Transformation of [^{14}C]-2,4-Dichlorophenol in Saskatchewan Soils

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The transformation of 0.75 μg/g ring-labeled [^{14}C]-2,4-dichlorophenol was investigated, under laboratory conditions, in soils at 85% field capacity and at 20 °C. Over a 14-day period in four soils with no recent herbicide history, 12–17% of the applied ^{14}C was released as carbon dioxide, 5–23% of the initial radioactivity was solvent recoverable as [^{14}C]-2,4-dichlorophenol, and 4–8% as [^{14}C]-2,4-dichloroanisole. Unknown volatile ^{14}C products and unidentified solvent-extractable ^{14}C compounds together accounted for 3–6% of the applied ^{14}C. Between 44 and 68% of the initial radioactivity was associated with soil organic matter.

INTRODUCTION

2,4-Dichlorophenol, in trace amounts, has been identified as a metabolite of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in laboratory soils (Ou et al., 1978; Ou, 1984; Smith, 1985), although its isolation and identification in 2,4-D-treated field soils remain to be demonstrated.

In soil, 2,4-dichlorophenol undergoes decomposition by both biological and nonbiological mechanisms (Baker and Mayfield, 1980; Bollag et al., 1980; Minard et al., 1981; Cheng et al., 1983; Smith, 1985; Soulas and Fournier, 1987; Somasundaram et al., 1989). Under laboratory conditions, ring-labeled [^{14}C]-2,4-dichlorophenol applied to the soil is degraded to [^{14}C]carbon dioxide (Cheng et al., 1983; Smith, 1985; Soulas and Fournier, 1987; Somasundaram et al., 1989). In soils, 2,4-dichlorophenol undergoes methylation to 2,4-dichloroanisole (Smith, 1985). It has also been reported (Cheng et al., 1983; Somasundaram et al. 1989) that considerable amounts of radioactivity remain associated with soil organic matter in a solvent nonextractable form.

Worldwide, 2,4-D is one of the most extensively used herbicides, and the fate of its degradation products in the soil is thus of particular interest. The present studies were undertaken to investigate the transformation of [^{14}C]-2,4-dichlorophenol in four soils by measuring amounts of [^{14}C]carbon dioxide evolved, quantifying solvent-extractable ^{14}C products, and determining the incorporation of radioactivity into soil organic matter.

MATERIALS AND METHODS

Soils. Four soils from Saskatchewan were used in the study: an Udic Boroll clay loam from Melfort; an Udic Boroll clay from Indian Head; a Typic Boroll clay from Regina; and a Typic Boroll sandy loam from White City. None of these soils had received any pesticide treatments for the previous 5 years. Soil samples were collected from the 0–5-cm soil horizon in September 1989 and stored at 4 ± 1 °C until use in the winter of 1989–1990. The physical characteristics of the soils are summarized in Table I.

Chemicals. 2,4-Dichloro[*ring*-U-^{14}C]phenol was obtained from Amersham Canada Ltd. (Oakville, ON, Canada) and was diluted with nonradioactive 2,4-dichlorophenol (Eastman Kodak Co., Rochester, NY; with a purity >98% according to the technical data sheet) to give a final concentration (in methanol) of 0.5 mg/mL and a specific activity of 691 kBq/mL. Radiochemical purity was over 97% as determined by two-dimensional thin-layer chromatography using benzene and a mixture of chloroform and acetic acid (9:1 v/v) as the two elution solvents followed by radiochemical analysis (see later). Methanolic solutions containing 2,4-dichlorophenol (2 mg/mL) and 2,4-dichloroanisole (20 mg/mL) were also prepared for comparative purposes in the TLC analyses.

