to ascertain whether the degradation of RH-0994 in soil results from metabolism by microorganisms or from abiotic mechanisms. Related studies in our laboratory have shown that RH-0994 does undergo decomposition, both by hydrolytic and by oxidative pathways, when held in sterile water in the dark (Ivie et al., 1981). It therefore seems likely that the decomposition of RH-0994 in soils is mediated by both biological and purely chemical mechanisms.

Although RH-0994 does not leach, it is degraded in soil to products that apparently do leach readily when there is adequate moisture. These residues apparently remain near the surface of contaminated soil and show no tendency to move downward. Thus, the use of RH-0994 should have low potential for contaminating subterranean water. Also, the apparent instability of RH-0994 in soil and water should preclude any serious problems with its contamination of ground water. Although rotation crops accumulate very low levels of residues, it seems unlikely that these include appreciable proportions of RH-0994 or its intact ester oxidative derivatives.

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LITERATURE CITED

Bartha, R.; Pramer, D. *Soil Sci.* **1965**, *100*, 68.
Bull, D. L.; Ivie, G. W. *J. Agric. Food Chem.* **1976**, *24*, 143.
Bull. D. L.; Ivie, G. W. *J. Agric. Food Chem.* **1981**, *29*, 121.
Bull, D. L.; Shaver, T. N. *J. Agric. Food Chem.* **1980**, *28*, 982.
Bull, D. L.; Stokes, R. A.; Coppedge, J. R.; Ridgway, R. L. *J. Econ. Entomol.* **1970**, *63*, 1283.
Helling, C. S. *Soil Sci. Soc. Am. Proc.* **1971**, *35*, 732.
Ivie, G. W.; Bull, D. L.; Ridlen, R. L. *J. Agric. Food Chem.* **1981**, *29*, 1146.
Mansager, E. R.; Still, G. G.; Frear, D. L. *Pestic. Biochem. Physiol.* **1979**, *12*, 172.

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Metabolism of *O*-Ethyl *O*-(4-Nitrophenyl) [^{14}C]Phenylphosphonothioate in Cotton and Soil

Robert L. Chrzanowski* and Robert E. Leitch

When phenyl-^{14}C-labeled EPN [*O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate] was foliarly applied to cotton plants, the half-life of the intact compound was 1 week. Initial metabolism was primarily through hydrolysis and oxidation to *O*-ethylphenylphosphonic acid. Further metabolism resulted in bound residues containing the [^{14}C]phenyl group and [^{14}C]phenylphosphonic acid. In soil, EPN degraded primarily to phenylphosphonic acid with lesser quantities of *O*-ethylphenylphosphonic acid, *O*-ethylphenylphosphonothioic acid, and CO_2. The half-life of EPN in soil was between 2 weeks and 1 month.

EPN [*O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate] insecticide has been used for over 25 years, and some information is available on metabolism in plants (Menn, 1971) and on soil residues. Residue analysis of soils (Wiersma and Sand, 1972) taken from cities near heavily farmed areas showed no EPN (<0.1 ppm), and no trace of EPN (<0.01–0.03 ppm) was found (Crockett, 1974) in crops and soils from sites in Mississippi and Arkansas using EPN. Since no detailed information was available on the breakdown of EPN, this study was undertaken to determine the metabolic fate of [^{14}C]EPN in cotton plants and soil under laboratory and field conditions. In a previous paper (Chrzanowski and Jelinek, 1981), the synthesis and metabolism of [^{14}C]EPN in rats and hens was described.

[^{14}C]EPN labeled in the phenyl ring was chosen for this study rather than in the 4-nitrophenyl ring since the fate of 4-nitrophenol (a likely EPN metabolite) in soils and plants is already known. Although nitrophenols may be reduced to aminophenols by fungi (Madhosingh, 1961), 4-nitrophenol is more commonly metabolized by soil microorganisms to inorganic nitrite (Germanier and Wuhrmann, 1963; Sethunathan, 1973) and 4-nitrocatechol (Raymond and Alexander, 1971). In some cases, enriched soil microorganism cultures partially decomposed 4-nitrophenol to CO_2 (Sudhakar-Barik and Sethunathan, 1978).

In *intact living* plants, 4-nitrophenol has been reported to accumulate unchanged (Dequidt et al., 1976; Schütte and Stock, 1978), while in plant cell cultures, 4-nitrophenol conjugates with glucose (Schütte and Stock, 1978).

EXPERIMENTAL SECTION

Equipment and Methods. Liquid scintillation counting (LSC), combustion analysis (CA) of solid samples, thin-layer chromatography (TLC), gas chromatography (GC), and gas chromatography–mass spectrometry (GC–MS) of metabolites was performed as described in the rat and hen metabolism study (Chrzanowski and Jelinek, 1981).

Chemicals. Authentic samples of phenylphosphonic acid (PPA), *O*-ethylphenylphosphonothioic acid (EPPTA), *O*-ethylphenylphosphonic acid (EPPA), *O,O*-dimethyl phenylphosphonate, *O*-ethyl *O*-methylphenylphosphonate, *O*-ethyl *O*-(4-nitrophenyl) phenylphosphonate ("oxon" EPN), and *O*-ethyl *O*-(4-aminophenyl) phenylphosphonothioate ("amino" EPN) for comparison with the EPN metabolites were prepared as described in the pre-

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vious paper (Chrzanowski and Jelinek, 1981).

