PRIMARY REVIEWER: Jess Rowland, M.S., Toxicologist 5/28/91
Section II, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head 6/3/91
Section II, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding-Nonrodent **GUIDELINE**: 82-1(b)

TOX.CHEM. No.: 315 **MRID No.:** 417373-01 **HED Project No.**1-0620

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid (2,4-D **acid)**

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

STUDY IDENTIFICATION: HLA Study No. 2184-115

TESTING LABORATORY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Subchronic Toxicity Study in Dogs with 2,4-Dichlorophenoxyacetic acid.

REPORT AUTHOR: Gene E. Schulze, Ph.D

REPORT DATE: December 14, 1990

SUMMARY: Groups of five male and five female dogs were given oral administration (via capsules) of **2,4-Dichlorophenoxyacetic acid** at 0, 0.3, 1.0, 3.0, or 10 mg/kg/day for 13 weeks. No treatment-related effects were observed at 0.3 mg/kg/day. At 1 mg/kg/day, the only compound-induced effects were transient changes in a few clinical chemistry parameters. However, the values for these parameters were well within the reference range for beagle dogs and therefore were not considered to be adverse effects. The dose level of 3 mg/kg/day was associated with altered hematological and clinical chemistry parameters, and histopathological changes in the kidneys of male dogs. At a dose of 10 mg/kg/day, 2,4-D acid increased mortality, lowered body weight gain, altered hematological and clinical chemistry parameters, decreased testicular weights and increased kidney weights, and induced histopathological changes in the kidneys of both sexes and testes of male dogs.

Under the conditions of this study, a NOEL of 1.0 mg/kg/day and a LEL of 3.0 mg/kg/day was established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid in male and female beagle dogs.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (82-1b) for a 90-day feeding study in nonrodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a subchronic toxicity study in dogs with **2,4-Dichlorophenoxyacetic acid.**

II. MATERIALS AND METHODS

1. Test and Control Articles

Test Chemical Name: 2,4-Dichlorophenoxyacetic acid.
Purity: 96.1%
Lot No.: 909
Description: Brown powder.
Control Material: Hard gelatin capsules, 1/4 oz.
Batch No.: 3403
Manufacturer: Topac Limited, Toronto, Canada.

2. Test Animals

Species: Dogs
Strain: Beagle
Sex: Males and females
Age: 4-6 months at initiation of study.
Weight: 5.6-8.2 kg (M), 4.5-6.1 kg,(F) at initiation.
Identification: Ear tags.
Acclimation: 14-days
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Canine Diet Meal #5007.
Water: Tap water ad libitum
Environment: Temperature- 70 - 77°F; Humidity- 40 - 85 %;
Light/dark cycles: 12 hr.light/12 dark cycle

3. Study Design

Group No.	Treatment	No. of Animals		Dose Level mg/kg/day[a]
		Males	Females	
1	Control	5	5	0
2	Low	5	5	0.3
3	Mid-1	5	5	1.0
4	Mid-2	5	5	3.0
5	High	5	5	10.0

[a] Adjusted based on most recent body weight.

4. **Test Material Formulation**

The amount of test material required for each dog was weighed out and then transferred into one 1/4 oz gelatin capsule for administration to the appropriate animal. The amount weighed for each dog was recorded with the project, week, and dog numbers, and the date.

5. **Treatment**

Dogs were given either one empty gelatin capsule (control, Group 1)) or the test material in hard gelatin capsule(s) (Groups 2, 3, 4, and 5) daily for 13 weeks.

6. **Experimental Procedures**

Mortality and moribundity checks were performed twice daily. Pharmacotoxic effects were monitored one-half hour post-dosing. Body weights and food consumption were measured once weekly. Ophthalmologic examinations were conducted prior to initiation and at termination. The following hematology, clinical chemistry, and urinalysis parameters were measured prior to initiation, and at weeks 4, 8 and 13.

