Metabolites of [^{14}C]-2,4-Dichlorophenoxyacetic Acid in Saskatchewan Soils

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The transformation of 2.0 μg/g ring-labeled [^{14}C]-2,4-dichlorophenoxyacetic acid was investigated, in the dark under controlled laboratory conditions, in four soils at 85% field capacity and at 20 °C. The rates of herbicide breakdown were reflected in the numbers of 2,4-D-degrading organisms isolated from the soils at the four sites. Over a 24-day period in soils with no recent herbicide history, more than 89% of the applied 2,4-D was metabolized with 25–31% of the applied ^{14}C being released as carbon dioxide, 2–10% being solvent recoverable as [^{14}C]-2,4-dichloroanisole, and 39–43% being associated with soil in a solvent-nonextractable form. In soils with prior 2,4-D treatments herbicide degradation was faster with approximately 50% of the applied radioactivity being released as carbon dioxide, 1–4% being solvent recoverable as [^{14}C]-2,4-dichlorophenol and 2–5% as [^{14}C]-2,4-dichloroanisole, and 22–30% of the initial ^{14}C associated with the soil in a solvent-nonextractable form.

INTRODUCTION

In North America, 2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most commonly used herbicides for the control of annual and perennial weeds and brush on cropland, rangeland, and rights-of-way, and in forests, turf, and lawns.

The microbiological breakdown of 2,4-D has been studied for over 40 years, and the findings have been extensively reviewed (Loos, 1975; Sandmann et al., 1988; Smith, 1989; Smith and Lafond, 1990). The transformation of 2,4-D in soils has not been studied to the same extent, although it has been reported that in moist nonsterile soils 2,4-D is transformed to 2,4-dichlorophenol (2,4-DCP) (Ou et al., 1978; Ou, 1984; Smith, 1985) which undergoes biological methylation to 2,4-dichloroanisole (2,4-DCA) (Smith, 1985; Smith and Aubin, 1991).

Studies have indicated that in soil 2,4-DCP is rapidly dissipated by both biological and nonbiological mechanisms (Baker and Mayfield, 1980; Bolag et al., 1980; Cheng et al., 1983; Soulas and Fournier, 1987; Smith and Aubin, 1991) so that such residues are unlikely to accumulate under field conditions. The fate of 2,4-DCA in soil is unknown, but given its high vapor pressure (boiling point 235 °C at atmospheric pressure) it has been assumed that it would be rapidly lost from field soils by volatilization (Smith, 1985).

Neither 2,4-DCA nor 2,4-DCP has been isolated from 2,4-D-treated field soils; however, it is of particular interest to obtain an indication of the amounts likely to be formed during herbicide degradation. The present studies were therefore conducted, using a closed system, to investigate the breakdown of ring-labeled [^{14}C]-2,4-D in four soils by measuring amounts of [^{14}C]carbon dioxide evolved, quantifying solvent-extractable [^{14}C]-2,4-D, [^{14}C]-2,4-DCA, and [^{14}C]-2,4-DCP, and determining amounts of solvent-nonextractable radioactivity associated with the soil. The numbers of 2,4-D-degrading organisms in each of the four soils were also determined.

MATERIALS AND METHODS

Soils. Four soils from Saskatchewan were used in this study: a Typic Boroll clay from Regina, a Typic Boroll sandy loam from White City, and two Udic Boroll clays from the vicinity of Indian Head. Neither of the Typic Borolls had received any herbicide treatments for at least 5 years. One of the Udic Boroll clays was from a farmer's field that had received recent applications of 2,4-D. The other Udic Boroll clay was collected from plots at the Agriculture Canada Experimental Farm, Indian Head, Saskatchewan, that had received annual spring treatments of ~1.1 kg/ha 2,4-D amine from 1947 until 1989 (Smith et al., 1989). Soil samples were collected from the 0–5-cm soil horizon in September 1990 and stored at 4 ± 1 °C until use in the fall of 1990. The physical characteristics of the soils were determined by the Saskatchewan Soil Testing Laboratory, Saskatoon, and are summarized in Table I.