Treatment of Plants. Cotton seedlings (cv. Coker 201) were grown in pots under greenhouse conditions until 8 weeks old. Each of 10 plants was then sprayed once to near runoff with 15 mL of a freshly prepared solution of 9 mg (45.8 μCi) of *O*-ethyl *O*-(4-nitrophenyl) [^{14}C]phenylphosphonothioate, [^{14}C]EPN, in acetone–water (1:1 v/v). The application was made uniformly with an atomizer held about 6 in. from the foliage and was equivalent to a use rate of 8 oz/100 gal. The plants were maintained in a greenhouse at 16–19 °C with a relative humidity range of 50–70%. At predetermined intervals (0, 7, 14, 21, and 28 days), two cotton plants were harvested by cutting at soil level. Plants were stored frozen at −20 °C until analyzed.

After being weighed, each plant was gently rinsed in 500 mL of distilled water for 1 min to dislodge ^{14}C-labeled residues from the surface of the leaves and stems. The aqueous wash was concentrated at 45 °C on a rotary vacuum evaporator to approximately 50 mL before EPN and its toluene-soluble metabolites were extracted with four successive 50-mL portions of toluene, leaving polar metabolites in the aqueous phase. Aliquots of all phases were counted by LSC, and the remainder was set aside for further fractionation and identification of metabolites.

After the aqueous wash, each cotton plant was successively macerated 4 times with 200-mL portions of a mixture of acetone–methanol–toluene (1:1:1 v/v/v) in a blender for 5-min periods. The extracts were combined, and the organic solvents were stripped from the mixture on a rotary evaporator at 45 °C to obtain approximately 50 mL of a primarily aqueous mixture. The toluene-soluble metabolites were extracted from this suspension with four 125-mL portions of toluene and counted by LSC, and the combined extracts were concentrated to 10 mL on a rotary evaporator. The radioactivity in the remaining aqueous phase was also counted by LSC.

The residual plant tissue was air-dried, and aliquots were analyzed for radioactivity by CA.

Plant Metabolite Identification. Several 14-day cotton plants were randomly selected for metabolite identification. After extraction by the above procedure, the ^{14}C-labeled residues in the toluene-soluble fractions were characterized according to procedures outlined in the animal metabolism study (Chrzanowski and Jelinek, 1981).

Polar ^{14}C-containing compounds at the origin of TLC plates were removed by scraping. After extraction with water, the extract was combined with the other aqueous phases. The polar metabolites in these combined fractions were isolated and identified by the following procedures.

TLC analysis of an aliquot with authentic standards on silica gel with the polar solvent system acetonitrile–H_2O–concentrated ammonium hydroxide (40:18:2 v/v/v) provided preliminary indication of the ^{14}C-labeled residues present. Additional experiments were performed to characterize the metabolites.

So that sufficient material for mass spectral confirmation of structure could be obtained, the remainder of the polar fraction was chromatographed on a 4.5 × 25 cm AG-1X (formate) anion-exchange column (Bio-Rad Laboratories). The column was eluted with 300 mL of distilled water to give a neutral fraction (free of radioactivity), followed by 500 mL of 2 N HCl to recover the radioactive acids. This acid fraction was evaporated to a residue on a rotary evaporator at 35 °C, and an aliquot was chromatographed along with standards on silica gel TLC plates developed in acetonitrile–water–concentrated ammonium hydroxide (40:18:2 v/v/v). The remainder of the sample was successively derivatized with excess diazomethane, purified by preparative TLC, and analyzed by GC–MS (Chrzanowski and Jelinek, 1981).

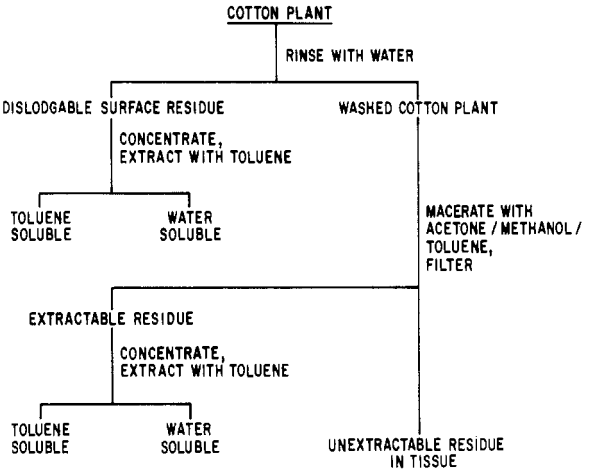

Figure 1. Extraction procedure for cotton plants.

Table I. Characteristics of Soils Used in Greenhouse, Field, and Laboratory Studies[a]

component	Fallsington sandy loam (Glasgow, DE)	Dundee silt loam (Scott, MS)	Keyport silt loam (Newark, DE)
sand, %	56	15	21
silt, %	29	67	62
clay, %	15	18	17
organic matter, %	1.4	1.3	2.75
nitrogen, %	0.085	0.049	0.97
pH	5.6	5.5	6.4
cation-exchange capacity, mequiv/100 g	4.8	14.3	8.2

[a] Soil analyses were performed by the College of Agricultural Sciences, University of Delaware, Newark, DE.