The checked (x) parameters were measured

Hematology	Clinical Chemistry
x Hematocrit (HCT)*	Alkaline phosphatase
x Hemoglobin (HGB)*	Cholinesterase)
x Leukocyte count (WBC)*	x Creatinine phosphokinase‡
x Erythrocyte count (RBC)*	Lactic acid dehydrogenase
x Leukocyte diff. count*	x Serum alanine aminotransferase*
Mean corpuscular HGB (MCH)	x Serum aspartate aminotransferase*
Mean corpuscular conc.(MCHC)	Gama glutamyl transferase
Mean corpuscular volume (MCV)	Glutamate dehydrogenase
x Platelet count*	x Albumin*
Reticulocyte count	x Albumin/globulin ratio
Blood clotting time	x Blood urea nitrogen*
	x Creatinine*
* Required for subchronic studies	Cholesterol
	x Globulins
	x Glucose*
	x Total bilirubin*
	x Total protein*
	Serum protein electrophoresis
	x Triiodothyronine T_3
	x Thyroxine T_4
	* Required for subchronic studies
	) Only for organophosphates

Urinalysis[@]

x Appearance*
x Glucose*
x Ketones*
Volume*
x Bilirubin*
x Specific gravity*
x pH
x Blood
x Protein*
x Sediment (microscopic)*
x Urobilinogen*
x Ova and parasites

@ Not required for subchronic studies.
* Required for chronic studies.

7. **Termination**

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium thiamylal, and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Brain	Kidneys	Liver with drained gall bladder
Ovaries	Heart	Testes with epididymides
Thyroid/parathyroids		

8. Histopathology

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Tongue x Salivary gland* x Esophagus* x Duodenum* x Jejunum* x Cecum* x Colon* x Rectum* x Liver*) x Gall bladder* x Pancreas Neurological System x Brain*) x Pituitary* Peripheral nerve*# x Spinal cord (3 levels)*# Eyes (optical nerve)*# Glandular System x Adrenals* Lacrimal glands# x Parathyroids*‡ x Thyroids*‡	x Trachea* x Lung* Pharynx+ Larynx+ Nose+ Cardiovascular/Hemo.System x Aorta (thoracic)* x Heart* x Bone marrow* x Lymph nodes* x Spleen* x Thymus* Urinogenital System x Kidneys*‡ x Urinary bladder* x Tests*) Epididymides x Seminal vesicle x Uterus* x Ovaries*) Others x All gross lesions and masses x Skeletal muscle*

* Required for subchronic and chronic studies.
\+ Required for chronic inhalation study.
\# In subchronic studies examined only if indicated by toxicity or target organ involvement.
) Organ weights required in subchronic and chronic studies.
‡ Organ weights required for nonrodent studies.

9. Statistical Analyses

Mean body weights and body weight changes, mean weekly food consumption, total food consumption, clinical pathology data (with the exception of urinalyses and cell morphology), and organ weight data of treated groups were compared statistically against data of the control group of same sex by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. All analyses were conducted using two-tailed tests with a minimum significance level of 5%.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Labs, and the Study Protocol.

III. RESULTS

1. Survival

No mortality occurred during the study. However, two males and one female at the 10 mg/kg/day group were sacrificed moribund during study Week 4, 13, and 9, respectively. Histopathology revealed inflammation in various areas of the skin and subcutis in the males and hepatic and splenic changes (which were compatible with severe anemia) in the female.

2. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for male and female dogs, respectively. A statistically significant ($p < 0.05$) reduction in mean body weight was observed for the high-dose (10 mg/kg/day) males at the Week 8 interval when compared with control males at this interval. Mean body weights of males in this group were consistently lower than the controls throughout the study. Mean body weights of the high-dose females were also lower than the controls throughout the study; however, the decreases were not statistically significant.

Table 1. **Mean Body Weights and S.D (kg) for Male Dogs.**

Dose Level (mg/kg/day)	Week: 0	Week: 4	Week: 8	Week: 13
0	6.7 ± 0.9	8.7 ± 1.1	9.7 ± 1.6	10.3± 1.7
0.3	7.0 ± 0.9	8.5 ± 1.2	9.3 ± 1.3	9.5 ± 1.5
1.0	7.1 ± 1.0	9.1 ± 1.2	10.0 ± 1.3	10.3± 1.2
3.0	6.6 ± 0.8	8.0 ± 0.6	8.5 ± 0.8	8.6 ± 0.8
10.0	6.6 ± 0.9	7.6 ± 0.8	**7.5* ± 0.8**	8.2 ± 0.3

* Significantly different from control value at $p < 0.05$.