Enumeration of 2,4-D-Degrading Organisms. The procedure used, based on that of the most probable number (MPN), was similar to those already described (Fournier, 1980; Cullimore, 1981; Ou, 1984). Enumeration was carried out on soils (three replicates) that had previously been moistened to 85% of field capacity and incubated in the dark at 20 ± 1 °C for 7 days. After prior incubation, soils (10 g) were shaken for 10 min with sterilized distilled water (90 mL) containing trisodium phosphate (0.1 g) and successive dilutions made (in sterile distilled water) ranging from 10^{-1} to 10^{-9}. Portions (1 mL) of these diluted soil suspensions were added to sterile mineral salt medium (9 mL) in capped glass tubes, with three replicates for each dilution. The mineral salt medium contained the following: K_2HPO_4 (1.17 g/L), KH_2PO_4 (0.54 g/L), NH_4NO_3 (0.38 g/L), $(NH_4)_2SO_4$ (0.04 g/L), $MgSO_4 \cdot 7H_2O$ (0.03 g/L), $FeSO_4 \cdot 7H_2O$ (0.007 g/L), and $ZnSO_4$ (0.004 g/L). The medium, which had a pH of 7.1, was enriched with 2,4-D (0.06 g/L) as sole carbon source.

The treated tubes were incubated in the dark at 28 ± 1 °C for 21 days after which time the 2,4-D remaining in each tube was determined spectrophotometrically (Loos et al., 1979; Cullimore, 1981). Breakdown was considered to have occurred when more than 25% of the applied 2,4-D had been metabolized in the dilution tubes, and the MPN of degrading organisms was then estimated according to standard tables (Cullimore, 1981).

Chemicals. 2,4-Dichloro[*ring*-UL-^{14}C]phenoxyacetic acid was obtained from Sigma Chemical Co. (St. Louis, MO) with a specific activity of 1.68 mBq/mg and a radiochemical purity >99% as determined by two-dimensional silica gel thin-layer chromatography (TLC), using mixtures of chloroform/acetic acid (9:1 v/v) and benzene/hexane/acetic acid (5:10:2) as the two elution solvents, followed by radiochemical analysis (see later). The [^{14}C]-2,4-D was dissolved in methanol (5 mL) to give a solution containing 0.74 mBq/mL and 0.44 mg of 2,4-D/mL. Methanolic solutions containing 2,4-D (2 mg/mL), 2,4-DCP (2 mg/mL), and 2,4-DCA (20 mg/mL) were also prepared. The nonradioactive compounds were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Degradation Studies. The experimental design was identical

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Table I. Composition and Physical Characteristics of Soils

location	soil type	composition, % clay	sand	silt	organic content, %	pH	field capacity, %
Indian Head (long-term)	clay	51	24	25	2.5	7.3	38
Indian Head (field)	clay	48	22	30	3.7	7.3	38
Regina	clay	62	2	36	3.3	7.6	40
White City	sandy loam	10	65	25	4.0	7.6	20

Table II. Identity of Radioactivity Recovered with Time from Soils Treated with 2.0 μg/g Ring-Labeled [^{14}C]-2,4-D following Incubation at 20 °C and 85% Field Capacity

	% of applied radioactivity[a]												
	Indian Head (long-term)			Indian Head (field)		Regina				White City			
^{14}C component	4 days	8 days	16 days	4 days	8 days	4 days	8 days	16 days	24 days	4 days	8 days	16 days	24 days
recovered as carbon dioxide	19 ± 3	30 ± 4	51 ± 1	38 ± 1	52 ± 2	2 ± 0	5 ± 0	14 ± 2	31 ± 3	1 ± 0	4 ± 1	17 ± 2	25 ± 1
recovered as unknown volatiles	<1	<1	<1	2 ± 0	2 ± 0	<1	<1	<1	<1	<1	<1	<1	<1
2,4-D	43 ± 8	18 ± 5	2 ± 0	15 ± 4	8 ± 1	73 ± 5	62 ± 1	39 ± 4	11 ± 3	76 ± 0	56 ± 9	19 ± 6	7 ± 3
2,4-DCP	4 ± 1	3 ± 1	1 ± 0	2 ± 1	<1	<1	<1	<1	<1	<1	<1	<1	1 ± 0
2,4-DCA	5 ± 1	5 ± 0	2 ± 0	2 ± 1	2 ± 0	6 ± 1	8 ± 1	10 ± 1	5 ± 1	2 ± 1	5 ± 2	8 ± 2	7 ± 1
unknowns	2 ± 2	5 ± 1	3 ± 1	2 ± 1	<2	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	2 ± 0	1 ± 0	3 ± 1
nonextractable, via soil combustion	23 ± 1	29 ± 2	30 ± 1	23 ± 2	22 ± 1	24 ± 5	18 ± 0	28 ± 2	39 ± 2	18 ± 1	27 ± 6	45 ± 7	43 ± 5
total ^{14}C recovered	96 ± 2	90 ± 1	89 ± 2	84 ± 1	86 ± 2	106 ± 6	94 ± 1	92 ± 1	87 ± 1	98 ± 1	94 ± 3	90 ± 3	86 ± 2

