatment. Total protein was decreased (decr 8-14%; p<=0.05) at Weeks 39 and 52 in the >=1280 ppm males and in the 10,240 ppm females. Albumin was decreased (decr 7-20%; p<=0.05) at 10,240 ppm in both sexes throughout treatment. Alkaline phosphatase was increased (incr 39-68%) throughout treatment in the 10,240 ppm males.

Alanine aminotransferase (ALT) was severely decreased (p<=0.01) in all treated groups of both sexes, with average values of <1 to 3 mU/mL in the treated groups compared to 18-27 mU/mL in the controls. 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 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.

Non-protein thiol concentration was increased (p<=0.05) compared to controls in the: (i) liver and kidneys of the 10,240 ppm males (incr 30-40%); (ii) kidneys in the >=160 ppm females (incr 30-80%); (iii) liver in the >=1280 ppm females (incr 60%); and (iv) stomach in the 10,240 ppm females (incr 20%). It was stated that these increases are likely due to the conjugation of Chlorothalonil with glutathione in these organs.

At >=1280 ppm, absolute liver weights in the males and absolute and adjusted liver weights in the females were increased (incr 9-21%), with the adjusted values in the females attaining significance. Increased absolute (incr 27%) and adjusted (incr 47%; p<=0.05) thyroid weights were noted in the 10,240 ppm males. However, there were no macroscopic or microscopic findings to corroborate an effect of treatment in either of these organs.

Brown pigment was observed in the epithelium of the cortical tubules of the kidneys in the males at >=1280 ppm (4/4 each treated vs 1/4 controls) and in the females at 1280 ppm (4/4 treated vs

2/4 controls) and 10,240 ppm (3/3 treated); however, the severity was trace to minimal. Increased incidence of moderate hypertrophy of the cells of the zona fasciculata was noted in the adrenals of the 10,240 ppm males (4/4 treated vs 1/4 controls).

Gross and microscopic findings in the stomach were evident at >=1280 ppm in both sexes. Thickened appearance of the stomach, often with a catarrhal adhesion to the mucosal surface, was noted macroscopically in both sexes at >=1280 ppm. The following microscopic findings were observed in the stomach (vs 0 controls): (i) prominent apoptotic bodies in the antrum in both sexes at >=1280 ppm; (ii) erosion of the lumenal surface of the epithelium in both sexes at 10,240 ppm; (iii) increased thickness of the mucosa with cellular hypertrophy in the >=1280 ppm males and in the 10,240 ppm females; (iv) congestion of the capillaries of the superficial mucosa, congestion of the submucosal vessels, mucus and cell debris adherent to the lumenal surface, and foci of mineralization in the mucosa in the >=1280 ppm males; and (v) inflammatory cell infiltration into mucosa in the >=1280 ppm females.

The only findings at 160 ppm included the following microscopic evidence of stomach irritation observed in 1/4 males (per finding): (i) congestion of the submucosal vessels; (ii) mucus and cell debris adherent to the lumenal surface; and (iii) foci of mineralization in the mucosa.

The LOAEL is 1280 ppm (equivalent to 43.3/45.3 mg/kg/day in M/F) based on macroscopic and microscopic pathological findings in the stomach including: thickened appearance of the stomach, often with a catarrhal adhesion to the mucosal surface, and prominent apoptotic bodies in the antrum of males and females; mucus and cell debris adherent to lumenal surface and foci of mineralization in the stomach mucosa of males. The NOAEL is 160 ppm (equivalent to 5.10/5.92 mg/kg/day in M/F).

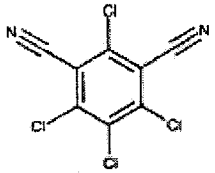
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chlorothalonil

Description:	White powder
Batch/Lot #:	NF 28/01
Purity (w/w):	99.28% a.i.
Stability:	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/weight at initiation of treatment:	24-29 weeks old; 8.0-10.8 kg
Source:	Interfauna UK Ltd, Abbots Ripton Rd, Wyton, Huntingdon, UK
Housing:	In kennels with sawdust bedding; in pairs from 5 p.m. to 9 a.m. and separately 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:	15-26°C
Humidity:	Not reported
Air changes:	Approximately 12/hr
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	12 weeks

B. STUDY DESIGN

1. In life dates - Start: 09/15/93 End: 09/19/94

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	5.10/5.92	4/4
Mid	1280	43.26/45.30	4/4
High	10,240	374/354	4/4

a Data were obtained from pages 18 and 57 of the study report.