Table 2. **Mean Body Weights and S.D (kg) for Female Dogs.**

Dose Level (mg/kg/day)	Week: 0	Week: 4	Week: 8	Week: 13
0	5.2 ± 0.4	6.7 ± 0.9	7.3 ± 1.0	7.7 ± 1.2
0.3	5.6 ± 0.5	7.0 ± 0.6	7.6 ± 0.7	8.0 ± 0.6
1.0	5.2 ± 0.4	6.5 ± 0.9	7.2 ± 0.8	7.6 ± 1.0
3.0	5.3 ± 0.3	6.7 ± 0.4	7.1 ± 0.4	7.4 ± 0.4
10.0	5.3 ± 0.4	6.2 ± 0.7	6.1 ± 1.3	6.6 ± 1.2

Body weight change data are presented in Tables 3 and 4 for male and female dogs, respectively. Statistically significant ($p < 0.05$) differences in body weight gains were seen in both males and females a the high-dose. Males exhibited lowered body weight gains during Weeks 0 through 4 interval and during the Weeks 4 through 8 interval and females during the Weeks 4 through 8 interval when compared to controls. In addition, the mean body weight changes for females at the high-dose were lower than control throughout the study.

Table 3. Body Weight Change (kg) in **Male Dogs.**

Dose Level (mg/kg/day	Weeks: 0 - 4	Weeks: 4 - 8	Weeks: 8 - 13
0	2.0 ± 0.38	1.0 ± 0.49	0.6 ± 0.28
0.3	1.5 ± 0.37	0.8 ± 0.30	0.2 ± 0.31
1.0	1.9 ± 0.48	0.9 ± 0.39	0.3 ± 0.37
3.0	1.3 ± 0.31	0.6 ± 0.26	0.1 ± 0.13
10.0	**0.9* ± 0.62**	**0.0* ± 0.52**	0.3 ± 0.21

* Significantly different from control at $p < 0.05$.

Table 4. Body Weight Change (kg) in **Female Dogs.**

Dose Level (mg/kg/day	Weeks: 0 - 4	Weeks: 4 - 8	Weeks: 8 - 13
0	1.5 ± 0.49	0.6 ± 0.13	0.4 ± 0.33
0.3	1.4 ± 0.54	0.6 ± 0.324	0.4 ± 0.20
1.0	1.4 ± 0.48	0.6± 0.29	0.5 ± 0.30
3.0	1.3 ± 0.26	0.4 ± 0.16	0.3 ± 0.15
10.0	0.9 ± 0.33	**0.1* ± 0.65**	0.0 ± 0.30

* Significantly different from control at $p < 0.05$.

3. Clinical Observations

Treatment-related clinical signs of toxicity observed in both sexes of treated dogs were thin appearance, languid appearance, anorexia, emesis, swollen paw, and swollen testes (males); these signs were most prevalent in the high-dose group and were especially prevalent in the three animals (2 males and 1 female) that were sacrificed moribund. Other signs were comparable to those seen in controls.

4. Ophthalmology Examination

No treatment-related ocular changes were seen at any dose level.

5. Food Consumption

Mean food consumption was significantly ($p < 0.05$) decreased in males at the 10 mg/kg/day during Weeks 4 (1.3 kg vs 2.0 kg in controls) and 8 (1.4 kg vs 2.0 kg). Total food consumption was also significantly ($p < 0.05$) reduced in the high-dose males throughout the study; 19.1 $\pm$ 1.9 kg vs 24.9 $\pm$ 3.27 in controls). Although no statistically significant differences were noted, mean and total food consumption values were also lower in the high-dose females.

6. Hematology and Clinical Chemistry

The significant, treatment-related hematological changes observed in dogs only at the 10 mg/kg/day are summarized Tables 5, 6, 7 and 8. Significant ($p < 0.05$) decreases in hemoglobin values were seen during Weeks 8 and 13 for male at the 10 mg/kg/day dose, and in hematocrit values in these dogs at Week 13. Male dogs at this dose also exhibited nonsignificant decreases in mean erythrocyte count, hemoglobin, and/or hematocrit values at most of the remaining treated intervals. Mean platelet counts were significantly ($p < 0.05$) decreased in the high-dose males and females at Week 4 and 8, respectively, with nonsignificant decreases at the remaining treated intervals.

The female dog (Animal # G27543) at the 10 mg/kg/day group that was sacrificed moribund showed severe anemia with a profound decline in erythrocyte count (1.39×10^{6} μL), hemoglobin (3.3 g/DL), and hematocrit (9.3%), leucocyte count (2.2×10^{3} μL), absolute segmented lymphocyte count (1.1×10^{3} μL) at Week 8. This dog also had prominent decreases in platelet counts at Week 4 (56×10^{3} μL) and Week 8 (11×10^{3} μL). The male dog (Animal # G27538) that was sacrificed moribund at the high-dose did not exhibit anemia, however, decreases in platelet count were seen at Weeks 4 through 13 (127×10^{3}, 41×10^{3}, 125×10^{3} μL, respectively).

