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TOXICITY OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXYACETIC ACID

A Report on Their Acute and Chronic Toxicity in Dogs

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DETROIT

BOTH 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) may act as plant hormones and herbicides¹ and are now being fairly widely used for the control of certain types of weeds on farm lands. They are being sprayed from airplanes on many acres of specialized farms and grazing land and are present in trademark preparations sold at retail for home gardeners. With the widespread use of these herbicides, possible toxic effects are important, as accidental ingestion may occur or residues may be inadvertently encountered in food. In an earlier report, 2,4-D was mentioned as being nontoxic to animals and man when administered orally.^{2,3} However, the injection of large doses of 2,4-D in animals produced symptoms similar to those seen in clinical myotonia.⁴ More detailed observations were reported by Hill and Carlisle who studied the acute and subacute effects of 2,4-D in various species of animals.⁵ There have been no reports concerning the toxic properties of 2,4,5-T. The present study concerns the acute oral toxicity and chronic oral toxicity of 2,4-D and 2,4,5-T in dogs.

EXPERIMENTAL PROCEDURES

Adult mongrel dogs of both sexes were used. Most of the animals were housed in the laboratory for a period of two to three months before the study was started. During this control period they were immunized against distemper with the Gervac vaccine. The dogs were fed a standard stock diet (Frickies) of Elixton. Both 2,4-D and 2,4,5-T were commercial materials, with a purity of 98.5 and 92.9% and freezing points of 132.8 and 150.6 C., respectively.

In the acute studies, the calculated dose of 2,4-D or 2,4,5-T was administered as a single oral dose in capsules. The dogs were observed for a period of 14 days, at which time the sur-

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1. (a) Hildebrand, E. M.: War on Weeds, Science 80:455-458, 1946. (b) van Overbeek, J., and Viller, L.: Use of 2,4-Dichlorophenoxyacetic Acid as a Selective Herbicide in the Tropics, Science 80:472-473, 1946. (c) Smith, F. G.; Hammer, C. L., and Carlson, R. F.: Control of Ragweed Pollen Production with 2,4-Dichlorophenoxyacetic Acid, Science 80:473-474, 1946. (d) Mark, P. C., and Nichol, J. W.: 2,4-Dichlorophenoxyacetic Acid as a Differential Herbicide, Bot. Gaz. 106:224-222, 1944.

2. Becker, M. L. R.: Effects of 2,4-Dichlorophenoxyacetic Acid on Experimental Animals, Proc. Soc. Exper. Biol. & Med. 63:204-205, 1946.

3. Hill, E. V., and Carlisle, H.: Toxicity of 2,4-Dichlorophenoxyacetic Acid for Experimental Animals, J. Indust. Hyg. & Toxicol. 29:83-85, 1947.

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 vivens were autopsied. In the chronic studies, the 2,4-D or 2,4,5-T was administered orally in capsules five days a week over a 13 week period. Each capsule was imbedded in a 4 to 5 gm. piece of commercial canned dog food, which the animals consumed readily, thus avoiding the continued trauma of the stomach tube. During the study the animals were weighed twice a week. Control blood counts were taken before the administration of the drug, on the 30th day, and on the 90th day at the completion of the study. Hemoglobin was determined with a photoelectric colorimeter.⁴ Upon death or at the completion of the study, the animals were autopsied. Tissues were taken from the lung, heart, liver, kidney, adrenals, spleen, thyroid, and ovary or testes, fixed in 10% formalin, and stained with hematoxylin and eosin.

TABLE 1.—Effect of Single Oral Doses of 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid in Dogs

