Degradation of the Herbicide Dichlorfop-Methyl in Prairie Soils

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The degradation of the herbicidal ester [^{14}C]dichlorfop-methyl was studied in moist nonsterile heavy clay, silty clay, and sandy loam at 20 ± 1 °C. The soils were extracted at regular intervals with aqueous acidic acetonitrile and radiochemical procedures were used to monitor the herbicide breakdown. On all soils an initial and very rapid hydrolysis to the corresponding acid was followed by a much slower hydrolytic process so that traces of the ester could still be detected after 10 weeks. In all cases the major breakdown product was the acid which degraded more quickly in the heavy clay and sandy loam than in the silty clay. With time, the acid appeared to undergo a strong binding or complexing to the soils from which it could be liberated only by treatment with hot alkali. Traces of 4-(2,4-dichlorophenoxy)phenol and its ethyl analogue were also recovered from the treated soils.

Dichlorfop-methyl [(1), methyl-2-(4-(2,4-dichlorophenoxy)phenoxy)propionate] is used on the Canadian prairies as a post emergence herbicide for the control of wild oats and other annual grasses in a variety of crops. Application rates range from 0.85 to 3.5 kg/ha.

Although the herbicide is applied to the growing crops some of the spray must inevitably come into contact with the soil thus making it necessary to determine the fate of dichlorfop-methyl residues in soils.

Analytical procedures for the extraction and gas chromatographic analysis of both the herbicidal ester and its probable soil degradation product, dichlorfop acid (2), have been reported (Smith, 1977), but little is known about the persistence of either chemical in soils. The studies to be described were undertaken to investigate the fate of [^{14}C]dichlorfop-methyl in three nonsterile soil types under laboratory conditions and to isolate and identify any degradation products.

MATERIALS AND METHODS

Soils. The soils used in these studies were collected from the 0–5 cm horizon, their composition and physical characteristics are indicated in Table I.

Chemicals. [^{14}C]Dichlorfop-methyl [methyl-2-(4-(2,4-dichlorophenoxy)phenoxy)propionate uniformly labeled in the dichlorophenyl ring moiety] was obtained from Hoechst Aktiengesellschaft, Frankfurt, Germany, (10 μCi/mg; purity >97%) as were the nonradioactive dichlorfop acid [(2), 2-(4-(2,4-dichlorophenoxy)phenoxy)-propionic acid] and the 4-(2,4-dichlorophenoxy)phenol (4). A methanolic solution of the [^{14}C]dichlorfopmethyl was prepared (100 μg/mL) with a specific activity of 0.95 μCi/mL.

4-(2,4-Dichlorophenoxy)phenetole (3) was prepared by heating a mixture of the dichlorophenoxyphenol [(4), 100 mg], anhydrous potassium carbonate (20 g), ethyl bromide (5 mL), and acetone (70 mL) under reflux conditions. After 1 h, the flask and contents were cooled and filtered, and the filtrate was evaporated to dryness to yield the substituted phenetole 3 as a white solid. The ethyl derivative was characterized by means of its mass spectrum (Finnigan 1015 mass spectrometer utilizing a solid probe) which indicated a molecular ion weight of 282 (equivalent to $C_{14}H_{12}O_2Cl_2$) and the presence of two chlorine atoms.

2,4-Dichlorophenol and 2,4-dichloroanisole were purchased from Aldrich Chemical Co. Inc., Milwaukee, Wis.

Short-Term Hydrolysis Study. Twenty-gram samples of moist silty clay, heavy clay, and sandy loam at 85 or 50% of their respective field capacity moisture levels were weighed into 70-mL capacity screw-capped glass bottles and treated with 0.5 mL (50 μg) of the [^{14}C]dichlorfop-methyl solution. This rate was equivalent to 2.5 ppm of the herbicide based on moist soil. After thorough mixing the bottles were capped and incubated in the dark at 20 ± 1 °C. Duplicate samples of the soils with the higher moisture content were analyzed after 0, 3, 6, and 24 h while duplicate samples of the soils having the lower water content were analyzed only after 24 h.