Table 5. Mean Hemoglobin (G/DL) Values in **Male Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	12.8 ± 0.7	12.7 ± 0.9	13.0 ± 0.5	14.5 ± 0.9
10 mg/kg/day	12.9 ± 0.5	12.4 ± 0.7	**11.9* ± 0.3**	**11.9* ± 0.5**

* Significantly different from control at p <0.05.

Table 6. Mean Hematocrit (%) Values in **Male Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	37.7 ± 1.6	38.0 ± 2.0	38.5 ± 2.1	41.4 ± 2.1
10 mg/kg/day	38.2 ± 1.5	36.1 ± 1.9	35.2 ± 1.5	**34.4* ± 1.6**

* Significantly different from control at p <0.05.

Table 7. Mean Platelet (TH/UL) Values in **Male Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	471 ± 36	389 ± 13	384 ± 30	354 ± 6
10 mg/kg/day	403 ± 85	**243* ± 91**	227 ± 127	252 ± 96

* Significantly different from control at p <0.05.

Table 8. Mean Platelet (TH/UL) values in **Female Dogs.**

Dose/ Week	-1	4	8	13
0 mg/kg/day	452 ± 85	374 ± 83	348 ± 46	330 ± 61
10 mg/kg/day	427 ± 86	261 ± 143	**211* ± 83**	281 ± 83

* Significantly different from control at p <0.05.

Treatment-related changes (significantly different from control value at $p < 0.05$) observed in clinical chemistry parameters are summarized in Table 9.

Table 9. Significant Changes in Clinical Chemistry Parameters.

Parameter Evaluated	Dose Level mg/kg/day	Sex	Study Week	Effect(s)
GLUCOSE	10	Males	13	Decreased
GLUCOSE	10	Females	13	Decreased
BUN	10	Males	4	Increased
BUN	10	Females	4	Increased
BUN	1,3 & 10	Males	8	Increased
BUN	1,3,& 10	Males	13	Increased
BUN	3 & 10	Females	13	Increased
CREAT	3	Males	13	Increased
CREAT	10	Males	4,8,13	Increased
CREAT	3	Females	13	Increased
CREAT	1, 3 & 10	Females	4	Increased
CREAT	1 & 3	Females	8	Increased
IN PHOS	0.3,1,3,10	Males	4	Decreased
T.BILI	1	Females	13	Increased
ALT	10	Females	4,13	Increased
CALCIUM	1 & 3	Females	13	Increased

Urinalyses were generally comparable between control and treated groups except for the lower specific gravity values for males and female dogs at the high dose during Week 13. These differences may reflect a loss of renal concentrating ability.

7. Gross Pathology

Treatment-related gross pathological changes observed at terminal sacrifice were limited to small testes in male dogs in the 3 and 10 mg/kg/day groups.

8. Organ Weights

Evaluation of the organ weight data showed a significantly ($p < 0.05$) lower absolute testis/epididymis weight for male dogs at the high dose and a significantly elevated relative kidney weight for females at the high dose. No other significant differences were observed in group mean absolute and relative organ weight values between the treated and control groups.

9. Histopathology

Moribund Sacrifices: Of the two males sacrificed moribund at the high dose, one (Animal # G27534) showed inflammation in the subcutis of the cervical region, extending around and into the salivary gland and thyroid gland. The lesion was compatible with bacterial infection.The dog had thymic lymphoid depletion which was considered to be secondary to severe stress. The inflammatory lesion in the subcutis was considered to be the cause of debility.

The other dog (G27538) had multiple ulcered and inflammatory lesions in the skin as well as thymic lymphoid depletion. The thymic lymphoid depletion was considered to be related to the in-life observations rather than the dermatitis.

The female dog (G27543) which was sacrificed had severe centrilobular necrosis in the liver, moderate extramedullary hematopoiesis in the spleen, and thymic lymphoid depletion. The combination of hepatic and splenic lesions was compatible with severe anemia, and the thymic lesion was considered to be secondary to severe stress.

All three dogs exhibited cellular alterations in the tubular epithelium of the kidneys.

Terminal Sacrifice: Treatment-related histopathological alterations observed in dogs sacrificed at the end of the study are were limited to kidneys and testes. No histopathological lesions were seen in the testes or kidneys of control males or in the kidneys of control females.The incidence are summarized in Table 10.

