Residual Aspects of 2,4,5-T and an Ester in Sheep and Cattle with Observations on Concomitant Toxicological Effects

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Sheep and cattle were given oral doses of 2,4,5-T and propylene glycol butyl ether esters of 2,4,5-T. The rate of appearance in blood, urinary elimination, and residue deposition in tissues was determined for both herbicides. In a ewe given a single oral dose of 25 mg per kg of Esteron 245 O.S., peak concentrations of 10 ppm of the unmetabolized ester were found at 3 and 4 hr posttreatment. Within 72 hr, approximately 86% of the administered dose was recovered from urine as the unmetabolized ester, and 1.4% was recovered as 2,4,5-T. Residual 2,4,5-T was less than 0.1 ppm in tissues of a sheep given a single oral dose of 25 mg per kg Esteron 245 O.S., or in omental fat of sheep given four daily oral doses of 0.15 or 0.75 mg per kg of the same formulation. Residues of 2,4,5-T as high as 368 ppm were found in tissues of sheep acutely poisoned by repeated oral doses to 250 mg per kg of 2,4,5-T or to the propylene glycol butyl ether esters of 2,4,5-T. Low levels of apparent residues were found in the omental fat of cattle given daily oral doses of 0.15 or 0.75 mg per kg Esteron 245 O.S. for up to 32 weeks of treatment. With the exception of the sheep dosed with 250 mg per kg of the herbicides, no toxic effects were observed.

To control undesirable vegetation, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their salts and esters have been used extensively. Chlorophenoxy herbicides are relatively less toxic to animals than many other pesticidal chemicals. Poisoning can occur in cases of gross negligence when all available water and/or feed are contaminated, or in instances in which animals are exposed to large amounts of concentrated technical material (Mitchell *et al.*, 1946; Palmer, 1963; Palmer and Radeleff, 1969; Willard, 1951).

St. John *et al.* (1964) reported that both 2,4,5-T and silvex [2-(2,4,5-trichlorophenoxy)propionic acid] given to dairy cows for 4 days at a rate of 5 ppm in feed were completely eliminated as soluble salts in the urine. When Kuron (propylene glycol butyl ether esters of silvex) was given to a dairy cow at the same rate, 67% was found to be eliminated as silvex in the urine. They suggested that the balance was eliminated as silvex in the feces. However, no fecal or tissue residue analyses were made.

Small amounts of residual C^{14}-labeled 2,4-D were found in tissues of a sheep following a single oral dose of 4 mg per kg of body weight (Clark *et al.*, 1964).

The objectives of this study were to determine absorption, metabolism, elimination, and residue deposition of 2,4,5-T herbicides in animals exposed to multiple doses and the tissue residue deposition in acutely poisoned animals. An additional objective was to determine the toxicity of 2,4,5-T formulations administered orally to sheep and cattle.

METHODS AND MATERIALS

Eight adult Delaine ewes purchased at a local livestock auction and nine 2-year-old Hereford heifer cattle from a government herd were used as test animals. All animals were in good health at the time of the trials.

All cattle were fed a maintenance ration consisting of a grain–protein concentrate each morning and a combination of good quality hegari and alfalfa hay each evening. For the sheep a ration of coarse ground alfalfa hay was added to the protein concentrate. This mixture was substituted for the bulk hay portion of the ration to a maximum of 3% of the body weight of each sheep. Water and mineral supplements were provided free-choice. All livestock were weighed at the start of the experiments. Dose rates were calculated for each animal on a mg per kg basis.

Animals were treated with 2,4,5-T (2,4,5-trichlorophenoxyacetic acid, 100% analytical standard), propylene glycol butyl ether esters of 2,4,5-T (99.2% technical grade), or Esteron 245 O.S. (65.3% EC, propylene glycol butyl ether esters of 2,4,5-T). All herbicides used in this study were obtained from Dow Chemical Co., Midland, Mich. In this paper, Esteron 245 O.S. will refer to the 65.3% emulsifiable concentrate; esteron will refer to the active ingredient of Esteron 245 O.S. (propylene glycol butyl ether esters of 2,4,5-T). All dosages were corrected to 100% active ingredient.

The ewes were placed in individual restrictive pens to facilitate continuous urine collections. Water and feed were made available in each pen.

In the first test a single 25 mg per kg oral dose of Esteron 245 O.S. was given to one ewe. Two others were given 0.15 and 0.75 mg per kg of the same formulation daily for a 4-day period. One ewe served as an untreated control.