Ester Extraction and Analysis. The soil from each bottle was placed in a 100-mL capacity glass stoppered flask and shaken on a wrist-action shaker for 1 h with 50 mL of a solution containing acetonitrile, water, and acetic acid in the proportions 80:18:2. Following centrifugation at 5000 rpm for 2 min, 10 mL of the clear extract, equivalent to 4 g of moist soil, was added to 40 mL of 5% aqueous sodium carbonate solution in a 250-mL separatory funnel and shaken with a 25-mL portion of *n*-hexane. The [^{14}C]dichlorfop-methyl extracted was calculated by determining the radioactivity present in a 5.0-mL aliquot of the *n*-hexane layer, while [^{14}C]dichlorfop acid was measured by examining 1.0-mL portions of the aqueous phase for radioactivity (see below). From these measurements, the percentages of the applied radioactivity present as the parent ester and acid hydrolysis product were determined.

Degradation Studies. Fifty-gram samples of all three soil types, moistened at 85% of their respective field capacities, were weighed into 300-mL volume waxed cardboard cartons with loose fitting caps and incubated for 7 days at 20 ± 1 °C to allow equilibration. Distilled water was added every 2 days to maintain the moisture content. Following the equilibration period, [^{14}C]dichlorfop-methyl solution (1.0 mL, 100 μg) was added and the soils were stirred to ensure a uniform herbicide concentration of 2 ppm, based on moist soil. This rate is approximately equivalent to 1 kg/ha assuming that under field conditions the chemical is contained in the top 5 cm of soil. The cartons were loosely capped to permit circulation of air, but to reduce water evaporation, and incubated in the dark at 20 ± 1 °C. Distilled water was added, with stirring, every 2 days to replace lost moisture. Duplicate samples were analyzed after 1, 7, 14, 21, 28, 35, 49, and 70 days.

Extraction Procedures for Degradation Studies. (See also Figure 1.) The soil from each carton was placed in a 250-mL glass-stoppered flask and sufficient extracting solvent added (acetonitrile, water, acetic acid in the ratio 80:18:2) so that the combined volume of the solvent together with the water present in the soil was equivalent to 100 mL. The flask was shaken on a wrist-action shaker for 1 h and then the contents were centrifuged at 5000 rpm

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Table I. Composition and Physical Characteristics of Soils

Soil type	Composition, %			Org. content	pH	Field capacity moisture, %
	Clay	Silt	Sand			
Jameson, sandy loam (SL)	6	9	85	3.2	7.5	11
Melfort, silty clay (SiC)	30	38	32	11.7	5.2	36
Regina, heavy clay (HvC)	69	26	5	4.2	7.7	40

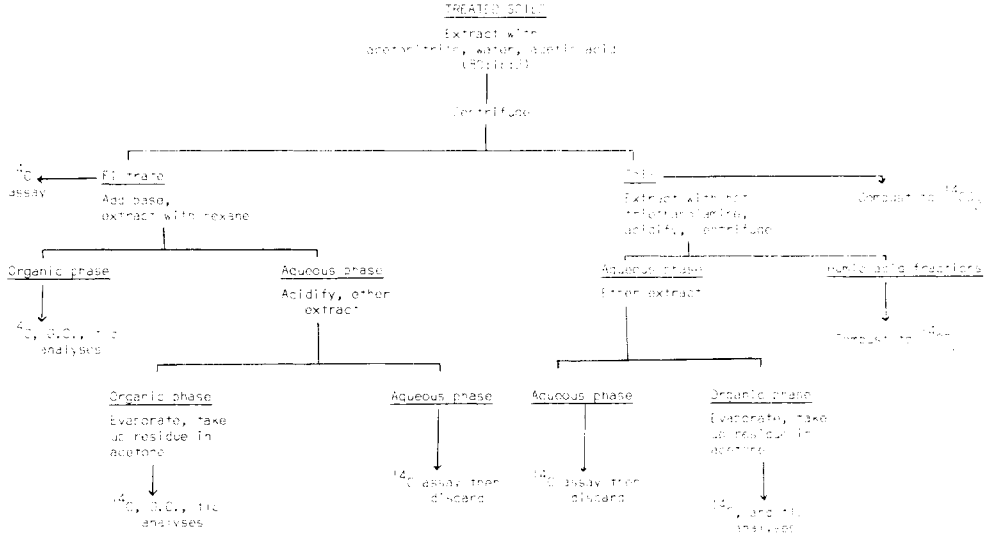

Figure 1. Outline of procedures used for the extraction and analysis of [^{14}C]dichlorfop-methyl and its degradation products from soils.

for 2 min. A 1.0-mL aliquot of the clear supernatant was examined for radioactivity so that the percentage of the applied ^{14}C solvent extracted could be determined.