A 10-g aliquot of the 14-day unextractable tissue fraction from the workup procedure (Figure 1) was heated under reflux for 15 h in 200 mL of 6 N HCl. The mixture was taken to dryness under vacuum, redissolved in 10 mL of water, and again evaporated to dryness to remove all traces of HCl. The residue was dissolved in 100 mL of water, and any material that failed to dissolve was separated by centrifugation. The nonhydrolyzable insoluble material was washed once with 50 mL of water, filtered, air-dried, and combusted for ^{14}C activity. Radioactive organic acids were separated by ion-exchange chromatography and prepared for analysis by GC–MS as previously described.

Aerobic Greenhouse Soil Study. A soil metabolism study with [^{14}C]EPN in the greenhouse was conducted on a light soil (Fallsington sandy loam, Glasgow, DE). The characteristics of this soil are given in Table I. The normal moisture capacity of the soil was determined to be 16% by weight by using the procedure described by Puri (1949).

Twelve 1000-mL glass beakers (ca. 12-in.^{2} surface area) were filled to a depth of 4 in. and firmed as for planting with the above sandy loam soil (ca. 1000 g dry weight). Onto each soil surface was distributed evenly by pipet 1.0 mL of a methanolic solution of 2.14 mg of [^{14}C]EPN (10.9 μCi) which was equivalent to 2.6 lb of active/A. The soil in each beaker was then wetted with 50 mL of water (ca. $^1/_4$ in.). One beaker was set aside immediately for analysis. The others were placed in the greenhouse, and additional water was added to each beaker to adjust the moisture content to 75% of normal holding capacity. Water was added as necessary to maintain this moisture level. Individual entire samples were taken 0, 1, 2, 3, 4, 8, 12, 24,

Table II. Distribution of ^{14}C-Labeled Residues in Cotton Plants

			% of total residue					
			dislodgeable surface residue		extractable residue			
days after spraying	total ^{14}C, calcd as ppm of EPN	av wt of plants, g	toluene soluble	water soluble	toluene soluble	water soluble	unextractable residue	EPN
0	38	75	ND[a]	ND	96	1	3	96
7	38	89	ND	ND	55	29	16	37
14	47	58	4	29	35	17	15	23
21	29	105	4	35	26	21	14	14
28	20	146	3	18	28	27	24	12

[a] ND = not determined. These plants were not prewashed.

36, 40, and 48 weeks after treatment and analyzed to determine the total ^{14}C remaining in the soil and specifically for [^{14}C]EPN and degradation products.

Each sample was ultrasonically extracted for 10 min 5 times with 600 mL of acetone–methanol–toluene (1:1:1 v/v/v) and then 3 times with 600 mL of methanol. The extracts were combined, and a 1.0-mL aliquot was taken to count for total ^{14}C by LSC after dilution with 15 mL of Aquafluor (New England Nuclear). Soil, after extraction, was air-dried and analyzed for residual activity by CA.

Nonpolar ^{14}C-labeled metabolites in the combined organic solvent extracts were analyzed as described previously. Recovery studies with soil (100 g) fortified with [^{14}C]EPN at the 2-ppm level and extracted within 1 h after treatment showed that 95–98% of the intact material was recovered by the above extraction procedure.

After a 36-week soil sample had been extracted with acetone–methanol–toluene as described above, a 60-g aliquot of the soil was Soxhlet extracted with 100 mL of pure methanol for 24 h. This solvent extracted only a fraction (ca. 13%) of the residual radioactivity. Soxhlet extraction of a separate aliquot with water removed slightly more (17%) of the radioactivity.

Consequently, a more rigorous extraction involving mild hydrolysis with dilute ammonium carbonate was employed. A sample (60 g) of the above soil was extracted once with 100 mL of 0.5 M $(NH_4)_2CO_3$ for 20 min by ultrasonic agitation and then heated on a steam bath for 1 h. The resulting mixture was clarified by centrifugation, the supernatant was decanted, and the soil was reextracted by the above procedure with distilled water. The aqueous extracts were combined, reduced to ca. 0.5 mL, counted for total ^{14}C by LSC, and analyzed by TLC developed in acetonitrile–water–ammonium hydroxide (80:18:2 v/v/v) with a nonradioactive standard of phenylphosphonic acid. The residual soil, after air-drying, was analyzed for total unextractable ^{14}C activity by CA. This method removed 50% of the bound radioactive residue.

Field Soil Studies. Studies to determine the fate of [^{14}C]EPN in soil under actual field conditions were conducted on test sites at Newark, DE (Keyport silt loam), and Scott, MS (Dundee silt loam). The soil characteristics are given in Table I.

The procedure at each location was identical. Eight stainless steel cylinders (4 in. diameter × 15 in. long) were driven into undisturbed soil, leaving approximately 1 in. of rim protruding above ground level to minimize runoff and splashing. The soil inside each cylinder was treated with [^{14}C]EPN (1.6 mg in 5 mL of acetone) at a rate equivalent to 1.8 lb of active/A. Water (50 mL) was added to each cylinder to distribute and settle the compound into the soil. All cylinders were fully exposed to normal weather conditions throughout the indicated test periods. Cylinders from each location were dug up at regularly scheduled intervals for laboratory analyses.

Soil was removed from each cylinder and divided into the following increments, as measured from the soil surface: 0–1, 1–3, 3–5, 5–8, 8–12, and 12–15 in. All aliquots were air-dried in a hood for 12 h and ground to a fine powder with a general-purpose mill. Duplicate 0.5-g aliquots of each increment were analyzed for total ^{14}C by CA.

