Residues of Chlorophenoxy Acid Herbicides and Their Phenolic Metabolites in Tissues of Sheep and Cattle

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Three chlorophenoxy acid herbicides, 2,4-D, 2,4,5-T, and silvex, were fed at four dosage levels (0, 300, 1000, and 2000 ppm) to adult sheep and cattle for 28 days. Some were killed and tissues were sampled 1 day after the last dose, others 1 week later. Residues of the chlorophenoxy acid herbicides and their phenol metabolites were determined in muscle, fat, liver, and kidney. Analytical methods for determining tissue residues of chlorophenoxy acids and chlorophenols are presented. Muscle and fat contained the least residues of the herbicides or their metabolites. Kidneys contained the highest residues of each of the three herbicides. Liver and kidney contained the highest levels of either 2,4-dichlorophenol or 2,4,5-trichlorophenol. Withdrawal from treatment for 1 week before killing resulted in significant reduction in tissue residue levels. No species difference in regard to chlorophenoxy herbicide residue deposition was observed. Anorexia, either partial or complete, accompanied by decreased weight gains was observed, especially at the highest dosage levels.

Herbicides are the only class of pesticides that are used extensively in range management. Chlorophenoxy herbicides, especially 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), are by far the most predominant materials used for the chemical control of broadleaf weeds and brush on rangeland. These compounds are usually applied as formulations of the esters, organic amine salts, or other salts rather than as the free acid, but evidence indicates that conversion to the free acid is required for herbicidal activity (Shaw et al., 1960). Enzymes from a wide variety of plant tissues hydrolyze esters of 2,4-D to the free acid (Shaw et al., 1960). In vivo studies have also shown rapid hydrolysis of 2,4-D esters to the free acid by several plant species (Menzie, 1969) and microorganisms (Lisk, 1968). Thus, the free acids 2,4-D, 2,4,5-T, and silvex [2-(2,4,5-trichlorophenoxy)-propionic acid] were chosen as test compounds for this study because of the likelihood that grazing animals would be exposed to the phenoxy acids rather than to their esters.

The levels of herbicides available for ingestion by grazing livestock following application to rangeland depend upon the nature and degree of cover, the rate and mode of application, time after application, and climatic conditions. Studies have shown that residues on grass immediately after application are not likely to exceed 100 to 150 ppm for each pound of actual herbicide applied per acre (Morton et al., 1967; Leng, 1972). The subsequent rapid decline of residues on plants results from photodecomposition, washoff, plant and microbial metabolism, and dilution by plant growth (Leng, 1972). In studies at four different geographical locations (Texas, California, North Carolina, and Michigan) in which phenoxy herbicides were applied directly to grass at 4 lb per acre, residues found immediately after application averaged 57 to 139 ppm/lb of herbicide applied per acre. These residues declined with a half-life of 1 to 2 weeks depending on geographical location (Leng, 1972). This rate of depletion agrees with that in a report of earlier work in Texas by Morton et al. (1967) in which phenoxy herbicides were applied to range forage grasses at 0.5 or 2.0 lb/acre; the highest residue level reported in grass samples collected 1 hr after application was 300 ppm, and residual half-life was 2 to 3 weeks.

The lowest dose rate in the present study (300 mg/kg feed for 28 days), therefore, represents an exposure in excess of that to be expected on forage under normal conditions. The higher levels fed are several-fold greater exposures and would represent gross negligence in the process of application.

The phenoxyacetic acids are relatively strong acids ($pK_a \simeq 3$) and are, therefore, rapidly excreted unchanged by animals in their urine. St. John et al. (1964) reported that both 2,4,5-T and silvex given to dairy cows for 4 days at a rate of 5 ppm in feed were completely eliminated in the urine as soluble salts. Clark et al. (1964) recovered, within 24 hr, 96% of an orally administered dose of 2,4-D-^{14}C unchanged from urine of a sheep.