[a] Mean and standard deviation from three replicates.

to that recently described for similar studies with [^{14}C]-2,4-DCP (Smith and Aubin, 1991). Thus, triplicate samples (50 g) of all soils at 85% of their field capacities in polystyrene foam cartons were placed in 2-L Mason jars and incubated for 7 days in the dark at 20 ± 1 °C when the soils were treated with [^{14}C]-2,4-D solution (50 μL, 22 μg, 37 kBq) and nonradioactive 2,4-D solution (40 μL, 80 μg) and thoroughly stirred. The added 2,4-D (2.0 μg/g of soil) is approximately equivalent to a field concentration of 1 kg/ha, assuming incorporation to a field depth of 5 cm (Smith, 1985). Following treatment, glass vials containing 15 mL of 0.1 M sodium hydroxide were added to the Mason jars, to absorb [^{14}C]carbon dioxide evolved, which were then reincubated at 20 ± 1 °C. [^{14}C]Carbon dioxide evolution was regularly monitored, and triplicate samples of all soil treatments were analyzed after 4, 8, 16, or 24 days.

Extraction and Analysis. The extraction and analysis were almost identical to that described for the degradation studies with [^{14}C]-2,4-DCP (Smith and Aubin, 1991). The sodium hydroxide solution in the vials was analyzed for [^{14}C]carbon dioxide and other ^{14}C products. The soils were extracted using a solvent mixture (acetonitrile/water/glacial acetic acid, 80:20:2.5 v/v/v) and centrifuged extracts added to aqueous sodium carbonate from which [^{14}C]-2,4-DCA was removed by shaking with hexane. The hexane extracts were analyzed by TLC and radiochemical techniques, and the identity of 2,4-DCA in the extracts was further confirmed by GC/MS techniques (see later). The aqueous solution, following acidification with hydrochloric acid, was extracted with diethyl ether (2 × 25 mL) (instead of dichloromethane that was used in the [^{14}C]-2,4-DCP study), and the extracts were dried and evaporated under nitrogen and analyzed for [^{14}C]-2,4-D, [^{14}C]-2,4-DCP, and other ^{14}C products using TLC and radiochemical techniques. After solvent extraction, the soils were collected by vacuum filtration, washed well, and, after drying, combusted to determine solvent-nonextractable radioactivity.

Thin-Layer Chromatography. For the evaporated hexane and diethyl ether extracts, silica gel TLC plates (60F-254, 0.25 mm, E. Merck, Darmstadt, Germany) were developed to a height of 10 cm above the origin using benzene, as reported (Smith, 1985). After development, the plates were dried in a fume hood and ^{14}C compounds quantified using a Model 2832 Berthold automatic TLC linear analyzer (Labserco Ltd., Oakville, ON, Canada). Nonradioactive standards of 2,4-D (R_f = 0.05), 2,4-DCP (R_f = 0.45), and 2,4-DCA (R_f = 0.82) were run for comparative purposes. The nonradioactive compounds were observed by viewing the developed chromatographic plates under short-wave ultraviolet radiation. In the hexane extracts, only [^{14}C]-2,4-DCA was noted. The ether extracts contained [^{14}C]-2,4-D and [^{14}C]-2,4-DCP as the major components, with unknown ^{14}C products being equivalent to less than 5% (0.1 μg) of the applied [^{14}C]-2,4-D. No attempts were made for further characterize these unknowns.

Measurement of Radioactivity. Radioactivity in the various solutions was measured using a Packard Tri-Carb 300 liquid scintillation spectrometer. Scinti-Verse II (15 mL, Fisher Scientific Co., Fair Lawn, NJ) was the scintillation solution, and counting efficiencies were determined using a ^{226}Ra standard. Radioactivity associated with the solvent-extracted soils was measured by combustion of samples (1.00 g) in a Harvey biological oxidizer, Model OX500 (R. J. Harvey Instrument Corp., Hillsdale, NJ).

Confirmation of 2,4-DCA as a Transformation Product. Evaporated hexane extracts and standard 2,4-DCA solutions (2 μL) were injected into a Hewlett-Packard 5890A gas chromatograph equipped with a 5970 mass ion detector and scanned from 10 to 200 amu. The column was of fused silica (25 m × 0.25 mm) coated with 0.33 μm of HP-1 Ultra (Hewlett-Packard Ltd.). Carrier gas was helium with a gas linear velocity of 25.3 cm/s. Initially the column temperature was held at 70 °C for 1 min after injection and then increased at a rate of 5 °C/min to a final temperature of 250 °C. The mass spectrum of the product in the hexane extracts with the same retention time (13.6 min) as authentic 2,4-DCA was identical to that of the latter with major ions at m/e 176, 161, and 133.

