Fate of Potassium 3,4-Dichloro-5-isothiazolecarboxylate in Soil

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In the laboratory, ^{14}C-labeled potassium 3,4-dichloro-5-isothiazolecarboxylate ([^{14}C]PDIC) was relatively stable over an extended period in three different soils (construction sand, Lufkin fine sandy loam, and Houston clay). For example, at 6 months posttreatment, respective recoveries of the parent compound were 81, 92, and 83% of the applied dose. A single decomposition product was detected and tentatively identified as 3,4-dichloroisothiazole (the decarboxylated derivative of PDIC). Also in the laboratory, comparative tests indicated that PDIC and acephate (*O,S*-dimethyl acetylphosphoramidothioate) leached readily in the same three soils and in Michigan muck, while diflubenzuron (*N*-[[(4-chlorophenyl)-amino]carbonyl]-2,6-difluorobenzamide) was essentially immobile. Postharvest residues in the soil of a plot used for treatment of cotton with [^{14}C]PDIC (single application of 1.12 kg/ha) declined to <0.1 ppm after 1 year, and residues of radiocarbon in rotational crops were insignificant.

The experimental plant growth regulator potassium 3,4-dichloro-5-isothiazolecarboxylate (herein referred to as PDIC) is being developed by the Pennwalt Corp. for use on cotton plants either as a preconditioner for defoliation or for selective termination of the fruiting cycle. In a previous study (Shaver et al., 1979), it was reported that foliar applications of PDIC were readily absorbed and freely translocated throughout the cotton plant. Relatively large concentrations of the chemical accumulated in different plant structures, especially the fruit, and changes attributable to its photodecomposition on foliar surfaces or to its metabolism within the plant were minimal (<5% of the applied dose).

The use of PDIC would probably result in some soil contamination from runoff during application or from the postharvest shredding and cultivation of treated plants. Therefore, it is important to know the fate of the chemical in that medium. This report describes studies of the fate of PDIC in different soils and its potential for uptake by rotational crops.

MATERIALS AND METHODS

Chemicals. Samples of nonradioactive and radioactive PDIC (19.32 mCi/mmol, dual labeled with ^{14}C at the 3 and carboxyl carbon positions of the molecule) were provided by the Pennwalt Corp., Tacoma, WA. Also provided were nonradioactive samples of two theoretical metabolites, 4-chloro-3-oxo-5-isothiazolidinecarboxylic acid (I) and 3,4-dichloroisothiazole (II). The radiochemical purity of [^{14}C]PDIC was >99%, as determined by thin-layer chromatography (TLC).

Analytical Methods. Samples were radioassayed by standard liquid scintillation counting (LSC) procedures. Radioactive compounds in different preparations were tentatively identified primarily by their cochromatography with authentic standards after two-dimensional development on precoated TLC plates (silica gel 60 F-254; Brinkmann Instruments, Houston, TX) with two solvent mixtures: (A) benzene, methanol, and diethylamine (10:4:2 v/v) and (B) benzene, methanol, and acetic acid (79:14:7 v/v). Nonradioactive compounds were located by exposure of plates to shortwave UV light, and radioactive compounds were located by autoradiography with X-ray film.

Residues of unextractable radiocarbon in soil were determined by combustion of samples (0.5–1 g) at 1000 °C in an oxygen atmosphere in a furnace (Bull et al., 1970); those in plants were analyzed with a standard combustion procedure (Bull and Ivie, 1976).

Fate of PDIC in Soil in the Laboratory. Ten grams of each of three different soils [construction sand, Lufkin fine sandy loam, and Houston clay; see Bull et al. (1970) for soil properties] was treated with 25 μg of [^{14}C]PDIC in a suufficient volume of water to completely wet the soil. Samples were contained in open 20-mL glass scintillation vials and held in the laboratory at 27 °C and ca. 90% RH; three replicates were prepared for each soil and for each collection time. At selected times posttreatment, samples of each soil were extracted 3 times with 50-mL portions of a 1% solution of hydrochloric acid in methanol. Soil–solvent mixtures were agitated vigorously for 30 min on a wrist-action shaker. The solvent was removed by centrifugation and decantation, and the three extracts of each sample were pooled for analysis. The combined extracts were radioassayed, and then the methanol was removed under vacuum. The remaining aqueous solution was diluted to 10 mL with water and partitioned twice with 50-mL portions of chloroform, and then both solvent fractions were analyzed by TLC. Extracted soil was dried at room temperature and analyzed to determine unextractable radioactivity. Also, after 1 month in the aerobic conditions, sufficient samples of treated loam were purged thoroughly with nitrogen, sealed, and held submerged in water for evaluation of possible anaerobic effects during the subsequent 2 months.