The literature contains little information regarding tissue residues resulting from ingestion of the phenoxy acids, especially in long term feeding trials. Clark et al. (1964) reported less than 0.05 ppm of 2,4-D in edible tissues of sheep resulting from a single oral dose of 4 mg/kg body weight. Clark and Palmer (1971) found 2,4,5-T residues of 0.08 ppm in omental fat of each of two sheep given four oral doses of either 0.15 or 0.75 mg/kg of the propylene glycol butyl ether esters of 2,4,5-T. In the same study, residues as high as 368 ppm of 2,4,5-T were found in the kidneys of animals killed by four daily 250 mg/kg doses of a 2,4,5-T ester.

Previous studies have not reported residues of metabolites of the phenoxy acid herbicides in livestock. Although high proportions of the phenoxy acids are rapidly eliminated in the urine, over an extended exposure period, some of the ingested herbicide could be hydrolyzed to the corresponding phenol by rumen microorganisms or by enzymatic processes within the animal. Wright et al. (1970) reported tissue residues of 2,4,5-trichlorophenol in sheep given oral doses of the herbicide erbon [2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate].

Increasing public concern regarding pesticides, their fate, and their potential hazards to all biological systems has resulted in requirements for more complete knowledge of the exogenous materials added to the environment. This study was designed to confirm and expand the knowledge of the fate and residues of the phenoxy herbicides 2,4-D, 2,4,5-T, and silvex in sheep and cattle.

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Table I. Levels of Chlorophenoxy Acid Herbicides Fed to Cattle and Sheep for 28 Days and the Scheduled Withdrawal from Treatment

	Treatment groups[a]		
	Herbicide in feed, ppm		
Animal	2,4-D	2,4,5-T	Silvex
Cattle	0		0
	300		300
	1000		1000
	2000		2000
	2000 + w[b]		2000 + w
Sheep	0	0	0
	2000	2000	2000
	2000 + w	2000 + w	2000 + w

[a] Three animals per group. [b] Seven-day withdrawal.

MATERIALS AND METHODS

Experimental Plan. The feeding and sampling was conducted at the U.S. Livestock Insects Laboratory, ARS, Kerrville, Tex. Thirty adult beef cattle and 27 adult sheep were purchased from local ranches that had no recent history of herbicide use. All animals appeared to be in good health at the onset and were under veterinary observation throughout the study. When the test began, the average weight of the cattle was 257 kg and that of the sheep was 36. The herbicides silvex (98% purity), 2,4,5-T (99% purity), and 2,4-D (99% purity) were supplied by Dow Chemical Co., Midland, Mich., and contained no detectable dioxin. The limit of detection was 0.5 ppm of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Getzendaner, 1971).

The animals were acclimated to their feed and surroundings, then randomly separated into groups of three and maintained throughout the test on a complete ration to which the prescribed amount of one of the herbicides had been added. Each animal was given feed up to 3% of its body weight daily, divided into morning and evening feedings. Any feed not consumed was weighed and then added to the next feeding. Individual animal weights were recorded weekly and rations adjusted accordingly. The experimental design is shown in Table I. Groups of cattle were given feed containing either silvex or 2,4-D for 28 days at four different levels—2000, 1000, 300, or 0 ppm; then the animals were killed on the 28th day and tissue samples collected. On the basis of each animal's consuming daily rations of 3% of its body weight, the levels of 300, 1000, and 2000 ppm feed are equivalent to 9, 30, and 60 mg/kg body wt per day, respectively. The sheep were treated with 2000 ppm of either silvex, 2,4,5-T, or 2,4-D for the same period. For information on disappearance of residue, additional groups of sheep and cattle given the highest level of each herbicide for 4 weeks were killed and sampled 7 days after the treated feed was replaced by untreated feed. Control animals (0 ppm) were given the same diet as the subjects but without added herbicide. Samples of muscle, liver, kidney, and fat were frozen immediately upon removal from the animal and kept in the frozen state until analyzed for residues of the phenoxy acids or the corresponding phenols.

Reagents. All solvents were distilled in glass except the 95% ethanol which was reagent grade. Boron trifluoride in methanol was prepared by passing BF_3 through pesticide grade methanol to the point of saturation. The solution was stored under refrigeration.