3. **Dose selection rationale** - The doses summarized in Table 1 were selected for the current study based on the results of a concurrently submitted 90-day study (MRID 45710206). In the 90-day study, Chlorothalonil was administered to 4 beagle dogs/sex/dose in the diet at nominal doses of 0, 160, 1600, or 16,000 ppm for 13 weeks. The LOAEL for the subchronic study was 16,000 ppm (equivalent to 597/570 mg/kg/day in M/F) based on decreased body weights and body weight gains in both sexes and decreased food consumption in females. Only minor findings occurred at 1600 ppm, but were not considered adverse, including: (i) minimal parenchymal foci of inflammatory cells in the liver; (ii) increased adjusted liver weights (↑25%; $p \leq 0.05$) in the females; (iii) decreased (↓5%; $p \leq 0.05$) albumin in both sexes at Week 13; and (iv) trace to minimal brown pigment in the epithelium of the cortical tubules of the kidneys in both sexes.

4. **Dose preparation and administration** - Each week, the appropriate amount of test substance was mixed with diet to form a pre-mix, and the required dietary concentrations were prepared by dilution of this pre-mix 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 in a concurrently submitted 90-day study (MRID 45710206) at 10, 2500, and 16,000 ppm. Homogeneity was also determined at 160 ppm in the subchronic study. Concentration of the test substance in the diet was verified for each dose level during Weeks 1, 6, 13, 26, 39, and 52 of the current study.

Results

Homogeneity (coefficient of variation): 1.8-4.8%

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

Concentration (% nominal): 94-104%

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 according to Fisher (1950) and Mantel (1963). Otherwise,

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	5.10/5.92	4/4
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4. **Treatment preparation and administration** - Each week, the appropriate amount of test substance was mixed with diet to form a pre-mix, and the required dietary concentrations were prepared by dilution of this pre-mix 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 in a concurrently submitted 90-day study (MRID 45710206) at 10, 2500, and 16,000 ppm. Homogeneity was also determined at 160 ppm in the subchronic study. Concentration of the test substance in the diet was verified for each dose level during Weeks 1, 6, 13, 26, 39, and 52 of the current study.

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- 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 according to Fisher (1950) and Mantel (1963). Otherwise,

- Barlett's test for homogeneity of variances was performed followed by analysis of variance (ANOVA) and Student's t-test or Williams' test if variances were homogeneous or by Kruskal-Wallis test and a non-parametric 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/week). Food efficiency was not reported. Group mean test substance intake (mg/kg/day) was calculated for each week and for the overall (Weeks 1-52) 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 Weeks 13, 26, 39, and 52, 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 vein of each dog prior to initiation of treatment and during Weeks 13, 26, 39, and 52 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* (PLT)	X	Reticulocyte count
	Blood clotting measurements*	X	Cell morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100

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	Globulin
X	Sodium*	X	Glucose (fasting)*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein *
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)	X	Ornithine carbamoyltransferase (OCT)
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyltransferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for chronic studies based on Guideline 870.4100

6. Urinalysis - Urine samples were collected from each dog prior to treatment and during Weeks 13, 26, 39, and 52 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.

