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

10938

APR 28 1994

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D), 2-ETHYLHEXYL ESTER (2-EHE) OF 2,4-D AND DIMETHYLAMINE (DMA) SALT OF 2,4-D - Subchronic Toxicity Studies in the Dog (§82-1B)

DP Barcode: D192640 **Case:** 818706
Submission: S443561 **PC Code:** 030001 (2,4-D)
Identification No.: 030001
Action: 627 GENERIC DATA SUBMISSION
MRID Nos.: 427800-
2,4-D = 01 (13-week); 04 (4-week)
2-EHE = 03 (13-week); 05 (4-week)
DMA = 02 (13-week); 06 (4-week)

FROM: Alan C. Levy, Ph.D., Toxicologist Alan C. Levy 4-26-94
Review Section IV, Toxicology Branch II
Health Effects Division (7509C)

TO: Walter Waldrop/Judith Coombs, PM 71
Special Review and Reregistration Division (7508W)

THRU: Jess Rowland, M.S. 4/26/94
Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C) 4/26/94

REQUEST: Review subchronic toxicity studies in the dog with 2,4-dichlorophenoxyacetic acid (2,4-D), the 2-ethylhexyl ester of 2,4-D and the dimethylamine salt of 2,4-D.

COMMENT: Attached to this Memorandum are the following:

1. Formula for 2,4-D
2. Summary of the findings for the three studies
3. Executive Summaries for the three studies

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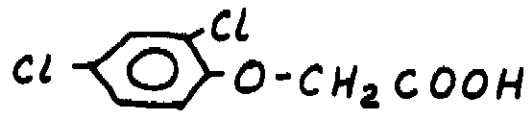
FORMULA FOR 2,4-DICHLOROPHENOXYACETIC ACID



A SUMMARY OF THE FINDINGS FROM 13-WEEK DOG STUDIES WITH 2,4-D, THE 2-ETHYLHEXYL ESTER (2-EHE) OF 2,4-D AND THE DIMETHYLAMINE (DMA) SALT OF 2,4-D

Parameter	1.0 mg/kg/day			3.75 mg/kg/day			7.5 mg/kg/day		
	2,4-D	2-EHE	DMA	2,4-D	2-EHE	DMA	2,4-D	2-EHE	DMA
MORTALITY	0	0	0	0	0	0	0	0	0
CLINICAL SIGNS	-	-	-	-	-	-	-	-	-
BODY WEIGHT CHANGE	-	-	-	↓	↓	?	↓	↓	↓
FOOD CONSUMPTION	-	-	-	↓	↓	-	↓	↓	↓
CLINICAL PATHOLOGY									
HEMATOLOGY	-	-	-	-	-	-	-	-	-
CLINICAL CHEMISTRY									
urea nitrogen	-	-	-	↑	↑	↑	↑	↑	↑
creatinine	-	-	?	↑	↑	↑	↑	↑	↑
alanine aminotransferase	-	-	-	↑	↑	↑	↑	↑	↑
aspertate aminotransferase	-	-	-	-	-	?	-	-	?
alkaline phosphatase	-	-	-	-	-	-	-	-	-
GROSS PATHOLOGY	-	-	-	-	-	-	-	-	-
ORGAN WEIGHTS									
testes	-	-	-	-	-	-	↓	↓	↓
thyroid	-	?	-	-	?	-	-	?	-
MICROSCOPIC PATHOLOGY	-	-	-	-	-	-	-	-	-
NOEL	NOEL								
LOEL				LOEL					

2,4-D = 2,4-Dichlorophenoxyacetic acid
2-EHE = 2-ethylhexyl ester of 2,4-Dichlorophenoxyacetic acid
DMA = Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid
NOTE: 2,4-D was also administered at a dose of 0.5 mg/kg/day; RESULTS = no apparent test article effect at this dose
- = no change
↑ = increase
↓ = decrease
? = questionable increase

EXECUTIVE SUMMARIES

2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) - MRID Nos. 427800-01 (13-week) and 04 (4-week)

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 52% 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.

2-ETHYLHEXYL ESTER (2-EHE) OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) MRID Nos. 427800-03 (13-week) and 05 (4-week)

In a subchronic toxicity study, the 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 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 67, 52 and 15% of the control value for males and 75, 38 and 50% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There were elevations in blood urea nitrogen, creatinine and alanine aminotransferase levels at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidneys. **[MRID Nos. 427800-03 and 05]**

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain 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.

DIMETHYLAMINE (DMA) SALT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)
MRID Nos. 427600-02 (13-week) and 06 (4-week)

In a subchronic toxicity study, the dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 1, 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 126, 126 and 68% of the control value for males and 78, 61 and 33% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There was decreased food consumption in 7.5 mg/kg/day females as well as elevated alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidneys. [MRID Nos. 427600-02 and 06]

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

Secondary reviewer: Jess Rowland, M.S. [signature] 4/25/94.
Section IV, Tox. Branch II

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.

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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.

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	155
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.

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):

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.

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.

M. 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]

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).

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	1	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, stabilit, and concentration were within acceptable limits.

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 aminctransferase 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 <u>moderate</u> chronic active perivascular inflammation of the liver compared with a less severe finding (minimal or slight) in controls or lower dose groups.

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.

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

Secondary reviewer: Jess Rowland, M.S. Jess Rowland 4/25/94.
Section IV, Tox. Branch II

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.

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.

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):

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.

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]

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).

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	1	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.

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 <u>moderate</u> chronic active perivascular inflammation of the liver compared with a less severe finding (minimal or slight) in controls or lower dose groups.

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.