To prepare the trimethylsilyl (Me_3Si) reagent, we placed 4.5 ml of hexane in a rubber septum stoppered vial and maintained the solution under nitrogen. One-half milliliter of TriSil Concentrate (Pierce Chemical Co., Rockford, Ill., No. 49005) was added to the vial. The Me_3Si reagent was removed from the vial through the septum with a 500-μl syringe and added to the reaction flask as required. The reagent prepared in this manner was usable for 2 days.

2,4-D, 2,4,5-T, and Silvex Residues. Tissue residue levels of the chlorophenoxy acid herbicides were determined by modification of gas chromatographic methods developed by Clark et al. (1967) and Clark (1969). Muscle, liver, and kidney samples were freeze dried, then homogenized with hot ethanol and refluxed for 1 hr. The solids were filtered out and the ethanol filtrate chilled in an ice bath. The fat, which precipitated upon chilling, was filtered out and discarded. The alcohol filtrate was then removed by evaporation with a rotary evaporator. The rest of the solid residue was digested for 1 or 2 hr with papain, a proteolytic enzyme, at pH 5.0 and 55° and then extracted with methylene chloride, which was subsequently removed by evaporation. The residue was transferred to a 10-ml volumetric flask and diluted to the mark with hexane. An aliquot of 0.5 to 5 ml was pipeted into another 10-ml volumetric flask and evaporated to dryness. The chlorophenoxy acid herbicides were then methylated with boron trifluoride in methanol as previously described (Clark, 1969).

Fat samples were dissolved in hot ethanol, refluxed, then chilled, and filtered to remove excess fat. Ethanol was removed by evaporation. Acetonitrile was added to the flask that contained the rest of the fat. The mixture was rotated to extract the herbicide residues. The acetonitrile solution was then transferred to another flask and the solvent removed by evaporation. The material remaining after evaporation was then dissolved in hexane and methylated by the same method as that used for other samples. Hexane solutions containing the methyl esters of the herbicides were injected into the chromatograph. Peaks were compared with appropriate standards for identification and quantitation. The instrument used was a Micro-Tek GC 220 gas chromatograph with a ^{63}Ni electron capture detector and fitted with a U-shaped glass column (6 ft × 3 mm i.d.) packed with 1.5% OV-17 plus 1.95% QF-1 on 80–100 Supelcoport (Tek Labs, Baton Rouge, La.). The system was operated at an oven temperature of 190°, injection port temperature of 220°, and detector temperature of 265°. Prepurified nitrogen was the carrier gas with a flow rate of 80 ml/min at 40 psi.

2,4-Dichlorophenol and 2,4,5-Trichlorophenol Residues. Residues of 2,4-dichlorophenol (2,4-D-phenol) were measured in animals treated with 2,4-D. Tissues from animals given either 2,4,5-T or silvex were analyzed for residues of 2,4,5-trichlorophenol (2,4,5-T-phenol), the hydrolysis product of both compounds.

Chlorophenol residues were determined by two methods. Initially, residues of the 2,4-D-phenol and 2,4,5-T-phenol were measured by a method that included acid but not alkaline hydrolysis (method I). Jensen (1972) observed that liver and kidney but not muscle or fat, from animals fed phenoxy herbicides, contained phenolic metabolites which did not yield to extraction without prior digestion in KOH. This observation was confirmed in our laboratory. Liver and kidney samples were subsequently reanalyzed by the same basic method as before, but a KOH digestion was included as the initial step (method II).

Chlorophenol Method I. Five-gram samples of liver, muscle, kidney, or fat were cut into small pieces and placed in a 500-ml round-bottomed distillation flask containing porcelain boiling chips and 300 ml of 6 *N* H_2SO_4. The flask was placed in a heating mantle and attached to a side-arm adapter and condenser. The 2,4-dichlorophenol or 2,4,5-trichlorophenol residues were carried over in the first 70 ml of distillate. The distillate was collected in a 125-ml separatory funnel containing 5 ml of 10% NaOH to

Table II. Average Residues of 2,4,5-Trichlorophenol or 2,4-Dichlorophenol Determined by Two Methods in Sheep and Cattle Fed 2000 ppm of Silvex, 2,4,5-T, or 2,4-D for 28 Days