Increments containing 10% or more of the applied ^{14}C were analyzed specifically for [^{14}C]EPN and degradation products by the extraction and TLC procedures described for the greenhouse soil study. Recovery studies with soils fortified with [^{14}C]EPN (2 ppm) showed that 98% of the added [^{14}C]EPN was recovered from these soils by this procedure.

Soil Biometer Study. The effect of soil microorganisms on the degradation of [^{14}C]EPN was determined by using the procedure and apparatus described by Bartha and Pramer (1965).

Duplicate samples of Keyport silt loam were used with each of the following treatments: control; sterile soil plus 2 ppm of [^{14}C]EPN; nonsterile soil plus 2 ppm of [^{14}C]-EPN; nonsterile soil plus 10 ppm of [^{14}C]EPN. Sterile samples were obtained by successively autoclaving the soils 3 times in the biometer flask for 15 min at a steam pressure of 15 psi.

Soil, equivalent to 50 g air-dry weight, was weighed into the 250-mL Erlenmeyer flask side of a biometer flask. [^{14}C]EPN in 1 mL of methanol was added to produce the above treatment levels (2 and 10 ppm), and then water was added to adjust the moisture content to 70% of the normal holding capacity. The normal moisture capacity of Keyport silt loam was determined to be 26% by weight by the method of Puri (1949). The biometer flasks, with 10 mL of 0.1 N sodium hydroxide in the 50-mL side tube, were closed and stored in the dark at 25 °C for the duration of the test. The NaOH in the side tube was changed periodically and analyzed for $^{14}CO_2$ by counting a 1-mL aliquot in Aquafluor scintillation solution. With several samples, a second 1-mL aliquot was treated with 5% barium chloride solution to precipitate $^{14}CO_3^{2-}$. The resulting suspensions were centrifuged for 10 min, and the ^{14}C activity remaining in solution was counted directly by LSC. Each time a sodium hydroxide solution was removed, the flask was flushed with oxygen to maintain aerobicity. After intervals of 1, 2, 4, 8, 12, 16, 24, and 36 weeks, a flask for each fortification level was removed from the incubator. The soil was extracted as described earlier except that 100 mL of solvent was used for each successive extraction. The extracts were analyzed by the TLC cochromatographic methods described earlier.

RESULTS AND DISCUSSION

Cotton Plants. When a group of cotton plants which had been treated with [^{14}C]EPN were analyzed according to the scheme shown in Figure 1, the distribution of ra-

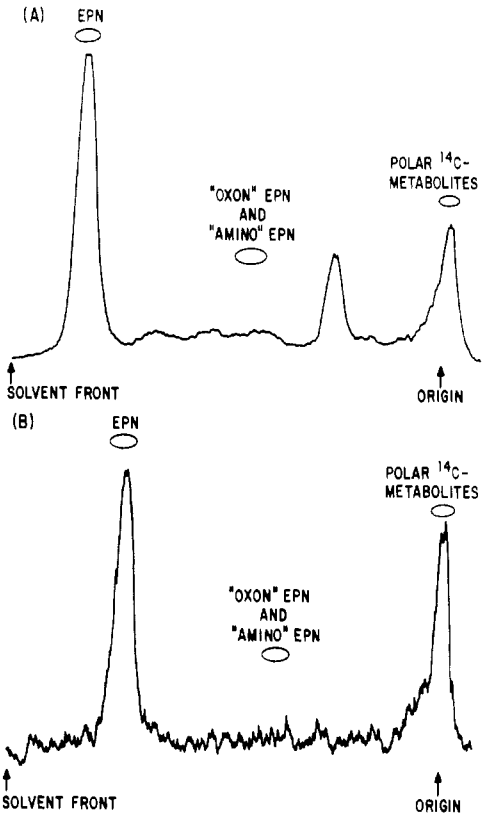

Figure 2. TLC of toluene-soluble ^{14}C-labeled residues from cotton plant 14 days after spraying. Solvent system: hexane–ethyl acetate (50:50 v/v). (A) Surface residues; (B) tissue residues.

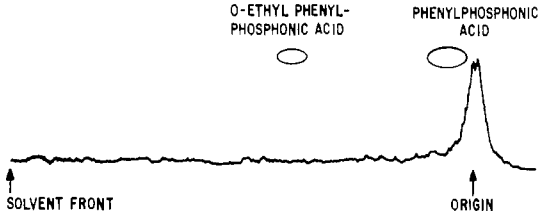

Figure 3. TLC of water-soluble residues from cotton plant 14 days after spraying. Solvent system: CH_3CN–H_2O–NH_4OH (80:18:2 v/v/v).

dioactivity shown in Table II was found.

TLC analysis of the toluene-soluble portion of both the aqueous wash and the plant extract (Figure 2) revealed EPN was the most significant ^{14}C-containing residue. No amino EPN or oxon EPN was observed (<0.01%). The levels of [^{14}C]EPN found in the cotton plants after the various time intervals is shown in Table II. This data supports a half-life for EPN on the cotton plant in the greenhouse of less than 1 week.