A 25-mL portion of the supernatant was added to 100 mL of 5% aqueous sodium carbonate solution in a separatory funnel and shaken with 25 mL of *n*-hexane. At every sampling, a 5-mL aliquot of the organic layer was analyzed for radioactivity, while after 49 and 70 days a 5-μL portion was subjected to gas chromatographic analysis to determine the amount of dichlorfop-methyl present. The remaining hexane solutions were evaporated under reduced pressure to approximately 0.5 mL and subjected to thin-layer chromatographic examination for the presence of the parent compound, the phenetole 3, and any other isolatable degradation products.

The alkaline aqueous phase following *n*-hexane extraction was acidified with 10 mL of concentrated hydrochloric acid and shaken with 2 × 50-mL volumes of ether. Samples (2.0 mL) of the aqueous layer were checked for ^{14}C before being discarded to ensure that all the radioactivity had been extracted into the ether. The combined ether extracts were evaporated to dryness under reduced pressure. Traces of water were removed from the flask by reduced pressure azeotropic distillation using a mixture of equal volumes of benzene and ethanol, and the residue was dissolved in 10.0 mL of acetone. At every sampling date a 1.0 mL volume of the acetone solution was assayed for radioactivity. After the 49- and 70-day incubations, a further 1.0-mL aliquot was evaporated to dryness using a hot water bath, and the residue, in ether, was methylated using a solution of diazomethane in ether as previously described (Smith, 1977). Following evaporation of excess reagent and solvent, the residue was dissolved in 10.0 mL of *n*-hexane when 5-μL samples were analyzed by gas chromatographic methods to quantitatively determine dichlorfop-methyl present.

The remaining acetone solutions were evaporated under reduced pressure to small volume (0.5 mL) and examined by thin-layer chromatography for dichlorfop acid (**2**), the dichlorophenoxy phenol (**4**), and any other radioactive degradation products.

Soil residues, following solvent extraction after the 1-, 35-, 49-, and 70-day incubation periods were collected and washed twice with methanol, the washings being discarded. The soils were then dried to constant weight in an oven set at 70 °C when 100-mg samples were combusted in oxygen (see below) and the [^{14}C]carbon dioxide released determined. From these data radioactivity still present in the soils and not recoverable by solvent extraction could be determined.

Alkaline Soil Extractions. Samples (20 g) of the above soils derived from the 35- and 70-day treatments and following solvent extraction, methanol washing, and oven drying were heated under reflux for 5 h with 50 mL of an aqueous solution containing 20% (by volume) of triethanolamine. After cooling, the contents of the flasks were centrifuged at 5000 rpm for 5 min and 25-mL portions added to 25 mL of 6 N hydrochloric acid in a 50-mL glass centrifuge tube. This treatment precipitated the humic acid fractions which could be separated from the clear, but colored, supernatant solutions by further centrifugations. The humic acid moieties were dried overnight at 70 °C to constant weight, when a 100-mg portion was combusted in an atmosphere of oxygen to liberate radioactivity as [^{14}C]carbon dioxide. From these data the percentage of initially applied radioactivity associated with the soil humic acids after 35- and 70-day incubation periods could be calculated.

Portions (2 mL) of the acidic supernatant (from above) were examined for radioactivity, and, following ether extraction (3 × 25-mL volumes), the aqueous phase was again checked to determine whether all the activity had been taken up into the ether. The aqueous layer was then discarded. The pooled ether extracts were evaporated to dryness under reduced pressure and traces of water removed by azeotropic distillation using benzene and eth-

Table II. R_f Values of Compounds Studied

Compound	R_f Ref a	Ref b	Ref c
Dichlorfop-methyl (1)	0.53	0.95	0.25
Dichlorfop acid (2)	0.00	0.17	0.00
4-(2,4-Dichlorophenoxy)phenetole (3)	0.89	0.97	0.75
4-(2,4-Dichlorophenoxy)phenol (4)	0.22	0.65	0.10
2,4-Dichlorophenol	0.50	0.70	0.35
2,4-Dichloroanisole	0.90	0.96	0.85

[a] Benzene. [b] Benzene-methanol (10:1). [c] Benzene-*n*-hexane (1:1).

anol. The residue was dissolved in 10 mL of acetone, 1-mL aliquots of which were analyzed for radioactivity. The remaining acetone extracts were evaporated to approximately 0.5 mL and examined by thin-layer chromatography for the presence of dichlorfop acid (2) and the dichlorophenoxy phenol 4.