Table 10. Histopathological Lesions in Dogs Given 2,4-D acid.

Lesions	Dose Level (mg/kg/day)	Incidence Males	Incidence Females
Kidneys: Cellular alterations, proximal tubules	3.0	3/5	0/5
	10.0	3/3	1/4
Testes: Hypo-spermatogenesis	10	1/3	
Giant cell formation	10	1/3	

Kidney lesions were characterized as cellular alterations in the proximal convoluted tubules. This subtle change consisted of a reduction in the size and reduction in cytoplasmic eosinophilia of epithelial cell lining some proximal convoluted tubules. The pathogenesis was similar to the changes seen in regenerative tubular epithelium.

Testicular lesions were characterized by testicular hypospermatogenesis and giant cell formation in the testes. Hypospermatogenesis consisted of a reduction in spermatogenic activity and seminiferous tubules, sometimes to the degree that individual tubules were lined only by Sertoli cells. Giant cells consisted of intra-tubular aggregations of multinucleated and/or karyomeglic cells which appeared to the derived from spermatogenic elements.

The other histopathological alterations observed were considered agonal or typical findings in clinically normal dogs of this strain and age.

IV. DISCUSSION

Oral administration of 0.3 mg 2,4-D acid/kg/day was not associated with any treatment-related changes.

At 1.0 mg/kg/day, the only compound-induced effect was transient changes in a few clinical chemistry parameters. However, as shown below, the values for these parameters were well within the reference range for beagle dogs and therefore were not considered to be toxicologically significant.

Sex	Parameter	1.0 mg/kg/day	Concurrent Controls	Reference Range for Hazleton dogs[a]
Males	BUN (MG/DL)	18 ± 4 (week 8)	12 ± 1.6	9 - 20
	PHOS (MG/DL)	6.7 ± 0.7 (week 4)	7.7 ± 0.6	3.1 - 8.4
Females	CREAT (MG/DL)	1.1 ± 0.9 (week 13)	0.9 ± 0.04	0.6 - 1.1
	CALCIUM (MG/DL)	11.5 ± 0.3 (week 13)	11 ± 0.15	9.8 - 11.6
	T.BILI (MG/DL)	0.2 ± 0.04	0.1 ± 0.0	0.2 - 0.5

[a] Source: Historical Control Data, Hazleton Labs 1984.

The dose level of 3.0 mg/kg/day was associated with altered hematological and clinical chemistry parameters, and microscopic changes in the kidneys of male dogs.

At a dose of 10 mg/kg/day, 2,4-D acid increased mortality, produced body weight loss, altered hematological and clinical chemistry parameters, decreased testicular weights and increased kidney weights, and induced histopathological changes in the kidneys of both sexes and testes of male dogs. The changes in BUN and creatinine levels seen in dogs given the 3.0 and 10 mg/kg/day doses were correlated with histopathological changes in the kidneys of these dogs. Kidney lesions in these animals consisted of a reduction in cytoplasmic eosinophilia of epithelial cells lining some proximal convoluted tubules of the kidney. The reduction in testicular weight noted in the high dose males was associated with testicular hypospermatogenesis and giant cell formation.

No mortality occurred during the study; however, 2 males and 1 female dog were sacrificed moribund at the 10 mg/kg/day group. Of the 2 males, 1 exhibited severe bacterial infection while the other animal was thin, languid, anorexic and displayed tremors. Microscopic lesions observed in this dog were multiple ulcerative inflammatory lesions in the skin, thymic lymphoid depletion and reduction in cytoplasmic eosinophilia of epithelial cells lining some proximal convoluted tubules of the kidneys similar to those observed in males at this dose level. The female dog exhibited severe signs of anemia which was associated with severe centrilobular hepatocellular necrosis, moderate extramedullary hematopoiesis in the spleen and thymic lymphoid depletion.

V. CONCLUSION

Under the conditions of this study, a NOEL of 1.0 mg/kg/day and a LEL of 3.0 mg/kg/day was established for the 90-day oral toxicity of 2,4-Dichlorophenoxyacetic acid to male and female dogs.

2,4-D: 13-Week Oral Toxicity Study in Dogs
Industry Task Force on 2,4-D Research Data. 1990. MRID No. 41737301.
HED Doc. No. 008400.

13544

R144311

Chemical: 2-4,D

PC Code: 030001
HED File Code: 13000 Tox Reviews
Memo Date: 5/28/1991
File ID: TX0008400
Accession #: *000-00-0119*

HED Records Reference Center
5/10/2007