Table I. Composition and Physical Characteristics of Soils

location	soil type	composition, % clay	sand	silt	organic content, %	pH	field cap, %
Indian Head	clay	51	24	25	2.5	7.3	32
Melfort	clay loam	30	30	40	11.7	6.0	35
Regina	clay	70	5	25	4.2	7.7	40
White City	sandy loam	10	65	25	4.0	7.6	20

Degradation Studies. Duplicate samples (50 g) of all soils at 85% of their field capacities were weighed into polystyrene foam cartons and placed in 2-L Mason jars equipped with spring clip lids. After a 7-day incubation in the dark at 20 ± 1 °C, soils were treated with 2,4-dichloro[*ring*-U-^{14}C]phenol (75 μL, 37.5 μg, 52 kBq) to yield a phenol concentration of 0.75 μg/g on the basis of moist soil. This is equivalent to a field concentration of approximately 375 g/ha, assuming incorporation to a depth of 5 cm (Smith, 1985), and represents levels of residues that might be expected in the topsoil following recommended 2,4-D field applications and assuming complete degradation of the herbicide to the phenol. The soils were thoroughly mixed, by use of a spatula, to distribute the chemical and the cartons replaced in the Mason jars. A glass vial containing 15 mL of 0.1 M sodium hydroxide was added to each jar to absorb any carbon dioxide evolved. Following treatment, all jars were reincubated in the dark at 20 ± 1 °C. During the incubation period, moisture loss from the soils was negligible. Samples of the sodium hydroxide solution were analyzed for radioactivity at regular intervals (see later), at which time the absorbing vials were replaced with others containing fresh alkaline solution.

Duplicate samples of all soil treatments were analyzed after 7 and 14 days. For control purposes, to assess nonbiological degradation, volatility from soil, and soil extraction recoveries, duplicate samples of all air-dry soils at ~10% field capacity were treated at the 0.75 and 0.10 μg/g levels with [^{14}C]-2,4-dichlorophenol and solvent extracted after 14 days of incubation at 20 ± 1 °C.

Extraction and Analysis. At each sampling, portions (1 mL) of the sodium hydroxide solution were analyzed by radiochemical analysis. To differentiate between [^{14}C]carbonate and possible volatile ^{14}C products, further portions (10 mL) of the trapping solution were treated with aqueous sodium carbonate (1 mL, 5 mg/mL) and aqueous barium chloride (2 mL, 0.2 g/mL) (Harvey et al., 1985). Precipitated barium carbonate was removed by centrifugation (2 × 1000*g* for 10 min). Portions (1 mL) of the clear supernatant were assayed for radioactivity. Prior experiments indicated that over 99% of the radioactivity in the caustic solution derived from [^{14}C]carbon dioxide was converted to insoluble barium [^{14}C]carbonate, whereas <1% of the radioactivity from [^{14}C]-2,4-dichlorophenol was similarly precipitated.

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Table II. Identity of Radioactivity Recovered from Soils Treated with 0.75 μg/g Ring-Labeled [^{14}C]-2,4-Dichlorophenol following Incubation at 20 °C and 85% Field Capacity for 7 and 14 Days

	% of applied radioactivity[a]							
	Indian Head (clay)		Melfort (clay loam)		Regina (clay)		White City (sandy loam)	
^{14}C components	7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
released as carbon dioxide	8	15	8	17	8	12	8	13
released as unknown volatiles	3	3	3	3	2	2	2	2
2,4-dichlorophenol	9	6[b]	39	23	16	10	9	5
2,4-dichloroanisole	14	8	7	5	10	8	8	4
unknowns	1	1	2	3	<1	2	1	1
nonextractable, via soil combustion	52	56	38	44	55	59	60	68
associated with fulvic acids	–[c]	16	–	13	–	20	–	18
associated with humic acids	–	8	–	7	–	8	–	16
associated with humins	–	32	–	24	–	31	–	34
total ^{14}C recovered	87	89	97	95	91	93	88	93

[a] Average of two replicates. [b] Recoveries of [^{14}C]-2,4-dichlorophenol from all air-dry soils incubated at 20 °C for 14 days were >98% of that applied. [c] Not determined.