010938

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

Secondary reviewer: Jess Rowland, M.S. Jess Rowland 4/25/94
Section IV, Tox. Branch II

DATA EVALUATION REPORT

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

TEST MATERIAL: 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid

SYNONYMS: 2-EHE of 2,4-D

PC Code: 030001 (2,4-D) **MRID Nos:** 13-Week Study = 427800-03
4-Week Rangefinding Study = 427800-05

STUDY NUMBERS: 13-Week Study = WHA 2184-127
4-Week Rangefinding Study = WHA 2184-122

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

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

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

AUTHOR: Dan W. Dalgard

REPORTS ISSUED: 13-Week = May 5, 1993; 4-Week = December 15, 1992

EXECUTIVE SUMMARY:

In a subchronic toxicity study, the 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 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 67, 52 and 15% of the control value for males and 75, 38 and 50% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There were elevations in blood urea nitrogen, creatinine and alanine aminotransferase levels at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidney. **[MRID Nos. 427800-03 and 05]**

The NOEL is 1.0 mg/kg/day. The LOEL of 3.75 mg/kg/day is based on decreased body weight gain 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-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D)

II. 4-WEEK EXPLORATORY RANGEFINDING STUDY (MRID No. 427800-05)

A. Test Article Description

Lot No.: 04KF54479
Storage: refrigerated
Physical Property: brown liquid
Purity: 62.7% 2,4-D acid equivalent

B. Test Article Homogeneity, Stability and Concentration

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 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	101	443	106	677	104
middle	362	101	443	104	677	106
bottom	362	101	443	105	677	105
DAY 7						
top	-	-	-	-	-	-
middle	362	97	443	98	677	99
bottom	-	-	-	-	-	-
WEEK 2						
top	349	100	523	98	697	99
middle	349	97	523	98	697	97
bottom	349	97	523	97	697	94
CONCENTRATION						
week 1	349	97	523	93	697	99
week 4	349	99	523	100	697	100

All % of target are means of duplicate samples.
Data extracted from Report Table 1, pages 24-28.

Homogeneity, stability and concentration data are within acceptable limits.

C. Dietary Admixes

After shaking, the amount of test article needed for one week's diets was weighed. The weighed material was mixed with a small amount of basal diet (Waring blender). This premix was added to appropriate amounts of basal food in order to obtain expected diets (10, 15 or 20 mg/kg/day of 2,4-D acid equivalent). 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. The dogs were individually housed in stainless-steel cages and exercised 3 times each week for at least 15 minutes. Room temperature and humidity were 64-79°F and 25-81%, 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 0, 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 dose-dependent increase was reported in the incidence of fecal abnormalities (orange/bile stained, mucoid, soft, at times with blood, diarrhea and few or no feces). One 15 mg/kg/day dog vomited food during week 1 and one 20 mg/kg/day dog had frothy emesis during week 3.

BODY WEIGHTS

These were recorded weekly. Table 2.

The two controls gained weight and all treated dogs lost weight in a dose-response fashion.

Table 2

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

Dog No.	mg/kg/day	Body Weight (kg) - Week					Gain or Loss
		1	2	3	4	5	
401	0	6.2	6.4	6.4	6.4	6.6	+0.4
402	0	7.8	7.9	7.8	7.7	7.9	+0.1
403	10	6.3	6.4	6.5	6.3	6.1	-0.2
404	10	6.8	6.9	6.6	6.6	6.7	-0.1
405	10	8.2	8.1	7.8	7.5	7.5	-0.7
406	15	6.6	6.3	6.3	6.0	6.0	-0.6
407	15	6.8	6.7	6.2	6.5	6.2	-0.6
408	15	8.2	8.1	7.5	7.3	7.2	-1.0
409	20	6.6	6.8	6.1	5.6	5.0	-1.6
410	20	7.4	7.1	6.0	5.8	5.5	-1.9
411	20	8.7	8.7	7.7	7.4	7.1	-1.6

Data extracted from Report Appendix 3, page 61.

The two controls gained weight and all treated dogs lost weight in a dose-response fashion.

FOOD CONSUMPTION

This was recorded weekly.

The amount of food consumed in the control and 3 treated groups during the 1st week was similar. During the last 3 weeks of the study, there was a dose-dependent decrease in food eaten so that, compared with control values, the decreases were about 20, 36 and 58% at 10, 15 and 20 mg/kg/day, respectively.

TEST ARTICLE CONSUMPTION

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

Table 3

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

mg/kg/day	No. of Dogs	Week			
		1	2	3	4
10	3	9.9	8.1	8.0	8.3
15	3	12.9	8.3	11.9	10.2
20	3	22.4	8.4	9.0	9.6

Data extracted from Report Table 5, page 36.

During the 1st week, when food consumption by treated groups was similar to the control group, 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 8.1, 10.1 and 9.0 mg/kg/day (target doses were 10, 15 and 20 mg/kg/day).

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 THE 2-ETHYLHEXYL ESTER OF 2,4-D

mg/kg/day	Urea Nitrogen (mg/DL)	Creatinine (mg/DL)	Alkaline Phos. (U/L)	Total Choles. (mg/DL)
0	14	0.8	85	125
10	32	1.3	43	222
15	40	1.3	37	158
20	35	1.2	50	180

No. of dogs: control = 2; treated = 3/group
Data extracted from Report Table 6, page 38.

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.

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 39 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 3/3 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 0.4 and 0.1 kg of body weight during the 4 weeks; whereas, the 10 and 15 mg/kg/day animals lost 0.1-1.0 kg, with the 20 mg/kg/day dogs losing 1.6-1.9 kg.