The leaching of [^{14}C]PDIC in construction sand, Lufkin fine sandy loam, Houston clay, and Michigan muck was evaluated with the soil TLC techniques described by Helling (1971). For this, 20 × 20 cm glass plates were coated with soil (500–750-μm layer). After the soil air-dried, [^{14}C]PDIC (2 μg) was applied to each soil plate. Samples of [^{14}C]acephate (*O,S*-dimethyl acetylphosphoramidothioate) and diflubenzuron (*N*-[[(4-chlorophenyl)-amino]carbonyl]-2,6-difluorobenzamide) were also included in each analysis as standards of reference. (Respective water solubilities of the chemicals were as follows: PDIC, 485 mg/mL; acephate, 65 mg/mL; diflubenzuron, 0.0002 mg/mL.) Treated plates were developed in water until the solvent fronts migrated 10 cm from the point of sample application. They were then dried and exposed to X-ray films for autoradiography of leaching patterns. Tests were replicated 3 times.

Fate of PDIC in Soil in the Field. The persistence of radiocarbon residues in a field treated with [^{14}C]PDIC was evaluated by the collection and analyses of core samples of soil from a plot (91 × 183 cm) previously used for

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Table I. Fate of [^{14}C]PDIC in Three Types of Soil in the Laboratory[a]

	μg equivalents of [^{14}C]PDIC recovered from:					
	sand		loam		clay	
time post-treatment[b]	extract	residue	extract	residue	extract	residue
0	25.2	0.1	23.9	0.2	22.9	0.7
1 d	24.9	0.1	24.1	0.2	22.0	0.7
3 d	25.0	0.1	25.0	0.3	23.1	0.6
1 wk	25.3	0.1	24.6	0.1	23.0	0.7
2 wk	24.8	0.1	24.6	0.3	22.6	1.0
3 wk	25.6	0.1	25.2	0.2	22.6	1.2
1 mon	25.1	0.1	24.4	0.2	22.2	0.7
2 mon	25.7	0.1	24.1	0.3	21.1	1.0
3 mon	25.0	0.3	23.4	0.6	20.8	1.3
4 mon	25.1	0.2	22.6	0.5	19.3	1.3
6 mon	23.2	0.7	22.7	0.7	19.7	1.5
		Anaerobic				
1 mon			23.9	0.3		
2 mon			23.4	0.6		

[a] Each sample consisted of 10 g of soil treated with ca. 25 μg of [^{14}C]PDIC. Standard errors for analyses of unextractable residues in soil were all ± < 0.1 μg. Standard error ranges for analyses of soil extracts were sand and loam ± 0.1–0.6 μg and clay ± 0.1–0.3 μg.

a study of the fate of the chemical in cotton plants (Shaver et al., 1979). This plot had been treated on August 9, 1977, with a single application of [^{14}C]PDIC at a rate of 1.12 kg of active ingredient per ha in 93.5 L of water. The seed cotton and plants were collected from the plot on September 21, 1977, divided into subsamples, and dried, and then all materials except the lint were ground to a fine powder in a Wiley mill. On October 27, 1977, ca. 90% of the powdered radioactive plant material was incorporated into the soil of the same plot to a depth of ca. 7.5 cm. Beginning on October 25, 1977, and continuing at ca. monthly intervals for ca. 1 year, we collected cores of soil (22.5 cm deep) from four random points within the plot. Each core was divided into three equal portions according to depth. Samples were air-dried and then analyzed by combustion to determine the concentration and vertical distribution of radiocarbon in the soil.

In another test, 20-g samples of Lufkin fine sandy loam were treated either directly by thorough mixture of the soil with sufficient [^{14}C]PDIC to produce an initial radiocarbon level of 2.5 ppm or indirectly by mixing the soil with enough of the powdered radioactive plant material to produce an initial radiocarbon level of 1.7 ppm. Each sample was placed in a 200-mesh stainless steel screen packet (ca. 8 × 8 cm) and buried in the field in the same soil type at a depth of ca. 10 cm. (Moisture readily penetrates these packets, but there is essentially no physical loss of soil.) At selected times posttreatment, triplicate samples were collected, extracted, and analyzed as described. Samples of untreated soil adjacent to the test area were collected for determination of moisture content.

Residues in Rotational Crops. Tests were conducted to determine if different plants grown in the plot previously used for treatments of cotton would accumulate radiocarbon from the soil. Native grasses were sampled, as were onions and cabbage planted in the spring of 1978. Plant materials were collected at selected times, divided into subsamples, dried for 24 h at 50 °C, and then analyzed for radiocarbon content by combustion in oxygen.