	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity*		Bilirubin
X	pH*		Occult blood*
X	Sediment (microscopic)		Nitrites
X	Protein*	X	Urobilinogen
		X	Total reducing substances
		X	Bile pigments
		X	Heme pigments

* Recommended for chronic studies based on Guideline 870.4100

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 using a modified Wrights stain, 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 periodic acid Schiff reagent (PAS). Non-protein thiol concentration was determined according to the method of Boyland and Chasseaud (1970) in fresh liver, kidney, and stomach samples from animals sacrificed at study termination. In the female sacrificed intercurrently (#370), sections of the liver and kidney were also stained with Perl's stain for iron.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (with optic nerve)*
X	Jejunum*	XX	Thymus [b]		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	XX	Thyroid*
XX	Liver*+	XX	Testes*+[a]		OTHER
X	Gall bladder*	XX	Epididymides*+[a]	X	Bone (sternum and/or femur)
XX	Pancreas*	XX	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	XX	Uterus*+	X	All gross lesions and masses*
XX	Lungs*++	X	Mammary gland*		
	Nasal structures*		Cervix		
	Pharynx*	X	Vagina		
	Larynx*				

* Required for chronic studies based on Guideline 870.4100.
\+ Organ weight required in chronic studies.
++ Organ weight required if inhalation route.
a The testes and epididymides were weighed together, although microscopic evaluation was only reported for testes.
b The thymus was weighed when present.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity** - Vasodilatation of the gums and/or ears was noted in all of the 10,240 ppm males and females compared to 0 controls, with males affected for 33-84 days and females affected for 30-191 days (Table 2). With the exception of a single female (#372), this effect tapered off after 26 weeks. Incidences and frequency of vasodilatation in the 160 and 1280 ppm animals were minimal. There were no other treatment-related clinical signs. Vomiting was noted in all treatment groups but was not dose-related in incidence or frequency.

Table 2. Incidences of vasodilatation in dogs fed 10,240 ppm Chlorothalonil in the diet for up to 52 weeks [a]

Animal #	Weeks				
	1-13	14-26	27-39	40-52	1-52 (Total) [b]
Males					
365	37	15	0	0	52
367	30	34	0	0	64
369	40	44	3	0	87
371	22	11	0	0	33
Females					
366	44	33	1	0	78
368	14	17	2	0	33
370	24	6	0	NA	30
372	70	78	42	1	191

a Data were summed by the reviewers from individual data in Appendix 1 on pages 125-128 of the study report.
b Total number of incidences for each dog were summed by the reviewers from data presented in this table.
NA Not applicable because female #370 was sacrificed in extremis during Week 31.

2. Mortality - One 10,240 ppm female (#370) was killed for humane reasons on the first day of Week 31. Vasodilatation of the gums was noted in this animal from Week 2; however, from Week 22, the gums were noted to be pale. Progressive weight loss was recorded from Week 16, with a 28% body weight loss between Week 16 and 22. Food consumption was decreased in this animal from Week 17. Hematology and clinical chemistry analyses revealed markedly lower red cell indices, reticulocytosis, increased total globulin, and a slight reduction in albumin concentration. Histopathological examination revealed changes in the heart (myocardial degeneration, intramyofibrillar hemorrhage and edema, and hemorrhage into myocardium), spleen (marked extramedullary hemopoiesis), bone marrow (myeloid hyperplasia), stomach (congestion of the capillaries of the superficial mucosa, mucus and cell debris adherent to the lumenal surface, and foci of mineralization in the mucosa), liver (moderate focal necrosis, pigmented macrophages/Kupffer cells/hepatocytes, inflammatory cell infiltration into the portal areas, extramedullary hematopoiesis, fat droplets in some areas of necrosis, and minimal generalized fat deposits in the hepatocytes), and kidneys (moderate brown pigment in the epithelium of cortical tubules). All other dogs survived to scheduled termination.

B. BODY WEIGHT AND WEIGHT GAIN - Body weights were decreased (↓4-11%; statistics not performed) in the 10,240 ppm males beginning at Week 2, resulting in significantly decreased ($p<0.05$) cumulative body weight gains beginning at Week 8 (↓36-47%) and an overall (Weeks 0-52) body weight gain decrease of 44% compared to controls (Table 3). In the females at this dose, body weights were decreased in all treated groups compared to controls at study initiation; thus, it is difficult to ascertain an effect of treatment by examining body weights. Cumulative body weight gains were decreased ($p\leq0.01$) in the high dose females at Week 4 (↓50%) and Week 8 (↓38%); however, overall body weight gains were comparable to controls. There were no other treatment-related differences in body weights or body weight gains. In the 1280 ppm males, cumulative body weight gains were decreased at Week 26 (↓36%) and Week 39 (↓33%). However, the decreased weight gain only resulted in minor decreases in body weights (≤3% compared to controls) during the second half of the study.