Dose dependent decreases in food consumption were reported for weeks 2, 3 and 4. During this same time period, test article intake was severly reduced from the target values, especially during the 2nd week of the study.

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

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

H. Conclusions

All three dose levels were poorly tolerated and were considered too high for longer studies.

III. 13-WEEK STUDY (MRID No.: 427800-03)

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 homogeneous, 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: 04KF54479
Storage: refrigerated
Physical Property: brown liquid
Purity: 62.7% (acid equivalent) - weight adjustment calculated to 100% purity

D. Test Article Homogeneity, Stability and Concentration

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

Report page 23 indicated that, during week 2, the initial results showed that the diets were about 60% of the desired amount, and a remix was done. Further investigation determined that a correction had been made for test article activity (62.7% of acid equivalent). It was concluded that the original mixes were correct and the remix diets were about 60% over target. Animals received the remix diet during the last 3 days of week 2.

Table 1 presents selected analytical data.

Table 1

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

Time/Location	1 mg/kg/day		3.75 mg/kg/day		7.5 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
PRETEST						
top M ..	-	-	-	-	334.13	98
middle M ..	-	-	-	-	334.13	96
bottom M ..	-	-	-	-	334.13	95
top F ..	34.87	96	-	-	-	-
middle F...	34.87	94	-	-	-	-
bottom F ..	34.87	91	-	-	-	-
PRETEST (7 day stabil. room temper						
M ..	-	-	-	-	334.13	95
F ..	34.87	93	-	-	-	-
30 DAY STABIL freezer						
M ..	-	-	-	-	334.13	93
F ..	34.87	95	-	-	-	-
WEEK 1						
M ..	44.55	97	167.07	97	334.13	95
F ..	34.87	95	130.77	98	261.54	95
WEEK 12						
M ..	43.48	96	194.95	96	410.69	99
F ..	45.80	95	208.30	98	400.39	97

Percent of targets are mean of duplicate samples.
M = male; F = female
Data extracted from Report Table 1, pages 39-42.

Data from all intervals indicated that concentrations were 91-100% of the targets (excludes week 2 data).

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, 1, 3.75 and 7.5 mg/kg/day.

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F. Dose Preparation

After shaking, an amount of test article for each dose level for one week's feed was weighed, added to about 200 g of basal diet and mixed in a Waring blender. This premix was then added to appropriate amounts of basal diet and mixed in a Patterson-Kelly blender. After week 1, fresh diets were prepared each week based on sex, body weight and food consumption.

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 the environment set to maintain a temperature of 64-84°F, humidity of 41-70% and 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).

Female G29543, from the 1.0 mg/kg/day group, was found dead on the 3rd study day. There had been no previous signs of illness. The Sponsor requested that a replacement dog be put on study (day 7) using the deceased's animal number. No data from the original dog were included in the Report. There was no mention of a necropsy. Report page 128 stated, "Presumably from an epileptic seizure." The death was not considered to have been test article related. All other dogs survived the 13 weeks.

There were no clinical signs which were considered to have been related to test article administration.

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 THE 2-ETHYLHEXYL ESTER OF 2,4-D

Week	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1.0	3.75	7.5	0	1.0	3.75	7.5
BODY WEIGHTS								
1	7.5	7.1	7.1	7.5	6.6	6.2	6.9	6.7
2	7.7	7.1	7.3	7.5	6.7	6.3	6.8	6.9
3	8.1	7.6	7.4	7.3	7.0	6.4	6.9	6.8
4	8.5	7.5	7.6	7.3	7.2	6.6	6.9	7.0
6	8.8	7.9	8.0	7.5	7.4	6.8	7.1	7.1
8	9.1	8.1	8.1	7.6	7.8	6.9	7.3	7.2
10	9.5	8.5	8.4	7.7	7.9	7.0	7.4	7.4
12	9.8	8.7	8.6	7.8	8.0	7.2	7.3	7.4
14	10.1	8.9	8.5	7.8	8.2	7.3	7.4	7.5
BODY WT GAIN								
1-4	1.0	0.4	0.5	-0.2	0.6	0.4	0	0.3
4-8	0.6	0.6	0.5	0.3	0.6	0.3	0.4	0.2
8-14	1.0	0.8	0.4	0.2	0.4	0.4	0.1	0.3
1-14	2.7	1.8	1.4*	0.4*	1.6	1.2	0.6*	0.8*

NOTE: 4 dogs/sex/group
Body weight gains were calculated by the Reviewer for all intervals except weeks 1-14 for which data were included in the Report.
Statistical Significance: * = $p \leq 0.05$; presented in the Report only for weeks 1-14 body weight gains.
Data extracted from Report Table 3, pages 46 and 47.

There were lower body weights in treated male and female groups during the study. Body weight gains for the 13 weeks of the study were significantly ($p \leq 0.05$) less than the controls for both sexes at 3.75 and 7.5 mg/kg/day. The 7.5 mg/kg/day dose males lost a group mean of 0.2 kg during the 1st four weeks; whereas, the controls gained a mean of 1.0 kg with the 1.0 and 3.75 mg/kg/day groups gaining a mean of 0.4 and 0.5 kg, respectively.

J. Food Consumption

Data for this parameter were recorded each week.

In males, there was relatively little difference in the amount of food consumed (g/dog/week) between the 1.0 mg/kg/day and the control group. The 3.75 mg/kg/day group means were about 9% less than controls and the 7.5 mg/kg/day group means, about 23% less.