Dog No. ^a	Dose, Mg./Kg.	Weight Loss, Kg.	Emaciation	Death Day	Mortality
2,4-D					
27.....	50	-0.1	+	2	2/3
28.....	50	-0.1	++	3	
29.....	50	-0.1	+++	4	2/3
30.....	50	-0.1	++	5	
31.....	50	-0.1	++	6	
32.....	50	-0.1	+	7	3/4
33.....	50	-0.1	+	8	
34.....	50	-0.1	+	9	
35.....	50	-0.1	0	10	
36.....	50	-0.1	0	11	
37.....	50	-0.1	0	12	
38.....	50	-0.1	0	13	
39.....	50	-0.1	0	14	
40.....	50	-0.1	0	15	
2,4,5-T					
41.....	50	-0.1	+	1	2/3
42.....	50	-0.1	+	2	2/3
43.....	50	-0.1	0	3	2/4
44.....	50	-0.1	0	4	
45.....	50	-0.1	0	5	
46.....	50	-0.1	0	6	
47.....	50	-0.1	0	7	
48.....	50	-0.1	0	8	
49.....	50	-0.1	0	9	
50.....	50	-0.1	0	10	
51.....	50	-0.1	0	11	
52.....	50	-0.1	0	12	
53.....	50	-0.1	0	13	
54.....	50	-0.1	0	14	
55.....	50	-0.1	0	15	
56.....	50	-0.1	0	16	
57.....	50	-0.1	0	17	
58.....	50	-0.1	0	18	
59.....	50	-0.1	0	19	
60.....	50	-0.1	0	20	
61.....	50	-0.1	0	21	
62.....	50	-0.1	0	22	
63.....	50	-0.1	0	23	
64.....	50	-0.1	0	24	
65.....	50	-0.1	0	25	
66.....	50	-0.1	0	26	
67.....	50	-0.1	0	27	
68.....	50	-0.1	0	28	
69.....	50	-0.1	0	29	
70.....	50	-0.1	0	30	
71.....	50	-0.1	0	31	
72.....	50	-0.1	0	32	
73.....	50	-0.1	0	33	
74.....	50	-0.1	0	34	
75.....	50	-0.1	0	35	
76.....	50	-0.1	0	36	
77.....	50	-0.1	0	37	
78.....	50	-0.1	0	38	
79.....	50	-0.1	0	39	
80.....	50	-0.1	0	40	
81.....	50	-0.1	0	41	
82.....	50	-0.1	0	42	
83.....	50	-0.1	0	43	
84.....	50	-0.1	0	44	
85.....	50	-0.1	0	45	
86.....	50	-0.1	0	46	
87.....	50	-0.1	0	47	
88.....	50	-0.1	0	48	
89.....	50	-0.1	0	49	
90.....	50	-0.1	0	50	
91.....	50	-0.1	0	51	
92.....	50	-0.1	0	52	
93.....	50	-0.1	0	53	
94.....	50	-0.1	0	54	
95.....	50	-0.1	0	55	
96.....	50	-0.1	0	56	
97.....	50	-0.1	0	57	
98.....	50	-0.1	0	58	
99.....	50	-0.1	0	59	
100.....	50	-0.1	0	60	
101.....	50	-0.1	0	61	
102.....	50	-0.1	0	62	
103.....	50	-0.1	0	63	
104.....	50	-0.1	0	64	
105.....	50	-0.1	0	65	
106.....	50	-0.1	0	66	
107.....	50	-0.1	0	67	
108.....	50	-0.1	0	68	
109.....	50	-0.1	0	69	
110.....	50	-0.1	0	70	
111.....	50	-0.1	0	71	
112.....	50	-0.1	0	72	
113.....	50	-0.1	0	73	
114.....	50	-0.1	0	74	
115.....	50	-0.1	0	75	
116.....	50	-0.1	0	76	
117.....	50	-0.1	0	77	
118.....	50	-0.1	0	78	
119.....	50	-0.1	0	79	
120.....	50	-0.1	0	80	
121.....	50	-0.1	0	81	
122.....	50	-0.1	0	82	
123.....	50	-0.1	0	83	
124.....	50	-0.1	0	84	
125.....	50	-0.1	0	85	
126.....	50	-0.1	0	86	
127.....	50	-0.1	0	87	
128.....	50	-0.1	0	88	
129.....	50	-0.1	0	89	
130.....	50	-0.1	0	90	
131.....	50	-0.1	0	91	
132.....	50	-0.1	0	92	
133.....	50	-0.1	0	93	
134.....	50	-0.1	0	94	
135.....	50	-0.1	0	95	
136.....	50	-0.1	0	96	
137.....	50	-0.1	0	97	
138.....	50	-0.1	0	98	
139.....	50	-0.1	0	99	
140.....	50	-0.1	0	100	
141.....	50	-0.1	0	101	
142.....	50	-0.1	0	102	
143.....	50	-0.1	0	103	
144.....	50	-0.1	0	104	
145.....	50	-0.1	0	105	
146.....	50	-0.1	0	106	
147.....	50	-0.1	0	107	
148.....	50	-0.1	0	108	
149.....	50	-0.1	0	109	
150.....	50	-0.1	0	110	
151.....	50	-0.1	0	111	
152.....	50	-0.1	0	112	
153.....	50	-0.1	0	113	
154.....	50	-0.1	0	114	
155.....	50	-0.1	0	115	
156.....	50	-0.1	0	116	
157.....	50	-0.1	0	117	
158.....	50	-0.1	0	118	
159.....	50	-0.1	0	119	
160.....	50	-0.1	0	120	
161.....	50	-0.1	0	121	
