sample contained 0.02 ppm. The six lemon pulp samples contained 0.01, 0.01, 0.03, 0.06, 0.07, and 0.09 ppm carbaryl with a mean ± SD value of 0.05 ± 0.03 ppm. Three lemon pulp samples analyzed with the omission of the alkaline hydrolysis step showed that the apparent carbaryl residues were not due to determinable amounts of 1-naphthol (0.01 ppm) in the final extract. No further efforts were made to confirm the identity of apparent carbaryl residues present due to the trace levels found and therefore the difficulty involved. Gunther et al. (1962) treated lemon trees at 7.5 and 15 lb of AI (1500 gal)$^{-1}$ acre^{-1} and orange trees at 12.5 and 25 lb of AI (2500 gal)$^{-1}$ acre^{-1} and reported no determinable amounts of carbaryl (0.2 ppm) or 1-naphthol (0.1 ppm) in the pulp of the fruit samples collected. Lower levels of detection were achieved here by use of additional sample cleanup.

Chlorobenzilate. Neither orange nor lemon pulp contained determinable amounts of chlorobenzilate (0.01 ppm). Gunther et al. (1955) treated lemon trees at 2.5 and 7.5 lb of AI (1000 gal)$^{-1}$ acre^{-1} and reported that 14- and 28-day samples analyzed colorimetrically contained trace amounts (<0.2 ppm) of chlorobenzilate. Subsequent samples of pulp were reported to be indistinguishable from control samples. Lower levels of detection were achieved here by use of additional sample cleanup and use of gas chromatography with electron-capture detection.

Dimethoate and Trichlorfon. None of the samples contained determinable amounts of dimethoate or trichlorfon (0.01) ppm). Gunther et al. (1965) treated orange trees with dimethoate at 11 and 22 lb of AI (2250 gal)$^{-1}$ acre^{-1} and analyzed pulp samples colorimetrically with a lower limit of detection of 0.08 ppm. Pulp residues for fruit sampled between 3- and 59-days postapplication were all no higher than levels reported for control samples (0.1–0.2 ppm). Woodham et al. (1974a) treated grapefruit trees with dimethoate at 2.5 lb/acre. The range of residue values reported was 0.01–0.19 ppm for pulp samples obtained 7- and 14-days postapplication; no determinable amount of dimethoxon was reported (0.05 ppm) in grapefruit pulp.

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Supplementary Material Available: Data used to plot Figures 1 and 2 and standard deviations for mean residue values (2 pages). Ordering information is given on any current masthead page.

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Transformation of [^{14}C]Diclofop-methyl in Small Field Plots

Allan E. Smith

The degradation of the herbicidal ester [^{14}C]diclofop-methyl, in soil, applied at a rate of 1.25 kg/ha, was studied under field conditions in small sandy loam plots. The plots, following treatment in May of 1978, were extracted at regular intervals using aqueous acidic acetonitrile and the extracts analyzed using radiochemical and gas chromatographic techniques to monitor the herbicide breakdown. In addition, the polar solvent extracted soils were oxidatively combusted to determine bound residue formation. During the growing season there was a loss of extractable, and an increase in nonextractable, radioactivity so that by October only 15% of the applied ^{14}C was solvent recoverable, while 37% was associated with the soil in a bound form. At all sampling dates the major soil transformation product was diclofop-acid, with 4-(2,4-dichlorophenoxy)phenol as a minor metabolite. Minute traces of 4-(2,4-dichlorophenoxy)phenetole and four other degradation products were also detected.

Diclofop-methyl [methyl 2-(4-(2,4-dichlorophenoxy)-phenoxy)propionate] is applied as a postemergence treatment for the control of wild oats and other annual grasses in a variety of crops.