RESULTS AND DISCUSSION

Fate of PDIC in Soil in the Laboratory. Little of the [^{14}C]PDIC (or its metabolites) was bound to any of the soils that were treated and held in the laboratory. The maximum concentration of unextractable radiocarbon (ca. 6% of the applied ^{14}C) was found in clay at 6 months posttreatment; bound residues in other soils never exceeded 0.01 ppm at any sample date (Table I). Except for a slight decrease in recoveries in clay samples at later sampling dates, recovery of the applied radioactivity was near-quantitative through 6 months posttreatment. Thin-layer chromatography analyses of soil extracts through 4 months revealed the presence of only the parent compound. However, at 6 months posttreatment, analyses revealed a product that cochromatographed with II, the decarboxylated derivative of PDIC. The proportions of PDIC and the degradation product were as follows: sand, 88:12; loam, 97:3, clay, 97:3. Results of analyses of samples of treated loam held in anaerobic conditions were virtually identical with those of samples held in aerobic conditions.

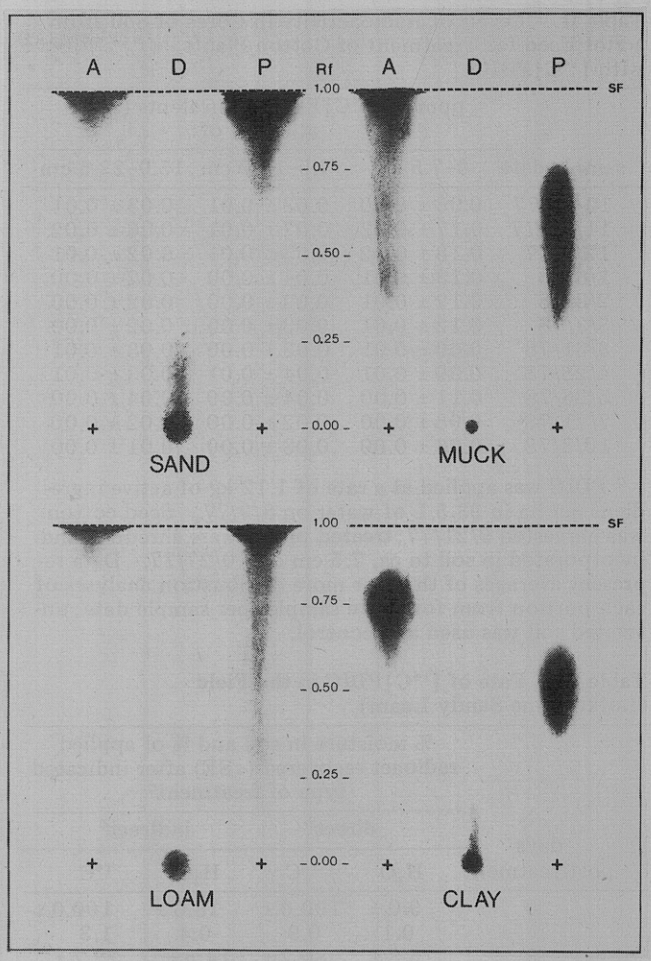

Figure 1. Soil TLC study of the leaching of [^{14}C]PDIC (P), acephate (A), and diflubenzuron (D) in four different soils with water. Solvent front (SF) was allowed to migrate 10 cm from the point of sample application. The figure is a copy made from representative autoradiograms of TLC plates.

Leaching studies with the soil TLC procedure indicated that PDIC was mobile in all the soils tested (Figure 1). In sand and loam, the leading edges of radioactivity associated with PDIC and acephate moved with the solvent front, though there was some tailing in both soils. Frontal R_f values for PDIC and acephate in muck were ca. 0.77 and 1.00 and in clay ca. 0.65 and 0.87, respectively; tailing of the two compounds was more evident in muck than in clay. Diflubenzuron was essentially immobile in all soils, though there was some streaking from the point of application in sand and clay. As anticipated, the most water-soluble compound (acephate) had the greatest apparent mobility in all four soils and the least water-soluble compound