A urinary retention catheter was placed in each ewe and a pretreatment urine sample collected. Posttreatment urine collections were made and volumes recorded at 1, 3, 5, 7, and 24 hr intervals each day from the multiple-treated sheep. Subsequent samples were collected at 15, 24, 28, 32, 34, 48, 52, 72, 96, and 100 hr after the fourth dose. In the ewe that was given only a single dose, urine samples were collected at 0.25, 0.5, 1, 2, 3, 5, 7, 15, 24, 28, 32, 48, 52, 72, and 76 hr posttreatment. Simultaneous with urine collection, blood samples were drawn from each animal, heparinized, and then frozen for later analysis. At the end of the test an omentectomy (Radeleff, 1950) was performed on each of the four sheep. The omental fat samples were frozen immediately and stored until analysis.

A second experiment involving four sheep was designed to compare tissue residues in sheep poisoned by each of three forms of 2,4,5-T. From toxicity screening tests (Palmer and

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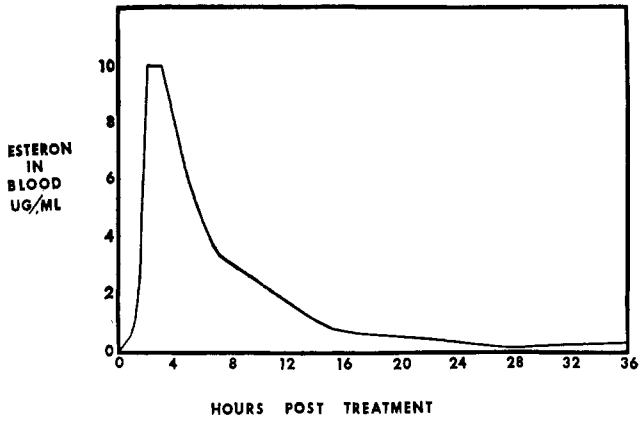

Figure 1. Propylene glycol butyl ether ester of 2,4,5-T in blood of a ewe given a single oral dose of 25 mg/kg

Table I. Propylene Glycol Butyl Ether Esters of 2,4,5-T and 2,4,5-T in Urine of a Sheep Given a Single Oral Dose of Esteron 245 O.S. at 25 mg/kg

Sample Hr Posttreatment	Recovery, mg	
	Ester	2,4,5-T
1	9.54	0.018
2	53.76	0.409
5	139.70	...
7	208.81	1.725
15	525.00	5.906
24	164.64	0.823
28	125.28	6.902
31	31.13	1.215
36	27.20	1.320
48	57.79	0.482
52	6.16	0.680
72	1.15	0.230

Radeleff, 1969) we estimated that six or seven doses of 250 mg per kg of esteron would be lethal to sheep.

One ewe (1216) was dosed with 250 mg per kg of the 100% ester equivalent of Esteron 245 O.S. A second ewe (1225) was dosed with 250 mg per kg of technical 2,4,5-T, and a third ewe (1221) was given 250 mg per kg of 99.2% analytical grade esteron. Each compound was given in gelatin capsules once each day until the sheep was poisoned or died. A fourth sheep was an untreated control.

Necropsies were performed at the end of the trials and samples of kidney, liver, skeletal muscle, renal fat, and omental fat were collected. Each sample was placed in a separate plastic bag, immediately frozen, and held at −60° C until analysis.

For the third experiment the cattle were divided into three groups of three animals each. In each group one animal was an untreated control. The other two cattle were treated daily with oral doses of Esteron 245 O.S., one at 0.15 mg per kg and one at 0.75 mg per kg body weight. The dose calculations were made on the assumption that each animal would consume approximately 3% of its weight in feed daily; therefore feed contaminated with 5 ppm or 25 ppm would be equivalent to a dose, by single oral capsule, of 0.15 and 0.75 mg per kg esteron, respectively. The animals were reweighed weekly and dosages recalculated. Prior to the start of dosing, an omental fat sample was collected from each of the nine cattle. Subsequent omentectomies were performed at 2-week intervals on the different groups so that each animal was subjected to surgery at 6-week intervals. Each heifer underwent a total of six such surgical procedures on alternate sides of the abdomen, over a period of up to 7 months of daily herbicide exposure.

