Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 4-25-94
Section IV, Tox. Branch II

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity Study - Dogs (§82-1B)

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid

SYNONYM: 2,4-D

PC Code: 030001 (2,4-D) **MRID Nos.:** 13-Week Study = 427800-01
4-Week Study = 427800-04

STUDY NUMBERS: 13-Week Study = HWA 2184-125
4-Week Rangefinding Study = HWA 2184-121

SPONSOR: Industry Task Force on 2,4-D Research

TESTING FACILITY: Hazleton Washington, Inc., Vienna, VA

TITLES OF REPORTS: 13-Week Dietary Toxicity Study of 2,4-D in Dogs
4-Week Exploratory Rangefinding Study in Dogs with 2,4-D

AUTHOR: Dan W. Dalgard

REPORTS ISSUED: 13-Week = May 6, 1993; 4-Week = September 17, 1992

EXECUTIVE SUMMARY:

In a subchronic toxicity study, 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 0.5, 1.0, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 89, 111, 50 and 61% of the control value for males and 74, 84, 53 and 58% for females at 0.5, 1.0, 3.75 and 7.5 mg/kg/day, respectively. Both sexes of dogs at 3.75 and 7.5 mg/kg/day exhibited elevated levels of blood urea nitrogen, creatinine and alanine aminotransferase. However, no corroborative histopathological changes were seen in the liver or kidneys. **[MRID Nos. 427800-01 and 04]**

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain and food consumption as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

THIS DER INCLUDES THE REVIEWS OF A 4-WEEK PALATABILITY AND TOXICITY STUDY IN FEMALE DOGS AND A 13-WEEK TOXICITY STUDY IN MALE AND FEMALE DOGS.

I. TEST ARTICLE

Name: 2,4-dichlorophenoxyacetic acid (2,4-D)

II. 4-WEEK EXPLORATORY RANGEFINDING STUDY (MRID No. 427800-04)

A. Test Article Description

Lot No.: 909
Storage: refrigerated
Physical property: off-white powder
Purity: 96.7%

B. Test Article Homogeneity, Stability and Concentration

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

Time/Location	10 mg/kg/day		15 mg/kg/day		20 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
DAY 0						
top	362	100	443	100	677	97
middle	362	99	443	98	677	99
bottom	362	100	443	99	677	100
DAY 7						
top	362	93	443	97	677	98
middle	362	96	443	96	677	95
bottom	362	99	443	97	677	98
CONCENTRATION						
week 1	349	99	523	98	697	99
week 4	349	97	523	99	697	108

All % of target are means of duplicate samples.
Data extracted from Report Table 1, pages 24-27.

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Homogeneity, stability and concentration data were within acceptable limits.

C. Dietary Admixes

After the test article was ground to a fine powder (mortar and pestle), weighed material was combined with about 200 g of basal diet and mixed in a Waring blender. This premix was added to appropriate amounts of basal food and mixed in a Patterson-Kelly twin-shell blender. Fresh diets were prepared weekly and made available to the dogs ad libitum 7 days/week for at least 28 days.

D. Animals

Female beagles were received from Hazleton Research Products, Inc., Cumberland, VA. There was a 2-week period of acclimation. Dogs were individually housed in stainless-steel cages and exercised 3 times each week for at least 15 minutes. Room temperature and humidity were 62-80°F and 20-85%, respectively. There was a 12-hour light/dark cycle. Food and water were available ad libitum.

There were 2 control dogs and 3/treated group. The doses were 10, 15 and 20 mg/kg/day.

E. Observations

SURVIVAL AND CLINICAL SIGNS

Animals were observed A.M. and P.M. each day. A physical examination was performed weekly.

There was no mortality.

Primarily during study weeks 3 and 4, a relative dose-dependent increase was reported in the incidence of fecal abnormalities: few or no feces, diarrhea, soft feces, mucoid feces (with or without blood) and discolored (orange) feces. Hypoactivity was reported in one 20 mg/kg/day dog and emesis in one 10 and one 20 mg/kg/day dogs. Both controls were without clinical signs.

BODY WEIGHTS

These were recorded weekly. Table 2.