Herbicide, metabolite, and animal	Residues found by indicated method,[a] ppm[b]							
	Muscle		Fat		Liver		Kidney	
	Method I	Method II	Method I	Method II	Method I	Method II	Method I	Method II
2,4-D (2,4-D-phenol)								
Sheep	<0.05	<0.05	<0.05	<0.05	0.08	0.16	0.08	0.26
Cattle	<0.05	<0.05	<0.05	<0.05	0.09	0.31	0.16	1.06
2,4,5-T (2,4,5-T-phenol)								
Sheep	<0.05	0.12	<0.05	<0.05	0.40	6.10	0.81	0.90
Silvex (2,4,5-T-phenol)								
Sheep	<0.05	<0.05	<0.05	<0.05	<0.05	0.37	0.08	0.17
Cattle	<0.05	<0.05	<0.05	<0.05	0.09	0.42	<0.05	0.10

[a] Details of methods in text. [b] Not corrected for recovery.

prevent loss of phenols by volatilization. The distillate was then acidified with 2 ml of 12 N H_2SO_4, and the chlorophenols were extracted with three 25-ml portions of methylene chloride. Each portion of the methylene chloride was passed through a sodium sulfate column into a boiling flask. After the third extraction, the column was washed with an additional 15 ml of methylene chloride. The flask was then attached to a rotary evaporator and the solvent removed. When the volume had been reduced to about 5 ml, the flask was removed and the final portion of solvent evaporated very carefully with a gentle stream of nitrogen; only hand heat was used. Just as the last drop of methylene chloride evaporated, the nitrogen stream was removed and the residue transferred to a 10-ml volumetric flask and diluted to the mark with hexane. An appropriate aliquot, usually 1 to 4 ml, was transferred to another 10-ml volumetric flask. The hexane again was evaporated down to 0.5 ml by hand as described above. The flask was then tightly stoppered to maintain a dry, nitrogen atmosphere. Two hundred microliters of the trimethylsilyl reagent was withdrawn from the rubber septum stoppered storage vial with a dry 500-μl syringe and quickly added to the 10-ml volumetric flask containing the phenolic residue. The flask was sealed with a ground glass stopper and placed in a 55° water bath for 5 min. The flask was then removed from the water bath, allowed to cool, and diluted to the mark with hexane. Appropriate aliquots were withdrawn with a microliter syringe and injected into the same gas chromatograph as was used for analysis of the phenoxy herbicides. The trimethylsilyl ethers of the chlorophenolic metabolites of the chlorophenoxy acid herbicides were chromatographed on a glass 6 ft × 0.25 in. i.d. column packed with 3% OV-1 on 80–90 mesh Chromosorb W. Nitrogen flow rate was 50 ml/min. Column temperature was 120° isothermal, injector temperature was 130°, and detector temperature was 270°. Under these conditions, 0.1 ng of 2,4-dichlorophenol produced about 35% of full-scale response on the 1-mV recorder.

Chlorophenol Method II. Five-gram samples of tissue were placed in a 50-ml flask containing 15 ml of 0.5 N KOH. The flask was then placed on an oscillating hot plate at 60° for 2 hr. The solution containing the dissolved tissue was allowed to cool and then transferred to a 500-ml boiling flask with 300 ml of 6 N H_2SO_4. Porcelain boiling chips were added, and the flask was placed in a heating mantle and attached to a side-arm adapter and condenser. Subsequent distillation, extraction, and gas chromatographic analysis were described for method I.

RESULTS AND DISCUSSION

Recovery of known amounts of the herbicides added to control tissue averaged 90% for 2,4-D, 93% for silvex, and 88% for 2,4,5-T. In every case, at least 80% of the added herbicide was recovered. Residues as low as 0.01 ppm for silvex, 0.03 for 2,4,5-T, and 0.035 for 2,4-D could be detected; however, 0.05 ppm was established as the point below which residues would not be reported. Hydrolysis with 6 N H_2SO_4 or 1 N KOH of tissue that had been previously extracted according to our method did not produce additional recovery of the phenoxy acid compounds. Residues as low as 0.02 ppm for 2,4-dichlorophenol and 0.01 for 2,4,5-trichlorophenol could be detected; however, 0.05 ppm was established as the point below which residues would not be reported. Average recovery of known amounts of either 2,4-dichlorophenol or 2,4,5-trichlorophenol added to raw control tissue before extraction was above 95% by either method I or method II. No background peaks interfered with the analysis of either of the phenolic compounds.