At all samplings the majority of the radioactivity in the sodium hydroxide vials was due to [^{14}C]carbon dioxide. Amounts of volatile ^{14}C products were always <3% of the ^{14}C initially applied to the soils. The concentrations of volatile ^{14}C products present in the caustic soda were always too low to allow characterization by thin-layer chromatographic analysis. The amounts of [^{14}C]-carbon dioxide released at each sampling time were calculated as a percentage of the total radioactivity applied to the soils.

Soils from the cartons were placed into 250-mL glass-stoppered flasks containing sufficient extraction solution (acetonitrile/water/glacial acetic acid, 80:20:2.5 v/v/v) so that the total volume of extraction solution together with water present in the soil was 100 mL. Following a 1-h shake on a mechanical shaker, samples were centrifuged at 2 × 1000*g* for 5 min. Aliquots of the clear supernatant (4 mL) were assayed for ^{14}C extracted. A 25-mL portion of the supernatant was added to a 250-mL separatory funnel containing aqueous sodium carbonate (25 mL, 50 mg/mL) and solvent extracted with hexane (2 × 10 mL). A sample of the combined hexane extracts (1 mL) was assayed for radioactivity. The hexane solution was dried over sodium chloride (5 g) and evaporated to ~0.3 mL at room temperature by using a stream of dry nitrogen. These extracts were then analyzed by thin-layer chromatographic and radiochemical techniques (see later). The aqueous solution was acidified with concentrated hydrochloric acid (3 mL) and extracted with dichloromethane (25 mL). The organic phase was dried over sodium chloride (40 g) and then decanted into a 50-mL centrifuge tube. The salt was washed with portions (2 × 10 mL) of dichloromethane which were also added to the centrifuge tube. To confirm complete extraction of the radioactivity after acidification and dichloromethane extraction, a portion (1 mL) of the remaining aqueous phase was assayed for ^{14}C. The dichloromethane extracts were evaporated to ~0.5 mL under nitrogen at room temperature in preparation for thin-layer chromatographic analysis (see later).

After solvent extraction, the soil was collected by vacuum filtration and successively washed with the aqueous acidic acetonitrile extraction solvent (100 mL), methanol (50 mL), and acetone (50 mL). All washings were discarded since radiochemical assay showed that they contained negligible amounts of radioactivity. The soil residues were dried at 90 °C for 2 h, and triplicate samples (1 g) of each soil were then subjected to combustion analysis (see later). To determine the amounts of radioactivity incorporated into fulvic, humic, and humin soil fractions after 14 days, a further portion (20 g) of dry soil residue was shaken with 1 M aqueous sodium hydroxide (50 mL) for 24 h on a wrist-action shaker and worked-up as described (Smith and Muir, 1980). An aliquot (1 mL), of the soluble fulvic acid fraction was assayed for ^{14}C. Radioactivity in the humic precipitate was determined by combustion analysis (see later). Radioactivity in the humin fraction was calculated as the difference between total ^{14}C remaining in the solvent-extracted soils and those in the fulvic and humic acid fractions.

Thin-Layer Chromatography. For the evaporated hexane and dichloromethane extracts, TLC plates coated with 0.25 mm of silica gel 60F-254 (E. Merck, Darmstadt, FGR) were used. Plates were developed to a height of 10 cm above the origin by using benzene, as previously reported (Smith, 1985). Following development, plates were air dried in a fume hood, and ^{14}C-containing compounds were detected and quantified by using a Model 2832 Berthold automatic TLC-linear analyzer (Labserco Ltd., Oakville, ON, Canada). Nonradioactive standards of 2,4-dichlorophenol (R_f = 0.48) and 2,4-dichloroanisole (R_f = 0.85) were run for comparative purposes. R_f values for the nonradioactive standards were determined by viewing the developed plate under a short-wave ultraviolet lamp. In the hexane extracts, only [^{14}C]-2,4-dichloroanisole was noted. The dichloromethane extracts contained [^{14}C]dichlorophenol as the major ^{14}C component. However, trace amounts of a second ^{14}C product with an R_f value of 0.36, and equivalent to <3% of the soil-applied ^{14}C, were also noted.