For females, the 1.0 mg/kg/day dogs ate the same or slightly less (generally no more than 5-8%; 5/13 intervals about equal and 8/13 intervals, less). The 3.75 mg/kg/day group means were about 13% less than controls and the 7.5 mg/kg/day group means about 10% less.

K. Test Article Intake (Report Table 5, pages 52 and 53)

This was calculated by body weight and food consumption data.

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

males = 1.1, 3.9 and 7.8 for 1.0, 3.75 and 7.5 mg/kg/day
females = 1.0, 3.9 and 7.7 for 1.0, 3.75 and 7.5 mg/kg/day

L. Ophthalmoscopic Examinations

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

There were no abnormalities noted in any animal at termination which were not reported prior to the beginning of dosing. Dog No. G29538, male, 1.0 mg/kg/day, had focal retinal fold of the right fundus (pretest and at termination) and No. G29558, female 7.5 mg/kg/day, had persistent hyaloid remnant of both lenses (pretest and 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 (food 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 conc.

* = 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

Those parameters which showed statistically significant ($p \leq 0.05$) differences between treated and control groups were urea nitrogen, creatinine and alanine aminotransferase. Table 3.

Table 3

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

mg/kg/day	Urea nitrogen MG/DL			Creatinine MG/DL			Alanine amino. U/L		
	-1a	4	13	-1	4	13	-1	4	13
MALES									
0	9	13	13	0.6	0.7	0.8	41	42	47
1.0	10	16	18*	0.6	0.9	1.0	32	46	45
3.75	11	21*	25*	0.6	1.0*	1.2*	22*	35	39
7.5	10	22*	27*	0.7	1.2*	1.3*	23*	70	71
FEMALES									
0	12	14	16	0.7	0.8	0.9	30	28	31
1.0	11	16	19	0.7	0.9	1.1*	28	35	36
3.75	12	22*	24*	0.8	1.1*	1.3*	30	55	66*
7.5	12	25*	25*	0.7	1.1*	1.3*	38	99*	127*

4 dogs/sex/group
a = week of determination
Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 7, pages 62-66.

Urea nitrogen and creatinine levels were elevated ($p \leq 0.05$) in males and females at 3.75 and 7.5 mg/kg/day at the weeks 4 and 13 intervals. Alanine aminotransferase was elevated (not significant) in 7.5 mg/kg/day males at weeks 4 and 13. In 3.75 and 7.5 mg/kg/day females at weeks 4 and 13, there were higher levels of this enzyme than in respective control dogs (all ≤ 0.05 except not significant for 3.75 mg/kg/day at week 4).

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. [The 1.0 mg/kg/day replacement dog that started on treatment day 7, was sacrificed with the other study dogs.] 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]

MACROSCOPIC

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

ORGAN WEIGHTS

Questionable, non-statistically significant ($p \leq 0.05$), organ weight changes were noted for the thyroid/parathyroid and testes. Table 4.

Table 4

GROUP MEAN ABSOLUTE AND RELATIVE THYROID/PARATHYROID AND TESTES WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH THE 2-ETHYLHEXYL ESTER OF 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	9775	0.77	.008	.010	7650	0.64	.008	.009
1.0	8750	1.05	.012	.014	7050	0.95	.013	.014
3.75	8450	0.97	.012	.014	7150	0.87	.012	.013
7.5	7575*	0.90	.012	.012	7100	0.92	.013	.013
TESTES								
0	9775	13.3	.14	.17				
1.0	8750	12.0	.14	.16				
3.75	8450	11.5	.14	.16				
7.5	7575*	9.4	.12	.12				

Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 9, pages 76-84.

At most, the increase in group mean thyroid/parathyroid and decrease in group mean testicular weights, are only suggestive of a test article effect. There was an overlapping of individual organ weights between the control and treated groups. In addition, because the group mean terminal body weights, especially in the 7.5 mg/kg/day groups, were lower than the controls, individual variation of organ weights between animals and groups might be expected.

MICROSCOPIC

There were no histopathological findings which distinguished treated from control dogs.

O. Discussion

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

Except for one female given 1.0 mg/kg/day which was found dead on study day 3 ("presumably from an epileptic seizure") and replaced on day 7, all dogs survived the 13-week study. There were no clinical signs which differentiated the treated from the control dogs.

During weeks 1-14, group mean body weight gains for males were 2.7, 1.8, 1.4 and 0.4 kg and for females, 1.6, 1.2, 0.6 and 0.8 kg for the 0, 1.0, 3.75 and 7.5 mg/kg/day groups. Food consumption for the 3.75 and 7.5 mg/kg/day males and females were at least 9% less than controls.

Test article intake (mg/kg/day) over the 13 weeks for the 1.0, 3.75 and 7.5 mg/kg/day groups was as follows: males = 1.1, 3.9 and 7.8; females = 1.0, 3.9 and 7.7.

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

Blood urea nitrogen and creatinine levels were elevated ($p \leq 0.05$) in males and females at both the 4 and 13 week intervals at doses of 3.75 and 7.5 mg/kg/day. Though not always statistically significant, alanine aminotransferase levels were elevated in males at 7.5 mg/kg/day and in females at 3.75 and 7.5 mg/kg/day at both 4 and 13 weeks. However, there were no corroborative histopathological changes in the kidneys or liver.

There were no macroscopic or microscopic pathology observations which were considered to have been related to test article administration. The suggestion of a decrease in testicular weights and an increase in thyroid/parathyroid

(both sexes) weights in one or more dose groups, appears to be mostly a reflection of body weights as there were no supporting microscopic findings.