Thin-Layer Chromatography. Precoated TLC plates (Silica Gel 60 F-254) were obtained from E. Merck, Darmstadt, Germany. Following development to a height of 10 cm above the origin, the plates were dried and examined for radioactive compounds using a PANAX Thin-Layer Paper Radiochromatogram Scanner (Panax Equipment Ltd., Redhill, England). Nonradioactive compounds run for comparative purposes were detected by viewing the developed chromatograms under a short-wave ultraviolet lamp. The R_f values of the compounds studied in three chromatographic solvent systems are shown in Table II.

Soil Combustions. Samples (100 mg) of oven-dried soils or humic acid fractions were combusted in a platinum holder using a Schöniger combustion flask filled with oxygen. The [^{14}C]carbon dioxide was absorbed in 15.0 mL of a solution of phenethylamine in methanol (1:1) and the radioactivity measured by adding a 5.0-mL aliquot to 15.0 mL of scintillation solution (toluene containing 0.4% PPO and 0.01% POPOP).

Radioactivity. The ^{14}C in the various solutions was measured using a Picker Nuclear Liquimat 200 liquid scintillation spectrometer. Except for the case of the soil combustions (see above), "Scinti Verse" (Fisher Scientific Co., Fair Lawn, N.J.) was scintillation solution. For the determination of counting efficiencies an external ^{137}Cs Standard was used.

Gas Chromatography. A Hewlett-Packard 5713 A gas chromatograph was used, equipped with on-column injection port and a radioactive nickel electron-capture detector operated at 300 °C. The glass column (1.5 m × 6 mm o.d.) was packed with 5% XE-60 on Chromosorb W-HP (80–100 mesh). Carrier gas was argon containing 5% methane at a flow rate of 40 mL/min. With a column temperature of 210 °C the retention time of dichlorfop-methyl was 14.3 min, while that of the phenetole 3 was 3.5 min. Standards and samples were injected as *n*-hexane solutions. Esters present in the samples were calculated by comparing sample peak heights, in the linear response region, with those of appropriate standards. Analysis of untreated soils confirmed the absence of interfering substances.

RESULTS AND DISCUSSION

The results of the 24-h hydrolysis study are summarized in Table III. Extraction and analysis of all soil types, at 85% of the field capacity moistures, immediately following herbicide treatment indicated that 100% of the applied radioactivity was being recovered as the ester, there being no ^{14}C in the aqueous phase. This confirmed that the extraction procedure was not contributing in any way to the hydrolysis of the dichlorfop-methyl to the acid. After 3 h on the moist soils ester hydrolysis was observed, the conversion to the acid being most noticeable on the heavy clay and least on the silty clay. This hydrolytic trend continued so that at the end of the 24-h incubation period 85% of the ester had undergone hydrolysis to the acid on the Regina heavy clay, 68% on the Jameson sandy loam, but only 40% on the Melfort silty clay. Similar results to these (Table III) were obtained when the herbicidal ester was incubated for 24 h on the various soils at 50% of their respective field capacities. Thus, even on soils with moistures approaching that of the wilting point, the extent of hydrolysis was very similar to that occurring on moister soils. On air-dried soils less than 5% of the ester treatments underwent conversion to the acid after 24 h. Comparatively, the soil hydrolysis of dichlorfop-methyl is almost as rapid as that for the isopropyl and *n*-butyl esters of dichlorophenoxyalkanoic herbicides, which are converted completely to their parent acids within 24 h on a variety of moist soils (Smith, 1972, 1976a).

Table III. Hydrolysis of [^{14}C]Dichlorfop-Methyl to [^{14}C]Dichlorfop Acid in Moist Soils at 20 ± 1 °C Over a 24 h Period

Time, h	Soil type	Remaining [a,b] 85% field capacity Ester %	Acid %	50% field capacity Ester %	Acid %
0	SL	100	0	c	
	HvC	106	0		
	SiC	104	0		
3	SL	82	18		
	HvC	57	43		
	SiC	90	10		
6	SL	59	41		
	HvC	29	71		
	SiC	81	19		
24	SL	32	68	39	61
	HvC	15	85	22	78
	SiC	60	40	71	29

[a] Initial herbicide concentration of 2 ppm. Average of two replicates. [b] Less than 5% hydrolysis to the acid occurred all on air-dried soils after 24 h. [c] Not determined.