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Laboratory studies with the ^{14}C-ring-labeled herbicide have shown (Smith, 1977; Martens, 1978) that in moist soil the ester underwent a rapid hydrolysis to diclofop, the corresponding propionic acid. Small amounts of 4-(2,4-dichlorophenoxy)phenol were also isolated from the treated soils (Smith, 1977; Martens, 1978) together with 4-(2,4-dichlorophenoxy)phenetole (Smith, 1977) and traces of other degradation products including [^{14}C]carbon di-

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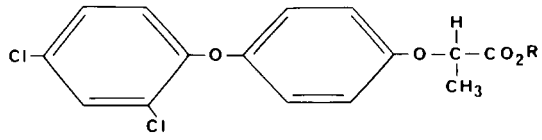

diclofop-methyl, R = CH_3
dichlofop-acid, R = H

oxide (Martens, 1978). Such studies have confirmed that diclofop-methyl is capable of being metabolized in soils with subsequent ring cleavage. However, the laboratory experiments demonstrated that with time there was an increase in the amount of radioactivity resistant to polar solvent extraction (Smith, 1977). Treatment of these soil-bound residues with hot aqueous triethanolamine solution was found to release small quantities of diclofop-acid held on the soil in a bound, or complexed, state (Smith, 1977).

Persistence experiments with diclofop-methyl carried out under field conditions have indicated (Smith, 1979) that no significant amounts of either the herbicidal ester or diclofop-acid were observed in any of the treated plots analyzed at the end of the growing season. At such a time it was also concluded that bound diclofop acid residues could only be present in negligible quantities.

In the present study the fate of [^{14}C]diclofop-methyl was studied in small sandy loam field plots after varying time intervals to determine the rate of herbicide breakdown under field conditions, to isolate and quantitate degradation products, and to measure the amounts of solvent nonextractable, or bound residues.

MATERIALS AND METHODS

Soils. The sandy loam had an average composition of 10% clay, 25% silt, and 65% sand, an organic content of 4.6%, a field capacity moisture level of 20%, and a soil pH value (in a 1:1 soil–water slurry) of 7.6.

Chemicals. Diclofop-methyl, diclofop-acid, 4-(2,4-dichlorophenoxy)phenol, and [^{14}C]diclofop-methyl (uniformly labeled in the dichlorophenyl ring moiety and with a specific activity of 10 μCi/mg), all with purities in excess of 97%, were obtained from Hoechst Aktiengesellschaft, Frankfurt, Germany, as was the sample of diclofop-methyl formulated as an emulsifiable concentrate (0.36 kg/L). Synthesis of the 4-(2,4-dichlorophenoxy)phenetole has been described already (Smith, 1977).

The [^{14}C]diclofop-methyl was diluted with the formulated herbicide, in ethanol, to give a solution containing 1.25 mg of ester/mL with a specific activity of 5.23 μCi/mL.

Field Studies. Unincorporated treatments of the ethanolic ester solution (1.0 mL) were made to small field plots (10 × 10 cm) using a procedure similar to that reported by Smith (1971). This rate, equivalent to 1.25 kg/ha, was approximately twice that recommended (0.7 kg/ha) for weed control in Saskatchewan. Seven plots were treated on May 15, 1978, which remained fallow and were hand-weeded as necessary. Treatments were sampled 1 day (one plot), 28 days (two plots), 56 days (two plots), 108 days (one plot), and 154 days (one plot) following application by removing the soil from the 0–5-cm level of each plot. The samples were dried in the laboratory at room temperature to constant weight (this ranged from 970 to 1200 g). Following air-drying, the soil samples were ground and thoroughly mixed in a laboratory mixer for 20 min.

Extraction of Unbound Residues. Duplicate soil subsamples (40 g) were extracted using 30% aqueous acetonitrile containing 2% of glacial acetic acid (100 mL), portions (25 mL) of which were partitioned into neutral and acidic extracts for examination using radiochemical procedures as described (Smith, 1977). These fractions were also analyzed gas chromatographically for diclofop-methyl and diclofop-acid content (Smith, 1977).