Following appropriate extraction and cleanup of tissue samples by a previously described method (Clark, 1969),

Table II. Propylene Glycol Butyl Ether Esters of 2,4,5-T and 2,4,5-T in Blood and Urine of a Sheep Given Repeated Oral Doses of Esteron 245 O.S. at 0.15 and 0.75 mg/kg

Sample		Urine						Blood			
		0.15 mg/kg			0.75 mg/kg			0.15 mg/kg		0.75 mg/kg	
Hr	After dose	Volume collected, ml	Recovery, μg Ester[a]	Recovery, μg 2,4,5-T	Volume collected, ml	Recovery, μg Ester	Recovery, μg 2,4,5-T	Ester μg/ml[a]	2,4,5-T μg/ml[a]	Ester μg/ml[a]	2,4,5-T μg/ml[a]
1	1	90	14.04	3.96	70	60.6	26.6	NDR	NDR	0.110	0.020
3	1	83	385.98	40.50	123	985.5	420.1	0.032	NDR	0.038	0.030
5	1	121	281.33	278.30	140	207.2	50.4	0.570	NDR	0.430	0.020
7	1	120	558.00	234.00	228	3135.0	3420.0	0.300	NDR	NDR	0.025
24	1	760	1748.00	315.00	930	3696.8	7858.5	NDR	0.005	0.090	NDR
1	2	25	57.50	64.57	45	28.1	189.0	NDR	0.065	NDR	NDR
3	2	57	370.50	201.78	44	234.0	682.0	NDR	NDR	0.040	0.010
5	2	51	629.85	178.50	55	536.3	1320.0	0.110	NDR	0.070	0.300
7	2	54	450.90	159.00	52	572.0	630.5	NDR	0.045	0.045	0.095
24	2	575	4312.50	694.60	935	10846.0	5937.3	NDR	0.040	NDR	0.045
1	3	23	24.15	9.96	32	176.0	28.8	NDR	0.015	NDR	0.040
3	3	41	57.40	18.04	42	195.9	945.0	0.015	0.040	0.035	0.035
5	3	39	19.50	...	24	171.0	936.0	NDR	0.015	0.015	0.015
7	3	64	33.28	28.80	25	345.6	956.3	NDR	0.010	0.784	0.025
24	3	450	84.60	83.70	...	...	...	0.215	0.025	...	...
1	4	35	NDR	0.35	41	1025.0	164.0	0.205	0.020	0.735	0.185
3	4	48	NDR	1.92	...	...	...	0.140	0.020	0.760	0.060
5	4	46	4.60	1.33	40	1020.0	163.0	0.175	0.015	1.068	0.030
7	4	41	2.87	34.85	37	980.5	712.3	0.115	0.080	0.768	0.575
24	4	399	15.16	56.26	527	10803.5	4479.5	0.250	0.040	0.435	0.015
32	4	166	10.30	103.09	160	1200.0	6140.0	...	...	...	...
48	4	430	NDR	49.02	800	680.0	3000.0	0.160	0.035	0.080	0.015
80	4	890	8.90	27.59	250	69.0	76.0	0.250	0.015	0.085	0.050

[a] NDR = No detectable residue.

Table III. Residues of 2,4,5-T Propylene Glycol Butyl Ether Esters and 2,4,5-T in Tissues of Sheep Given Oral Doses of 2,4,5-T Herbicides

Animal	Treatment	Omental fat		Muscle		Liver		Kidney		Renal Fat	
		Ester[a] ppm	2,4,5-T[a] ppm	Ester[a] ppm	2,4,5-T[a] ppm	Ester[a] ppm	2,4,5-T[a] ppm	Ester[a] ppm	2,4,5-T[a] ppm	Ester[a] ppm	2,4,5-T[a] ppm
1312	Control	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR
1309	Esteron 245 O.S., 0.15 mg/kg, four daily doses	NDR	0.08	...	...	...	...	...	...	...	...
1311	Esteron 245 O.S., 0.75 mg/kg, four daily doses	NDR	0.08	...	...	...	...	...	...	...	...
1310	Esteron 245 O.S., 25 mg/kg, 1 dose	NDR	0.014	NDR	0.005	NDR	0.013	NDR	0.013	...	...
1216	Esteron 245 O.S., 250 mg/kg (active), six daily doses	NDR	33.0	1.00	100.0	0.35	100.0	1.25	240.0	0.15	113.0
1221	Esteron; 250 mg/kg, 99.2%, four daily doses	0.25	65.0	0.75	80.0	0.20	80.0	0.60	368.0	0.10	80.0
1225	2,4,5-T; 250 mg/kg, four daily doses	...	34.0	...	40.0	...	20.0	...	176.0	...	60.0

[a] NDR = No detectable residue.

the herbicide residues were converted to the methyl ester of 2,4,5-T. Samples were then analyzed by microcoulometric gas chromatography. The analytical column was stainless steel, 5 ft long and 1/8 in. in diameter. The column was packed with 15% Dow 710 on Chromport XXX and operated at 210° C with purified nitrogen as the carrier gas.