162.....	50	-0.1	0	122	
163.....	50	-0.1	0	123	
164.....	50	-0.1	0	124	
165.....	50	-0.1	0	125	
166.....	50	-0.1	0	126	
167.....	50	-0.1	0	127	
168.....	50	-0.1	0	128	
169.....	50	-0.1	0	129	
170.....	50	-0.1	0	130	
171.....	50	-0.1	0	131	
172.....	50	-0.1	0	132	
173.....	50	-0.1	0	133	
174.....	50	-0.1	0	134	
175.....	50	-0.1	0	135	
176.....	50	-0.1	0	136	
177.....	50	-0.1	0	137	
178.....	50	-0.1	0	138	
179.....	50	-0.1	0	139	
180.....	50	-0.1	0	140	
181.....	50	-0.1	0	141	
182.....	50	-0.1	0	142	
183.....	50	-0.1	0	143	
184.....	50	-0.1	0	144	
185.....	50	-0.1	0	145	
186.....	50	-0.1	0	146	
187.....	50	-0.1	0	147	
188.....	50	-0.1	0	148	
189.....	50	-0.1	0	149	
190.....	50	-0.1	0	150	
191.....	50	-0.1	0	151	
192.....	50	-0.1	0	152	
193.....	50	-0.1	0	153	
194.....	50	-0.1	0	154	
195.....	50	-0.1	0	155	
196.....	50	-0.1	0	156	
197.....	50	-0.1	0	157	
198.....	50	-0.1	0	158	
199.....	50	-0.1	0	159	
200.....	50	-0.1	0	160	
201.....	50	-0.1	0	161	
202.....	50	-0.1	0	162	
203.....	50	-0.1	0	163	
204.....	50	-0.1	0	164	
205.....	50	-0.1	0	165	
206.....	50	-0.1	0	166	
207.....	50	-0.1	0	167	
208.....	50	-0.1	0	168	
209.....	50	-0.1	0	169	
210.....	50	-0.1	0	170	
211.....	50	-0.1	0	171	
212.....	50	-0.1	0	172	
213.....	50	-0.1	0	173	
214.....	50	-0.1	0	174	
215.....	50	-0.1	0	175	
216.....	50	-0.1	0	176	
217.....	50	-0.1	0	177	
218.....	50	-0.1	0	178	
219.....	50	-0.1	0	179	
220.....	50	-0.1	0	180	
221.....	50	-0.1	0	181	
222.....	50	-0.1	0	182	
223.....	50	-0.1	0	183	
224.....	50	-0.1	0	184	
225.....	50	-0.1	0	185	
226.....	50	-0.1	0	186	
227.....	50	-0.1	0	187	
228.....	50	-0.1	0	188	
229.....	50	-0.1	0	189	
230.....	50	-0.1	0	190	
231.....	50	-0.1	0	191	
232.....	50	-0.1	0	192	
233.....	50	-0.1	0	193	
234.....	50	-0.1	0	194	
235.....	50	-0.1	0	195	
236.....	50	-0.1	0	196	
237.....	50	-0.1	0	197	
238.....	50	-0.1	0	198	
239.....	50	-0.1	0	199	
240.....	50	-0.1	0	200	
241.....	50	-0.1	0	201	
242.....	50	-0.1	0	202	
243.....	50	-0.1	0	203	
244.....	50	-0.1	0	204	
245.....	50	-0.1	0	205	
246.....	50	-0.1	0	206	
247.....	50	-0.1	0	207	
248.....	50	-0.1	0	208	
249.....	50	-0.1	0	209	
250.....	50	-0.1	0	210	
251.....	50	-0.1	0	211	
252.....	50	-0.1	0	212	
253.....	50	-0.1	0	213	
254.....	50	-0.1	0	214	
255.....	50	-0.1	0	215	
256.....	50	-0.1	0	216	
257.....	50	-0.1	0	217	
258.....	50	-0.1	0	218	
259.....	50	-0.1	0	219	
260.....	50	-0.1	0	220	
261.....	50	-0.1	0	221	
262.....	50	-0.1	0	222	
263.....	50	-0.1	0	223	
264.....	50	-0.1	0	224	
265.....	50	-0.1	0	225	
266.....	50	-0.1	0	226	
267.....	50	-0.1	0	227	
268.....	50	-0.1	0	228	
269.....	50	-0.1	0	229	
270.....	50	-0.1	0	230	
271.....	50	-0.1	0	231	
272.....	50	-0.1	0	232	
273.....	50	-0.1	0	233	
274.....	50	-0.1	0	234	
275.....	50	-0.1	0	235	
276.....	50	-0.1	0	236	
277.....	50	-0.1	0	237	
278.....	50	-0.1	0	238	
279.....	50	-0.1	0	239	
280.....	50	-0.1	0	240	
281.....	50	-0.1	0	241	
282.....	50	-0.1	0	242	
283.....	50	-0.1	0	243	
284.....	50	-0.1	0	244	
285.....	50	-0.1	0	245	
286.....	50	-0.1	0	246	
287.....	50	-0.1	0	247	
288.....	50	-0.1	0	248	
289.....	50	-0.1	0	249	
290.....	50	-0.1	0	250	
291.....	50	-0.1	0	251	
292.....	50	-0.1	0	252	