Table 2

INDIVIDUAL BODY WEIGHTS AND WEIGHT GAINS IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

Dog No.	mg/kg/day	Body Weight (kg) - Week					Gain or Loss
		1	2	3	4	5	
390	0	7.2	7.4	7.6	7.7	7.5	+0.3
391	0	7.9	7.7	7.7	8.0	8.1	+0.2
392	10	7.7	7.7	7.8	7.6	7.7	0
393	10	8.4	8.4	8.1	7.8	7.7	-0.7
394	10	9.7	10.3	9.6	9.5	9.4	-0.3
395	15	7.4	7.6	7.3	7.0	6.8	-0.6
396	15	8.6	8.7	8.6	8.6	8.5	-0.1
397	15	9.0	8.8	8.8	8.6	8.5	-0.5
398	20	6.7	6.8	6.3	6.0	5.6	-1.1
399	20	8.0	7.8	7.0	6.2	6.0	-2.0
400	20	9.6	9.3	8.9	8.3	8.0	-1.6

Data extracted from Report Appendix 3, page 64.

The two controls gained weight and all but one 10 mg/kg/day treated dogs lost weight in a dose-response fashion.

FOOD CONSUMPTION

This was recorded weekly.

All treated groups ate less food than the controls, particularly during weeks 2,3 and 4. The 20 mg/kg/day dogs ate 1/3 to 1/2 as much as controls during this time period. There was a decrease in the amount of food eaten in each of the 4 groups from week 1 to week 4 (% decrease: 5, 20, 32 and 61 for 0, 10, 15 and 20 mg/kg/day).

TEST ARTICLE CONSUMPTION

This was calculated weekly and was based on body weight and food eaten. Table 3.

During the 1st week, when food consumption by treated groups was not much below the control value, test article intake was near the target doses. For the last 3 weeks, when food intake was reduced, there was a concomitant decrease in test article consumed so that during this 3-week period, the dogs actually received an average of 7.2, 10.5 and 9.0 mg/kg/day (target doses were 10, 15 and 20 mg/kg/day).

Table 3

GROUP MEAN TEST ARTICLE CONSUMPTION (mg/kg/day) IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

mg/kg/day	No. of Dogs	Week			
		1	2	3	4
10	3	8.7	7.1	7.1	7.4
15	3	13.6	11.3	10.9	9.4
20	3	18.5	11.2	7.3	8.4

Data extracted from Report Table 5, page 35.

E. Clinical Pathology

Although none was originally scheduled for this study, the Report indicated that, because of results from other studies, limited serum chemistry parameters were to be examined. Blood was taken from the jugular vein of fasted (food and water withheld - no time stated) animals during the last treatment week. The following were measured: alkaline phosphatase, urea nitrogen, creatinine and total cholesterol. Table 4.

Table 4

GROUP MEAN CLINICAL CHEMISTRY VALUES IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH 2,4-D

mg/kg/day	Urea nitrogen (MG/DL)	Creatinine (MG/DL)	Alkaline Phos. (U/L)	Total Choles. (MG/DL)
0	12	0.6	65	156
10	28	1.3	50	195
15	37	1.2	38	180
20	33	1.2	23	202

No. of dogs: control = 2; treated = 3/group
Data extracted from Report Table 6, page 37.

There were increases in urea nitrogen, creatinine and total cholesterol group mean values of treated dogs compared with control. Alkaline phosphatase levels in animals administered the test article were lower than in the control group.

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F. Sacrifice and Pathology

Following 4 treatment weeks, all dogs were fasted, weighed, anesthetized with sodium thiamylal (intravenous) and exsanguinated. Gross necropsies were performed and about 38 tissues were fixed in 10% formalin. No tissues were processed/ examined microscopically.

The only apparent test article related finding was a distended gallbladder in 0/2 controls, 2/3 at 10 mg/kg/day, 3/3 at 15 mg/kg/day and 2/3 at 20 mg/kg/day.

G. Discussion

No dogs died during the 4-week study. A dose-dependent increase in fecal abnormalities was noted during the 3rd and 4th weeks.

The 2 control dogs gained 0.3 and 0.2 kg of body weight during the 4 weeks; whereas, the 10 and 15 mg/kg/day animals lost 0-0.7 kg, with the 20 mg/kg/day dogs losing 1.1-2.0 kg.

Decreases in food consumption were noted for all 3 treated groups during weeks 2, 3 and 4, with the 20 mg/kg/day dogs eating 1/3 to 1/2 as much as the controls.