IV. CONCLUSIONS

In a subchronic toxicity study, the 2-ethylhexyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 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 67, 52 and 15% of the control value for males and 75, 38 and 50% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There were elevations in blood urea nitrogen, creatinine and alanine aminotransferase levels at 3.75 and 7.5 mg/kg/day. 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 as well as alterations in clinical chemistry parameters.

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

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

Secondary reviewer: Jess Rowland, M.S. 4/25/94
Section IV, Tox. Branch II

DATA EVALUATION REPORT

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

TEST MATERIAL: Dimethylamine salt of 2,4-dichlorophenoxyacetic acid

SYNONYM: DMA salt of 2,4-D

PC Code: 030001 (2,4-D) **MRID Nos.:** 13-Week Study = 427800-02
4-Week Study = 427800-06

STUDY NUMBERS: 13-Week Study = HWA 2184-126
4-Week Rangefinding Study = HWA 2184-123

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

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

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

AUTHOR: Dan W. Dalgard

REPORTS ISSUED: 13-Week = May 7, 1993; 4-Week = December 15, 1992

EXECUTIVE SUMMARY:

In a subchronic toxicity study, the dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 1, 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 126, 126 and 68% of the control value for males and 78, 61 and 33% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There was decreased food consumption in 7.5 mg/kg/day females as well as elevated alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine at 3.75 and 7.5 mg/kg/day. However, no corroborative histopathological changes were seen in the liver or kidneys. **[MRID Nos. 427800-02 and 06]**

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: Dimethylamine salt of 2,4-dichlorophenoxyacetic acid (DMA salt of 2,4-D)

II. 4-WEEK EXPLORATORY RANGEFINDING STUDY (MRID No. 427800-06)

A. Test Article Description

Lot No.: 04FD31349
Storage: refrigerated
Physical property: brown liquid
Purity: 55.45% 2,4-D acid equivalent ("dietary formulations adjusted accordingly")

B. Test Article Homogeneity, Stability and Concentration

Table 1

TEST ARTICLE HOMOGENEITY, STABILITY AND CONCENTRATION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 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	96	443	94	677	98
middle	362	95	443	96	677	93
bottom	362	97	443	93	677	99
DAY 7						
top	362	91	443	94	677	92
middle	362	91	443	89	677	93
bottom	362	93	443	106	677	94
CONCENTRATION						
week 1	349	102	524	102	697	100
week 5	349	104	524	102	697	100

All % of target are means of duplicate samples.
Data extracted from Report Table 1, pages 24-26

Homogeneity, stability and concentration data are within acceptable limits.

C. Dietary Admixes

After shaking, the amount of test article needed was weighed, added to about 200 g of basal diet and mixed in a Waring blender. This premix was added to appropriate amounts of basal food in order to obtain expected diets (10, 15 or 20 mg/kg/day of the test article). 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. The dogs were individually housed in stainless-steel cages and exercised 3 times each week for at least 15 minutes. Room temperature and humidity were 68-84°F and 12-82%, 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 0, 10, 15 and 20 mg/kg/day (2,4-D acid equivalent).

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 the last 10 dosing days, a dose-dependent increase was reported in the incidence of fecal abnormalities (diarrhea, orange and/or bloody feces and mucoid feces). No controls had any of these signs.

One 20 mg/kg/day dog was hypoactive, thin (last week of the study), had decreased food consumption and lost weight.

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 THE DIMETHYLAMINE SALT OF 2,4-D

Dog No.	mg/kg/day	Body Weight (kg) - Week					Gain or Loss
		1	2	3	4	5	
412	0	6.6	6.7	6.7	6.7	6.8	+0.2
413	0	7.3	7.7	7.5	7.6	7.7	+0.4
414	10	6.8	7.0	6.9	6.9	6.8	0
415	10	7.3	7.3	7.2	7.2	7.2	-0.1
416	10	8.9	9.1	9.0	8.9	8.8	-0.1
417	15	6.7	6.7	6.6	6.2	6.1	-0.6
418	15	8.5	8.5	8.1	7.9	7.6	-0.9
419	15	8.7	8.7	8.2	8.0	8.1	-0.6
420	20	6.4	6.3	5.7	4.9	4.5	-1.9
421	20	7.4	7.4	7.0	6.6	5.9	-1.5
422	20	8.6	8.6	8.0	7.5	6.7	-1.9

Data extracted from Report Appendix 3, page 60.

The two controls gained a small amount of weight with one 10 mg/kg/day dog having the same terminal as initial weight and all other treated animals losing weight in a dose-response fashion.

FOOD CONSUMPTION

This was recorded weekly. Table 3.

With the exception of one 20 mg/kg/day dog (No. 420), animals in treated groups ate about the same amount of food as did controls during week one. During the 4-week study, the same amount of food was consumed each week by controls, 2/3 dogs at 10 mg/kg/day, 1/3 at 15 mg/kg/day and 0/3 at 20 mg/kg/day.

Table 3

INDIVIDUAL FOOD CONSUMPTION IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Dog No.	mg/kg/day	Weekly Food Consumption (g)			
		1	2	3	4
412	0	1087	1229	1057	1109
413	0	1639	1599	1592	1650
414	10	1343	1191	1080	989
415	10	1341	1528	1316	1539
416	10	1703	1637	1386	1406
417	15	1278	1037	671	669
418	15	1683	1104	1085	1006
419	15	1550	1300	1318	1469
420	20	919	382	52	128
421	20	1339	985	801	234
422	20	1437	942	686	351

Data extracted from Report Appendix 4, page 62.

TEST ARTICLE CONSUMPTION

This was calculated weekly and was based on body weight and food eaten. Table 4.

