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Identification of 2,4-Dichloroanisole and 2,4-Dichlorophenol as Soil Degradation Products of Ring-Labelled [^{14}C]2,4-D

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Several metabolites of 2,4-D (2,4-dichlorophenoxyacetic acid) have been isolated and identified from solutions of the herbicide incubated with microbial cultures, and the subject has been comprehensively reviewed (Loos 1975). A major degradation pathway involves the conversion of the phenoxyalkanoate acid to 2,4-dichlorophenol, which undergoes further breakdown (Loos 1975).

Although it has been established (Foster and McKercher 1973; Fournier et al. 1981; Kunc and Rybarova 1983; McCall et al. 1981) that the aromatic ring and side chain carbon atoms of 2,4-D can be rapidly converted to carbon dioxide in moist soils, the isolation and identification of other breakdown products in treated soils have received relatively little attention. Extraction and analysis of soils fortified with ring-labelled [^{14}C]2,4-D failed to indicate any 2,4-dichlorophenol (Smith and Muir 1980). Another study has revealed (McCall et al. 1981) that whereas both 2,4,5-trichloroanisole and 2,4,5-trichlorophenol could be isolated from soils treated with ring-labelled [^{14}C]2,4,5-T, analagous metabolites were not observed in soils treated with ring-labelled [^{14}C]2,4-D. The anisole was considered to have been formed from the corresponding phenol through a microbial methylation reaction. It has also been reported (Paasivirta et al. 1983) that the herbicide MCPA undergoes degradation in the soil to 4-chloro-2-methylphenol.

Both 2,4-dichloroanisole and 2,4-dichlorophenol are volatile compounds, the boiling point of the former being 233°C at 740 mm, while that of the latter is 210°C at 760 mm (cf. Dictionary of Organic Compounds 1982). Moreover, the phenol with a pKa value of 7.89 (cf. Dictionary of Organic Compounds 1982), will exist in most soils in the more volatile phenolic form, rather than as the nonvolatile phenate anion. Thus, failure to isolate 2,4-dichlorophenol and 2,4-dichloroanisole from soils treated with 2,4-D may result from either prior volatilization, or, from volatility losses during workup of soil extracts.

The present work was undertaken for three reasons. The first was to attempt to isolate both [^{14}C]2,4-dichlorophenol and [^{14}C]2,4-dichloroanisole from soils incubated with ring-labelled

[^{14}C]2,4-D. A second objective was to determine whether [^{14}C]2,4-dichloroanisole could be formed in moist soils treated with ring-labelled [^{14}C]2,4-dichlorophenol. The third reason was to ascertain whether [2-^{14}C]2,4-D could be converted to [^{14}C]2,4-dichloroanisole in soils as a result of a decarboxylation process. In order to reduce losses of degradation products by volatility, all soil incubations were conducted in Bartha and Pramer flasks (Bartha and Pramer 1965). These flasks allow air into the system, yet offer an almost closed environment.

MATERIALS AND METHODS

The 2,4-dichloro[ring-U-^{14}C]phenoxyacetic acid, 2,4-dichlorophenoxy[2-^{14}C]acetic acid and the 2,4-dichloro[ring-U-^{14}C]phenol were all purchased from Amersham Corp., Oakville, Ontario, Canada. The radiochemical purities of all three compounds was determined by thin-layer chromatography, using the solvent systems described in Table 1. After development, the chromatograms were subjected to radiochemical analysis using a radiochromatogram scanner (see later). The radiochemical purities of the materials were 98% or greater.

The radioactive materials were diluted with nonradioactive compounds, in methanol, to give solutions containing 0.5 mg 2,4-D or 2,4-dichlorophenol per mL. The specific activities of the solutions containing the [ring-U-^{14}C]2,4-D, [2-^{14}C]2,4-D and [ring-U-^{14}C]2,4-dichlorophenol were 5.23, 5.60 and 13.24 uCi/mL respectively.

Table 1. Rf values of compounds studied.

Compound	Rf I	 II
2,4-D	0.05	0.0-0.2
2,4-Dichlorophenol	0.50	0.41
2,4-Dichloroanisole	0.87	0.78

I Benzene.
II Benzene : n-hexane : acetone (25 : 25 : 1).

