Metabolic and Residue Studies with 2-(2,4,5-Trichlorophenoxy)-ethyl 2,2-Dichloropropionate (Erbon) Herbicide in Sheep

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Erbon was absent from the blood, urine, and feces of an adult ewe dosed with the compound at 50 mg per kg. Two metabolites, 2-(2,4,5-trichlorophenoxy)-ethanol and 2,4,5-trichlorophenol, were in blood, urine, and feces shortly after administration of erbon orally as a drench. Approximately 70% of the erbon dose was eliminated in the urine and feces within 96 hr after dosing. Erbon was metabolized to 2-(2,4,5-trichlorophenoxy)ethanol when incubated with fresh rumen fluid or homogenized sheep liver. Sheep were given daily oral doses of active erbon at 25, 50, and 100 mg per kg levels for 10 days or until death. Tissue samples were analyzed for erbon, 2-(2,4,5-trichlorophenoxy)ethanol, and 2,4,5-trichlorophenol by gas chromatography. No erbon was found in any of the tissues analyzed. High levels of both metabolites were found in the liver, kidney, and omental fat of the sheep given 100 mg per kg. The highest residue of 2-(2,4,5-trichlorophenoxy)ethanol was 6.35 ppm in the omental fat and the highest residue level for 2,4,5-trichlorophenol was 5.54 ppm found in the kidney.

Erbon [2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate] is a herbicide used in noncrop areas to control weeds and grass. In oral toxicity studies with cattle and sheep at this laboratory, erbon has been more toxic than other members of the 2,4,5-T herbicide family (Palmer, 1968). The possibility exists that livestock might accidentally ingest this herbicide while consuming previously sprayed weeds and grass. To our knowledge, no previous work has been done on its metabolism and elimination. Preliminary work has indicated that two metabolites of erbon are 2,4,5-trichlorophenol and 2-(2,4,5-trichlorophenoxy)ethanol. The chemical structures for these compounds are shown in Figure 1. Therefore, our objectives were to determine the metabolism rate of erbon and determine the elimination rate for the parent compound and metabolites.

The secondary purpose of the study was to determine the amount of deposition of erbon and metabolites in sheep tissues following oral daily doses of the herbicide at three different levels.

MATERIALS AND METHODS

Reagents. ERBON. (Baron) 2-(2,4,5-trichlorophenoxy)-ethyl 2,2-dichloropropionate 41.0% Emulsifiable Concentrate and technical standard (Dow Chemical Co., Midland, Mich.).

METABOLITES. 2-(2,4,5-trichlorophenoxy)ethanol, 6.34% OH, and 2,4,5-trichlorophenol, 98.0% analytical standard (Dow Chemical Co., Midland, Mich.).

Elimination Studies. An adult ewe was weighed and confined in a stall with adequate food and water supplied. The ewe was catheterized and pretreatment urine, fecal, and blood samples collected. The ewe was given 50 mg per kg of active erbon as an oral drench. Total post-treatment urine and feces were collected. Blood samples were obtained at predetermined intervals by jugular vein puncture. The collected samples were extracted with organic solvents, reacted to produce trimethylsilyl derivatives of the metabolites, *i.e.*, 2-(2,4,5-trichlorophenoxy)ethanol and 2,4,5-trichlorophenol, and analyzed by gas chromatography (Wright *et al.*, 1969).

Metabolic Studies. BLOOD, URINE AND FECES. Samples of blood, urine, and feces were collected from an untreated adult ewe as described above. These samples were fortified with known amounts of erbon, 2,4,5-trichlorophenol, or 2-(2,4,5-trichlorophenoxy)ethanol at 25° C. The stability of the metabolites was studied by extracting aliquots of the metabolite-fortified samples at predetermined time intervals. The extracted samples were analyzed by gas chromatography as mentioned earlier.

RUMEN FLUID. Fresh rumen fluid was obtained from an adult sheep immediately after slaughter. The metabolism of erbon in the presence of rumen fluid was studied. Forty ml of fresh incubating rumen fluid (37° C) was fortified with 100 μg of active erbon (2.5 ppm). Two ml aliquots of the rumen fluid, including a prefortification sample, were extracted at various time intervals and analyzed for erbon and metabolites as for urine (Wright *et al.*, 1969).