Table 4

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

mg/kg/day	No. of Dogs	Week			
		1	2	3	4
10	3	9.4	9.3	8.2	8.5
15	3	14.1	11.0	10.0	10.4
20	3	16.3	10.4	7.1	3.7

Data extracted from Report Table 5, page 34.

During the 1st week, when food consumption by treated groups was similar to the control group, test article intake was relatively near the target doses. For the last 3 weeks, especially in the 15 and 20 mg/kg/day groups, the amount of test article received was considerably less than the amount targeted (decreased food consumption and body weight decrease).

E. Clinical Pathology

During the last treatment week, blood was taken from the jugular vein of food-fasted dogs and the following clinical chemistry parameters were examined: alkaline phosphatase, urea nitrogen, creatinine and total cholesterol. Table 5.

Table 5

GROUP MEAN CLINICAL CHEMISTRY VALUES (± S.D.) IN A 4-WEEK RANGEFINDING STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

mg/kg/day	Urea nitrogen (mg/DL)	Creatinine (mg/DL)	Alkaline Phos. (U/L)	Total Choles. (mg/DL)
0	16±0.7	0.8±0.07	72±1.4	149±27.6
10	31±10.4	1.3±0.20	40±9.6	165±39.7
15	33±4.0	1.3±0.00	31±1.7	164±4.5
20	40±5.5	1.3±0.12	38±25.7a	161±46.4

a = Individual values were: 18, 29 and 67.
No. of dogs: control = 2; treated = 3/group
Data extracted from Report Table 6, page 36.

In treated groups, there were increases in group mean urea nitrogen and creatinine values. Alkaline phosphatase levels of 2/3 treated dogs in the 20 mg/kg/day group were below the control. The "sick" dog (No. 420) had a value of 18 U/L.

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 40 tissues were fixed in 10% formalin. No tissues were processed or examined microscopicaly.

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 3/3 at 20 mg/kg/day.

G. Discussion

No dogs died during the 4 week study. A dose dependent increase in fecal abnormalities was noted primarily during the last 10 dosing days. Dog No. 420 (20 mg/kg/day) appeared to be "sick" throughout the study.

The 2 control dogs gained 0.2 and 0.4 kg of body weight during the 4 weeks; whereas, the 10 mg/kg/day animals lost 0-0.1 kg, the 15 mg/kg/day lost 0.6-0.9 kg and the 20 mg/kg/day lost 1.5-1.9 kg.

Most treated animals ate less food than did controls during the last 1 or 2 or 3 weeks of the study. The 20 mg/kg/day dogs, during week 4, ate only about 20% of the amount of food consumed by them during week one. The Report indicated that palatability was probably not the cause of decreased food intake as treated and control dogs ate about the same amount during the 1st study week.

In treated animals, there were increases in urea nitrogen and creatinine values as well as decreases in alkaline phosphatase levels.

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

G. Conclusions

All three dose levels were poorly tolerated and were considered too high for longer studies.

III. 13-WEEK STUDY (MRID No.: 427800-02

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, "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: 04FD31349

Storage: refrigerated

Physical Property: brown liquid

Purity: 55.45%, acid equivalent ("weight adjustment was calculated to 100% purity")

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.

Data from all intervals indicated that concentrations were 89-115% 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 THE DIMETHYLAMINE SALT OF 2,4-D

Time/Location	1.0 mg/kg/day		3.75 mg/kg/day		7.5 mg/kg/day	
	Target ppm	% of Target	Target ppm	% of Target	Target ppm	% of Target
PRETEST						
top M ..	-	-	-	-	334.13	98
middle M ..	-	-	-	-	334.13	99
bottom M ..	-	-	-	-	334.13	97
top F ..	34.87	95	-	-	-	-
middle F ..	34.87	96	-	-	-	-
bottom F ..	34.87	100	-	-	-	-
PRETEST (7 day stabil. room temper						
M ..	-	-	-	-	334.13	93
F ..	34.87	94	-	-	-	-
32 DAY STABIL freezer						
M ..	-	-	-	-	334.13	98
F ..	34.87	105	-	-	-	-
WEEK 1						
M ..	44.55	97	167.07	95	334.13	91
F ..	34.87	97	130.77	93	261.54	96
WEEK 12						
M ..	57.42	91	204.33	95	474.19	96
F ..	54.89	96	212.71	102	422.82	94

M = male; F = female
% of targets are means of duplicate samples
Data extracted from Report Table 1, pages 39-43.

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, 1, 3.75 and 7.5 mg/kg/day.

F. Dose Preparation

After shaking, an amount of test article for each dose level was weighed, added to about 200 g of basal diet and mixed in a Waring blender. The premix was then added to appropriate amounts of basal diet and mixed in a Patterson-Kelly blender.

Fresh diets were prepared each week based on sex, body weight and food consumption.

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 where the temperature was 68-77°F and the humidity was 42-77%. 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. Before the start of the study, males weighed 7.7-10.1 kg and females, 5.4-7.5 kg.

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).

The Report (page 23) indicated that fecal abnormalities consisted of discoloration, mucoid, bloody, soft, diarrhea, few or none and that these were observed in both treated and control animals.

Because of the fecal "abnormalities" reported in the 4-week rangefinding study, these incidences in the 13-week study dogs are presented in Table 2.

Table 2

SUMMARY INCIDENCE OF FECAL "ABNORMALITIES" IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Observation	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
diarrhea	0	2	1	1	0	1	1	1
discoloration	2	2	1	2	0	2	0	3
few	1	1	0	1	1	1	2	0
mucoid	3	1	2	3	3	2	1	4
none	0	0	0	1	0	0	0	0
soft	4	4	4	4	4	4	3	4

4 dogs/sex/group
Data extracted from Report Table 2, page 45.