In treated animals, there were increases in urea nitrogen, creatine and total cholesterol levels and a decrease in alkaline phosphatase.

Distended gallbladders were noted only in treated dogs (2/3, 3/3 and 2/3 at 10, 15 and 20 mg/kg/day).

H. Conclusions

It was considered by the Report Author that doses greater than 10 mg/kg/day exceeded the maximum tolerated dose and levels below that would be appropriate for longer studies.

III. 13-WEEK STUDY (MRID No.: 427800-01)

A. Statistical Analyses

The following were subjected to statistical analysis: body weight changes, total feed consumption, clinical pathology data and organ weight data.

LEVENE'S test of homogeneity of variances

Homogeneous - ANOVA - if not significant "stop"; if significant, Dunnett's control versus treatment comparisons (for equal variances, for unequal variances, if heterogeneous)

Heterogeneous - Log 10 transformation, to square transformation, to square root transformation, to reciprocal transformation, to angular (arcsine) transformation, to rank transformation and to ANOVA. If any transformation is heterogeneous, directly to ANOVA.

B. Regulatory Compliance

A Good Laboratory Practice Compliance Statement, Quality Assurance Statement and a list of Quality Assurance inspections were included in the Report.

A signed statement of no confidentiality claim was provided.

The Report stated that, "The criteria for flagging studies, stipulated in 40 CFR 158.34, do not apply to this study." This was signed by the Testing Facility Study Director, but not by the Sponsor (person's name typed, but no signature or date).

C. Test Article Description

Lot Number: 909
Storage: refrigerated
Physical Property: off-white powder
Purity: 96.7%

D. Test Article Homogeneity, Stability and Concentration

Analyses were performed on all concentrations for male and female diets at weeks 1, 2, 3, 4, 8 and 12. Table 1 presents selected analytical data.

Data from all intervals indicated that concentrations were 93-105% of the targets.

Data for homogeneity, stability and concentration were within acceptable limits.

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

Time/ Location	0.5 mg/kg/day		1.0 mg/kg/day		3.75 mg/kg/day		7.5 mg/kg/day	
	Targ ppm	% of Targ	Targ ppm	% of Targ	Targ ppm	% of Targ	Targ ppm	% of Targ
PRETEST								
top M	-	-	-	-	-	-	334.13	102
mid M	-	-	-	-	-	-	334.13	102
bot M	-	-	-	-	-	-	334.13	109
top F	17.44	98	-	-	-	-	-	-
mid F	17.44	96	-	-	-	-	-	-
bot F	17.44	99	-	-	-	-	-	-
PRETEST 7 day stabil. rm temp								
M	-	-	-	-	-	-	334.13	97
F	17.44	97	-	-	-	-	-	-
30 DAY STABIL freezer								
M	-	-	-	-	-	-	334.13	96
F	17.44	97	-	-	-	-	-	-
WEEK 1								
M	22.28	97	44.55	102	167.07	97	334.13	96
F	17.44	102	34.87	103	130.77	96	261.54	101
WEEK 12								
M	20	100	40	101	160	100	240	98
F	20	101	30	103	140	99	230	93

% of targets are mean of duplicate samples;
M = male; F = female
mid = middle; bot = bottom
rm temp = room temperature; stabil. = stability; Targ = Target
Data extracted from Report Table 1, pages 41-44.

Data from all intervals indicated that concentrations were 93-105% of the targets.

Data for homogeneity, stability and concentration were within acceptable limits.

E. Dose Selection

The doses for this 13-week study were selected based upon the results of the 4-week rangefinding study (reviewed earlier in this Data Evaluation Report). The doses in this 13-week study are 0, 0.5, 1.0, 3.75 and 7.5 mg/kg/day.

F. Dose Preparation

After the test article was ground to a fine powder (mortar and pestle), weighed material was combined with about 200 g of basal diet and mixed in a Waring blender. This premix was added to appropriate amounts of basal food and mixed in a Patterson-Kelly twin-shell blender. Fresh diets were prepared weekly and made available to the dogs ad libitum 7 days/week for 13 weeks.