This experiment also demonstrated that aqueous acidic acetonitrile is capable of efficiently extracting both dichlorfop-methyl and dichlorfop acid from various soils as reported (Smith, 1976b).

Results from the 70-day degradation experiment are displayed in Table IV. The percentage of applied radioactivity recovered from the soils using the aqueous acidic acetonitrile (as obtained by direct assay of this extract) gradually decreased with time so that by the end of the 70-day period only 20% of the initial ^{14}C was extracted from the sandy loam and heavy clay, while 50% was recoverable from the silty clay. At all samplings and in all soils the radioactivity in the hexane phases was shown to be almost exclusively [^{14}C]dichlorfop-methyl as indicated by TLC examinations using benzene as developing solvent. No evidence for any other degradation

Table IV. Radioactivity Recovered from Moist Soils Treated with 2 ppm [^{14}C]Dichlorfop-Methyl Following Incubation at 20 ± 1 °C

Time, days	Soil type	% of applied ^{14}C solvent extracted[a]	% of applied ^{14}C extracted as: Dichlorfop-methyl	Dichlorfop acid	Other products[b]	Total	% of applied ^{14}C recov. by combustion of ext. soils	Total % of applied ^{14}C accountable
	SL	100	23	82	0	105	0	105
1	HvC	95	9	90	0	99	0	99
	SiC	97	66	35	0	101	0	101
	SL	81	8	72	0	80	*c*	
7	HvC	77	5	72	0	77		
	SiC	81	20	60	0	80		
	SL	67	8	58	<1	66		
14	HvC	65	4	61	<1	65		
	SiC	76	16	62	<1	78		
	SL	58	7	49	<1	56		
21	HvC	56	4	48	<1	52		
	SiC	74	12	60	<1	72		
	SL	49	6	41	<1	47		
28	HvC	47	4	40	<1	44		
	SiC	63	10	51	<1	61		
	SL	45	5	39	<1	44	32	76
35	HvC	39	4	34	<1	38	52	90
	SiC	65	10	54	<1	64	22	86
	SL	38	4 (3)[d]	33 (28)	<1	37	35	72
49	HvC	30	3 (2)	27 (25)	<1	30	42	72
	SiC	57	8 (8)	50 (53)	<1	58	25	83
	SL	22	3 (2)	19 (15)	<1	22	40	62
70	HvC	19	2 (1)	17 (12)	<1	19	52	71
	SiC	54	8 (5)	50 (45)	<1	58	38	96

[a] Acetonitrile-water-acetic acid (80:18:2). [b] Phenol (4) and phenyl ether (3). [c] Not determined. [d] Figures in parenthesis represent percent of ester or acid extracted, as determined by gas chromatographic analysis.

products could be observed on any of the radiochromatogram scans. In addition, the gas chromatographic analyses of the hexane extracts for the herbicidal ester after the 49- and 70-day incubation periods (Table IV) were in very close agreement with the amounts of [^{14}C]dichlorfop-methyl remaining as determined by radiochemical analysis, thus confirming the absence of significant amounts of radioactive degradation products.

The aqueous phase following hexane extraction, acidification, and subsequent shaking with ether showed the absence of any ^{14}C, which demonstrated that all of the radioactivity not recovered from alkaline solution with *n*-hexane was of acidic nature. Radiochromatogram scans derived from the concentrated acetone extracts which had been subjected to TLC analysis using the benzene–methanol (10:1) developing solvent (Table II) indicated that with all soils after every sampling period the majority of the extracted radioactivity could be attributed to [^{14}C]dichlorfop acid. The specific gas chromatographic analyses of the methylated extracts derived from the 49- and 70-day incubations gave figures for the remaining dichlorfop acid in very close agreement with the data obtained by radiochemical means (Table IV). This indicated that no significant quantities of other radioactive degradation products were present in the acetone solutions. However, in all soils after the 14-day incubation and at all subsequent samplings, a very small amount of ^{14}C (less than 1% of that applied) always cochromatographed with the nonradioactive dichlorophenyl **4** added to the acetone extracts for marker purposes, indicating that 4-(2,4-dichlorophenoxy)phenol (**4**) was formed in trace amounts in all three soils.