I. Body Weights

Body weights were recorded before treatment and weekly during the study. Table 3.

Table 3

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

Week	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
BODY WEIGHTS								
1	9.2	9.0	8.6	8.8	6.2	6.5	6.2	6.2
2	9.7	9.3	9.1	9.0	6.5	6.8	6.5	6.4
3	10.0	9.7	9.4	9.2	6.8	7.1	6.6	6.4
4	9.9	9.7	9.5	9.6	6.9	7.1	6.7	6.5
6	10.4	10.2	10.0	9.3	7.3	7.4	7.0	6.6
8	10.7	10.6	10.5	9.7	7.6	7.7	7.2	6.7
10	10.8	11.1	10.7	10.1	8.0	7.8	7.3	6.5
12	10.6	11.2	10.9	9.9	8.0	8.0	7.3	6.7
14	11.1	11.4	11.0	10.1	8.0	7.9	7.3	6.8
BODY WT GAIN								
1-4	0.7	0.7	0.9	0.8	0.7	0.6	0.5	0.3
4-8	0.8	0.9	1.0	0.1	0.7	0.6	0.5	0.2
8-14	0.4	0.8	0.5	0.4	0.4	0.2	0.1	0.1
1-14	1.9	2.4	2.4	1.3	1.8	1.4	1.1	0.6*

4 dogs/sex/group
Body weight gains were calculated by the Reviewer for all intervals except weeks 1-14 which were included in the Report.
Statistical Significance: * = ≤ 0.05; presented in the Report only for weeks 1-14 body weight gains.
Data extracted from Report Table 3, pages 47 and 48.

The only statistically significant ($p \leq 0.05$) difference between a treated group and the control, was a lesser body weight gain in the 7.5 mg/kg/day female group. Individual animal weight gains (kg) in the female groups were (mg/kg/day): 0 = 2.4, 1.9, 1.7 and 1.4; 1 = 1.8, 1.6, 1.3 and 0.9; 3.75 = 1.4, 1.1, 1.1 and 0.8; and 7.5 = 1.5, 0.4, 0.3 and 0.1.

J. Food Consumption

Data for this parameter were recorded each week.

For males, there was essentially no difference in the group mean g of food consumed by treated or control groups. An accurate evaluation regarding the 7.5 mg/kg/day males is not considered possible because of the number of weeks that 3 of 4 dogs had "exclude" in place of a value.

Though not statistically significant, the grams of food consumed/week by the 7.5 mg/kg/day females was less than the control at most weekly measurements. The 1 and 3.75 mg/kg/day female group mean values were more similar to the control.

K. Test Article Intake (Report Table 5, pages 53 and 54)

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):

males = 1.1, 4.0 and 8.3 for 1.0, 3.75 and 7.5
females = 1.0, 3.8 and 7.7 for 1.0, 3.75 and 7.5

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 at termination which were not reported prior to the beginning of dosing. Dog No. 29577, male, 3.75 mg/kg/day, had hemorrhage of the right fundus (pretest only). Dog No. 29578, male, 3.75 mg/kg/day, had pignment spots on the right fundus (pretest and at termination). Dog No. 29589, female, 7.5 mg/kg/day, had aberrant eyelashes, both conjunctiva (pretest and at termination). Dog No. 29590, female, 7.5 mg/kg/day, had had a non-tapetal hypopigmented area of the right fundus (pretest and 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 (food 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*	Total bilirubin*
Aspartate aminotransferase*	Total cholesterol
Alkaline phosphatase	Total protein*
Albumin*	Glucose*
Globulin	Inorganic phosphorus*
Urea nitrogen*	Potassium*
Creatine kinase	Sodium*
Creatinine*	Calcium*
	Chloride*

* = EPA Guideline Requirement

Those parameters which appeared to be affected by test article administration (some, $p \leq 0.05$) were alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine. [Table 4] The Report stated that alkaline phosphatase levels which decline normally with age, declined more rapidly in treated animals than in controls. [Table 5]

Table 4

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

mg/kg/day	Alanine amino. U/L			Aspartate amino. U/L			Urea nitrogen MG/DL			Creatinine MG/DL		
	-1a	4	13	-1	4	13	-1	4	13	-1	4	13
MALE												
0	25	28	35	33	34	35	10	11	12	0.6	0.7	0.9
1	30	39	51*	32	35	39	12	19*	17	0.6	1.0*	1.2*
3.75	28	61*	88*	33	35	43	11	18*	22*	0.6	1.0*	1.2*
7.5	34	223*	388*	46	41	48	12	22*	25*	0.7	1.1*	1.3*
FEM												
0	29	36	41	28	31	34	12	16	13	0.6	0.7	0.8
1	32	36	36	37	47	40	11	19	18	0.6	0.9	1.0
3.75	30	50	47	36	40	42	11	20	22*	0.6	1.0*	1.2*
7.5	30	74*	87*	35	42	49*	13	22	25*	0.7	1.1*	1.3*

4 dogs/sex/group
a = week of determination
Statistical Significance; * = $p \leq 0.05$
Data extracted from Report Table 7 and Appendix 7, pages 62-67 and 154-165.

The 7.5 mg/kg/day group mean alanine aminotransferase value of 388 U/L at week 13 (control = 35) consisted of the following 4 values: 1,081, 215, 136 and 118 (controls of 31-37). The 3.75 mg/kg/day group mean value for this enzyme at week 13 was 88 and consisted of the following 4 values: 199, 57, 54 and 43.