The composition and physical properties of the clay, clay loam and sandy loam used in these studies have already been described (Smith and Muir 1980). Samples (50 g) of the three soils at 85% of their field capacity moisture levels were weighed into Bartha and Pramer flasks (Bartha and Pramer 1965), and incubated in the dark at 20±1°C for 7 days. The moisture levels were monitored, by weighing, every second day and distilled water added if necessary. Following the equilibration period, the soils were treated separately with portions of the [^{14}C]2,4-D and [^{14}C]2,4-dichlorophenol solutions (100 uL, 50 ug) to yield a 2,4-D or 2,4-dichlorophenol concentration, based on moist soil weight, of 1.0 ug/g. This concentration is equivalent to a field rate of approximately 0.5

kg/ha, assuming incorporation in the field to a depth of 5 cm. The soils were thoroughly stirred to effectively distribute the chemicals. Two replicate flasks were treated with each labelled compound for each soil type. To the side arm of the flasks were added 25 mL of 0.2N aqueous sodium hydroxide to absorb radioactive carbon dioxide evolved (Fournier et al. 1981); this solution was replaced with fresh hydroxide every second day. After treatement, the flasks were incubated in the dark at 20±1°C.

Samples (1.0 mL) of the aqueous sodium hydroxide solution were assayed every day for radioactivity, and the cumulative amounts released calculated as a percentage of the total radioactivity originally applied to the soils.

Soils treated with the radioactive phenol were analysed after 7 days while those treated with [^{14}C]2,4-D were extracted and analysed after 10 days. The soil from each duplicate treatment flask was placed in a 250 mL glass-stoppered flask and shaken, on a wrist-action shaker for 1 hour, with sufficient 20% aqueous acetonitrile containing 2% of glacial acetic acid so that the total volume of extractant together with the water present in the soil was equal to 100 mL. Following centrifugation at 2000 g for 4 minutes, aliquots (5 mL) of the supernatant were assayed for radioactivity extracted. A further portion (25 mL) of the supernatant was added to 5% aqueous sodium chloride solution (100 mL) containing N-hydrochloric acid (2 mL) and vigorously shaken with dichloromethane (10 mL). The organic phase was dried over anhydrous sodium chloride, and gently evaporated at room temperature to approximately 0.25 mL using a stream of dry nitrogen. The evaporated extracts were examined by thin-layer chromatographic and radiochemical techniques to detect and quantify the various [^{14}C] contaning compounds present.

For confirmatory purposes a portion (25mL) of the supernatant was added to aqueous sodium chloride solution containing hydrochloric acid (as above), and extracted with n-hexane (10 mL). Aliquots of the organic phase were then examined gas chromatographically for the presence of 2,4-dichloroanisole and 2,4-dichlorophenol.

The recovery of [^{14}C]2,4-D from the three soils using the aqueous acidic acetonitrile is about 96% (Smith and Muir 1980). Air-dried samples of all three soils, fortified at the 1.0 ug/g level with [^{14}C]2,4-dichlorophenol, were extracted as described after a 96 hour equilibration period. In all cases, over 95% of the applied radioactivity was recovered, and thin-layer chromatographic and radiochemical analysis confirmed that all the activity extracted could be attributed to [^{14}C]2,4-dichlorophenol.

The radioactivity in the various solutions was measured by adding aliquots to scintillation solution (15 mL) consisting of an equivolume mixture of toluene and 2-methoxymethanol containing 0.4% of PPO and 0.1% of POPOP. Samples were counted on a Packard TRI-CARB 300C liquid scintillation spectrometer, with counting efficiences being determined using an external [^{226}Ra]-standard.

Precoated TLC plates (Silica Gel 60F-254) were obtained from E. Merck, Darmstadt, Germany. Following development to a height of 10 cm above the origin, the plates were air dried and examined for radioactive compounds using a PANAX Thin-layer 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 Rf values of the compounds studied in two chromatographic solvent systems are compared in Table 1.

By comparing the peak areas from the radiochromatogram scans, and knowing the amount of radioactivity extracted from each soil, quantification of the various radioactive compounds present in each particular soil extract was achieved.

A Hewlett-Packard 5713A gas chromatograph was used, equipped with on-column injection facilities and a radioactive nickel electron-capture detector operated at 350°C. The glass column (1.5 m x 4 mm i.d.) was packed with 2% LAC-2R-446 modified with 0.4% phosphoric acid on Chromosorb W HP (80-100 mesh). The carrier gas was argon containing 5% methane at a flow rate of 40 mL/min. With a column temperature of 130°C, the retention time for 2,4-dichlorophenol was 1.92 min, while at 100°Cthat for 2,4-dichloroanisole was 2.85 min. To maintain good resolution of the phenolic peaks on the chromatograms, injections of 0.3% phosphoric acid in acetone (2 x 5 uL) were made at the start and end of each day.