Table II. Levels of Radioactivity in Cores of Soil from a Plot Used for Treatment of Cotton Plants with [^{14}C]PDIC[a]

	ppm of [^{14}C]PDIC equivalents (±SE) at depth of:		
sample date	0-7.5 cm	7.5-15.0 cm	15.0-22.5 cm
10/25/77	0.06 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
11/10/77	0.17 ± 0.02	0.07 ± 0.01	0.06 ± 0.02
12/9/77	0.18 ± 0.02	0.05 ± 0.01	0.02 ± 0.01
1/6/78	0.13 ± 0.01	0.04 ± 0.00	0.02 ± 0.00
2/3/78	0.12 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
3/3/78	0.12 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
3/31/78	0.09 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
4/28/78	0.09 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
5/26/78	0.11 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
7/21/78	0.08 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
10/3/78	0.06 ± 0.00	0.03 ± 0.00	0.01 ± 0.00

[a] PDIC was applied at a rate of 1.12 kg of active ingredient per ha in 93.5 L of water on 8/9/77. Seed cotton was harvested 9/21/77; treated plants were shredded and incorporated in soil to ca. 7.5 cm on 10/27/77. Data represent averages of three or more combustion analyses of each portion from four core samples per sample date; untreated soil was used as a control.

Table III. Fate of [^{14}C]PDIC in the Field (Lufkin Fine Sandy Loam)

	% moisture in soil and % of applied radioact recovered (±SE) after indicated type of treatment[a]			
	direct[b]		indirect[c]	
days posttreatment	H_2O	^{14}C	H_2O	^{14}C
0	9.0 ± 0.1	100.0 ± 0.9	18.6 ± 0.4	100.0 ± 1.3
3	6.3 ± 0.3	80.2 ± 1.2	14.5 ± 0.8	72.7 ± 3.0
7	18.7 ± 0.2	27.2 ± 0.1	15.6 ± 0.3	34.4 ± 0.9
14	19.0 ± 0.1	5.1 ± 0.5	11.5 ± 0.1	31.7 ± 0.7
21	18.9 ± 0.1	4.2 ± 0.8	10.3 ± 0.1	27.9 ± 1.3
28			15.6 ± 0.4	26.4 ± 0.5

[a] Each value is an average of three replicates; radiocarbon data represent combined extractable and unextractable material. [b] Test initiated 1/6/78; unextractable radioactivity at respective times was 0.02, 0.02, 0.01, <0.01, and <0.01 ppm. [c] Test initiated 2/13/78; unextractable radioactivity at respective times was 0.29, 0.29, 0.25, 0.27, 0.25, and 0.28 ppm.

(diflubenzuron) had the least.

Fate of PDIC in Soil in the Field. The results of combustion analyses of core samples of soil taken from the plot used for treatments of cotton plants with [^{14}C]PDIC are shown in Table II. The maximum level of radiocarbon did not exceed 0.2 ppm in any sample. Residues were highest during the first few weeks after (10/27/77) the radioactive plant material was mixed with the soil. Thereafter, they decreased progressively until <0.1 ppm remained at ca. 1 year posttreatment. At all sample dates, the amounts of radioactivity were greatest at the 0–7.5-cm soil depth.

In the test with soil treated directly with [^{14}C]PDIC, the radiocarbon leached rapidly from soil packets; only ca. 5% of the applied dose remained at 14 days posttreatment, and the soil bound very little radioactive material (Table III). Since PDIC is highly soluble in water and leaches readily in loam, the rapid loss can probably be attributed to leaching by the large amounts of rain that fell after the third day posttreatment.

Tests of soil treated indirectly with [^{14}C]PDIC via contaminated plant tissue were also conducted under conditions of high moisture in field soil. Again the applied radioactivity was rapidly lost from the packets (Table III). At 14 days posttreatment, 31.7% of the applied radioactivity remained in the soil packets; however, ca. 16% of this was unextractable. Because results of the laboratory studies indicated that bound residues were minimal in soil treated directly with PDIC and because indirectly treated samples at "0 h" also had high levels of bound radiocarbon residues, the relatively large concentrations of unextractable radiocarbon material in all samples from indirect treatments of soil were probably associated primarily with the radioactive plant material.

Rotational Crops. Results of combustion analyses of native grasses, onions, and cabbage grown as rotational crops in the plot previously treated with [^{14}C]PDIC are shown in Table IV. Concentrations of radioactive material were highest (ca. 4.6 ppm) in grass that began growing in plots when ^{14}C-labeled residues were highest in the soil (Table II). However, levels of radioactive material in all samples diminished with time.