(c) **Symptoms:** In dogs that died the effect produced by 2,4-D varied from a mild ataxia and stiffness in the hind legs to a definite myasthenia. Initial signs were often noted six hours after the oral administration of 2,4-D. At this time the animals were more quiet than normal, and a slight ataxia was occasionally present. The hind legs were always affected first; the forelegs later or not at all. There was usually a progressive increase in spasm in the hind legs associated with increasing ataxia, and some dogs spontaneously extended the hind limbs in a spastic movement lasting a few seconds. The knee jerk was either normal or hyperactive.

In the early stages of acute poisoning with 2,4-D, the animals were usually quiet. If the animal was lifted and made to walk, the spasm and ataxia were clearly noticed, but after it had walked for a while, the spasm and ataxia tended to decrease. In the later stages of intoxication, the animal, when placed on its feet, was unable to stand and would then sit or lie in an awkward position with spastic limbs marked particularly by spasm of the hind legs. Other dogs stumbled and tumbled on the floor in attempts to right themselves. An occasional dog showed evidence of irritation or pain when the skin over the back of the neck was grazed. Some dogs, when held up by the shoulders, would show extension and crossing of the hind limbs. As the toxic effects of the drug progressed, the dogs eventually refused the various types of food offered. Occasionally, sneezing, rubbing of the eyes, and diarrhea were observed, but not vomiting.

Such signs were also seen in one dog that survived a lower dose of 2,4-D for 14 days (Table 1). In this instance the changes were relatively mild and were limited to spasm of the hind legs and a slight ataxia.

Marked effects were not observed in the dogs receiving 2,4,5-T. The changes were limited to a slight or moderate stiffness in the hind legs, with the development of ataxia in the two dogs receiving the highest doses. No symptoms were evident in the one dog that died after a single dose of 100 mg./kg. (Table 1).

(d) **Pathology:** Changes observed were limited to the gastrointestinal tract and lungs. Four dogs (1, 2, 4, and 6) receiving 2,4-D and two dogs (13 and 17) fed 2,4,5-T showed mild to diffuse redness of the mucosa in the small intestine. In some dogs pneumonia was present. Histologically, the above dogs showed some necrosis and inflammation of the intestinal mucosa. One dog (13) had a moderate, diffuse hepatic necrosis. Two dogs (13 and 16) had a mild degree of renal tubular degeneration. In most dogs that died the findings were limited to nonspecific changes, such as hepatic congestion. In many cases death was apparently due to pneumonia, which followed the development of anorexia, weight loss, and myotonia.

II. Chronic Oral Toxicity.—(a) **Mortality:** All dogs receiving 2, 5, or 10 mg. of 2,4-D or 2,4,5-T per kilogram of body weight survived the 90-day test period. The dogs receiving 20 mg./kg. of these compounds died during the study. The administration of 20 mg./kg. in divided doses twice a day produced death in both dogs receiving 2,4,5-T and in one dog receiving 2,4-D (Table 2).