Table 3. Selected mean body weights and cumulative body weight gains (kg) in dogs treated with Chlorothalonil in the diet for up to 52 weeks [a]

Study week	Dose (ppm)			
	0	160	1280	10,240
Males				
0	9.5	10.3	10.2	9.5
2	9.8	10.6	10.7	9.4
13	11.4	12.4	11.8	10.5
52	12.6	13.4	12.4	11.2
Weeks 0-8 weight gain	1.5	1.5	1.1	0.8* (↓47)
Weeks 0-13 weight gain	2.0	2.1	1.6	1.1* (↓45)
Weeks 0-52 weight gain	3.2	3.1	2.2	1.8* (↓44)
Females				
0	10.0	9.2	9.4	9.6
13	11.8	10.5	11.0	10.9
52	12.6	11.6	12.0	12.1
Weeks 0-4 weight gain	0.6	0.5	0.6	0.3** (↓50)
Weeks 0-8 weight gain	1.3	0.9	1.3	0.8** (↓38)
Weeks 0-13 weight gain	1.8	1.3	1.6	1.3
Weeks 0-52 weight gain	2.6	2.4	2.6	2.8

a Data were obtained from Table 1 on page 50-51 of the study report; n = 4, except in the 10,240 ppm females after Week 31, where n = 3. 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 $p \leq 0.01$.

C. FOOD CONSUMPTION - At 10,240 ppm, weekly food consumption was decreased during the first six months of the study in the males and generally throughout treatment in the females, with greater decreases in the females (↓ ≤18% compared to controls) than in the males (↓ ≤8%; Table 4). Average food consumption for the overall (Weeks 1-52) study was decreased in both sexes by 2% compared to controls, with the difference attaining significance ($p \leq 0.05$) in the females. There were no other treatment-related differences in food consumption.

Table 4. Selected mean weekly and overall food consumption (g) in dogs treated with Chlorothalonil in the diet for up to 52 weeks [a]

Study week	Dose (ppm)			
	0	160	1280	10,240
Males				
3	2800	2800	2800	2580 (↓8)
27	2800	2800	2800	2753 (↓2)
1-52 (Overall)	2800	2800	2800	2758 (↓2)
Females				
1	2800	2800	2800	2300 (↓18)
13	2783	2800	2800	2738 (↓2)
26	2800	2800	2678	2458 (↓12)
39	2763	2800	2800	2673 (↓3)
52	2800	2800	2668	2683 (↓4)
1-52 (Overall)	2796	2800	2777	2728* (↓2)

a Data were obtained from Table 2 on page 54-55 of the study report; n = 4, except in the 10,240 ppm females after Week 31, where n = 3. Percent difference from controls, calculated by the reviewers, is included in parentheses.

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

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

E. BLOOD ANALYSES

1. Hematology - At 10,240 ppm, mean corpuscular hemoglobin concentration (MCHC) was decreased (3-4%; $p \leq 0.05$) at Weeks 26 and 52 in the males and at Week 52 in the females. Additionally in the females at this dose, mean corpuscular volume (MCV) was increased (↑5%; $p \leq 0.05$). However, these differences were considered toxicologically unimportant because they were minor. Significant differences from controls in all other parameters were considered unrelated to treatment because they were transient, occurred prior to treatment, and/or were not dose related. Furthermore, bone marrow smears showed no abnormalities in cellularity, distribution, or morphology.