LIVER HOMOGENATE. Fresh liver was obtained from an untreated adult sheep immediately after slaughter. Five grams of fresh sheep liver previously ground in a Latapie tissue homogenizer were blended with 35 ml of isotonic saline. The incubating homogenate (25° C) was fortified with 100 μg of erbon (20 ppm in relation to tissue) and 2-ml aliquots were extracted at predetermined time intervals and analyzed for erbon and metabolites as described earlier for urine (Wright *et al.*, 1969).

Residue Deposition Studies. Four adult ewe sheep in good physiological condition obtained at the local auction were confined in a small pen with adequate food and water supplied. After the sheep had become accustomed to their surroundings, they were weighed. One sheep served as a control and was given no erbon, one was given erbon at 25 mg per kg, another at 50 mg per kg, and the last at 100 mg per kg. Dosing given by oral drench was continued for 10 daily dosages or until the sheep died. On the 11th day, the control and principals were killed and representative tissue samples were obtained. The following tissues were selected for residue analysis: brain, kidney, liver, muscle, and omental fat. Total tissue samples were ground in an electric meat grinder and frozen until extraction. A modified micro-technique was used for the extraction of erbon and metabolites and was followed by gas chromatographic analysis (Wright *et al.*, 1969).

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Table I. Elimination of Erbon as Metabolites in Urine and Feces of Sheep Treated with 50 mg per kg of Parent Compound[a]

Time of Collection (Hr)	Urine			Feces			Total Erbon Eliminated
	Metabolite 1[b]	Metabolite 2[c]	Total 1 and 2	Metabolite 1[b]	Metabolite 2[c]	Total 1 and 2	
0–7	1.98	3.81	5.79	0.00	0.02	0.02	5.81
7–23	27.37	26.32	53.69	0.27	1.05	1.32	55.01
23–48	3.62	4.82	8.44	0.09	0.22	0.31	8.75
48–72	0.16	0.29	0.45	0.02	0.06	0.08	0.53
72–96	0.02	0.03	0.05	0.00	0.01	0.01	0.06
Totals	33.15	35.27	68.42	0.38	1.36	1.74	70.16

[a] All figures represent percent of total erbon administered. [b] 2-,4,5-trichlorophenol. [c] 2-(2,4,5-trichlorophenoxy)ethanol.

Table II. Average Concentration of Erbon Metabolites in Sheep Urine and Feces at Time Intervals Following Oral Drench with 50 mg per kg of Parent Compound[a]

Time of Collection (Hr)	Urine		Feces	
	Average Concentration (ppm)		Average Concentration (ppm)	
	Metabolite 1[b]	Metabolite 2[c]	Metabolite 1[b]	Metabolite 2[c]
0–7	49.1	115.2	0.6	4.1
7–23	322.1	379.2	11.7	54.8
23–32	59.9	136.0	...	...
32–48	19.5	16.0	3.5	11.0
48–56	2.9	5.8	...	...
56–72	2.8	6.1	0.7	2.9
72–80	0.5	1.3	...	...
80–96	0.1	0.3	0.1	0.6

[a] Any feces collected during a period indicated as a blank in the table was combined with the next time interval, *i.e.*, feces collected for time intervals 23 to 32 and 32 to 48 were combined, and the total represented under time interval 32 to 48. [b] 2,4,5-trichlorophenol. [c] 2-(2,4,5-trichlorophenoxy)ethanol.