Table I. R_f Values of Compounds Studied

compound	solvent, R_f: benzene	benzene–methanol (10:1)
diclofop-methyl	0.40	0.95
diclofop-acid	0.00	0.17
4-(2,4-dichlorophenoxy)-phenol	0.22	0.65
4-(2,4-dichlorophenoxy)-phenetole	0.84	0.97

Determination of Degradation Products. Further soil samples (40 g) were shaken with 30% aqueous acetonitrile solution containing 2% of glacial acetic acid (100 mL) for 1 h on a wrist-action shaker and then filtered under suction. The filtrate was added to water (150 mL) and 12 N hydrochloric acid (10 mL), in a 500-mL capacity separatory funnel, and shaken with 2 × 100 mL portions of diethyl ether. The pooled ether extracts were evaporated under reduced pressure and traces of water removed by azeotropic distillation following the addition of equal volumes (25 mL) of 2-propanol and benzene. The residue was dissolved in ether (5 mL) and the evaporated solution examined by thin-layer chromatography for radioactive degradation products. Precoated TLC plates (silica gel 60 F-254) were obtained from E. Merck, Darmstadt, Germany. After development to a height of 10 cm above the origin, the plates were dried and examined for radioactive compounds by placing the plates in contact with X-ray film (Kodak RP Royal X-OMAT) for 3 weeks.

The R_f values of the compounds studied in the two solvent systems are displayed in Table I.

Determination of Bound Residues. The soil residua following solvent extraction were collected by filtration, washed with a further portion of aqueous acidic acetonitrile, then with methanol, and finally acetone. After drying to constant weight at 80 °C samples of the soils (700 mg) were oxidized in a Packard Model 306 sample oxidizer and the [^{14}C]carbon dioxide evolved measured to determine the nonextractable, or bound, radioactivity remaining on the soil. The [^{14}C]carbon dioxide evolved was absorbed into Carbosorb (6 mL) and then mixed with scintillation solution (Permafluor 5, 12 mL). Using this system the efficiency of oxidation of standards to [^{14}C]carbon dioxide was 98%.

Treatment of Bound Residues with Hot Alkali. Duplicate samples (20 g) of the soils after solvent extraction, washing, and drying were heated under reflux for 5 h with 20% aqueous triethanolamine solution and the centrifuged alkaline extracts separated into humic and fulvic acid components exactly as described (Smith, 1977, 1979). Samples (100 mg) of the precipitated humic acid fractions were combusted using a platinum holder and Schöniger flask filled with oxygen to liberate associated radioactivity as [^{14}C]carbon dioxide (Smith, 1977). The aqueous fulvic acid fractions were ether extracted and the ^{14}C in the extracts measured prior to thin-layer and autoradiographic examination.

Radioactivity. The ^{14}C in the various solutions was determined using a Picker Nuclear Liquimat Model 200 liquid scintillation spectrometer. Unless otherwise stated Scinti-Verse (15 mL) was the scintillation solution used. For the determination of counting efficiencies an external ^{137}Cs standard was used.

Table II. Recovery of Radioactivity from the Top 5 cm of Treated Field Plots with Time

sampling date (1978)	days	% of applied ^{14}C solvent extracted (unbound)	extracted ^{14}C identified as[a] nonacidic ^{14}C	diclofop-methyl[b]	acidic ^{14}C	diclofop-acid[b]	% of applied ^{14}C by combustion of extracted soils (bound)	total % ^{14}C extracted (bound and unbound)
16 May	1[c]	85	33	35	52	52	8	93
12 Jun	28[d]	44	5	5	36	33	26	70
10 Jul	56[d]	30	3	<2	25	22	36	66
31 Aug	108[c]	29	3	<2	26	18	35	64
16 Oct	154[c]	15	2	<2	15	8	37	52

[a] Expressed as percent of applied [^{14}C]diclofop-methyl. [b] Diclofop-methyl and -acid in nonacidic and acidic fractions, respectively, determined gas chromatographically. [c] Average of duplicate analyses carried out on soil from a single plot. [d] Average of single analyses carried out on soils from duplicate plots.

Gas Chromatography. A Hewlett-Packard Model 5713 A gas chromatograph was used equipped with a radioactive nickel electron-capture detector operated at 300 °C and with on-column injection. The glass column (1.5 m × 4 mm i.d.) was packed with 100–200 mesh Ultrabond 20M. Carrier gas was argon containing 5% methane, at a flow rate of 40 mL/min. With a column temperature of 220 °C the retention time for diclofop-methyl was 3.1 min. Standards and samples were injected in hexane and the ester present in sample solutions was calculated by comparing their peak heights with those of appropriate standards. Analysis of untreated soils confirmed that no interfering substances were present.