Measurement of Radioactivity. Radioactivity present in various solutions was determined by using a Packard Tri-Carb 300 liquid scintillation spectrometer. Scintillation solution was Scinti-Verse II (15 mL, Fisher Scientific Co., Fair Lawn, NJ). Counting efficiencies were determined by using a ^{226}Ra standard. Radioactivity associated with solvent-extracted soils and the humic precipitates was measured by combustion of samples in a Harvey biological oxidizer, Model OX500 (R. J. Harvey Instrument Corp., Hillsdale, NJ). [^{14}C]Carbon dioxide evolved as a result of combustion was trapped in Harvey carbon-14 cocktail (15 mL). Recoveries of ^{14}C as [^{14}C]carbon dioxide from soils fortified with ^{14}C standards, immediately before combustion, were greater than 95%.

RESULTS AND DISCUSSION

After 14 days in all air-dry soils at ~10% field capacity and 20 °C, >98% of the applied radioactivity was solvent recoverable as [^{14}C]-2,4-dichlorophenol, confirming lack of any loss or transformation under such conditions. In contrast, soils at 85% field capacity showed a rapid loss of [^{14}C]-2,4-dichlorophenol accompanied by [^{14}C]carbon dioxide evolution, formation of the metabolite [^{14}C]-2,4-dichloroanisole, and production of solvent-nonextractable radioactivity. The results are summarized in Table II, and there was excellent agreement between the results from the two replicates for each soil at the various sampling times.

Release of [^{14}C]carbon dioxide from all soils was similar, with 8% of the applied ^{14}C being thus liberated after 7 days and 12–17% after 14 days (Table II). At all sampling times small amounts of ^{14}C (<3% of that applied) from sources other than [^{14}C]carbon dioxide were noted in the sodium hydroxide traps. This material was considered to consist of such products as [^{14}C]-2,4-dichlorophenol and perhaps [^{14}C]-2,4-dichloroanisole, both of which are very

volatile (Smith, 1985). Amounts present were too low to allow positive identification.

The majority of the solvent-extractable radioactivity in the treated soils was identified as [14C]-2,4-dichlorophenol and [14C]-2,4-dichloroanisole, with unknown 14C products accounting for <3% of the initial radioactivity. No attempts were made to characterize these unknown products. Loss of the [14C]-2,4-dichlorophenol from the clays and sandy loam was rapid (Table II) and similar, with 9–16% remaining after 7 days and 5–10% after 14 days. Breakdown was slower in the clay loam from Melfort with 39 and 23% of the [14C]-2,4-dichlorophenol remaining after 7 and 14 days. In all soils, [14C]-2,4-dichloroanisole accounted for 4–14% of the applied 14C.

Combustion of the solvent-extracted soils indicated considerable amounts of 14C in the soils in a nonrecoverable form. After 14 days, 44–68% of the applied radioactivity was in such a form (Table II). The lowest figure (44%) for the clay loam from Melfort probably reflects the slower rate of [14C]-2,4-dichlorophenol transformation in this soil. In all soils the majority of the solvent-nonextractable radioactivity appeared to be contained in the humin fraction, with moderate amounts in the fulvic acid fraction, and the least in the humic acid fraction.

At all sampling times, the total accountable 14C ranged from 87 to 97% of that applied (Table II). Some 14C losses may have occurred as a result of the escape of volatile products, such as [14C]carbon dioxide and [14C]dichloroanisole, when the Mason jars were opened for sampling and the exchange of trapping vials.