(b) **Body Weight:** The only significant change in body weight occurred in the animals that failed to survive the study (Table 2). Loss of weight in these dogs began 7 to 12 days before the death of the dog.

(c) **General Symptoms:** Dogs that survived the oral administration of 2,4-D or 2,4,5-T for 90 days were free of any symptoms.

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(c) **Symptoms:** In dogs that died the effects produced by 2,4-D varied from a mild ataxia and stiffness in the hind legs to a definite myeloma. Initial signs were often noted six hours after the oral administration of 2,4-D. At this time the animals were more quiet than normal, and a slight ataxia was occasionally present. The hind legs were always affected first; the forelegs later or not at all. There was usually a progressive increase in spasm in the hind legs associated with increasing ataxia, and some dogs spontaneously extended the hind limbs in a spastic movement lasting a few seconds. The knee jerk was either normal or hyperactive.

In the early stages of acute poisoning with 2,4-D, the animals were usually quiet. If the animal was lifted and made to walk, the spasm and ataxia were clearly noticed, but after it had walked for a while, the spasm and ataxia tended to decrease. In the later stages of intoxication, the animal, when placed on its feet, was unable to stand and would then sit or lie in an awkward position with spastic limbs marked particularly by spasm of the hind legs. Other dogs stumbled and rolled on the floor in attempts to right themselves. An occasional dog showed evidence of irritation or pain when the skin over the back of the neck was grazed. Some dogs, when held up by the shoulders, would show extension and crossing of the hind limbs. As the toxic effects of the drug progressed, the dogs eventually showed the various types of food offered. Occasionally, sneezing, rubbing of the eyes, and diarrhea were observed, but not vomiting.

Such signs were also seen in one dog that survived a lower dose of 2,4-D for 14 days (Table 1). In this instance the changes were relatively mild and were limited to spasm of the hind legs and a slight ataxia.

Marked effects were not observed in the dogs receiving 2,4,5-T. The changes were limited to a slight or moderate stiffness in the hind legs, with the development of ataxia in the two dogs receiving the highest doses. No symptoms were evident in the one dog that died after a single dose of 100 mg./kg. (Table 1).

(d) **Pathology:** Changes observed were limited to the gastrointestinal tract and lungs. Four dogs (1, 2, 4, and 6) receiving 2,4-D and two dogs (13 and 17) fed 2,4,5-T showed mild to diffuse redness of the mucosa in the small intestine. In some dogs pneumonia was present. Histologically, the above dogs showed some necrosis and inflammation of the intestinal mucosa. One dog (13) had a moderate, diffuse hepatic necrosis. Two dogs (13 and 16) had a mild degree of renal tubular degeneration. In most dogs that died the findings were limited to nonspecific changes, such as hepatic congestion. In many cases death was apparently due to pneumonia, which followed the development of anorexia, weight loss, and anoxemia.

II. Chronic Oral Toxicity.—(a) Mortality: All dogs receiving 2, 3, or 10 mg. of 2,4-D or 2,4,5-T per kilogram of body weight survived the 90-day test period. The dogs receiving 20 mg./kg. of these compounds died during the study. The administration of 20 mg./kg. in divided doses twice a day produced death in both dogs receiving 2,4,5-T and in one dog receiving 2,4-D (Table 2).

(b) **Body Weight:** The only significant change in body weight occurred in the animals that failed to survive the study (Table 2). Loss of weight in these dogs began 7 to 12 days before the death of the dog.

(c) **General Symptoms:** Dogs that survived the oral administration of 2,4-D or 2,4,5-T for 90 days were free of any symptoms.

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The three dogs that died while receiving the highest dose of 2,4-D showed signs differing somewhat from those observed in the acute studies. The dogs were quiet, weaker, and less responsive than normal. Muscle mass was higher in the hind legs, particularly on passive extension. The hind legs were held more stiffly than usual when walking, and a slight stasis was present. During the last two to three days of their survival two of the dogs showed difficulty in chewing or swallowing and eventually even refused a small bolus of canned dog food (usually readily consumed). There was also some oozing of blood from the gums and buccal mucosa.