G. Animals

Male and female beagle dogs were received from Hazleton Research Products, Inc., Cumberland, VA. Animals were acclimated for at least 2 weeks. Dogs were individually housed in stainless-steel cages in a room with temperature and humidity at 69-76°F and 35-70%, respectively. There was a 12-hour light/dark cycle. The animals were placed in an exercise pen, with members of the same sex and group, 3 days/week for about 15 minutes/day. Food and water were available ad libitum. After physical and ophthalmic examinations as well as clinical laboratory tests, the dogs were stratified by weight and assigned to groups by a computerized randomization program.

H. Survival and Clinical Observations

Dogs were observed twice each day for mortality, moribundity and clinical signs. Physical examinations were conducted once each week at the time of weighing.

All animals survived the 13-week study. No clinical signs were considered to have been the result of test article administration. Fecal changes (discoloration, mucoid, soft, diarrhea, few and none) were noted in dogs of all treated and control groups.

Male No. 29650 (3.75 mg/kg/day) lost 1.5 kg body weight (with a decrease in food consumption) during study week 11. The dog was examined by a veterinarian and was observed to be: hypoactive, hunched, abdominal discomfort, salivating and mucoid/bloody feces. There was an increase in the leukocyte count and a firm swelling (presumed a swollen lymph node) was noted on the neck. A repeat leukocyte count several days later was also elevated. An antibiotic was given for 5 days, after which appetite improved and the swelling subsided. Body weights increased at the week 13 and 14 weighings.

I. Body Weights

Body weights were recorded before treatment and weekly during the study. Table 2.

Table 2

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

Week	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0.5	1.0	3.75	7.5	0	0.5	1.0	3.75	7.5
B.W.										
1	8.0	8.3	8.0	9.2	7.5	6.9	6.6	6.7	7.3	6.9
2	8.0	8.8	8.2	9.1	7.6	7.0	6.7	7.0	7.5	6.9
3	8.3	8.8	8.6	9.4	7.9	7.3	7.1	7.0	7.5	7.0
4	8.9	9.2	9.0	9.8	8.1	7.4	7.2	7.1	7.7	7.1
6	9.3	9.6	9.8	10.2	8.5	7.8	7.5	7.6	7.9	7.3
8	9.9	10.0	10.1	10.4	8.7	8.2	7.8	7.9	7.9	7.5
10	10.4	10.3	10.4	10.3	8.9	8.5	7.8	8.0	8.3	7.7
12	10.7	10.7	10.9	10.2	9.2	8.6	8.0	8.1	8.3	7.8
14	10.8	10.8	11.1	10.6	9.2	8.8	8.0	8.3	8.3	8.0
B.W. GAIN										
1-4	0.9	0.9	1.0	0.6	0.6	0.5	0.6	0.4	0.4	0.2
4-8	1.0	0.8	1.1	0.6	0.6	0.8	0.6	0.8	0.2	0.4
8-14	0.9	0.8	1.0	0.2	0.5	0.6	0.2	0.4	0.4	0.5
1-14	2.8	2.5	3.1	1.4	1.7	1.9	1.4	1.6	1.0	1.1

4 dogs/sex/group
Body weight gains were calculated by the Reviewer for all intervals (the Report stated the gains for the weeks 1-14 interval).
Data extracted from Report Table 3, pages 48 and 49.

For both males and females at 3.75 and 7.5 mg/kg/day, group mean body weight gains were lower (not statistically significant) than control values.

J. Food Consumption

Data for this parameter were recorded each week.

In males, there appeared to be a decrease in group mean food consumption at 3.75 and 7.5 mg/kg/day compared with the control amounts. For females, there were lower group mean values at most measurement intervals in all treated groups versus the control group.

K. Test Article Intake (Report Table 5, pages 54 and 55)

This was calculated from body weight and food consumption data.

The group means for the entire 13-week period were as follows (mg/kg/day):

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males = 0.5, 1.0, 3.8 and 7.8 for 0.5, 1.0, 3.75 and 7.5 mg/kg/day
females = 0.5, 1.0, 3.8 and 7.7 for 0.5, 1.0, 3.75 and 7.5 mg/kg/day

L. Ophthalmoscopic Examinations

Using indirect ophthalmoscopy, the eyes of all dogs were examined before the start of treatment and at study termination.