CONCLUSIONS

Although PDIC has been found to be relatively stable in the biological and soil media studied thus far, the compound is not unusually persistent in the field and residues in conventional rotational crops appear to be insignificant. However, residues of PDIC or its products apparently

Table IV. Levels of Radioactivity in Rotational Crops Grown in Plot Used for Treatment of Cotton with [^{14}C]PDIC

	ppm of [^{14}C]PDIC equivalents (±SE) at harvest dates:[a]				
part	11/28/77	2/9/78	4/28/78	5/30/78	7/21/78
			Native Grass		
top		0.37 ± 0.07	0.25 ± 0.00		0.00 ± 0.00
root		0.39 ± 0.06	0.22 ± 0.01		0.11 ± 0.01
whole	4.64 ± 0.21				
			Onion		
top			0.79 ± 0.04	0.34 ± 0.04	
bulb			0.26 ± 0.04	0.19 ± 0.03	
root			0.43 ± 0.03	0.18 ± 0.06	
			Cabbage		
leaves			0.41 ± 0.02	0.05 ± 0.01	0.01 ± 0.00
stem			0.17 ± 0.00	0.03 ± 0.01	0.00 ± 0.00
root			0.05 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
head					0.00 ± 0.00

[a] Data are based on the dry weight of plants; values shown are averages of four or more analyses of each plant part from three or more plants per sample time; plants grown in untreated soil were used as controls.

reach significant levels in treated cotton plants and in plants (i.e., native grass) grown immediately after post-harvest cultivation in treated area. If such residues are subsequently judged to be potentially harmful, then some limitation on posttreatment use of treated fields may be advisable.

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Capillary Gas Chromatograms of Leaf Volatiles. A Possible Aid to Breeders for Pest and Disease Resistance

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The chemical pest and disease defenses in plants must have been selected among spontaneously emitted substances and substances released or produced after the plant is wounded. Leaf volatiles are the first defense wall which meets an attacker of higher plants. Wound-emitted leaf volatiles from seven tomato cultivars and two wild tomato species were isolated and concentrated by adsorption on Tenax GC. A capillary gas chromatograph, adapted to give reproducible retention time values, has been used to separate the emitted volatiles. Two different data programs were used to graphically present a comparison of the chromatograms. As expected from the breeding history of the tomato, the recorded chemical characters were found to be very homogenous in varieties of *Lycopersicon esculentum*, but *Lycopersicon peruvianum* and *Lycopersicon hirsutum* showed very different component patterns. Resistance breeders work blindly without knowing the biochemical basis of resistance. Methods are needed to screen the chemical diversity in plants as guidance for the broadening of the genetic base in new crop varieties by the incorporation of pest resistance factors. Such chemical emission patterns could be used in breeding programs without identification of the different components.

It is safe to predict that resistant varieties will play an increasingly important part in the control of crop pests and diseases. The gene pool contains sources of resistance to all major groups of plant attackers, even birds and parasitic weeds (Russel, 1978). Most resistant varieties are produced with empirical methods without knowledge of the nature of the resistance or the genetic background. It seems clear that the causes of resistance are dominated by biochemical characters. Without understanding of the basis of resistance, the breeders work blindly. This is a serious problem from several points of view. They can only indirectly select for chemical characters of resistance; i.e., they have to wait for the results of spontaneous and induced attacks. It is especially difficult to breed for a quantitative polygenetic resistance without knowing the causes of the resistance. For the same reason, a valuable and durable horizontal resistance can be masked and lost during the selection for a monogenetically inherited total resistance. Demands have been raised that plant breeders should specify the chemical changes in new varieties to avoid negative health consequences for man and animals from toxic or antinutritional resistance chemicals. Extreme suggestions to stop resistance breeding do not solve this problem as all breeding probably involves chemical changes in the plants. The conclusion is instead that plant breeders need methods to follow the chemical changes which are produced. Such methods can in the first step be designed as pattern comparisons without knowing the chemical identity of the various components. The goal is to screen the patterns of spontaneously emitted substances and substances released or produced after the plant is wounded. The chemical parasite defenses in the plants must have been selected among these substances. There is a need for short words for such recordings. In analogy with terms such as "antennogram" and "encephalogram", we have suggested the somewhat popular words "leakogram" and "woundogram" (Andersson et al., 1979). No technique is available to cover the whole chemical emission pattern of a plant. As a beginning, we have focused our interest on leaf volatiles which are the first defense wall which meets an attacker of higher plants. Capillary gas chromatography is a natural choice of tool to screen plant volatiles.

The pretreatment of the plant samples is very important, in order not to exclude interesting chemicals or not to introduce artefacts. A practical procedure has been designed to isolate and concentrate plant volatiles by adsorption (Andersson et al., 1979; Andersson et al., in press). This paper reports the results of an investigation of wound-emitted leaf volatiles from seven tomato cultivars

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