Analysis of the aqueous fraction by TLC with reference samples of PPA and EPPA suggested that the polar ^{14}C-labeled residue was more polar than these acids since it remained at the origin (Figure 3). However, passage of this material through an anion-exchange column liberated a single ^{14}C-labeled component, which did cochromatograph with EPPA (Figure 4). Additionally, this ^{14}C-labeled material was converted by diazomethane to a single ^{14}C-labeled derivative, the methyl ester of EPPA (Figure 5), which was confirmed by GC–MS analysis in comparison with an authentic sample of the compound (Figure 6). EPPA, as a complex or salt, therefore accounted for 59% of the radioactive residue in the cotton plant 14 days after treatment with [^{14}C]EPN.

The completely deesterified acid, PPA, was not found in any fraction as the free acid. However, it was recovered

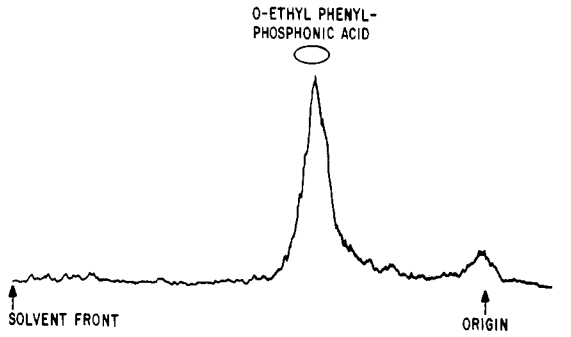

Figure 4. TLC of water-soluble residues after elution from the anion-exchange column. Solvent system: CH_3CN–H_2O–NH_4OH (80:18:2 v/v/v).

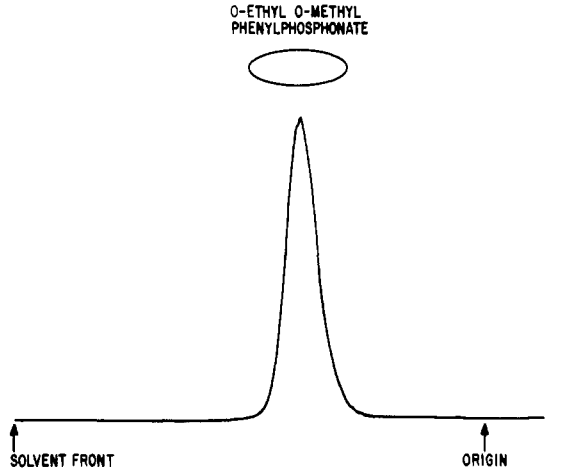

Figure 5. TLC of *O*-ethyl *O*-methyl [^{14}C]phenylphosphonate from methylated polar residues in the 14-day water fraction. Solvent: ethyl acetate.

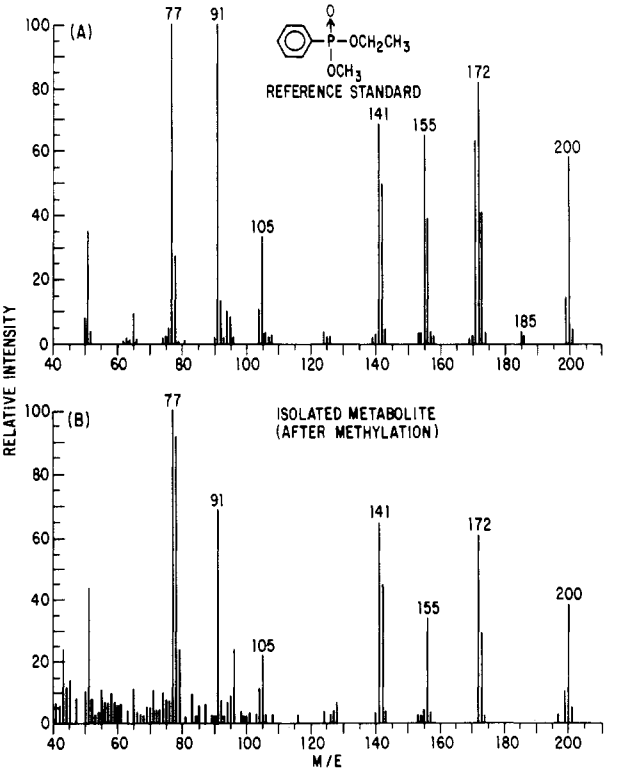

Figure 6. Mass spectrum of *O*-ethyl *O*-methyl phenylphosphonate. (A) Reference standard; (B) isolated metabolite.

as the principal soluble product (70%) after vigorous acid hydrolysis of the totally unextractable ^{14}C-labeled fraction

Table III. Radioactive Materials in Cotton Plants 14 Days after Treatment with [^{14}C]EPN

compds identified or compd classes	% of ^{14}C-labeled residues in cotton plant
EPN	23
O-ethylphenylphosphonic acid (EPPA)[a]	59
phenylphosphonic acid (PPA)[b]	11
nonhydrolyzable insoluble fraction	4
unidentified nonpolar compounds	2
total	99

[a] Found in the water-soluble portion of the plant extract as the salt or complex; liberated by ion-exchange chromatography. [b] Found in the unextractable residue of the plant tissue; liberated only by strong acid hydrolysis.

in the plant tissue. The [^{14}C]PPA derived by hydrolysis from this fraction accounted for 11% of the ^{14}C content in the plant. The structure of this compound was confirmed by derivatization with diazomethane and GC/MS study of the resultant *O,O*-dimethylphenylphosphonic acid (Figure 7).