Prior experiments confirmed that untreated soils contained no interfering substances.

RESULTS AND DISCUSSION

Rapid breakdown of ring-labelled [^{14}C]2,4-D occurred in all soils, being over 70% complete within 10 days (Table 2). During this time, between 30 and 42% of the soil-applied radioactivity was released as [^{14}C] carbon dioxide confirming ring fission of the herbicide. Small amounts of radioactive compounds were recovered from all soils whose Rf values, in both solvent systems (Table 1), were identical to those for 2,4-dichloroanisole and 2,4-dichlorophenol (Fig. 1). The presence of the phenol and anisole in soil extracts were also confirmed by gas chromatographic analysis, as described. In each soil type, total amounts of these compounds accounted for less than 10% of the applied radioactivity (Table 2). Between 66 and 72% of the total radioactivity could be attributed to radioactively labelled compounds (Table 2), and it was assumed that at least some of the remaining activity had been converted into soil organic matter as previously reported for these soils (Smith and Muir 1980).

After 7 days in the moist clay and sandy loam soils, the ring-labelled [^{14}C]2,4-dichlorophenol had undergone over 80% loss, while on the clay loam 40% of the original phenol still remained (Table 3). Between 10 and 14% of the applied radioactivity was released over the period as [^{14}C]carbon dioxide (Table 3),

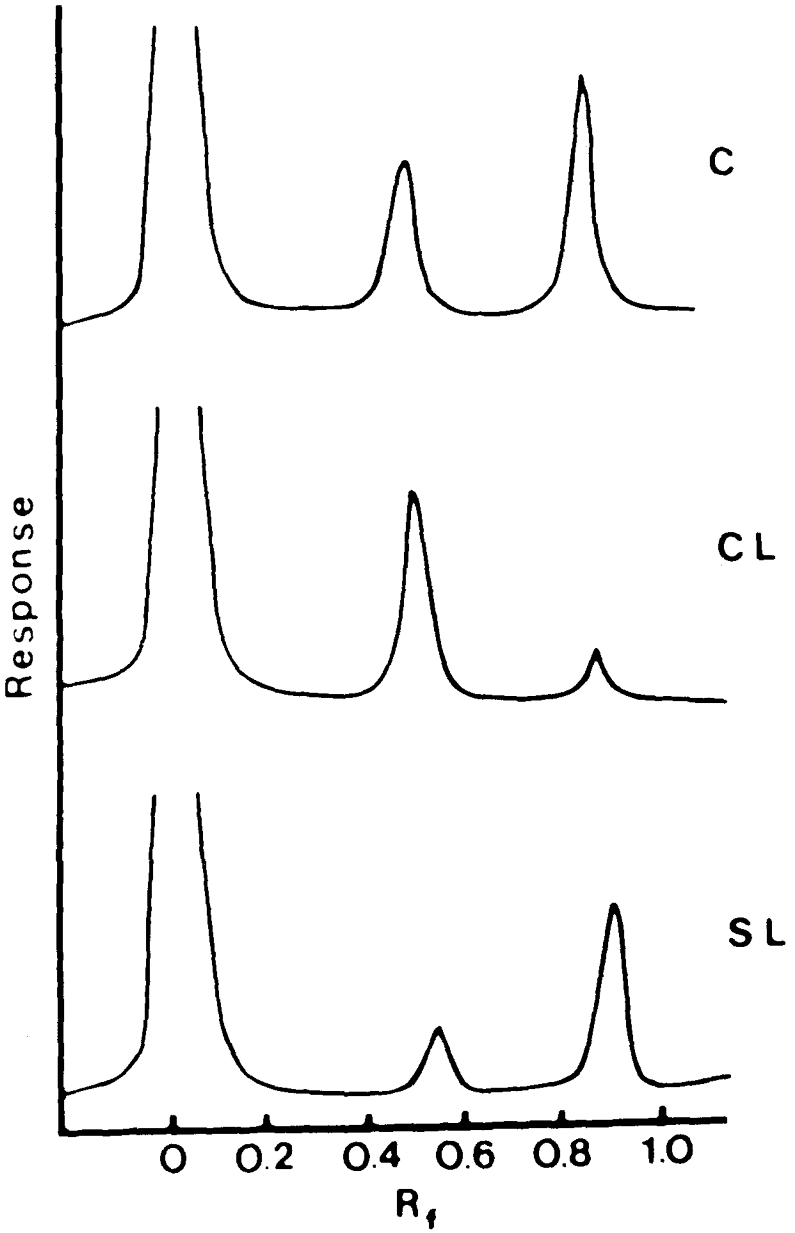

Figure 1. Radioscans of chromatograms, developed with benzene, of extracts from clay (C), clay loam (CL) and sandy loam (SL) soils treated 10 days previously with [*ring*-U-^{14}C]2,4-D.