Studies with single soils (Cheng et al., 1983; Soulas and Fournier, 1987; Somasundaram et al., 1989) have indicated that the degradation of 2,4-dichlorophenol is dependent upon its concentration and also upon whether the soils have received phenol pretreatments. Evolution of [14C]-carbon dioxide from a silty clay loam treated with 0.24 and 24 mg/kg [14C]-2,4-dichlorophenol accounted for 33 and 25% of the applied 14C, respectively, after 12 weeks (Soulas and Fournier, 1987). In a silty loam, 31% of the 14C from a 10 mg/kg treatment of [14C]-2,4-dichlorophenol was released as [14C]carbon dioxide after 11 weeks, with 64% of the radioactivity being associated with the soil (Cheng et al., 1983). Under the same conditions, but with a [14C]-2,4-dichlorophenol concentration of 100 mg/kg, 59% of the 14C was evolved as [14C]carbon dioxide and 33% incorporated into the soil after 11 weeks (Cheng et al., 1983).

Incubation of 5 mg/kg [14C]-2,4-dichlorophenol in a clay loam for 21 days resulted in 28% of the applied 14C being released as [14C]carbon dioxide and 54% being associated with the soil in a nonextractable form (Somasundaram et al., 1989). After four prior treatments (5 mg/kg) with nonradioactive phenol, a similar application of [14C]-2,4-dichlorophenol released 54% of the applied 14C as [14C]-carbon dioxide over a 21-day period, with 32% of the radioactivity being incorporated into the soil (Somasundaram et al., 1987).

2,4-Dichlorophenol has been shown to stimulate soil bacteria responsible for its degradation (Baker and Mayfield, 1980). Thus, the increased rate of [14C]carbon dioxide evolution noted from high [14C]phenol application rates, or repeat treatments, probably resulted from an increase in the numbers of 2,4-dichlorophenol-degrading organisms. As noted, nonbiological mechanisms are also important processes for the soil breakdown of 2,4-dichlorophenol (Baker and Mayfield, 1980; Soulas and Fournier, 1987). The various studies with 2,4-dichlorophenol indicate that biological degradation results in carbon dioxide evolution, and presumably the formation of 2,4-dichloroanisole, whereas its nonbiological breakdown leads to incorporation of the phenol, or modified phenol, into soil components (Baker and Mayfield, 1980; Cheng et al., 1983; Smith, 1985; Somasundaram et al., 1989). The present data (Table II) are in agreement with the previous findings.

It is assumed that in soil methylation of [14C]-2,4-dichlorophenol to the anisole is a result of microbiological processes. In culture solution, *Arthrobacter* spp. have been shown to convert 2,4-D to 2,4-dichloroanisole via 2,4-dichlorophenol (Loos, 1975). Similarly, biological mechanisms have been implicated in the conversion of 2,4,5-trichlorophenol and 2,4,6-trichlorophenol to their corresponding anisoles (McCall et al., 1981; Tindale et al., 1989).

The fate of 2,4-dichloroanisole in the soil is unknown, but given its high vapor pressure it has been concluded (Smith, 1985) that under field conditions it would rapidly be lost from the soil by volatilization so that residue would not accumulate.

Metabolism of 2,4-D in culture media by *Arthrobacter* and *Pseudomonas* sp. involves the cleavage of the acetic acid side chain to yield 2,4-dichlorophenol, which is then converted to a catechol that undergoes ring fission (Loos, 1975). However, the significance of the phenol as a soil metabolite of 2,4-D is unclear since only trace amounts of this compound have been detected in 2,4-D-treated soils under controlled laboratory conditions (Ou et al., 1978; Ou, 1984; Smith, 1985).

It has been suggested (Stott et al., 1983) that since 2,4-D is taken up into the cells of degrading microorganisms, and degraded intracellularly, any 2,4-dichlorophenol formed by metabolic processes would be likely to remain within the degrading cells. The present studies (Table II) indicate that should any 2,4-dichlorophenol come into contact with the soil as a result of 2,4-D metabolism, such residues would be rapidly dissipated.

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