After acid hydrolysis, a limited amount of insoluble ^{14}C-labeled material was recovered. This material accounted for 4% of the radioactivity in the 14-day plant. Although no further characterization was carried out, this may represent [^{14}C]phenyl incorporation into insoluble plant constituents such as lignin.

Table III summarizes the quantitative analysis of ^{14}C-labeled residues found in the 14-day posttreatment cotton plant, and Figure 8 summarizes the metabolic pathway.

Soils. Under greenhouse conditions, [^{14}C]EPN had a half-life in soil of 5–6 weeks as shown by the data in Table IV. TLC analyses of the acetone–methanol–toluene extracts showed that the primary extractable ^{14}C-labeled material was EPN (>99%). Although the TLC system (hexane–ethyl acetate, 4:1 v/v) used to quantitate the EPN level did not completely separate oxon EPN and amino EPN from the origin, the use of a slightly more polar system (hexane–dioxane, 4:1 v/v) clearly showed that both of these materials were absent (<0.2%). The radioactivity in the area of the TLC plate corresponding to EPN was confirmed by direct-probe mass spectrometry.

Analysis of the methanol and water extracts of the polar ^{14}C-labeled residues in the 36-week soil showed that they consisted primarily (ca. 60%) of PPA with lesser (ca. 20% each) quantities of EPPA and EPPTA. The levels found were not entirely reproducible (depending upon extraction conditions), probably because of ease of oxidation and hydrolysis.

Analysis of the slightly alkaline 0.5 M $(NH_4)_2CO_3$ extract for ^{14}C-labeled compounds showed by LSC that 50% of the residual ^{14}C had been removed, and TLC showed that it consisted entirely of PPA. A representative scan, cochromatographed with standards, is shown in Figure 9.

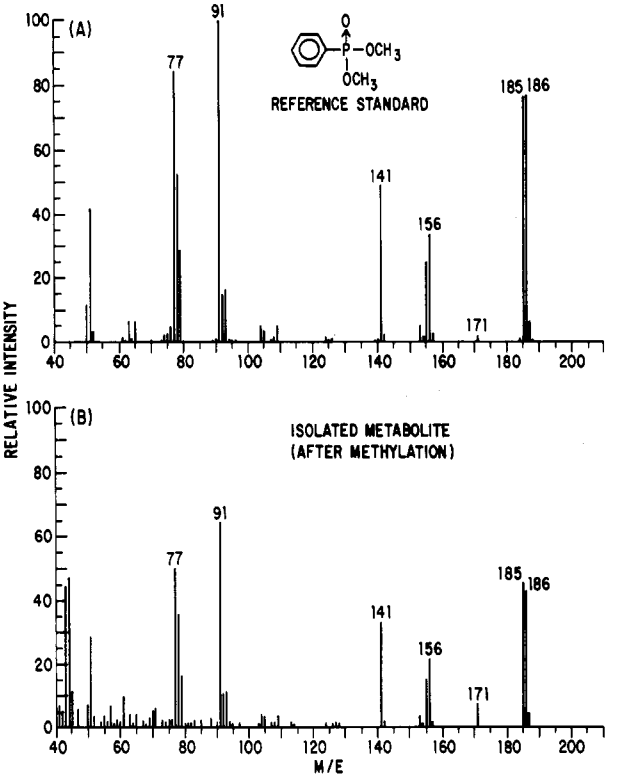

Figure 7. Mass spectrum of *O,O*-dimethyl phenylphosphonate. (A) Reference standard; (B) isolated metabolite.

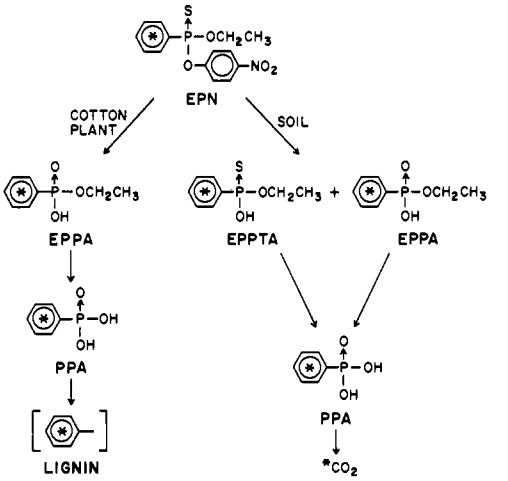

Figure 8. Route of degradation of [^{14}C]EPN in cotton plants and soil. The asterisk denotes the position of the ^{14}C label.

The remaining 50% of radioactivity in the soil is probably more bound PPA which would be released upon more rigorous hydrolysis.

During the 48-week test period, 65% of the total applied ^{14}C activity was lost from the soil (Table IV). The major metabolic pathway appears to be hydrolysis and oxidation to PPA, followed by complete degradation of the phenyl

Table IV. Analysis of [^{14}C]EPN-Treated Fallsington Sandy Loam

	% applied [^{14}C]EPN remaining (2-ppm treatment) for greenhouse exposure, weeks, of										
	0	1	2	3	4	8	12	24	36	40	48
extracted[a]	102	71	62	73	58	43	30	25	37	15	9.6
unextracted[b]	0.4	7.2	12	12	21	23	40	49	20	26	25
total	102	78	74	85	85	79	70	74	57	41	35

[a] >99% of extracted ^{14}C by acetone-methanol-toluene was intact EPN at all time intervals. [b] At least 50% of this material consisted of bound EPPA, EPPTA, and PPA.