RESULTS AND DISCUSSION

The recovery of radioactivity from the top 5 cm of the [^{14}C]diclofop-methyl treated field plots with time is summarized in Table II. Sampling was not carried out at depths greater than 5 cm since previous work had shown that neither diclofop-methyl nor diclofop-acid were leached under field conditions (Smith, 1979).

Because the amounts of [^{14}C]diclofop-methyl were limited, only seven plot treatments could be made. Thus, sampling of duplicate plots was restricted to two occasions 28 and 56 days following application. However, on both dates analysis of the duplicate soils showed the variation in the amounts of bound and unbound residues to be less than 5%, indicating errors resulting from analysis of single plots to be unlikely.

The results (Table II) show that over the 154-day period there was a gradual decline in the amount of solvent extractable radioactivity which was accompanied by an increase in the quantity of solvent nonextractable, or bound, ^{14}C residues. The concentration and composition of solvent extractable radioactivity after 56 and 108 days (Table II) were identical and this was attributed to lack of breakdown of diclofop acid during the hot, dry months of July and August, when the moisture of the top 5 cm of soil was below that of the wilting point. Such conditions would not favor biological decomposition, the most likely means of diclofop degradation (Martens, 1978). By the end of the study, in October, only 15% of the applied radioactivity could be extracted using aqueous acidic acetonitrile, while 37% of the ^{14}C treatment was associated with the sandy loam in an unextractable, or bound, form.

Within experimental error, at all sampling dates the radioactivity in the acidic and nonacidic fractions was equal to the ^{14}C originally recovered from the soils using aqueous acidic acetonitrile (Table II), which confirmed that no loss of radioactivity had occurred during the work-up stages. Gas chromatographic analysis indicated the major components of the nonacidic and acidic extracts to be diclofop-methyl and diclofop-acid, respectively (Table II), thus implying there were no significant concentrations of further soil degradation products. The carry-over of 8% diclofop-acid at the end of the growing season was in good agreement with field persistence data obtained during 1976 and 1977, when less than 5% carry-over of the acid was recorded (Smith, 1979).

Chromatographic analysis of the various acetonitrile extracts for radioactive degradation products showed that the field sample collected after 1 day contained only diclofop-acid and the parent ester, while at all other sampling dates the soils contained [^{14}C]diclofop-acid as major component, as well as traces of diclofop-methyl and six other compounds, five of which were discernible in barely detectable quantities. There appeared to be no buildup of any of these breakdown products in the soil plots with time as judged by the intensity of the various bands on the corresponding autoradiograms.

The major breakdown product (R_f in benzene of 0.21) derived from diclofop-acid was identified as 4-(2,4-dichlorophenoxy)phenol which has also been reported as a primary metabolite from laboratory studies (Smith, 1977; Martens, 1978). A compound with R_f in benzene of 0.84 was eluted from the chromatograms and identified as 4-(2,4-dichlorophenoxy)phenetole using cochromatographic and gas chromatographic techniques already described (Smith, 1977). Other metabolites with R_f values in benzene of 0.55, 0.43, 0.12, and 0.08 were present in amounts too low to allow structural investigation, but may include products formed through fission of the aromatic ring systems as has been reported (Martens, 1978).

Thus, the degradation of diclofop-methyl under field conditions would appear to parallel that in the laboratory with diclofop-acid as main breakdown product and 4-(2,4-dichlorophenoxy)phenol as a very minor metabolite. The latter was never present in significant amounts since extractable radioactivity attributable to such a source (Table II) could never be more than approximately 5% of the initial herbicide treatment. Other degradation products were present as insignificant traces.

Oxidative combustion of the soil residua following the polar solvent extraction confirmed the formation, with time, of increasing amounts of solvent nonextractable, or bound, radioactivity derived from the [^{14}C]diclofop-methyl applications (Table II). Similar bound residues were also reported during laboratory studies with the ^{14}C herbicide (Smith, 1977).