TABLE 2—Body Weight and Survival of Dogs Fed 2,4-Dichlorophenoxyacetic Acid and 2,4,5-Trichlorophenoxyacetic Acid for 90 days

Dog No.	Sex, Age/No.	Weight			Days Surv.
		Initial, kg.	Final, kg.	Diff., kg.	
207	Male	24.9	26.1	+1.2	8
208	Male	24.9	26.9	+2.0	8
209	Female	24.9	26.9	+2.0	8
210	Female	24.9	26.9	+2.0	8
211	Female	24.9	26.9	+2.0	8
212	Female	24.9	26.9	+2.0	8
213	Female	24.9	26.9	+2.0	8
214	Female	24.9	26.9	+2.0	8
215	Female	24.9	26.9	+2.0	8
216	Female	24.9	26.9	+2.0	8
217	Female	24.9	26.9	+2.0	8
218	Female	24.9	26.9	+2.0	8
219	Female	24.9	26.9	+2.0	8
220	Female	24.9	26.9	+2.0	8
221	Female	24.9	26.9	+2.0	8
222	Female	24.9	26.9	+2.0	8
223	Female	24.9	26.9	+2.0	8
224	Female	24.9	26.9	+2.0	8
225	Female	24.9	26.9	+2.0	8
226	Female	24.9	26.9	+2.0	8
227	Female	24.9	26.9	+2.0	8
228	Female	24.9	26.9	+2.0	8
229	Female	24.9	26.9	+2.0	8
230	Female	24.9	26.9	+2.0	8
231	Female	24.9	26.9	+2.0	8
232	Female	24.9	26.9	+2.0	8
233	Female	24.9	26.9	+2.0	8
234	Female	24.9	26.9	+2.0	8
235	Female	24.9	26.9	+2.0	8
236	Female	24.9	26.9	+2.0	8
237	Female	24.9	26.9	+2.0	8
238	Female	24.9	26.9	+2.0	8
239	Female	24.9	26.9	+2.0	8
240	Female	24.9	26.9	+2.0	8
241	Female	24.9	26.9	+2.0	8
242	Female	24.9	26.9	+2.0	8
243	Female	24.9	26.9	+2.0	8
244	Female	24.9	26.9	+2.0	8
245	Female	24.9	26.9	+2.0	8
246	Female	24.9	26.9	+2.0	8
247	Female	24.9	26.9	+2.0	8
248	Female	24.9	26.9	+2.0	8
249	Female	24.9	26.9	+2.0	8
250	Female	24.9	26.9	+2.0	8
251	Female	24.9	26.9	+2.0	8
252	Female	24.9	26.9	+2.0	8
253	Female	24.9	26.9	+2.0	8
254	Female	24.9	26.9	+2.0	8
255	Female	24.9	26.9	+2.0	8
256	Female	24.9	26.9	+2.0	8
257	Female	24.9	26.9	+2.0	8
258	Female	24.9	26.9	+2.0	8
259	Female	24.9	26.9	+2.0	8
260	Female	24.9	26.9	+2.0	8
261	Female	24.9	26.9	+2.0	8
262	Female	24.9	26.9	+2.0	8
263	Female	24.9	26.9	+2.0	8
264	Female	24.9	26.9	+2.0	8
265	Female	24.9	26.9	+2.0	8
266	Female	24.9	26.9	+2.0	8
267	Female	24.9	26.9	+2.0	8
268	Female	24.9	26.9	+2.0	8
269	Female	24.9	26.9	+2.0	8
270	Female	24.9	26.9	+2.0	8
271	Female	24.9	26.9	+2.0	8
272	Female	24.9	26.9	+2.0	8
273	Female	24.9	26.9	+2.0	8
274	Female	24.9	26.9	+2.0	8
275	Female	24.9	26.9	+2.0	8
276	Female	24.9	26.9	+2.0	8
277	Female	24.9	26.9	+2.0	8
278	Female	24.9	26.9	+2.0	8
279	Female	24.9	26.9	+2.0	8
280	Female	24.9	26.9	+2.0	8
281	Female	24.9	26.9	+2.0	8
282	Female	24.9	26.9	+2.0	8
283	Female	24.9	26.9	+2.0	8
284	Female	24.9	26.9	+2.0	8
285	Female	24.9	26.9	+2.0	8
286	Female	24.9	26.9	+2.0	8
287	Female	24.9	26.9	+2.0	8
288	Female	24.9	26.9	+2.0	8
289	Female	24.9	26.9	+2.0	8
290	Female	24.9	26.9	+2.0	8
291	Female	24.9	26.9	+2.0	8
292	Female	24.9	26.9	+2.0	8
293	Female	24.9	26.9	+2.0	8
294	Female	24.9	26.9	+2.0	8
295	Female	24.9	26.9	+2.0	8
296	Female	24.9	26.9	+2.0	8
297	Female	24.9	26.9	+2.0	8
298	Female	24.9	26.9	+2.0	8
299	Female	24.9	26.9	+2.0	8
300	Female	24.9	26.9	+2.0	8

* F in this column means female; M, male.
† Only those dogs died and administered one half to 1 g. m. and one-half to 1 g. m.
‡ In this column means survived to day test period.