Table V. Percent of Applied Radioactivity and Intact EPN Remaining in Treated Field Soils

soil depth, in.	% for exposure time, months, of 0	0.5	1	2	4	6	12	18
				Dundee Silt Loam (Scott, MS)				
0-1	71	71	63	56	34	34	24	3.1
1-3	23	3.0	6.9	5.3	5.3	1.3	3.7	2.5
3-5	0.1	0.6	1.4	1.5	2.7	0.3	0.5	0.3
5-8	0.8	0.3	1.3	1.6	0.7	0.0	0.2	0.1
8-12	0.2	0.5	0.2	1.6	<0.1	0.1	0.1	0.3
12-15	<0.1	0.1	0.1	0.7	<0.1	0.0	0.0	0.1
total recovery	95	76	73	67	43	36	28	6.4
intact EPN	86	52	31	17	12	6.2	4.4	1.2
total rainfall, in.	0.0	5.5	7.0	9.8	15.3	23.0	50.8	71.0
				Keyport Silt Loam (Newark, DE)				
0-1	84	79	87	76	61	28	23	8.0
1-3	0.4	0.4	0.5	0.5	2.7	4.7	5.8	14
3-5	<0.1	0.3	0.8	<0.1	0.4	0.5	3.8	1.0
5-8	0.2	0.3	1.0	<0.1	0.2	0.4	0.6	0.2
8-12	0.2	0.4	1.0	<0.1	<0.1	0.2	0.8	0.1
12-15	0.2	0.4	0.4	<0.1	<0.1	0.1	1.6	0.0
total recovery	85	81	91	77	64	34	36	23
intact EPN	74	57	53	43	38	14	11	7.1
total rainfall, in.	0.00	0.07	2.4	4.8	10.7	15.6	45.6	70.7

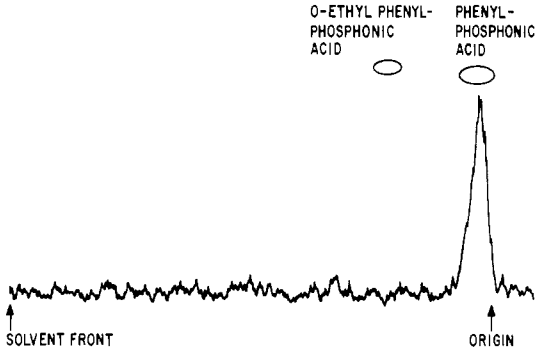

Figure 9. TLC of ammonium carbonate extract from greenhouse soil. Treatment was 10 ppm; aged 36 weeks. Solvent system: CH_3CN–H_2O–NH_4OH (80:18:2 v/v/v).

ring to $^{14}CO_2$ as described later in the biometer results.

Results of analyses for total ^{14}C and [^{14}C]EPN remaining in the field soils are given in Table V. The half-life for EPN ranged from about 2 weeks in the Mississippi test to 1 month in the Delaware test. The half-life for total ^{14}C-labeled residue remaining in the soil was ca. 3 months in Mississippi and ca. 5 months in Delaware. Greater than 90% of the residual ^{14}C was in the 0–3-in. soil increment after 18-months exposure in both Delaware and Mississippi, indicating no significant leaching of EPN or its degradation products.

EPN was again found to degrade primarily to soil-bound metabolites that would yield PPA upon extraction with hot ammonium carbonate. No oxon or amino EPN was found (<0.2%). Ninety-nine percent of the radioactivity in the acetone–methanol–toluene extract consisted entirely of [^{14}C]EPN. From 44 to 58% of the soil-bound ^{14}C activity consisted of metabolites from which PPA could be released by hydrolysis. The identity of EPN and PPA isolated from the 18-month cylinders was confirmed by GC–MS.

Results of analyses for total ^{14}C and [^{14}C]EPN remaining in the biometer flasks are presented in Table VI. The half-life for EPN in Keyport soil was 5 weeks at 2 ppm and 6 weeks at a 10-ppm fortification level. As in the other soil studies, no oxon EPN or amino EPN could be detected. Greater than 99% of the ^{14}C activity extracted with organic solvents from the biometer soil at any interval was [^{14}C]EPN. Greater than 99% of the radioactivity in the sodium hydroxide trap solutions at all sampling intervals was $^{14}CO_3^{2-}$. No unprecipitable ^{14}C was detected in the trap solutions after treatment with barium chloride, indicating that only $^{14}CO_3^{2-}$ from $^{14}CO_2$ was present. The results in Table VI show that after an initial lag period (ca. 4 weeks), the rate of $^{14}CO_2$ evolution increased. This indicated that the soil microorganisms were able to utilize EPN and/or its hydrolysis products as a carbon source. After 36 weeks, 8–25% of the original ^{14}C had been evolved as $^{14}CO_2$, but the sterile controls evolved only 6.1% of the original ^{14}C as $^{14}CO_2$ at 2 ppm and 1.1% at 10 ppm. Most of the adsorbed ^{14}C activity could be liberated from soil as PPA (82% from 36 week soil) by treatment with hot ammonium carbonate.