Since very small quantities of the phenol **4** had been detected in all soils from the second week of incubation onwards, the hexane phases containing the neutral soil extracts were assiduously analyzed for the presence of the phenyl ether **3**. This phenetole **3** could be formed in soils from dichlorfop acid by a decarboxylation process and then undergo dealkylation in the soil to the already detected phenol **4**. Such a soil dealkylation of the herbicide dicamba has been similarly reported (Smith, 1973). Clearly the phenyl ether **3**, if formed, could only exist in small amounts since the present radiochemical studies have already precluded the presence of significant quantities of ^{14}C degradation products in the hexane phases.

Accordingly, following TLC analysis (benzene as developing solvent) of the hexane residues derived from the 42- and 70-day soil incubations, the silica gel areas of the TLC plates in the region ranging from R_f 0.7 to 1.0 were carefully removed, shaken with benzene, and 2-μL portions examined gas chromatographically for the presence of the phenyl ether **3**. Traces of a compound with the correct retention time were recorded, suggesting that, in all soils, less than 1% of the applied radioactivity could be attributed to ^{14}C present as the phenetole **3** at both sampling periods. Further confirmation for the identity of the phenyl ether **3** was obtained by adding nonradioactive substituted phenetole **3** to the benzene extracts when the mixture was rechromatographed using benzene and *n*-hexane (1:1) as developing solvent (cf. Table II). After development, the TLC plates were viewed under the ultraviolet lamp and the areas corresponding to the dichlorophenoxy phenetole **3** carefully removed and eluted with methanol (5 mL) which was then checked for the presence of radioactivity. In all cases the ^{14}C in the methanol was approximately 20 to 30 times those recorded by a blank prepared by eluting silica gel with methanol. The presence of the phenyl ether **3** as a soil transformation product of dichlorfop-methyl, albeit in trace amounts, was thus tentatively established.

[^{14}C]Dichlorfop acid appeared to be the major degradation product from moist nonsterile soils incubated with [^{14}C]dichlorfop-methyl (Table IV). Despite the rapid hydrolysis of the herbicidal ester during the first 24 h (Tables III and IV), thereafter the hydrolysis was much slower so that even after 70 days small amounts of the parent ester (less than 10%) could still be recovered from the treated soils. As previously noted, ester hydrolysis was

Table V. Radioactivity Liberated by Treatment of Solvent Extracted Soils with Boiling Aqueous Triethanolamine Solution

Time, days	Soil type	% of applied ^{14}C not solvent ext. from soils[a]	% of applied ^{14}C recovered from soils by base as				
			Dichlorfop acid	Phen 1 (4)	Assoc. with humic substs	Remains in aq. solution	Total
35	SL	32	5	<1	29	3	37
	HvC	52	15	<1	2	7	24
	SiC	22	4	<1	8	2	14
70	SL	40	5	<1	30	7	42
	HvC	52	13	<1	3	12	28
	SiC	38	2	<1	21	2	25

[a] Not extracted using acetonitrile-water-acetic acid (80:18:2) and determined by soil combustion techniques.

similar on the sandy loam and heavy clay and slower on the silty clay (cf. Tables III and IV). Consequently, on the heavy clay and sandy loam build up of [^{14}C]dichlorfop acid residues reached a maximum during the first week following ester treatment, whereupon the residues slowly declined. At the end of 70 days approximately 20% of the applied radioactivity in these soils could be solvent extracted as [^{14}C]dichlorfop acid. On the silty clay the ester hydrolysis was slower than on the other two soils, thus maximum build up of the acid was not reached until between 7 to 21 days following ester treatment. Subsequently, the acid residues slowly declined.

Although all the radioactivity recovered from the treated soils using the acidic aqueous acetonitrile could be attributed to either the ^{14}C ester or ^{14}C acid, a considerable portion of the applied ^{14}C could not be accounted for (cf. Table IV). Samples of the dried soils following solvent extraction were combusted to [^{14}C]carbon dioxide and the percentage of the applied radioactivity remaining thus calculated. The results (Table IV) confirmed that after 35, 49, and 70 days a considerable portion of initial radioactivity was resisting solvent extraction and remaining in intimate contact with all soil types. In these experiments, it was possible to account for at least 60% of the radioactivity initially applied to all the soils (cf. Table IV). No attempts were made to monitor [^{14}C]carbon dioxide evolution from the cartons, and since work at Hoechst (private communication) has indicated a partial breakdown of the [^{14}C]dichlorfop-methyl to [^{14}C]carbon dioxide, some of the radioactivity not accounted for may have been thus degraded.