Average recoveries of 2,4,5-T added, prior to extraction, to fat, lean tissue, urine, and blood at levels from 0.05 to 20.0 ppm were 89.3, 89.6, 93.0, and 93.6%, respectively. Recovery of esteron added to fat, lean tissue, urine, and blood at levels from 0.5 to 20.0 ppm averaged 77.9, 70.5, 94.5, and 92.5%, respectively. However, data reported in this paper were not corrected for extraction loss.

RESULTS AND DISCUSSION

Absorption, Metabolism, and Excretion of Esteron 245 O.S. by Sheep. In the ewe treated with a single oral dose of 25 mg per kg Esteron 245 O.S. (total dose 1590 mg), 0.13 ppm of the herbicide was found in the blood sample drawn 15 min posttreatment. Peak concentrations of 10 ppm were found in blood drawn at 3 and 4 hr posttreatment (Figure 1). Blood samples collected at 3, 5, and 7 hr contained the metabolite 2,4,5-T at 0.27, 0.60, and 0.57 ppm, respectively.

Within 72 hr approximately 86% of the administered dose was recovered from the urine as the unmetabolized ester, with about 1.4% recovered as the metabolite 2,4,5-T. Most of the herbicide was recovered from samples collected between 7 and 15 hr posttreatment (Table I).

When Esteron 245 O.S. was given to a ewe at the daily rate of 0.15 mg per kg over a 4-day period (total esteron fed was 24.2 mg), 9.27 mg was recovered from the urine as unchanged esteron and 2.59 mg (3.75 mg esteron equivalent) was recovered as the metabolite 2,4,5-T. This was a total recovery (corrected to esteron equivalent) of 53.76% of the total esteron fed. Most of the recovered herbicide was excreted unchanged until after the fourth dose, suggesting an increasing ability of the animal to hydrolyze the ester (Table II). Absorption of esteron and appearance of 2,4,5-T in venous blood are shown in Table II.

From the ewe given four daily oral doses of esteron at 0.75 mg per kg (total esteron fed was 137 mg), 37.08 mg was recovered as the ester and 38.78 mg (56.12 mg esteron equivalent) was recovered as 2,4,5-T. This was a total corrected recovery of 68.0% of the total esteron fed (Table II).

Tissue Residues. 2,4,5-T residues of 0.08 ppm were found in the omental fat of each of two sheep given four oral doses of either 0.15 or 0.75 mg per kg Esteron 245 O.S. (Table III). Residues of the unmetabolized ester were not found in the omental fat of either animal. In ewe 1310 dosed with a single oral capsule of Esteron 245 O.S. at the rate of 25 mg per kg, less than 0.015 ppm of 2,4,5-T were found in the tissues analyzed. No residues of the unmetabolized ester were found (Table III).

Residues of 2,4,5-T were very high in the three animals which were poisoned by 250 mg per kg of the various 2,4,5-T herbicides (Table III). In tissues from poisoned animals relatively small residues of the unhydrolyzed ester were found. Since 2,4,5-T is eliminated primarily in the urine, residues in the kidney were much higher than in other tissue.

Results of residue analyses of omental fat samples from cattle given daily doses of Esteron 245 O.S. at 0.15 and 0.75 mg per kg are shown in Table IV. A background peak with retention characteristics similar to those of the methyl ester of 2,4,5-T was present, particularly in gas chromatograms of samples collected after 6 weeks of treatment. We were unable to separate the interfering contaminant from the 2,4,5-T methyl ester by gas chromatography. Therefore the peaks were measured and are reported here as apparent 2,4,5-T residues. The extent of this difficulty is reflected in the apparent residues reported for untreated animals, in the pretreatment levels observed in treated animals, and in subsequent observations within the same treated animals (Table IV). The highest apparent residues found were 1.6 ppm esteron in the control animal group B (Table IV) in the sixth week of the test and 1.16 ppm 2,4,5-T in an animal which had been exposed to 0.75 mg per kg Esteron 245 O.S. daily for 6 weeks. In both instances the next samples collected from the same animals had apparent residues near or below the lower limit of quantitation (0.025 ppm).