Table 5

GROUP MEAN ALKALINE PHOSPHATASE VALUES (U/L) IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

Week	Males (mg/kg/day)				Females (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
-1	104	126	113	101	134	100*	131	144
4	86/17	113/10	86/24	70/31	112/16	78/22	93/29	86/40
13	62/40	74/41	63/44	68/33	89/34	49*/51	63*/52	78/46

#/# = enzyme value Units per Liter/% reduction from week -1 value
4 dogs/sex/group
Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 7, page 63.

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.

M. 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 percents as well as organ-to-brain weight ratios were calculated: adrenals, brain with stem, heart, kidneys, liver with gallbladder, ovaries, pituitary, testes without epididymides and thyroid with parathyroids.

The following tissues were removed, preserved in 10% neutral-buffered formalin and the "x" organs were 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*	xThyroids*	Gross lesions and masses*
Eyes (with optic n.)*		

* = EPA Guideline Requirements

MACROSCOPIC

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

ORGAN WEIGHTS

Testicular weights (absolute, to-body weight and to-brain weight) of the 7.5 mg/kg/day dogs were lower (not statistically significant) than controls. Table 6.

Table 6

GROUP MEAN ± S.D. TESTICULAR WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

	mg/kg/day			
	0	1	3.75	7.5
absolute g	17.0±3.9	15.7±3.0	15.1±4.4	9.8±2.3
to-body wt %	0.16±0.03	0.14±0.02	0.14±0.05	0.10±0.02
to-brain wt ratio	0.21±0.05	0.20±0.04	0.20±0.06	0.13±0.03

Data extracted from Report Table 9, page 85.

The group mean absolute brain weight of 7.5 mg/kg/day male dogs was lower ($p \leq 0.05$) than the control mean. The Report Author did not consider this to be of biological significance. Table 7.

Table 7

GROUP MEAN BRAIN WEIGHTS IN A 13-WEEK STUDY IN DOGS WITH THE DIMETHYLAMINE SALT OF 2,4-D

	Male (mg/kg/day)				Female (mg/kg/day)			
	0	1	3.75	7.5	0	1	3.75	7.5
absolute g	82.1	80.3	76.2	72.7*	71.1	74.4	69.0	67.2
to-body wt %	0.79	0.74	0.71	0.76	0.93	1.00	0.99	1.06

Terminal body weights: males = 10.5, 11.0, 10.8 and 9.8 kg (0, 1, 3.75 and 7.5 mg/kg/day)
females = 7.7, 7.5, 7.0 and 6.4 kg (0, 1, 3.75 and 7.5 mg/kg/day)
Statistical Significance: * = $p \leq 0.05$
Data extracted from Report Table 9, page 82.

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As the brain-to-body weight values for the control and 7.5 mg/kg/day are similar, the lower ($p \leq 0.05$) absolute weight is probably a reflection of a lower group mean terminal body weight.

MICROSCOPIC

There were no definitive histopathologic findings which were attributed to test article administration.

The Pathology Report (Report page 36) stated, "There was a minimal increase in the average severity of perivascular, chronic active inflammation in the liver of Group 4 (7.5 mg/kg/day) males and females when compared to Group 1 (control) males and females. This increase in severity was due to a slight severity in two males and a moderate severity in one female. Minimal perivascular, chronic active inflammation of the liver noted in controls, across all male treated groups, and Group 4 females, and the single slight occurrence in a Group 3 female, is considered unrelated to treatment."

O. Discussion

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

There was no mortality.

An increased incidence of fecal "abnormalities" (as noted in the 4-week Rangefinding study) was not observed in this 13-week study.

The only statistically significant ($p \leq 0.05$) decrease in body weight gain occurred in the 7.5 mg/kg/day females, although in males, the controls gained a group mean of 1.9 kg and the 7.5 mg/kg/day dogs, 1.3 kg.

Food consumption in all treated male groups was similar to the control, although it was not possible to evaluate this parameter accurately because 3 of 4 dogs at 7.5 mg/kg/day did not have food consumption values for most of the weeks (no explanation was given for the lack of data). Females given 7.5 mg/kg/day ate less (not statistically significant) than the controls during most weeks.

Test article intake (mg/kg/day) over the 13 weeks for the 1, 3.75 and 7.5 mg/kg/day groups was as follows: males = 1.1, 4.0 and 8.3; females = 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.

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Statistically significant ($p \leq 0.05$) or apparent (not significant) increases in alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine were reported for males and females of the 7.5 mg/kg/day group at the 4 and 13 week intervals. The 3.75 mg/kg/day group also had values for some of these parameters increased over controls. There were no liver or kidney organ weights or histopathology findings which clearly corroborated these clinical chemistry results.

Testicular (absolute, to-body weight and to-brain weight) weights of the 7.5 mg/kg/day group were lower (not statistically significant) than the control. There were no histopathological findings.

The Pathology Report indicated that there was a minimal increase in the average severity of perivascular, chronic active inflammation in 7.5 mg/kg/day male and female livers (primarily due to 2 males and 1 female), but that this was not considered related to treatment.

IV. CONCLUSIONS

In a subchronic toxicity study, the dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D) was administered to Hazleton beagle dogs as a dietary admix at nominal doses of 0, 1, 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 126, 126 and 68% of the control value for males and 78, 61 and 33% for females at 1, 3.75 and 7.5 mg/kg/day, respectively. There was decreased food consumption in 7.5 mg/kg/day females as well as elevated alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine at 3.75 and 7.5 mg/kg/day. 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.

END