Table 2. Radioactivity recovered from soils treated with 1 ug/g [ring-U-^{14}C]2,4-D following incubation at 20°C for 10 days.

Soil	% of applied ^{14}C extracted as*				
	2,4-D	2,4-Dichlorophenol	2,4-Dichloroanisole	CO_2	Total
Clay	30	3	5	30	68
Clay loam	23	5	2	42	72
Sandy loam	22	2	4	38	66

* Average from 2 replicates.

indicating that some fission of the phenolic ring had occurred. Thin-layer chromatographic and gas chromatographic analysis indicated that in all soils small quantities of [^{14}C]2,4-dichloroanisole were present. This tends to suggest that 2,4-dichlorophenol, like 2,4,5-trichlorophenol (McCall et al. 1981), can undergo methylation in soils to the corresponding anisole.

Table 3. Radioactivity recovered from soils treated with 1 ug/g [ring-U-^{14}C]2,4-dichlorophenol following incubation at 20°C for 7 days.

Soil	% of applied ^{14}C extracted as*			
	2,4-Dichlorophenol	2,4-Dichloroanisole	CO_2	Total
Clay	13	7	11	31
Clay loam	40	10	10	60
Sandy loam	10	6	14	30

* Average from 2 replicates.

Of the total activity applied to the soils, between 30 and 60% could be acounted for (Table 3). Possible losses of radioactivity could have occurred as a result of volatilization both of the phenol and anisole from the Bartha and Pramer flasks during the daily sampling of the alkaline solution, or, during exchange of the sodium hydroxide with fresh solution. Similar losses of these compounds could have occurred during workup of the soil extracts, especially during the evaporation stage of the dichloromethane solutions with nitrogen.

[2-^{14}C]2,4-D underwent considerable breakdown in the moist soils over a 10-day period, with between 27 and 45% of the radioactivity being released as [^{14}C]carbon dioxide (Table 4). No [^{14}C] containing material was detected in any of the soils whose Rf values were equivalent to those for 2,4-dichloroanisole. Neither was any anisole detected in soil extracts following gas chromatographic analysis.

Table 4. Radioactivity recovered from soils treated with 1 ug/g [2-^{14}C]2,4-D following incubation at 20°C for 10 days.

Soil	% of applied ^{14}C extracted as*			
	2,4-D	2,4-Dichloroanisole	CO_2	Total
Clay	35	< 0.5	33	68
Clay loam	44	< 0.5	27	71
Sandy loam	19	< 0.5	45	64

* Average from 2 replicates.

These results confirm the conclusions of others (Foster and McKercher 1973; Kunc and Rybarova 1983) that decarboxylation of the herbicide to 2,4-dichloroanisole does not occur in soils, and that fission of the 2,4-D side chain ocurs between the oxygen atom and the carbon atom at the 2-position. Of the soil-applied radioactivity, 64 to 71% (Table 4) could be attributed to parent [^{14}C]2,4-D and to [^{14}C]carbon dioxide production. At least some of the unaccounted for radioactivity will have been converted into soil organic matter (Smith and Muir 1980).

Although mass spectral data is necessary to confirm definitively the identify of the degradation products isolated in the present study, TLC analysis in conjunction with the gas chromatographic data strongly indicated that 2,4-dichlorophenol and 2,4-dichloroanisole are formed in soil from 2,4-D. Thus, the investigation tends to suggest that in soils 2,4-D can be converted to 2,4-dichlorophenol by a fission between the oxygen atom and the carbon atom at the 2-position of the side chain; and that this phenol can then undergo methylation in the three soils to 2,4-dichloroanisole. Degradation of 2,4-dichlorophenol via ring fission to carbon dioxide occurred in all soils.

It must be assumed that in the previously reported experiments (McCall et al. 1981; Smith and Muir 1980), where the degradation of [ring-U-^{14}C]2,4-D was being monitored, volatility losses of the phenol and anisole from the experimental systems occurred, so that their presence was not observed. It is only under systems where volatility losses can be reduced that the phenol and anisole can be isolated and detected.

Given the volatile nature of both 2,4-dichlorophenol and 2,4-dichloroanisole, together with the high pKa value for the former and the fact that most agricultural soils range in pH from 5.5 to 7.5, it is doubtful whether buildup of these degradation products of 2,4-D would occur in soils under field conditions.

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