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Identification of 4-Chloro-2-methylphenol as a Soil Degradation Product of Ring-labelled [^{14}C]Mecoprop

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Although it has been established (Foster and McKercher 1973; Fournier et al. 1981; Kunc and Rybarova 1983; McCall et al. 1981) that the aromatic and side chain carbon atoms of 2,4-D can be rapidly converted to carbon dioxide in moist nonsterile soils, the isolation and identification of other breakdown products derived from soils treated with phenoxyalkanoic acid herbicides have received relatively little attention.

In microbiological culture solutions a major degradation pathway involves the conversion of substituted phenoxyalkanoate acids to the correspondingly substituted phenols, which then undergo further breakdown (Loos 1975). Evidence suggests that a similar pathway exists for the breakdown of such compounds in soils. Thus, it has been reported (McCall et al. 1981) that under laboratory conditions ring-labelled [^{14}C]2,4,5-T can undergo degradation in soils to 2,4,5-trichlorophenol which is then converted by a microbiological methylation reaction to 2,4,5-trichloroanisole. Analogous metabolites were not observed in soils treated with ring-labelled [^{14}C]2,4-D (McCall et al. 1981). However, it has been noted (Smith 1984) that both 2,4-dichlorophenol and 2,4-dichloroanisole are very volatile compounds so that failure to isolate these compounds from soils treated with 2,4-D may result from their being lost by volatility either during the soil incubation, or during workup of soil extracts. When the degradation of ring-labelled [^{14}C]2,4-D was investigated in Bartha and Pramer flasks, which allow air into the incubation system while offering an almost closed environment, both 2,4-dichlorophenol and 2,4-dichloroanisole were identified as degradation products (Smith 1984). Under nonlaboratory conditions, traces of 4-chloro-2-methylphenol have been recovered (Paasivirta et al. 1983) from soil in locations where MCPA was used for brushwood control.

In the work to be described, the degradation of ring-labelled [^{14}C]mecoprop was investigated in three soil types to determine whether 4-chloro-2-methylphenol could be isolated as a degradation product. Because the boiling point of this phenol has been reported (cf. Dictionary of Organic Compounds 1982) to be 222-5°C at 760 mm, the experiments were conducted in Bartha and Pramer flasks (Barth and Pramer 1965) to reduce volatility losses of degradation products during the soil incubation period.

MATERIALS AND METHODS

DL-2-(4-chloro-2-methyl[ring-U-^{14}C]phenoxy)propanoic acid was purchased from Amersham Corp., Oakville, Ontario, Canada. The radiochemical purity was determined by thin-layer chromatography using the solvent systems shown in Table 1; and after development the chromatograms were subject to radiochemical analysis using a radiochromatogram scanner. The radiochemical purity of the ring-labelled [^{14}C]mecoprop was over 99%.

The radioactive acid was diluted with nonradioactive mecoprop and a methanolic solution prepared having a specific activity of 23.2 μCi/mL and a herbicide concentration of 1 mg/mL.

Table 1. Rf of compounds studied.

	Rf	
Solvent	Mecoprop	4-Chloro-2-methylphenol
Benzene	0.05	0.40
Benzene : acetone (10 : 1)	0.07	0.72
Chloroform	0.09	0.50
Dichloromethane	0.05	0.64

The composition and physical characteristics of the clay, clay loam and sandy loam used in this study have already been described (Smith and Muir 1980). The experimental design was exactly as stated (Smith 1984) for similar studies involving ring-labelled [^{14}C]2,4-D. Thus, duplicate samples (50 g) of the three soil types at 85% of their field capacity moisture levels were incubated in the dark for 7 days at 20°C in Bartha and Pramer flasks before being treated with the [^{14}C]mecoprop solution (50 μL, 50 μg herbicide). After treatment, the flasks were incubated in the dark at 20°C for 20 days before extraction and analysis. As before (Smith 1984), 0.2N sodium hydroxide solution (25 mL) was added to the side arm of the flasks to absorb [^{14}C]carbon dioxide evolved; the solution being replaced with fresh every second day.

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

The recovery of mecoprop from the three soils under test using aqueous acidic acetonitrile is over 95% (Smith and Hayden 1981). Thus, for extraction, 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 equivalent 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 $[^{14}C]$compounds present.

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 Packard TRI-CARB 300C scintillation spectrometer, with counting efficiencies 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 compounds studied in four chromatographic solvent systems are compared in Table 1. By comparing 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 soil was achieved.

RESULTS AND DISCUSSION

Breakdown of the ring-labelled $[^{14}C]$mecoprop was rapid in all soils, being over 70% complete in 20 days (Table 2). During this time, between 20 and 29% of the soil-applied radioactivity was released as [^{14}C]carbon dioxide, indicating that fission of the aromatic nucleus was occurring. In addition to [^{14}C]mecoprop, small amounts of a single radioactive compound were recovered from all soils, whose Rf values in all four solvent systems (Table 1) were identical to those for 4-chloro-2-methylphenol. There was no trace of any compound with a higher Rf value that could be attributed to 4-chloro-2-methylanisole. Between 48 and 52% of the total activity could be attributed to radioactively labelled compounds (Table 2).

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

	% of applied radioactivity extracted as*			
Soil	Mecoprop	4-Chloro-2-methylphenol	CO_2	Total
Clay	30	2	20	52
Clay loam	21	3	26	50
Sandy loam	16	3	29	48

*Average from 2 replicates.

It was assumed that at least some of the remaining activity had been converted into soil organic matter, since this is known to occur with 2,4-D (Smith and Muir 1980; Stott et al. 1983; Wilson and Cheng 1978), with carbon dioxide or small carbon-containing fragments formed by breakdown of the herbicide being incorporated into the soil biomass.

Losses of [^{14}C]carbon dioxide and the [^{14}C]phenol could have occurred as a result of volatilization 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 the phenol could also have occurred during workup of the soil extracts, especially during the evaporation stage of the dichloromethane solutions with nitrogen.

Although mass spectral data are necessary to definitively confirm the identity of the degradation product isolated in the present study, the TLC results strongly indicate that 4-chloro-2-methylphenol is formed in soils from mecoprop. Thus, the investigation suggests that in soil mecoprop can be converted to the corresponding phenol by a fission between the oxygen atom and the carbon atom at the 2-position of the side chain. In this regard mecoprop behaves in a similar manner in the soil to MCPA, 2,4-D and

2,4,5-T (Paasivirta et al. 1983; McCall et al. 1981; Smith 1984). In the present study there was no indication that the 4-chloro-2-methylphenol underwent methylation to the corresponding anisole, as seems to be the case with the chlorinated phenols derived from 2,4-D and 2,4,5-T (McCall et al. 1981; Smith 1984).

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