Experiments were conducted to determine if the phenolic residues found could have resulted from hydrolysis of the phenoxy acids during the extraction process or by tissue enzymes in vitro. Control tissue samples were spiked with known amounts of either 2,4-D, silvex, or 2,4,5-T. The samples were homogenized and allowed to stand for several hours before analysis. Recovery of 2,4-D, silvex, or 2,4,5-T from spiked control tissue in no way indicated that phenols were generated by hydrolysis of the chlorophenoxy acids by either 1 N KOH, 6 N H_2SO_4, or by tissue enzymes. In no instance were phenols resulting from hydrolysis of the parent phenoxy acids detected. In each instance, essentially all of the added phenoxy acid was recovered.

Selected liver, kidney, muscle, and fat samples from test animals were analyzed by method I. The material remaining in the 500-ml round-bottomed flask after distillation of the H_2SO_4 solution was made strongly alkaline with KOH, then processed according to method II. Additional phenolic residues were recovered from liver and kidney but not from muscle or fat.

Average residues of 2,4-D-phenol and 2,4,5-T-phenol detected in tissues of the test animals are presented in Table II. Residues in each sample were determined by both methods previously described. The data represent analysis of tissues of animals fed at the 2000-ppm treatment level for each herbicide in order to show the higher residues detected with alkaline hydrolysis (method II) of the tissue samples. Except for low levels of 2,4,5-T-phenol recovered by the alkaline hydrolysis method from muscle of sheep fed 2000 ppm of 2,4,5-T, residues recovered by either method were <0.05 in both fat and muscle for all three herbicides. Recovery of residues of the phenolic me-

Table III. Residues of 2,4-D and 2,4-Dichlorophenol in Sheep and Cattle Fed 2,4-D for 28 Days[a]

		Residues found, ppm[b,c]							
		Muscle		Fat		Liver		Kidney	
Animal	2,4-D in feed, ppm	2,4-D	2,4-D-phenol	2,4-D	2,4-D-phenol	2,4-D	2,4-D-phenol	2,4-D	2,4-D-phenol
Sheep	2000	0.06	<0.05	0.10	<0.05	0.98	0.16	9.17	0.26
	2000(+ w)[d]	<0.05	<0.05	0.15	<0.05	0.29	0.15	0.37	0.07
Cattle	300	<0.05	<0.05	0.13	<0.05	<0.05	0.11	2.53	0.56
	1000	<0.05	<0.05	0.45	<0.05	0.14	0.59	8.67	1.17
	2000	0.07	<0.05	0.34	<0.05	0.23	0.31	10.9	1.06

[a] Average of three animals per group; data for individual animals are available on microfilm (see Supplementary Material Available paragraph at end of paper). [b] Not corrected for recovery. [c] 2,4-D phenol residues were determined by method II. [d] (+ w) = 7-day withdrawal.

Table IV. Residues of 2,4,5-T and 2,4,5-Trichlorophenol in Sheep Fed 2,4,5-T for 28 Days[a]

	Residues found, ppm[b,c]							
	Muscle		Fat		Liver		Kidney	
2,4,5-T in feed, ppm	2,4,5-T	2,4,5-T-phenol	2,4,5-T	2,4,5-T-phenol	2,4,5-T	2,4,5-T-phenol	2,4,5-T	2,4,5-T-phenol
2000	1.00	0.13	0.27	<0.05	2.29	6.1	27.2	0.90
2000 (+ w)[d]	<0.05	0.05	<0.05	<0.05	<0.05	4.4	0.06	0.81

[a] Average of three animals per group. Data for individual animals are available on microfilm (see paragraph at end of paper regarding supplementary material). [b] Not corrected for recovery. [c] 2,4,5-T-phenol residues were determined by method II. [d] (+ w) = 7-day withdrawal.

tabolites from liver and kidney was increased by as much as 20-fold by alkaline hydrolysis. These data thus confirm the observation of Jensen (1972).