2. Clinical chemistry - Selected clinical chemistry findings are presented in Tables 5a and 5b. Total protein was decreased (↓8-14%; $p \leq 0.05$) at Weeks 39 and 52 in the ≥1280 ppm males and in the 10,240 ppm females. Total protein was also decreased (↓7-8%; $p \leq 0.05$) in the 160 and 1280 ppm females at Week 39; however, these decreases were considered unrelated to treatment because they were transient. Albumin was decreased (↓7-20%; $p \leq 0.05$) at 10,240 ppm in both sexes throughout treatment. Alkaline phosphatase was increased (↑39-68%) throughout treatment in the 10,240 ppm males, with increases attaining significance ($p \leq 0.05$) at Weeks 26 and 52. Cholesterol was increased (↑7-48%) in the ≥1280 ppm males throughout treatment, with increases attaining significance at 1280 ppm at Week 13 and at 10,240 ppm at Weeks 13, 26, and

52. Alanine aminotransferase (ALT) was severely decreased ($p \leq 0.01$) in all treated groups of both sexes, with average values of <1 to 3 mU/mL in the treated groups compared to 18-27 mU/mL in the controls. There were no other treatment-related effects on clinical chemistry.

Table 5a. Selected mean clinical chemistry parameters in male dogs treated with Chlorothalonil in the diet for up to 52 weeks [a]

Parameter	Week	Dose (ppm)			
		0	160	1280	10,240
Total protein (g/dL)	-2	4.9	5.0	5.1	5.1
	13	5.0	5.2	5.1	4.7
	26	5.3	5.3	5.2	5.1
	39	5.7	5.3	5.1* (111)	4.9** (114)
	52	5.2	5.2	4.8* (18)	4.7** (110)
Albumin (g/dL)	-2	2.5	2.5	2.6	2.5
	13	2.6	2.7	2.5	2.3* (112)
	26	2.6	2.7	2.6	2.3* (112)
	39	2.7	2.8	2.6	2.2** (119)
	52	2.7	2.7	2.5	2.2** (119)
Alkaline phosphatase (mU/mL)	-2	261	237	244	290
	13	206	174	201	306 (149)
	26	156	135	144	262* (168)
	39	146	114	121	203 (139)
	52	130	119	125	189* (145)
Alanine aminotransferase (mU/mL)	-2	24	20	17	23
	13	27	3**	1**	<1**
	26	25	3**	<1**	<1**
	39	24	3**	<1**	<1**
	52	27	4**	1**	<1**
Cholesterol (mg/dL)	-2	106	114	121	119
	13	95	115	132** (139)	141** (148)
	26	109	111	130 (117)	137* (126)
	39	115	112	141 (123)	142 (123)
	52	109	111	128 (117)	153** (140)

a Data were obtained from Table 6 on pages 75-78 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 $p \leq 0.01$.

Table 5b. Selected mean clinical chemistry parameters in female dogs treated with Chlorothalonil in the diet for up to 52 weeks [a]

Parameter	Week	Dose (ppm)			
		0	160	1280	10,240
Total protein (g/dL)	-2	5.0	5.0	5.0	5.2
	13	5.4	5.3	5.3	5.3
	26	5.8	5.5	5.5	5.6
	39	5.9	5.4* (↓8)	5.5* (↓7)	5.3* (↓10)
	52	5.7	5.5	5.6	5.0* (↓12)
Albumin (g/dL)	-2	2.6	2.7	2.6	2.8* (↑8)
	13	2.8	2.9	2.7	2.6** (↓7)
	26	3.0	2.9	2.7	2.4** (↓20)
	39	3.0	2.9	2.8	2.6** (↓13)
	52	3.0	2.9	2.9	2.6** (↓13)
Alanine aminotransferase (mU/mL)	-2	21	27	22	27
	13	21	3**	1**	<1**
	26	18	3**	<1**	<1**
	39	20	3**	<1**	<1**
	52	21	3**	1**	<1**

a Data were obtained from Table 6 on pages 79-82 of the study report; n = 4, except at 10,240 ppm after Week 31, where n = 3. 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 - There were no treatment-related effects on any urinalysis parameter. A minor decrease in specific gravity was noted in the 10,240 ppm males at Week 39; however, this parameter was similarly decreased prior to treatment. Decreased (↓86%; $p \leq 0.01$) urine volume was noted in the 10,240 ppm females at Week 26; however, this finding was considered unimportant because it was transient. All other significant differences ($p \leq 0.05$) from controls occurred prior to treatment.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - At ≥1280 ppm, absolute liver weights in the males and absolute and adjusted liver weights in the females were increased (↑9-21%), with the adjusted values in the females attaining significance (Table 6). Increased absolute (↑27%) and adjusted (↑47%; $p \leq 0.05$) thyroid weights were noted in the 10,240 ppm males. 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 52 weeks [a]