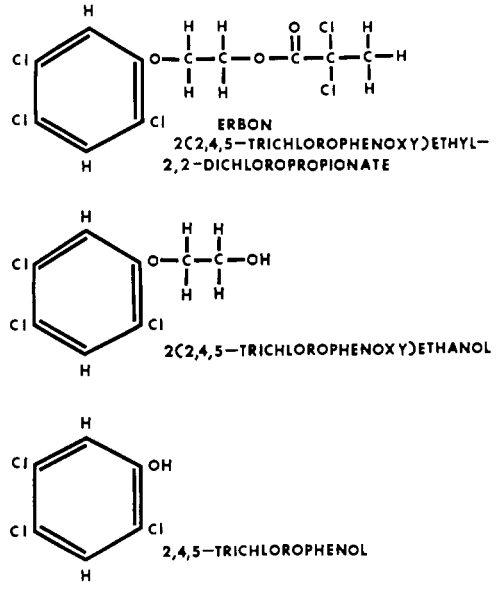

Figure 1. Chemical structure of erbon and metabolites

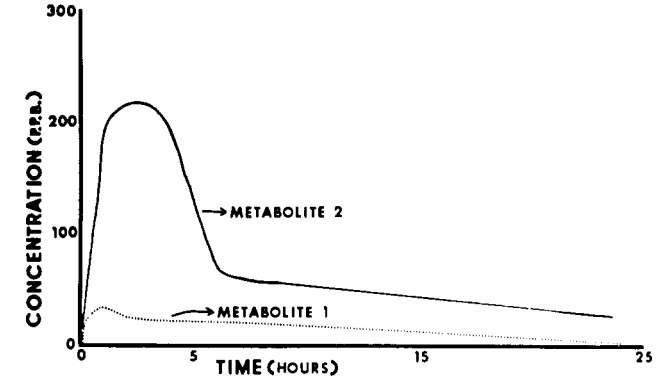

Figure 2. Formation and disappearance of Metabolite 1 (2,4,5-trichlorophenol) and Metabolite 2 [2(2,4,5-trichlorophenoxy)ethanol] in the blood of a sheep given 50 mg per kg of active erbon as a drench

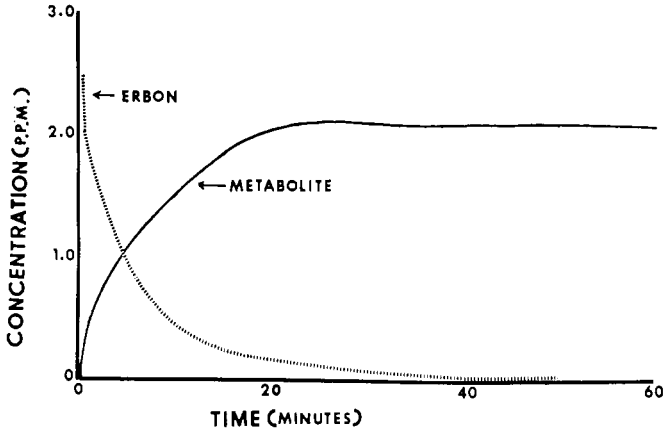

Figure 3. Disappearance of the herbicide and production of 2-(2,4,5-trichlorophenoxy)ethanol in fortified rumen fluid

RESULTS

After an oral drench, no residues of the parent compound were detected in blood, urine, or feces of an adult ewe at any time during the test period. Analysis of all three samples indicated the presence of both metabolites, *i.e.*, 2-(2,4,5-trichlorophenoxy)ethanol and 2,4,5-trichlorophenol.

Figure 2 illustrates the appearance and disappearance of the metabolites from blood as a function of time following an oral drench with 50 mg of active erbon per kg of sheep body weight.

Table I presents the rate of elimination of an oral dose of erbon (50 mg per kg) as the metabolites. Results are calculated as percent of total erbon administered.

Table II indicates the average concentration (ppm) of the metabolites, at various time intervals, in urine and feces of an adult ewe dosed with 50 mg per kg of the herbicide.

Erbon was completely metabolized to 2-(2,4,5-trichlorophenoxy)ethanol in blood, urine, and feces in less than 1 hr. The metabolites were stable in blood, urine, and feces even after being allowed to stand for at least 18 hr at room temperature.

Figures 3 and 4 illustrate the disappearance of the herbicide

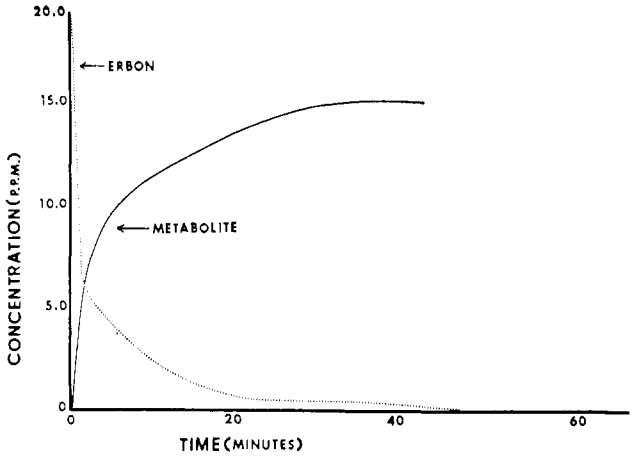

Figure 4. Disappearance of the herbicide and production of 2-(2,4,5-trichlorophenoxy)ethanol in a fortified liver homogenate

and concurrent formation of the metabolite 2-(2,4,5-trichlorophenoxy)ethanol, in a liver homogenate and fresh incubated rumen fluid, respectively, fortified with the herbicide.