In order to be recovered from either method I or method II, phenolic residues had to be in a form distillable with water from an acid solution. Thus, residues detected with method I would include free chlorophenol or conjugates which are hydrolyzed to the free chlorophenol by the 6 *N* H_2SO_4. It is doubtful that conjugated phenols as such would be distillable. Since method II included alkaline hydrolysis followed by distillation from a strongly acid solution, residues recovered would include both free residues or those bound but hydrolyzed to free phenols by either the alkaline or acid conditions of the procedure.

The residues of 2,4-dichlorophenol or 2,4,5-trichlorophenol reported in Tables III, IV, and V were determined by method II.

Tissue Residues. *2,4-D.* Residues of 2,4-D and 2,4-dichlorophenol detected in sheep and cattle fed rations containing 0, 300, 1000, or 2000 ppm of 2,4-D daily for 28 days are presented in Table III. With the exception of the kidneys, 2,4-D residues averaged less than 1 ppm in the tissues analyzed. Because phenoxy acid herbicides are excreted unchanged in the urine, the higher residue levels detected in renal tissue might be expected (Clark, 1969). 2,4-Dichlorophenol was not detected in fat and muscle of any animals. As with 2,4-D, the highest residue levels of the phenol were detected in the kidneys. Replacement of the test diet for 7 days with a diet that did not contain 2,4-D resulted in reduced residues of both 2,4-D and the metabolite, 2,4-dichlorophenol (Table II).

2,4,5-T. Residues of 2,4,5-T and 2,4,5-trichlorophenol detected in tissues of sheep fed rations containing 2000 ppm of 2,4,5-T for 28 days are presented in Table IV. Residues of 2,4,5-T averaged 1 ppm in muscle and 0.25 ppm in fat in sheep that were killed on the same day as the final dose. Levels of 2,4,5-T found in muscle or fat collected from the animals that were held for a week on untreated feed did not exceed 0.05 ppm. Residues of the metabolite 2,4,5-trichlorophenol were not detected in fat of any of the animals. With the exception of one sheep in which 0.25 ppm was detected, muscle from neither the withdrawal nor the nonwithdrawal group contained more than 0.05 ppm of 2,4,5-trichlorophenol. 2,4,5-T residues ranged from 0.5 to 5.5 ppm in liver and from 5 to 70 ppm in kidney of the nonwithdrawal group. After withdrawal from exposure for 7 days, residues in both tissues were, with exception of kidney from one sheep, less than 0.05 ppm. 2,4,5-Trichlorophenol residues averaged over six times greater in liver than kidney. After the withdrawal period, kidney residues were unchanged, whereas liver residues were about one-third less than those in the nonwithdrawal group.

Silvex. Residues of silvex and its metabolite 2,4,5-trichlorophenol detected in tissues of sheep and cattle fed rations containing 0, 300, 1000, or 2000 ppm of silvex daily for 28 days are shown in Table V. Except for the group of sheep and the group of cattle fed the highest level of silvex with no withdrawal period before slaughter, silvex residues in muscle did not exceed 0.1 ppm. Silvex residues were higher in fat than in muscle, but the levels did not suggest accumulation in fat. 2,4,5-Trichlorophenol levels were below the established detection limit of 0.05 ppm in muscle and fat in all groups. Silvex was detected in livers and kidneys in all animals in each nonwithdrawal group. Levels of silvex in liver and kidney were reduced very efficiently by the 1-week withdrawal. 2,4,5-Trichlorophenol residues were slightly higher in liver than in kidney and were reduced by withdrawal, but not to the degree as was silvex.

Feed Consumption and Weight Gain. Sheep and cattle given feed containing the 2,4,5-T, silvex, or 2,4-D general-

Table V. Residues of Silvex and 2,4,5-Trichlorophenol in Sheep and Cattle Fed Silvex for 28 Days[a]