Organ		Dose (ppm)			
		0	160	1280	10,240
Males					
Liver -	absolute	396.1	392.8	436.6 (110)	445.5 (112)
Thyroid -	absolute	0.89	0.90	0.94	1.13 (127)
	adjusted	0.86	0.80	0.95	1.26* (147)
Females					
Liver -	absolute	382.4	378.8	449.0 (117)	415.5 (19)
	adjusted	370.3	390.5	449.1* (121)	416.0* (112)

a Data were obtained from Table 8 on pages 93-95 of the study report; n = 4, except in the 10,240 ppm females, where n = 3. Percent difference from controls, calculated by the reviewers, is included in parentheses.

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

2. Gross pathology - Thickened appearance of the stomach, often with a catarrhal adhesion to the mucosal surface, was noted at 1280 ppm (2/8 dogs) and 10,240 ppm (4/7 dogs). There were no other macroscopic findings that could be attributed to treatment.

3. Microscopic pathology - Selected microscopic findings are presented in Table 7. Trace to minimal brown pigment was observed in the epithelium of the cortical tubules of the kidneys in the males at ≥1280 ppm (4/4 each treated vs 1/4 controls) and in the females at 1280 ppm (4/4 treated vs 2/4 controls) and 10,240 ppm (3/3 treated). Increased incidence of minimal to moderate hypertrophy of the cells of the zona fasciculata was noted in the adrenals of all treatment groups in the males (2-4/4 treated vs 1/4 controls); however, the incidences at 160 and 1280 ppm (2/4 each treated vs 1/4 controls) were only increased by one animal over controls, and the severity was minimal.

Incidences of the following microscopic findings were observed in the stomach (vs 0/4 controls): (i) prominent apoptotic bodies in the antrum in the males at ≥1280 ppm (4/4 each treated) and in the females at 1280 (3/4) and 10,240 (2/3) ppm; (ii) erosion of the lumenal surface of the epithelium at 10,240 ppm in the males (1/4) and females (1/3); (iii) increased thickness of the mucosa with cellular hypertrophy in the males at 1280 (1/4) and 10,240 (3/4) ppm and in the females at 10,240 ppm (3/3); (iv) congestion of the capillaries of the superficial mucosa in the 1280 (2/4) and 10,240 (1/4) ppm males; (v) congestion of the submucosal vessels in the 160 (1/4), 1280 (1/4), and 10,240 (3/4) ppm males; (vi) mucus and cell debris adherent to the lumenal surface in the 160 (1/4), 1280 (4/4), and 10,240 (4/4) ppm males; (vii) foci of mineralization in the mucosa in the 160 (1/4), 1280 (2/4), and 10,240 (2/4) ppm males; and (viii) inflammatory cell infiltration into mucosa in the 1280 (2/4), and 10,240 ppm (2/3) females. 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 52 weeks [a]

Microscopic finding			Dose (ppm)			
			0	160	1280	10,240
Males						
Kidneys	Brown pigment in the epithelium of cortical tubules -	Total	1	2	4	4
		Trace	1	1	1	2
		Minimal	0	1	3	2
Adrenals	Hypertrophy of cell of zona fasciculata -	Total	1	2	2	4
		Minimal	1	2	2	0
		Moderate	0	0	0	4
Stomach	Prominent apoptotic bodies in the antrum		0	0	4	4
	Erosion of lumenal surface of epithelium		0	0	0	1
	Increased thickness of the mucosa with cellular hypertrophy		0	0	1	3
	Congestion of the capillaries of the superficial mucosa		0	0	2	1
	Congestion of the submucosal vessels		0	1	1	3
	Mucus and cell debris adherent to lumenal surface		0	1	4	4
	Foci of mineralization in the mucosa		0	1	2	2
	Mucosal edema		0	0	0	1
Females						
Kidneys	Brown pigment in the epithelium of cortical tubules -	Total	2	2	4	3
		Trace	2	2	1	1
		Minimal	0	0	3	2
Stomach	Prominent apoptotic bodies in the antrum		0	0	3	2
	Erosion of lumenal surface of epithelium		0	0	0	1
	Increased thickness of the mucosa with cellular hypertrophy		0	0	0	3
	Inflammatory cell infiltration into mucosa		0	0	2	2