The dose given each sheep in the residue study is shown in Table III. The sheep receiving the highest dose of the herbicide was given seven daily doses and died on the 8th day. Tissue samples were obtained at necropsy. Neither of the other dosed sheep had any signs of poisoning, but the sheep given the lowest dose level was inappetent near the end of the dosing period. Tissue samples were obtained when control and principals were killed on the 11th day. No erbon was found in any of the tissues analyzed. The residues of the metabolites found in the tissues of the dosed sheep are shown (Tables IV and V).

DISCUSSION

Upon oral dosing of a sheep with erbon, the compound is metabolized rapidly to 2,4,5-trichlorophenol and 2(2,4,5-trichlorophenoxy) ethanol. The peak concentration of the metabolites in urine, feces, and blood occurs within the first 23 hr after oral dosing. The metabolite, 2-(2,4,5-trichlorophenoxy)ethanol, is produced in the largest quantity. Most of the erbon (59.48% of the total administered) is eliminated in the urine as both metabolites in the first 23 hr after treatment. Traces of the metabolites are present in both the urine and feces for as long as 96 hr after treatment. Only trace amounts of the metabolites are eliminated in the feces during the test period, accounting for 1.75% of the total dose of erbon administered. In 96 hr, 70.16% of the total dose of erbon administered is eliminated in the urine and feces as the metabolites. In blood the metabolites have practically disappeared within 25 hr.

In vitro metabolism of erbon occurs rapidly in both a liver homogenate and fresh incubated rumen fluid. No erbon was found in the fortified samples after 50 min. Only one metabolite, 2(2,4,5-trichlorophenoxy) ethanol, was detected in these studies; after 50 min, the quantity of the metabolite present accounted for the total amount of erbon used in the fortification.

Table III. Dosage and Amount of Erbon Given Each Sheep

Animal No.	Dose Level mg per kg	No. of Doses	Total Amount Received (g)	Remarks
1[a]	0	0	0	Control
2[a]	25	10	6.825	NIE[b]
3[a]	50	10	15.250	NIE[b]
4[c]	100	7	21.630	Died on 8th day

[a] Killed on morning of 11th day. [b] No ill effects apparent. [c] Sheep necropsied after death on 8th day.

Table IV. Residues of 2-(2,4,5-Trichlorophenoxy)ethanol Found in Tissues of Sheep Given Daily Oral Doses of Erbon

Dose Level (mg per kg)	Residues in Tissues (ppm)				
	Brain	Kidney	Liver	Omental Fat	Muscle
None	...	...	...	...	...
25	...	0.76	0.45	0.54	...
50	...	0.42	0.54	0.18	...
100	0.80	4.88	5.14	6.35	0.60

Table V. Residues of 2,4,5-Trichlorophenol Found in the Tissues of Sheep Given Daily Oral Doses of Erbon

Dose Level (mg per kg)	Residues in Tissues (ppm)				
	Brain	Kidney	Liver	Omental Fat	Muscle
None	...	...	...	...	...
25	...	0.84	...	...	...
50	...	...	...	...	...
100	0.21	5.54	3.14	2.06	1.00

Results show that erbon is primarily deposited as 2-(2,4,5-trichlorophenoxy)ethanol. The primary sites of deposition for this metabolite are omental fat, liver, and kidneys. The levels of deposition vary but do not appear to be deposited according to dosage level, since residues in the sheep given 25 mg per kg were higher than in the sheep given 50 mg per kg. This difference in deposition is most likely due to individual animal differences.

The sheep given 100 mg per kg of erbon also had high residue levels of 2,4,5-trichlorophenol in the kidney, liver, and omental fat. The only other sheep to have any deposition of this metabolite was given 25 mg per kg and had residues in the kidney.

LITERATURE CITED

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