In dogs that died while receiving 2,4,5-T, the prominent effects were weakness, slight stiffness in the hind legs, difficulty in swallowing food, and, in one dog, bleeding from the gums.

(d) Blood Count: The administration of 2,4-D or 2,4,5-T did not have any significant effect on the hemoglobin, red cell count, or total white cell count of animals that survived or died during the study. The differential blood count remained normal in the surviving animals. In three animals that died a terminal fall in the percentage of lymphocytes was observed (Table 3).

(e) Organ Weights: There was no significant change in the weight of the thyroid gland, adrenal gland, heart, liver, or kidney in animals that survived the 90-day period of study. In two of the three dogs that died during the administration

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of 2,4-D, there was a slight increase in heart and kidney weight. A similar change was noted in two of the four dogs that died as a result of 2,4,5-T.

(f) Gross Pathology: Two dogs receiving 2,4-D (26 and 34) showed areas of redness in the duodenum. With the administration of 2,4,5-T there developed in dog 42 a diffusely reddened duodenum and jejunum, while in dog 48, autopsied on the 59th day, a generalized icterus was demonstrated.

TABLE 2.—Effect of 2,4-D and 2,4,5-T on Hemoglobin, Total Blood, and Differential Counts

Dog No.	Sex	Age	Hb. Gm. %	HCT. ml.	WBC	Differential Count					Death Day
						Neut. %	Lymph. %	Mon. %	Eos. %	Bas. %	
Control Dogs											
27	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
28	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
29	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
30	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
LAD											
31	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
32	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
33	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
34	Male	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
LADY											
35	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
36	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
37	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
38	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
39	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
40	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
41	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
42	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—
43	Female	0	12.0	3.0	12,000	50	40	0	0	0	—
		10	12.0	3.0	12,000	50	40	0	0	0	—
		20	12.0	3.0	12,000	50	40	0	0	0	—

* P in this column means female; M, male.

† Daily dose divided and administered one-half in a. m. and one-half in p. m.
No animals spent, only data on animal dogs and the highest a. m. dose given.

(g) Microscopic Changes: The heart, lungs, thyroid, adrenal, ovary, or testes did not demonstrate any significant changes. Three of the dogs receiving 2,4,5-T and two receiving 2,4-D showed an occasional area of focal necrosis in the liver. This finding, however, was not related to either the dose of the compounds or the death of any animal (Table 4) and was judged to be of no significance. The duodenum of dogs 26, 34, 42, and 48 showed varying degrees of hyperemia and some early infiltration of cells in the mucosa. The submucosa and serosa were normal.

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A slight increase in the number of casts in kidney sections was noted in some dogs (Table 4), but again this was unrelated to dosage and was of doubtful significance.

COMMENT

In the acute studies, single large oral doses of 2,4-D administered to dogs may produce signs characteristic of clinical myotonia. This effect of 2,4-D was similar to that observed after various experimental species had received injections.^{2,3} In addition, some dogs showed signs of irritation and pain when the skin of the back of the neck was grasped, which may indicate meningeal irritation. The extension and crossing of the hind limbs, when lifted by the shoulders, may point to a spiral or

TABLE 4.—Histological Changes in Liver and Kidney of Dogs Fed 2,4-D or 2,4,5-T for 90 Days