a Data were obtained from Table 10 on pages 114, 116, and 117 of the study report; n = 4, except in the 10,240 ppm females, where n = 3.

4. Non-protein thiol concentration - Non-protein thiol concentration was increased ($p \leq 0.05$) compared to controls in the: (i) liver and kidneys of the 10,240 ppm males (↑30-40%); (ii) kidneys in the ≥160 ppm females (↑30-80%); (iii) liver in the ≥1280 ppm females (↑60%); and (iv) stomach in the 10,240 ppm females (↑20%; 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 52 weeks [a]

Organ	Dose (ppm)		
	160	1280	10,240
Males			
Liver	1.0	1.3	1.4*
Kidney	0.9	1.1	1.3*
Stomach (antrum)	1.0	1.1	1.1
Females			
Liver	1.1	1.6**	1.6**
Kidney	1.3**	1.5**	1.8**
Stomach (antrum)	0.9	1.1	1.2*

a Data were obtained from Summary Tables 1 and 2 on pages 318-319 of the study report; n = 4, except in the 10,240 ppm females, where n = 3.
* Significantly different from the controls at $p \leq 0.05$.
** Significantly different from the controls at $p \leq 0.01$.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - It was concluded that the LOAEL was 10,240 ppm based on the decreased body weights, body weight gains, and food consumption in both sexes. Additionally, the death of a single female at this dose was most likely due to treatment. Gastric mucosal irritation was noted at this dose, and to a lesser extent at 1280 ppm. Brown pigment in the cortical tubules of the kidneys was noted at ≥1280 ppm, and hypertrophy of the cells of the zona fasciculata was observed in the adrenals at 10,240 ppm.

B. REVIEWER COMMENTS - Vasodilatation of the gums and/or ears was noted in all of the 10,240 ppm males and females compared to 0 controls, with males affected for 33-84 days and females affected for 30-191 days. With the exception of a single female (#372), this effect tapered off after 26 weeks.

One 10,240 ppm female (#370) was killed for humane reasons on the first day of Week 31, and the death was considered to be treatment-related. The following were noted in this animal prior to death: vasodilatation of gums later followed by paleness of gums; decreased body weight, food consumption, red cell indices, and albumin; reticulocytosis; and increased total globulin. Post-mortem examination revealed microscopic changes in the heart, spleen, bone marrow, stomach, liver, and kidneys.

At 10,240 ppm, body weights were decreased (14-11%; statistics not performed) in the males beginning at Week 2, resulting in significantly decreased ($p \leq 0.05$) cumulative body weight gains beginning at Week 8 (136-47%) and an overall (Weeks 0-52) body weight gain decrease of 44% compared to controls. In the females at this dose, cumulative body weight gains were decreased ($p < 0.01$) early in treatment, with decreases of 50% compared to controls at Week 4 and decreases of 38% at Week 8. However, overall body weight gains were comparable to controls.

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At 10,240 ppm, weekly food consumption was decreased during the first six months of the study in the males and generally throughout treatment in the females, with greater decreases in the females (↓≤18% compared to controls) than in the males (↓≤8%). Average food consumption for the overall study was decreased in both sexes by 2% compared to controls, with the difference attaining significance ($p \leq 0.05$) in the females.

Cholesterol was increased (↑7-48%) in the ≥1280 ppm males throughout treatment. Total protein was decreased (↓8-14%; $p \leq 0.05$) at Weeks 39 and 52 in the ≥1280 ppm males and in the 10,240 ppm females. Albumin was decreased (↓7-20%; $p \leq 0.05$) at 10,240 ppm in both sexes throughout treatment. Alkaline phosphatase was increased (↑39-68%) throughout treatment in the 10,240 ppm males.