Treatment of the solvent extracted soils with boiling 20% aqueous triethanolamine for 5 h, followed by acidification of the centrifuged extracts was used to separate acid insoluble humic substances from acid-soluble fulvic material. Analysis indicated that in both fractions there was an increase in radioactivity content with time (Table III). In all cases total ^{14}C measured in the fulvic acid and

Table III. Distribution of Radioactivity in the Solvent Extracted Soils as Determined by Treatment with Boiling Aqueous Triethanolamine

time, days	% of applied ^{14}C in bound form, by combustion	% of bound ^{14}C associated with[a]		
		fulvic substances	humic substances	total
1	8	<2	<2	<2
28	26	3	6	9
56	36	5	14	19
108	35	5	12	17
154	37	12	18	30

[a] Average of duplicate analyses, expressed as percent of applied [^{14}C]diclofop-methyl.

humic fractions was less than that determined by direct oxidative combustion of the soils prior to alkaline extraction (Table III). This implied that not all of the radioactivity was being extracted by the hot triethanolamine, and that a portion was remaining with the alkali insoluble humin fraction of the soil.

Chromatographic analysis of the ether extracts derived from the fulvic acid moiety confirmed diclofop-acid to be the only identifiable ^{14}C-containing products, as reported for the previous laboratory studies (Smith, 1977). The amounts of [^{14}C]diclofop-acid released from the soil at the end of the growing season seemed to be slightly greater than those observed from earlier field studies (Smith, 1979).

The identity of the bound radioactivity has not been established. It has been inferred that a portion of this ^{14}C may be attributed to diclofop-acid which, with time becomes irreversibly bound, or complexed, to soil components and from which it can only be released by treatment with hot triethanolamine (Smith, 1977, 1979). The earlier studies have also shown that the recoveries of diclofop-acid from fortified soils using hot triethanolamine is low, a fact attributable to adsorption of diclofop-acid to precipitated humic substances during workup (Smith, 1979). Thus, the radioactivity associated with the humic fraction could include contributions from the bound diclofop-acid, as well as from incorporation of small radioactive fragments (resulting from fission of the herbicide ring systems) into soil organic matter.

The main interest regarding bound pesticide residues is whether they will affect the growth of, or be taken up into, future crops (Kaufman et al., 1976; Helling and Krivonak, 1978). Experiments are therefore in progress to determine the phytotoxic significance, if any, of bound residues originating from diclofop-methyl applications.

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Metabolism and Selectivity of Fluchloralin in Soybean Roots

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Fluchloralin [*N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-(trifluoromethyl)aniline] is metabolized rapidly by soybean [*Glycine max* (L.) Merr.] roots to several chloroform-soluble and water-soluble metabolites and high levels of methanol-insoluble residue. No single metabolite represented more than 4% of the total ^{14}C in the roots. The posttreatment solution contained fluchloralin and all chloroform-soluble and possibly all water-soluble metabolites found in the root tissues. The metabolites in the posttreatment solution were formed by root tissues and not by chemical, photochemical, or microbial degradation. Four chloroform-soluble metabolites were isolated from the posttreatment solution and characterized by mass spectrometry. Soybean roots metabolized fluchloralin at a higher rate than corn (*Zea mays* L.) roots. This correlated with the greater resistance displayed by soybean roots (relative to corn roots) to fluchloralin injury.

Fluchloralin [*N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-(trifluoromethyl)aniline] is a relatively new substituted dinitroaniline herbicide and is similar structurally to trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), a major dinitroaniline herbicide. The fate of these compounds in plants is important because of the increased use of dinitroanilines to control annual grasses and broadleaf weeds in major crops.

Dinitroaniline herbicides undergo several biotransformation reactions including N-dealkylation, nitro reduction, and cyclization when exposed to ultraviolet light (Leitis and Crosby, 1974; Newsom and Woods, 1973; Nilles and Zabik, 1974; Plimmer and Klingebiel, 1974), incorporated in the soil (Golab et al., 1970, 1975; Golab and Althaus,

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