Dog No.	Dose, mg./kg.	Death Day	Liver		Kidney	
			Cholestasis	Focal Necrosis	Casts	Tub. Degrad.
2,4-D						
1	None	--	0	0	0	0
2	None	--	0	0	0	0
3	1	--	0	0	0	0
4	1	--	0	0	1+	0
5	1	--	1+	1+	0	0
6	1	--	1+	0	1+	1+
7	10	--	0	0	0	0
8	10	--	0	0	0	0
9	10	--	1+	0	0	0
10	10	--	0	0	0	0
11	10	20	1+	1+	0	0
12	10	20	1+	0	0	0
2,4,5-T						
13	None	--	0	0	0	0
14	None	--	0	0	0	0
15	1	--	0	0	1+	0
16	1	--	0	0	0	0
17	1	--	0	0	0	0
18	1	--	0	1+	1+	1+
19	10	--	1+	0	1+	0
20	10	--	0	0	0	0
21	10	20	1+	1+	0	0
22	10	20	1+	1+	1+	0
23	10	20	1+	0	0	0
24	10	20	1+	1+	0	0
25	10	20	1+	1+	1+	0
26	10	20	1+	0	0	0

* Also a 14. Deaths. Throms graded on basis of 0, 1+, 2+, 3+, and 4+.

central lesion. Other effects occasionally observed were sneezing, rubbing of the eyes, and diarrhea. The effects of 2,4,5-T, previously studied, were not as severe, being limited to stiffness in the hind legs and ataxia. The higher doses of either compound produced anorexia and weight loss. Although initial effects may be seen six hours after the compounds are given, the syndrome developed slowly in the dogs, and death was delayed, occurring several days after the administration of the compounds.

In the chronic studies, the dogs survived doses of 2, 5, or 10 mg. of 2,4-D or 2,4,5-T per kilogram of body weight without apparent ill effects. However, the daily oral administration of 20 mg./kg. of 2,4-D produced death in three of four dogs within 18 to 49 days. This delayed death may indicate a cumulative effect of 2,4-D. The signs observed in animals dying from the 20 mg./kg. dose of 2,4-D

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differed somewhat from those seen in the acute studies. With the chronic administration, the animals exhibited chiefly a stiffness of the hind legs and ataxia, weakness, difficulty in chewing or swallowing, and occasionally bleeding from the gums. All four dogs which received 20 mg./kg. of 2,4,5-T succumbed between the 11th and the 75th day of the study. Toxic effects in these animals consistently included slight muscle spasms and difficulty in swallowing food. Thus, the chronic oral toxic dose for 2,4,5-T in dogs seems to be in the same range as that obtained with 2,4-D. With both drugs, there was a terminal loss in body weight.

Dogs surviving the chronic doses of 2,4-D or 2,4,5-T did not show any significant change in hemoglobin, total blood counts, or differential blood count. In three of the dogs (64, 57, and 105) which died while receiving 20 mg./kg. of 2,4-D or 2,4,5-T, there was a definite decrease in the percentage of lymphocytes in the peripheral blood. Hill and Carlisle⁸ have observed a fall in the polymorphonuclear leukocyte count in one dog and a decrease in the per cent of lymphocytes in two dogs to which 30 mg./kg. of 2,4-D was administered intravenously each day for six days. In mice which received chronic injections there was no alteration in the peripheral blood picture.⁹

Large doses of 2,4-D administered intravenously or, in the present study, orally occasionally produced some necrosis in the liver. However, the administration of chronic oral doses produced only a slight and inconsistent local necrosis of doubtful significance (Table 4). Deaths during the chronic oral administration of 2,4-D or 2,4,5-T were not correlated with significant lesions in the liver, kidney, or other organs examined.

SUMMARY

The acute oral L.D.₅₀ of 2,4-dichlorophenoxyacetic acid (2,4-D) in dogs was approximately 100 mg. per kilogram of body weight. Doses in this range or higher produced a definite myotonia accompanied by anorexia and weight loss. The acute oral L.D.₅₀ for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was in the range of 100 mg. per kilogram or higher. Such toxic doses produced only signs of a mild spasticity.

All dogs survived the oral feeding of 2, 5, or 10 mg. per kilogram of 2,4-D or 2,4,5-T 5 days a week for a period of 90 days. These doses did not produce any symptoms or changes in body weight, organ weights, or blood count.

Three of the four dogs receiving repeated doses of 20 mg. per kilogram of 2,4-D died, and all four dogs receiving 20 mg. per kilogram of 2,4,5-T died during the study. Toxicity to 2,4-D at the dosage level was accompanied by bleeding from the gums, necrotic changes in the buccal mucosa, and some difficulty in chewing and swallowing, but there was only little evidence of clinical myotonia. A terminal fall in the percentage of lymphocytes was observed in three of the animals.

Death during the repeated administration of 2,4-D or 2,4,5-T was not related to pathological changes in the liver, kidney, or other organs examined.

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