RESULTS AND DISCUSSION

Aqueous acidic acetonitrile was selected as extraction solvent since it has been shown to be an efficient extractant of 2,4-D, 2,4-DCP, and 2,4-DCA from soils (Smith and Muir, 1980; Smith and Aubin, 1991). In all soils there was a rapid loss of [^{14}C]-2,4-D with time which was accompanied by evolution of [^{14}C]carbon dioxide, the formation of small quantities of [^{14}C]-2,4-DCP and [^{14}C]-2,4-DCA, and the production of solvent-nonextractable radioactivity (Table II).

In all four soils herbicide loss followed first-order kinetics ($R^2 > 0.92$) with half-life values (time for 50% of the applied 2,4-D to be degraded) of 1 and 3 days, respectively, in the soils from the two Indian Head sites and of 7 and 9 days, respectively, in the soils from White City and Regina. Thus, losses of [^{14}C]-2,4-D in the soils collected from the Regina and White City locations that had no recent pesticide

treatments were significantly slower ($P < 0.05$) than those in soils from the other two sites that had received prior 2,4-D applications. The rates of breakdown were reflected in the number of 2,4-D-degrading organisms isolated from the soils at the four sites. In the three replicate soil samples from the Regina location the number of degrading organisms ranged from 59 to 662 with a mean of 193 organisms/g of oven-dry soil, while for the White City soil the numbers ranged from 52 to 322, with a mean of 127 2,4-D degrading organisms/g of oven-dry soil. Soil from the Indian Head long-term treatments, which had received no 2,4-D for 18 months prior to the enumeration, contained herbicide-degrading organisms ranging from 367 to 1396 (mean of 710) organisms/g of oven-dry soil. In the Indian Head field soil, which exhibited the fastest degradation rate (Table II), the greatest numbers of 2,4-D-degrading organisms were observed, ranging from 36 750 to 661 500 (mean of 254 800) organisms/g of oven-dry soil.

Greater overall release of [^{14}C]carbon dioxide (equivalent to ~50% of the applied radioactivity) was recorded from the Indian Head soils than from the Regina clay (31% of the initial ^{14}C) and the White City sandy loam (25%). At all samplings, >96% of the radioactivity in the sodium hydroxide traps was attributable to [^{14}C]carbon dioxide.

Only trace amounts of [^{14}C]-2,4-DCP (<4% of the applied radioactivity) were recoverable from the treated soils, whereas amounts of radioactivity attributable to [^{14}C]-2,4-DCA ranged from 2 to 10% of that applied. Other solvent-extractable radioactivity due to unknown ^{14}C products accounted for <5% of the initial ^{14}C.

Combustion of the solvent-extracted soils indicated that when over 89% of the 2,4-D had been degraded, 22–43% of the applied radioactivity was associated with the soil. The data for the Regina clay (39%) and White City sandy loam (43%) are very similar to those (36 and 35%, respectively) observed during an earlier study with ring-labeled [^{14}C]-2,4-D after 21 days in soils collected from the same field locations (Smith and Muir, 1980).

It would appear that in both field soils from Indian Head not only is breakdown faster but almost twice as much [^{14}C]carbon dioxide was released during the experiment than from the Regina and White City soils, while significantly smaller amounts of radioactivity were associated with the soil in a nonextractable form. Such differences are probably a result of the greater numbers of 2,4-D-degrading organisms to be found in the former soils.

At all sampling times, the total accountable ^{14}C ranged from 84 to 106% with a mean and standard deviation from the 39 separate sets of analyses of 92 ± 6%. There was a tendency for the total accountable ^{14}C to decrease with time; this was particularly apparent in the case of the Indian Head field soil, where [^{14}C]-2,4-D breakdown was most pronounced. Given the volatile nature of 2,4-DCA, it is possible that, during the experiments, vapors of this metabolite, or other volatile ^{14}C products, gradually diffused from the soils into the air space and so escaped from the closed system when the Mason jars were opened for sampling and the exchange of the sodium hydroxide trapping vials. The likelihood of unidentified volatile compounds accounting for the missing 4–21% of the applied radioactivity suggests the need for additional experiments with provision for trapping and analysis of 2,4-DCA and other volatile metabolites. Also, field studies are required to determine the extent of 2,4-DCA formation following 2,4-D field applications.

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