Table IV. Apparent Residues (ppm) of Propylene Glycol Butyl Ether Esters of 2,4,5-T and 2,4,5-T in Omental Fat from Yearling Cattle Given Repeated Daily Oral Doses of 0.15 and 0.75 mg/kg Esteron 245 O.S.

Weeks of treatment	Animal group[a] sampled	Daily Dose Rate: Untreated		0.15 mg/kg		0.75 mg/kg	
		Ester[b,c]	2,4,5-T[b,c]	Ester[b,c]	2,4,5-T[b,c]	Ester[c]	2,4,5-T[b,c]
0	A,B,C	<0.025	0.06	<0.025	0.04	0.03	0.09
4	A	NDR	NDR	NDR	<0.025	NDR	<0.025
6	B	1.60	<0.025	0.52	<0.025	0.80	1.16
8	C	0.10	NDR	NDR	0.10	0.20	<0.025
10	A	NDR	NDR	0.06	NDR	0.06	NDR
12	B	0.03	0.09	0.03	0.05	NDR	<0.025
14	C	NDR	<0.025	0.04	<0.025	0.03	0.03
16	A	0.03	<0.025	NDR	<0.025	NDR	0.04
18	B	<0.025	0.035	<0.025	NDR	NDR	<0.025
20	C	NDR	0.03	...	0.10	NDR	0.04
22	A	NDR	NDR	NDR	<0.025	NDR	0.10
24	B	NDR	NDR	NDR	...	NDR	0.035
26	C	...	...	NDR	<0.025	NDR	NDR
28	A	...	<0.025	NDR	NDR	NDR	<0.025
30	B	NDR	NDR	NDR	0.40	NDR	NDR
32	C	NDR	0.035	...	...	NDR	0.125

[a] Each group was made up of three animals; one untreated, one dosed daily at 0.15 mg/kg, and one dosed daily at 0.75 mg/kg. [b] The symbol <0.025 indicates the presence of apparent trace residue below the lower limit of quantitation. [c] NDR = No detectable residue.

Recovery tests were conducted in which known quantities of 2,4,5-T and esteron were added to omental fat samples from untreated animals. The spiked samples were analyzed by the same procedure as samples from test animals.

Toxicologic Effects. No ill effects were observed in any of the sheep due to treatment with 0.15, 0.75, or 25 mg per kg of Esteron 245 O.S. The ewe given the single 25 mg per kg dose died from a surgical complication 4 days after the omentectomy. Death was due to a strangulated intestine that had penetrated the abdominal musculature.

Three sheep were poisoned by the 250 mg per kg dosages of the 2,4,5-T compounds. Sheep 1221 was moribund after four doses of the 99.2% esteron while sheep 1225 exhibited muscular spasms, dehydration, and anorexia after four doses of the pure 2,4,5-T acid. Both sheep were submitted to euthanasia. At necropsy, signs of acute hepatitis, rumen stasis, and enteritis were observed. One sheep, 1216, dosed with 250 mg per kg Esteron 245 O.S., was dehydrated and extremely depressed after six daily doses and was found dead the following day. On necropsy, signs of nephritis, hepatitis, and lung congestion were evident. Control sheep 1215 was subsequently sacrificed, and no gross pathological lesions were observed at necropsy.

As with the sheep dosed at 0.15 and 0.75 mg per kg Esteron 245 O.S. daily, no ill effects were observed in the cattle from daily herbicide ingestion of 0.15 and 0.75 mg per kg Esteron 245 O.S. for the extended treatment period. The average weight gain for the three untreated cattle was 15% of pretreatment weights over the 7-month period, while the cattle treated daily at 0.15 and 0.75 mg per kg gained an average of 24 and 9% of their pretreatment weight, respectively. Near the end of the experiment some of the cattle developed localized omental adhesions due to previous surgery. Thus it was impossible to collect fat samples in sufficient quantity for analysis.

There was no toxicologic effect observed from repeated daily dosing of 0.15 or 0.75 mg per kg of the 2,4,5-T ester to cattle or sheep.

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