To determine the nature of the nonsoluble radioactivity associated with the soils, samples of all three soil types after 35 and 70 days of incubation, and following solvent extraction, were subjected to hydrolysis with aqueous base. Shaking the soils with aqueous 1 N sodium hydroxide at room temperature for 1 h did not result in any significant extraction of ^{14}C, and heating the soils under reflux with aqueous triethanolamine was necessary for the recovery of substantial amounts of radioactivity. Following acidification, to precipitate the humic acid fractions, the aqueous phase was shaken with ether which extracted most of the ^{14}C into the organic solvent (Table V). TLC analysis of these ether soluble extracts indicated that the major radioactive component extracted from all soils at both times was [^{14}C]dichlorfop acid with a trace of [^{14}C]dichlorophenoxyphenol (4) also being detected (Table V). Separate experiments showed that the phenol 4 was not formed when [^{14}C]dichlorfop-methyl was heated with hot aqueous triethanolamine. It would therefore appear that both the acid 2 and phenol 4 can undergo a binding or complexing (either physical or chemical) to the various soils necessitating hot alkaline treatments for their recovery. The nature of this binding is not known but could be similar to that reported for the herbicide benzoylprop ethyl, which following breakdown in soil to the corresponding carboxylic acid became firmly bound to the soil

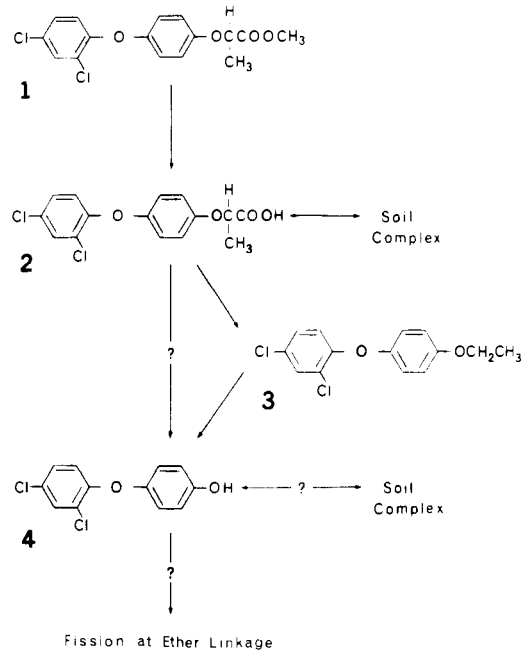

Figure 2. A possible degradation scheme for dichlorfop-methyl in moist nonsterile soils.

(Beynon et al., 1974). Smith (1976b) has noted that dichlorfop acid has the property of undergoing complete methylation in methanolic solution at room temperature within 14 days, though esterification in ethanol and *n*-butanol was not observed. Thus, in soils, it is possible that some chemical reaction could occur involving the acid carboxyl grouping so that the dichlorfop acid could become chemically linked to soil components.

Combustion experiments indicated (Table V) that considerable amounts of radioactivity were associated with the precipitated soil humic acid fractions derived from the sandy loam and silty clay and lesser amounts with those from the heavy clay. However, this disparity may lie in the fact that in the latter case the humic materials precipitated on acidification of the basic extracts amounted to just over 100 mg; whereas approximately 1 and 2 g were precipitated respectively from the extracts derived from the sandy loam and silty clay. The nature of the radioactivity associated with the humic acid fractions is unknown and could be due to complexed or adsorbed ^{14}C degradation compounds or result from [^{14}C]dichlorfop acid adsorption from the acid solution during workup. It should also be noted that the ^{14}C recovered by hot alkaline soil treatments did not appear to account for all the radioactivity present in the solvent extracted heavy clay and silty clay, as determined by direct combustion of these soils (Table V).