Alanine aminotransferase (ALT) was severely decreased ($p \leq 0.01$) in all treated groups of both sexes, with average values of <1 to 3 mU/mL in the treated groups compared to 18-27 mU/mL in the controls. 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 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.

Non-protein thiol concentration was increased ($p \leq 0.05$) compared to controls in the: (i) liver and kidneys of the 10,240 ppm males (↑30-40%); (ii) kidneys in the ≥160 ppm females (↑30-80%); (iii) liver in the ≥1280 ppm females (↑60%); and (iv) stomach in the 10,240 ppm females (↑20%). It was stated that these increases are likely due to the conjugation of Chlorothalonil with glutathione in these organs.

At ≥1280 ppm, absolute liver weights in the males and absolute and adjusted liver weights in the females were increased (↑9-21%), with the adjusted values in the females attaining significance. Increased absolute (↑27%) and adjusted (↑47%; $p \leq 0.05$) thyroid weights were noted in the 10,240 ppm males. However, there were no macroscopic or microscopic findings to corroborate an effect of treatment in either of these organs.

Brown pigment was observed in the epithelium of the cortical tubules of the kidneys in the males at ≥1280 ppm (4/4 each treated vs 1/4 controls) and in the females at 1280 ppm (4/4 treated vs 2/4 controls) and 10,240 ppm (3/3 treated). However, the severity of this finding was trace to minimal. Increased incidence of moderate hypertrophy of the cells of the zona fasciculata was noted in the adrenals of the 10,240 ppm males (4/4 treated vs 1/4 controls).

Gross and microscopic findings in the stomach were evident at ≥1280 ppm in both sexes. Thickened appearance of the stomach, often with a catarrhal adhesion to the mucosal surface, was noted at 1280 ppm (2/8 dogs) and 10,240 ppm (4/7 dogs). Incidences of the following microscopic findings were observed in the stomach (vs 0/4 controls): (i) prominent apoptotic

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bodies in the antrum in the males at ≥1280 ppm (4/4 each treated) and in the females at 1280 (3/4) and 10,240 (2/3) ppm; (ii) erosion of the lumenal surface of the epithelium at 10,240 ppm in the males (1/4) and females (1/3); (iii) increased thickness of the mucosa with cellular hypertrophy in the males at 1280 (1/4) and 10,240 (3/4) ppm and in the females at 10,240 ppm (3/3); (iv) congestion of the capillaries of the superficial mucosa in the 1280 (2/4) and 10,240 (1/4) ppm males; (v) congestion of the submucosal vessels in the 1280 (1/4) and 10,240 (3/4) ppm males; (vi) mucus and cell debris adherent to the lumenal surface in the 1280 (4/4) and 10,240 (4/4) ppm males; (vii) foci of mineralization in the mucosa in the 1280 (2/4) and 10,240 (2/4) ppm males; and (viii) inflammatory cell infiltration into mucosa in the 1280 (2/4), and 10,240 ppm (2/3) females.

The only findings at 160 ppm included the following microscopic evidence of stomach irritation observed in 1/4 males (per finding): (i) congestion of the submucosal vessels; (ii) mucus and cell debris adherent to the lumenal surface; and (iii) foci of mineralization in the mucosa.

The LOAEL is 1280 ppm (equivalent to 43.3/45.3 mg/kg/day in M/F) based on macroscopic and microscopic pathological findings in the stomach including: thickened appearance of the stomach, often with a catarrhal adhesion to the mucosal surface, and prominent apoptotic bodies in the antrum of males and females; mucus and cell debris adherent to lumenal surface and foci of mineralization in the stomach mucosa of males. The NOAEL is 160 ppm (equivalent to 5.10/5.92 mg/kg/day in M/F).

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

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

- Standard deviations were not presented with the summary data.
- Mean corpuscular HGB (MCH) and magnesium were not measured.
- Appearance of urine and presence of occult blood in urine were not reported.
- 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.
- Nasal structures, pharnyx, and larynx were not examined microscopically.

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