		Residues found, ppm[b,c]							
		Muscle		Fat		Liver		Kidney	
Animal	Silvex in feed, ppm	Silvex	2,4,5-T-phenol	Silvex	2,4,5-T-phenol	Silvex	2,4,5-T-phenol	Silvex	2,4,5-T-phenol
Sheep	2000	1.40	<0.05	0.74	<0.05	6.23	0.22	9.33	0.17
	2000 (+ w)[d]	<0.05	<0.05	0.06	<0.05	0.08	0.63	0.14	0.10
Cattle	300	0.07	<0.05	0.66	<0.05	5.73	0.06	22.4	0.06
	1000	0.09	<0.05	1.36	<0.05	11.5	0.48	24.4	<0.05
	2000	0.70	<0.05	3.77	<0.05	8.37	0.42	23.6	0.10
	2000 (+ w)[d]	0.12	<0.05	0.67	<0.05	0.55	0.13	1.13	0.06

[a] Average of three animals per group. Data for individual animals are available on microfilm (see paragraph regarding supplementary material at end of paper). [b] Not corrected for recovery. [c] 2,4,5-T-phenol residues were determined by method II. [d] (+ w) = 7-day withdrawal.

Table VI. Effect of Ingested Chlorophenoxy Acid Herbicides on Feed Consumption, Feed Conversion, and Weight Gain of Sheep and Cattle

	Treatment		28-day herbicide feeding period			7-day withdrawal (no herbicides)		
Animal	Herbicide	Level, ppm	Wt gain[a]	Feed consumption[b]	Feed conversion[c]	Wt gain[a]	Feed consumption[b]	Feed conversion[c]
Cattle	Silvex	2000	−0.05[e]	2.15		*d*	3.00	
		1000	0.29	2.55	9.82			
		300	0.28	2.65	10.74			
	2,4-D	2000	0.34	2.84	8.50	*d*	3.00	
		1000	0.37	2.89	8.25			
		300	0.25	2.62	10.81			
	Control	0	0.40	3.18	8.41			
Sheep	Silvex	2000	0.18	2.50	13.71	0.80	2.88	3.89
	2,4,5-T	2000	0.35	2.99	7.35	0.43	2.95	6.37
	2,4-D	2000	0.33	2.92	9.04	0.42	2.84	7.28
	Control	0	0.39	3.10	8.30			

[a] Average percentage of body weight gained per day. [b] Average percentage of body weight consumed per day. [c] Average pounds of feed consumed per pound of weight gained. [d] Data unavailable. [e] Highly significant, $P = 0.01$.

ly ate less feed, gained less weight, and had less efficient feed conversion ratios than controls (Table VI).These effects were most evident with silvex at the highest levels.

Anorexia, either partial or complete, was evident in all cattle groups given 2,4-D or silvex at all dosage levels. For those given 2,4-D, the refusal of feed was occasional. However, for those given silvex, anorexia began during the first or second day and continued throughout the 28-day trial. Anorexia resulted in decreased weight gains in the lower levels and a loss of weight in the 2000-ppm group.

Anorexia in the eight test groups of sheep was apparent for all three herbicides at all dosage levels, especially during the first week of the trials. Anorexia resulted in an initial loss of weight, but this was subsequently regained in the following 3 weeks. During the 7-day withdrawal period, feed consumption in all groups of sheep and cattle, regardless of previous treatment, approximated that of the controls. The temporary anorexia and decreased weight gains were possibly due to decreased palatability of the herbicide-containing feed.

CONCLUSION

The methods for the analysis of chlorophenoxy acid herbicide residues produced high recoveries. Additional extraction following digestion with KOH or H_2SO_4 failed to increase residues recovered from tissues of treated animals. The analytical technique for chlorophenol residues is simple, fast, and reproducible. Codistillation of chlorophenols with water from an acid system provides a very efficient cleanup procedure that could probably be used for residue analysis of other volatile phenols.

Chlorophenoxy herbicide residues are not likely to be greater than 300 ppm in or on forage immediately after treatment with these compounds at rates recommended for control of weeds and brush in pastures or rangeland. Residues decline rapidly with a half-life of 1 to 2 weeks, depending on geographical location (Leng, 1972). The studies reported here indicate that proper agricultural uses of 2,4-D, silvex, or 2,4,5-T will not result in more than minimal residues of the phenoxy herbicides or their phenolic metabolites in meat of sheep or cattle. No significant differences were observed in residues between sheep and cattle. Residues in meat may be expected only under the highly unlikely circumstances in which animals would be slaughtered during continuous ingestion of freshly treated vegetation. No adverse effects, other than decreased weight gain due to anorexia, were observed for any of the herbicides at any level of ingestion. Withdrawal of animals from treated feed for 1 to 2 weeks before slaughter would drastically reduce any residues present. Proper agricultural use of 2,4-D, silvex, or 2,4,5-T should not result in either harmful effects to the animals consuming treated forage or in more than minimal residues in meat.