Table VI. Microbial Degradation of [^{14}C]EPN in Keyport Silt Loam

weeks after treatment (2 ppm)	% of applied radioactivity: CO_2 (cumulative)	EPN	polar materials
0	0	92	2
1	0.2	74	12
2	0.5	67	18
4	1.4	55	30
8	4.5	44	38
12	6.1	34	43
16	19	28	37
24	20	23	41
36	25	19	35

weeks after treatment (10 ppm)	% of applied radioactivity: CO_2 (cumulative)	EPN	polar materials
0	0	92	0.8
1	<0.1	79	11
2	0.1	71	18
4	0.2	58	29
8	0.6	44	40
12	1.2	49	39
16	6.5	32	43
24	7.0	27	47
36	8.5	19	45[a]

[a] 82% of polar material was extracted from soil as [^{14}C]PPA with hot ammonium carbonate.

Overall, these data show that microbial degradation contributes to the decomposition of EPN in soil and that the phenyl ring was broken to liberate $^{14}CO_2$. The remaining metabolites were adsorbed to the soil as compounds which release PPA or were possibly incorporated into the humic acid fraction as lignin-like compounds.

CONCLUSIONS

EPN has been shown to rapidly disappear when applied to cotton. Breakdown of residual EPN in both cotton plants and soil proceeds through hydrolysis and oxidation to phenylphosphonic acid which ultimately is converted to CO_2 through soil microbial action. The observed rate of breakdown in soil was fastest in the field, especially in a warm climate such as Mississippi where the half-life of the intact compound was found to be about 2 weeks.

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LITERATURE CITED

Bartha, R.; Pramer, D. *Soil Sci.* **1965**, *100*, 68.
Chrzanowski, R. L.; Jelinek, A. G. *J. Agric. Food Chem.* **1981**, *29*, 580.
Crockett, A. B. *Pestic. Monit. J.* **1974**, *8*, 69.
Dequidt, J.; Erb, F.; Colein, P.; Bernard, R. *Bull. Soc. Pharm. Lille* **1976**, *32*, 55.
Germanier, R.; Wuhrmann, K. *Pathol. Microbiol.* **1963**, *26*, 569.
Madhosingh, C. *Can. J. Microbiol.* **1961**, *7*, 553.
Menn, J. J., presented at the International Symposium on Pesticide Terminal Residues, IUPAC, Tel Aviv, Israel, Feb 17–19, 1971.
Puri, A. N. "Soils: Their Chemistry and Physics"; Reinhold: New York, 1949; p 341.
Raymond, D. G. M.; Alexander, M. *Pestic. Biochem. Physiol.* **1971**, *1*, 123.
Schütte, H. R.; Stock, M. *Int. Congr. Ser.—Excerpta Med.* **1978**, *No. 440*, 225.
Sethunathan, N. *J. Agric. Food Chem.* **1973**, *21* (4), 602.
Sudhakar-Barik; Sethunathan, N. *J. Environ. Qual.* **1978**, *7* (3), 349.
Wiersma, G. B.; Sand, P. F. *Pestic. Monit. J.* **1972**, *6*, 126.

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Hydrolytic Release of Tightly Complexed 4-Chloroaniline from Soil Humic Acids: An Analytical Method

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Research was conducted to develop a method to release and quantitate "bound" or tightly complexed 4-chloroaniline (4-CA) from soil humic acids. An evaluation of several techniques to release tightly complexed [^{14}C]-4-CA from soil humic acids resulted in the development of an analytical method using alkaline hydrolysis and quantitation of the released 4-CA as its heptafluorobutyryl derivative detected by electron-capture gas–liquid chromatography (EC-GLC). Soil humic acids were treated with [^{14}C]-4-CA at 477 ppm. Two percent of the applied radioactivity was uncomplexed, 10% was extractable into benzene and acetone (loosely complexed), and 88% remained tightly complexed. The alkaline hydrolysis released 80% of the applied radioactivity (91% of tightly complexed residue), with 46% of the applied 4-CA reproducibly quantitated as its *N*-(heptafluorobutyryl)-4-chloroaniline derivative. Other extraction methods such as acid hydrolysis, methylation, heptafluorobutyrylation, sonication, or pyrolysis were not as efficient in releasing tightly complexed 4-CA.

Substituted chloroanilines are derived from several pesticides but especially phenylureas, phenylcarbamates, and anilide herbicides. It has been shown, for example, that herbicide-derived 4-chloroaniline (4-CA) and 3,4-dichloroaniline are bound abundantly and tenaciously to soil and particularly to soil humic acids (Hsu and Bartha, 1974a, 1976). A soil-bound residue has been defined as the chemically unidentified pesticide residue or its degradation product remaining in fulvic acid, humic acid, and humin soil fractions after exhaustive sequential extraction with nonpolar and polar solvents (Kaufman et al., 1976). A precise definition of a bound residue has yet to be wholly agreed upon; therefore, the term tightly complexed will be used to describe residues recalcitrant to extraction: this removes any ambiguity and connotation of covalent bonding.

Several toxicological risks have been evaluated for chlorinated anilines: phytotoxicity (Hoffman and Allen, 1960), carcinogenicity (Neish et al., 1964; Hall, 1979), mammalian toxicity (Bartha and Prammer, 1967; Weisburger and Weisburger, 1966), and microbial toxicity (Corke and Thompson, 1970). Analysis of tightly complexed chloroaniline soil residues is of paramount importance because of their innate toxicity and because of their potential to accumulate in soil organic matter, possibly affecting soil physical and biochemical properties such as water-holding capacity, soil structure, and soil organisms and the processes of nitrification, ammonification, and

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