There were no abnormalities noted in any animal which were considered to be the result of test article administration. Dog No. 29628, male, control, had multifocal retinal fold of the left fundus only at termination. Dog No. 29634, male, 0.5 mg/kg/day, had multifocal retinal fold of the left fundus only at pretest. Dog No. 29642, male, 1.0 mg/kg/day, had multifocal retinal fold of the right fundus only at termination. Dog No. 29649, male, 3.75 mg/kg/day, had persistent hyaloid remnant of the left lens only at pretest. Dog No. 29658, male, 7.5 mg/kg/day, had pigment in the right cornea at pretest and right corneal opacity at termination.

M. Clinical Pathology

Blood was collected from the jugular vein of overnight-fasted dogs prior to treatment as well as during weeks 4 and 13 for hematology and clinical chemistry determinations. Urine samples were taken by catheterization prior to study initiation and during week 13 (feed and water available prior to the collection of urine).

HEMATOLOGY

The following parameters were examined:

Erythrocyte count*	Leukocyte count*
Hematocrit*	Corrected leukocyte count
Hemoglobin*	Leukocyte differential*
Platelet count*	Cell morphology
Reticulocyte count	Mean corpuscular volume
Absolute retic. count	Mean corpuscular hemoglobin
	Mean corpuscular hemoglobin concentration

* = EPA Guideline Requirement

There were no hematology parameters which appeared to be altered by test article administration.

CLINICAL CHEMISTRY

The following parameters were examined:

Alanine aminotransferase*
Aspartate aminotransferase*
Alkaline phosphatase
Albumin*
Globulin
Urea nitrogen*
Creatine kinase
Creatinine*
Total bilirubin*
Total cholesterol
Total protein*
Glucose*
Inorganic phosphorus*
Potassium*
Sodium*
Calcium*
Chloride*

* = EPA Guideline Requirement

The parameters which appeared to be altered by test article administration were urea nitrogen, creatinine and alanine aminotransferase. In addition, the Report stated that alkaline phosphatase levels, which usually decline with age, declined more rapidly in treated dogs than in controls. Table 3.

Table 3

SELECTED GROUP MEAN CLINICAL CHEMISTRY PARAMETERS IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

mg/kg/day	Urea nitrogen MG/DL			Creatinine MG/DL			Alanine amino U/L			Alk. phos. U/L		
	-1a	4	13	-1	4	13	-1	4	13	-1	4	13
MALES												
0	10	11	13	0.7	0.7	0.8	24	28	31	109	118	77
0.5	11	11	16	0.7	0.8	1.0	28	266b	40	107	180c	70
1.0	11	14	17	0.7	0.9*	1.1	29	41	41*	122	124	81
3.75	11	18*	20*	0.8	1.0*	1.3*	32	89*	96*	151	115	91
7.5	9	19*	22*	0.7	1.0*	1.3*	36	101*	87*	123	82	71
FEMALES												
0	9	11	13	0.7	0.7	0.8	30	31	37	135	116	83
0.5	11	12	15	0.7	0.8	0.9	26	30	36	120	98	78
1.0	10	13	17	0.7	0.9	1.1*	31	34	37	122	111	82
3.75	12	19*	23*	0.8	1.1*	1.4*	28	49*	56	124	81	59
7.5	11	19*	22*	0.8	1.0*	1.2*	29	63*d	76*e	151	97	80

4 dogs/sex/group
a = week of determination
individual values - **b** = 30, 970, 34 and 29; **c** = 101, 447, 93 and 79
d = 131, 35, 47 and 38; **e** = 143, 41, 69 and 52
NOTE: Dog male 634 (0.5 mg/kg/day) at week 4, alkaline phosphatase = 447, aspartate aminotransferase = 116 (other 3, 26-39) and alanine aminotransferase, 970
Statistical Significance: * = ≤0.05
Data extracted from Report Table 7 and Appendix 7, pages 70-79 and 175-186.

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URINALYSIS (not an EPA requirement)

The following parameters were examined:

Appearance	Occult blood	Bilirubin
pH	Glucose	Protein
Ketones	Specific gravity	Microscopic examination of sediment
Urobilinogen		

There were no parameters which appeared to be altered by test article administration.