Supplementary Material Available. Tables III-V corresponding to those in the text but listing data for individual animals will appear following these pages in the microfilm edition of this volume of the journal. Photo-

copies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-573.

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Comparative Adsorption, Desorption, and Mobility of Dipropetryn and Prometryn in Soil

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Adsorption-desorption isotherms with dipropetryn [2-(ethylthio)-4,6-bis(isopropylamino)-*s*-triazine] and prometryn [2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine] were determined using six adsorbent materials possessing a wide range in cation exchange capacities (CEC), percent organic matter (OM), clay levels, and pH values. Dipropetryn and prometryn adsorption-desorption isotherms showed increasing adsorption with increasing clay content, cation exchange capacity, and organic matter levels, and decreasing pH values. With all adsorbents, more dipropetryn was adsorbed than prometryn. After eight successive desorption extractions, more dipropetryn than prometryn remained adsorbed. Soil thin-layer chromatography was used to evaluate herbicide mobility. Fluometuron [1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea], a more mobile herbicide, was included as a standard. Dipropetryn showed less movement in the soil than prometryn or fluometuron, with fluometuron showing the greatest movement. As CEC, percent OM, and clay content decreased, herbicide mobility increased for all three herbicides. Soil mobility of dipropetryn and prometryn correlated well with adsorption-desorption isotherm parameters.

Prometryn [2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine], a *methyl*thio-*s*-triazine, has been used in cotton (*Gossypium hirsutum* L.) for weed control for a number of years. Its use on sandy soils has frequently resulted in crop injury. Dipropetryn [2-(ethylthio)-4,6-bis(isopropylamino)-*s*-triazine], an *ethyl*thio-*s*-triazine, is a new herbicide being evaluated for weed control in cotton grown on sandy soils. Initial reports indicate that this ethylthio analog may be less phytotoxic to cotton than the methylthio analog.

Herbicide adsorption by the soil matrix has been used by many investigators to explain different herbicide phytotoxicity levels for various soils (Scott and Weber, 1967). Adsorption characteristics of the *s*-triazines have been shown to be affected by soil organic matter content (Harris and Warren, 1964; Scott and Weber, 1967; Talbert and Fletchall, 1965), soil pH (Harris and Warren, 1964; Swanson and Dutt, 1973; Weber et al., 1965), clay content and type (Scott and Weber, 1967; Talbert and Fletchall, 1965; Weber et al., 1965), soil temperature (Harris et al., 1968; Harris and Warren, 1964; Talbert and Fletchall, 1965), as well as charcoal (Weber et al., 1968) and type of exchange resins (Harris and Warren, 1964; Scott and Weber, 1967; Weber et al., 1965). Harris et al. (1968) and Harris and Warren (1964) have shown a lack of correlation between water solubility and adsorption for three similar chloro-*s*-triazines. Scott and Weber (1967) have shown that adsorption on organic materials correlated well with phytotoxicity. When the adsorption of several *s*-triazines was evaluated using a given adsorbent, prometryn was shown to be adsorbed in larger quantities (Gilmour and Coleman, 1971).

Herbicide mobility in the soil is, in part, a factor which influences the effectiveness of a herbicide. The extent to which herbicides move in the soil is directly related to the adsorption-desorption characteristics of each herbicide with the soil. Davidson et al. (1972) have shown that the nonsingularity between herbicide adsorption and desorption was responsible for the shape of the total herbicide concentration distribution in the soil. The nonsingularity tends to decrease the maximum herbicide solution concentration as well as increase the length of soil profile over which the distribution occurs. This skewing of the concentration distribution noted for fluometuron [1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea], picloram (4-amino-3,5,6-trichloropicolinic acid), and prometryn in a water-

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