These studies suggest that in moist soils [^{14}C]dichlorfop-methyl undergoes extensive hydrolysis to the acid 2 which can then complex or bind to soil components (see Figure 2). The acid would also appear to be able to

undergo decarboxylation to the phenyl ether 3 which in turn is transformed to the phenol 4. The direct conversion of the acid 2 to the phenol 4 may also be possible. The phenol 4 may then undergo complexing or binding to the soils and/or be subjected to further degradative processes involving ring fission to give such products as *m*-dichlorobenzene or 2,4-dichlorophenol. The former compound is very volatile with a boiling point of 172 °C and could thus be expected to volatilize very quickly from the moist soils; for this reason no attempts were made to effect its isolation; however, efforts were made to determine the presence of the latter compound in the appropriate extracts before and after methylation to 2,4-dichloroanisole, but none were observed. From these data it would also seem that the phenol 4 and the phenetole 3 do not build up to any great extent in the soils under investigation and are possibly broken down almost as fast as they are formed.

The persistence and fate of dichlorfop-methyl under field conditions is presently under study and will be reported.

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Metabolism of 2,4-Dichlorophenoxyacetic Acid. 11. Herbicidal Properties of Amino Acid Conjugates

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Twenty amino acid conjugates of 2,4-dichlorophenoxyacetic acid (2,4-D) were tested for their herbicidal effect upon green beans, sunflowers, peas, and soybeans in field and growthroom experiments. All conjugates possessed herbicidal properties. The most active compounds were the less polar amino acid conjugates such as leucine, isoleucine, valine, alanine, and methionine. In general the aromatic and polar amino acid conjugates exhibited poor herbicidal properties.

Previous investigations have demonstrated that amino acid conjugates of 2,4-dichlorophenoxyacetic acid (2,4-D) are formed in plants and in plant tissue cultures (Andreae and Good, 1957; Klämbt, 1961; Feung et al., 1971, 1972, 1973b, 1975). Amino acid conjugates of 2,4-D have been isolated from at least six plant callus tissue cultures (carrots, jackbean, sunflower, tobacco, corn, and soybean) (Feung et al., 1973b, 1975) and seven conjugates (Asp, Glu, Ala, Val, Leu, Phe, and Trp) have been found in soybean callus tissue cultures (Feung et al., 1973b). Nearly all of the potential 20 amino acid conjugates of 2,4-D have been shown to be biologically active and stimulate plant cell division and elongation (Feung et al., 1974).

Since 2,4-D is a widely used herbicide and its amino acid conjugates have been shown to be present in the tissue and have biological activity, it is important to investigate the toxicological effects of these conjugates on both plants and animals. Thus, this paper presents the herbicidal effects of 20 amino acid conjugates of 2,4-D on four plant species in field and growthroom studies.

MATERIALS AND METHODS

The 20 L-amino acid conjugates of 2,4-D used in these studies were synthesized previously in this laboratory (Feung et al., 1973a). The purified amino acid conjugates and 2,4-D were dissolved in 0.0005 M NaOH solution containing 0.2% Tween 20 immediately before spraying. Plants were sprayed with four levels of conjugates (0.5 lb/acre, 0.25 lb/acre, 0.125 lb/acre, and 0.1 lb/acre) with a compressed air hand sprayer in the field and an aerosol pressurized sprayer in the growthroom (conjugates were stable under these conditions).

Growthroom Study. Seeds of four plant species (Long Tender Green Bean, Russian Mammoth Sunflower, Alaska Pea, Amsoy-71 Soybean) were planted in six rows in a peat–vermiculite commercial potting mixture (Pro-Mix) in a 12 × 8 in. tray and grown in the greenhouse. All seeds were presoaked for 6 h prior to planting. Seedlings of each species were thinned to six plants per row 4 to 5 days following germination. Plants were treated 2 weeks following germination. An aerosol can sprayer was used to spray the plants with three different concentrations (0.5 lb/acre, 0.25 lb/acre, and 0.1 lb/acre) of each tested chemical and the control plants were sprayed only with diluent solution. Two growthroom experiments were conducted; both contained two replicates for each treatment. The treated plants were carefully watered to avoid washing off the tested chemicals. Injury was evaluated on the 7th, 11th, and 14th day following treatment by two individuals. Plants were arbitrarily judged as follows: 0, no effect; 1, slight effect; 2, moderate effect; 3, severe effect; and 4, dead. The ratings from the two replicates were averaged.

Field Studies. Seeds of the same four plant species tested in the growthroom were planted in six rows in a 6 × 6 ft field plot. All seeds were presoaked for 10 h prior

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