N. Sacrifice and Pathology

At the end of the 13-week treatment period, the animals were weighed, anesthetized with sodium thiamylal and exsanguinated. Complete necropsies were performed. The following organs were weighed and organ-to-body weight as well as organ-to-brain weight ratios were calculated: adrenals, brain with stem, heart, kidneys, liver without gallbladder, ovaries, pituitary, testes without epididymides and thyroid with parathyroid.

The following tissues were removed, preserved in 10% neutral buffered formalin and the "x" organs weighed:

DIGESTIVE	RESPIRATORY	UROGENITAL
Salivary gland*	Trachea*	xKidneys*
Esophagus*	Lung*	Urinary bladder*
Stomach*		xTestes*
Duodenum*	CARDIOVASC/HEMAT	xOvaries
Jejunum*	Aorta*	Epididymides
Ileum*	xHeart*	Prostate
Cecum*	Bone marrow*	Uterus*
Colon*	Lymph nodes*	Lacrimal gland
xLiver*	Spleen*	Vagina
Pancreas*	Thymus*	
Gallbladder*		
Tongue		
NEUROLOGIC	GLANDULAR	OTHER
xBrain*	xAdrenals*	Bone*
Peripheral nerve*	Mammary gland*	Skeletal muscle*
Spinal cord (3 levels)*	xParathyroids*	Skin
xPituitary*	xThyroid*	Gross lesions and masses*
Eyes (with optic n.)*		

* = EPA Guideline Requirements [rectum required but not examined]

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MACROSCOPIC

There were no gross pathology findings that were considered to have been related to test article administration.

ORGAN WEIGHTS

Statistically significant ($p \leq 0.05$) or apparent differences were noted in the weights of thyroids, heart and testes of treated versus control groups. Table 4.

Table 4

GROUP MEAN ABSOLUTE AND RELATIVE THYROID/PARATHYROID, HEART AND TESTES WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

mg/kg/day	Males				Females			
	Final B.W. (g)	Absolute (g)	to BW (%)	to brain (ratio)	Final B.W. (g)	Absolute (g)	to BW (%)	to brain (ratio)
THYROID								
0	10350	0.95	.009	.013	8325	0.70	.008	.010
0.5	10250	0.80	.008	.011	7600	0.73	.010	.010
1.0	10550	1.03	.010	.013	7950	0.75	.010	.011
3.75	10175	1.06	.010	.014	8225	0.75	.009	.011
7.5	8875	0.83	.009	.011	7425	1.03	.014*	.014
HEART								
0	10350	91.6	0.89	1.22	8325	76.9	0.92	1.13
0.5	10250	87.0	0.85	1.14	7600	74.7	0.98	1.07
1.0	10550	87.2	0.83	1.12	7950	72.1	0.91	1.04
3.75	10175	73.4*	0.73*	0.96*	8225	62.3	0.76*	0.90
7.5	8875	71.4*	0.81	0.99*	7425	67.8	0.91	0.93
TESTES								
0	10350	17.0	0.16	0.23				
0.5	10250	16.0	0.16	0.21				
1.0	10550	16.1	0.15	0.21				
3.75	10175	13.2	0.13	0.17				
7.5	8875	10.6*	0.12	0.15				

4 dogs/sex/group
Statistical Significance: * =$p \leq 0.05$
Data extracted from Report Table 9, pages 88-97.

Only female thyroid (absolute, to-body weight and to-brain weight) weights at 7.5 mg/kg/day appeared to be heavier than controls. This observation did not seem to be of toxicological significance.

Heart weights for males and females in the 3.75 and 7.5 mg/kg/day groups appeared to be below respective control values (except for female to-body weight at 7.5 mg/kg/day).

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The Report Author indicated that, "The significance of the lower heart weights in Groups 4 and 5 [3.75 and 7.5 mg/kg/day] is not known."

Group mean testicular weights were observed to be lower than controls in the 3.75 and 7.5 mg/kg/day groups.

MICROSCOPIC

Report pages 38 and 39 (Pathology Report, Histopathology), indicated that there were no consistent lesions which were attributed to test article administration. One male and one female at 7.5 mg/kg/day were reported to have moderate perivascular, chronic active inflammation of the liver (male No. 658, female No. 661). Table 5.

Table 5

SEVERITY OF LIVER INFLAMMATION IN A 13-WEEK STUDY IN DOGS WITH 2,4-D

Parameter		Males (mg/kg/day)					Females (mg/kg/day)				
		0	0.5	1.0	3.75	7.5	0	0.5	1.0	3.75	7.5
No. Examined		4	4	4	4	4	4	4	4	4	4
No. not remarkable		0	0	0	0	0	0	1	0	0	1
Inflammation, chronic	->	0	0	1	0	1	0	2	0	0	1
	1>	4	4	3	4	2	3	2	3	4	3
	2>	0	0	0	0	1	1	0	1	0	0
	TL>	4	4	4	4	4	4	4	4	4	4
	MN>	1.0	1.0	0.8	1.0	1.0	1.3	0.5	1.3	1.0-	0.8
Inflammation, chronic active, perivascular	->	0	2	2	0	1	1	2	1	1	1
	1>	3	1	1	4	1	3	2	3	2	2
	2>	1	1	1	0	-	0	0	0	1	0
	3>	0	0	0	0	1	0	0	0	0	1
	TL>	4	4	4	4	4	4	4	4	4	4
	MN>	1.3	0.8	0.8	1.0	1.5	0.8	0.5	0.8	1.0	1.3

-> = finding not present
1> = minimal
2> = slight
3> = moderate
TL> = total
MN> = mean of graded findings
Data extracted from Report Table 11, pages 106-107.

O. Discussion

Data for homogeneity, stability and concentration were within acceptable limits.

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All dogs survived the 13-week study. There were no clinical signs which differentiated treated from control dogs. One 3.75 mg/kg/day male presumably had a swollen lymph node with accompanying leukocytosis (lost 1.5 kg body weight during week 11). The dog was given an antibiotic for 5 days and improved.

Body weight gains for both sexes at 3.75 and 7.5 mg/kg/day, were lower than controls, but were not statistically significant. Group body weight gains were 89, 111, 50 and 61% of the control values for males at 0.5, 1.0, 3.75 and 7.5 mg/kg/day, respectively. The corresponding values for females were 74, 84, 53 and 58%.

Food consumption was reduced in males at 3.75 and 7.5 mg/kg/day. In females, lower group mean values were noted at most intervals for all treated groups compared with the control group.

Test article intake (mg/kg/day) over the 13 weeks for the 0.5, 1.0, 3.75 and 7.5 mg/kg/day groups was as follows: males = 0.5, 1.0, 3.8 and 7.8; females = 0.5, 1.0, 3.8 and 7.7.

There were no ophthalmic, hematology or urinalysis changes which appeared related to the administration of the test article.

Blood urea nitrogen, creatinine and alanine aminotransferase values (with one exception) were significantly ($p \leq 0.05$) above control levels at the 4- and 13-week intervals for 3.75 and 7.5 mg/kg/day dogs of both sexes. Alkaline phosphatase levels declined with age more rapidly in treated groups than in the control groups. However, there were no corroborative histopathological changes in the kidneys or liver.

Group mean thyroid weights (absolute, to-body weight and to-brain weight) of 7.5 mg/kg/day females only, appeared to be heavier than the control value. The Report Author stated that the significance of the lower heart weights at 3.75 and 7.5 mg/kg/day was unknown. There were lower group mean testicular weights in the 3.75 and 7.5 mg/kg/day groups compared with the control group. No histopathological changes were seen in the thyroids or hearts. Therefore, these weight changes were not considered to be treatment related or of toxicological significance.

One male and one female at 7.5 mg/kg/day were reported to have moderate chronic active perivascular inflammation of the liver compared with a less severe finding (minimal or slight) in controls or lower dose groups.

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IV. CONCLUSIONS

In a subchronic toxicity study, 2,4-dichlorophenoxyacetic acid (1,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 0.5, 1.0, 3.75 and 7.5 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry. urinalysis, macroscopic pathology, organ weights and microscopic pathology.

Group mean body weight gains were 89, 111, 50 and 61% of the control value for males and 74, 84, 53 and 58% for females at 0.5, 1.0, 3.75 and 7.5 mg/kg/day, respectively. Both sexes of dogs at 3.75 and 7.5 mg/kg/day exhibited elevated levels of blood urea nitrogen, creatinine and alanine aminotransferase. However, no corroborative histopathological changes were seen in the liver or kidneys.

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain and food consumption as well as alterations in clinical chemistry parameters.

This study is core classified as **Minimum** and satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

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