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# Fate of *O*-[4-[(4-Chlorophenyl)thio]phenyl] *O*-Ethyl *S*-Propyl Phosphorothioate (RH-0994) in Soil

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Under laboratory conditions, [$^{14}$C]RH-0994 [*O*-[4-[(4-chlorophenyl)thio]phenyl] *O*-ethyl *S*-propyl phosphorothioate] is degraded in construction sand, Lufkin fine sandy loam, and Houston clay soils by oxidation and hydrolysis. Laboratory tests indicated that [$^{14}$C]RH-0994 does not leach in loam and clay; however, the compound decomposes readily in the field to more water-soluble products that do leach. Residues in soil that resulted from the treatment of cotton with [$^{14}$C]RH-0994 reached maximum levels (ca. 1.0 ppm) just after the last of 10 spray applications (ca. 5-day intervals) and then declined rapidly. $^{14}$C-Labeled residues in soil resulting from [$^{14}$C]RH-0994 sprays and the postharvest incorporation of treated plant material into the soil declined progressively to a level of ca. 0.16 ppm after 14 months. Residues associated with [$^{14}$C]RH-0994 treatments were detected in rotation crops, but levels were generally low (ca. 0.1 ppm or less) in the plant parts that would be consumed.

The experimental organophosphorus insecticide *O*-[4-[(4-chlorophenyl)thio]phenyl] *O*-ethyl *S*-propyl phosphorothioate (RH-0994) is being developed for use in controlling phytophagous pests of crops and is especially effective against the *Heliothis* spp. that attack cotton. Bull and Ivie (1981) reported that foliar applications of RH-0994 were absorbed readily by foliage of the cotton plant and then metabolized within the plant to the sulfoxide and sulfone derivatives of the intact ester and to three substituted phenolic products that were found in both free and conjugated forms. These authors also reported that 10 spray applications of RH-0994 at 5-day intervals led to an appreciable accumulation of insecticide-related residues in the foliage of cotton. There is therefore a good potential for contamination of soil by RH-0994 or its degradation products as a result of the runoff of sprays or as a result of shredding and cultivation of treated foliage. This report describes studies to assess the fate of RH-0994 after application to different kinds of soil.

## MATERIALS AND METHODS

**Chemicals.** Technical-grade and emulsifiable concentrate formulations of RH-0994 (I) that were unlabeled or uniformly radiolabeled with $^{14}$C in the *P*–*O*–phenyl moiety (respective specific activities of 2.48 and 1.30 mCi/mmol), were supplied by the Rohm and Haas Co., Spring House, PA. The technical [$^{14}$C]RH-0994 was purified (>99%) via thin-layer chromatography (TLC) before use in soil treatments. Also provided were pure unlabeled samples of known (Bull and Ivie, 1981) metabolites of I: II, *O*-[4-[(4-chlorophenyl)sulfinyl]phenyl] *O*-ethyl *S*-propyl phosphorothioate; III, *O*-[4-[(4-chlorophenyl)sulfonyl]-phenyl] *O*-ethyl *S*-propyl phosphorothioate; IV, 4-[(4-chlorophenyl)thio]phenol; V, 4-[(4-chlorophenyl)-sulfinyl]phenol; VI, 4-[(4-chlorophenyl)sulfonyl]phenol. Structures of these chemicals are shown in Figure 1.

**Soils.** Three types of soil were used for tests: construction-grade sand and Lufkin fine sandy loam and Houston clay obtained from cotton fields near College Station and Waco, TX, respectively. The properties of these soils, which were determined by the Soil Testing Laboratory at Texas A&M University, are listed in Table I. Prior to use in different tests, samples of these soils were sieved to pass a 40-mesh screen.

**Analytical Methods.** The radioactivity in different samples was determined by standard liquid scintillation counting procedures. Corrections for quenching in certain samples were made through the use of internal standards.

TLC was done with precoated glass plates (silica gel 60 F-254, 0.25 mm thick; EM Laboratories, Inc., Elmsford, NY). Tentative identifications of radioactive materials in different extracts were based upon their cochromatography with authentic standards after two-dimensional development on TLC plates in different combinations of the four solvent systems reported by Bull and Ivie (1981): (A) benzene and methanol, 9:1; (B) chloroform, hexane, and acetone, 6:3:2; (C) heptane, chloroform, and methanol, 9:4:1; (D) benzene, ethanol, and acetic acid, 93:7:1. Nonradioactive analytical standards were visualized under short-wave UV light, and radioactive compounds were located by autoradiography with X-ray film.

The levels of unextractable radioactive materials in treated soil were determined by combusting samples in a furnace at 1000 °C in an oxygen atmosphere (Bull et al., 1970) and radioassaying trapped radiocarbon by liquid scintillation. Radioactive residues in plant materials were analyzed by a standard oxygen combustion procedure (Bull and Ivie, 1976).

**Fate of [$^{14}$C]RH-0994 in Soil in the Laboratory.** Samples (10 g each) of the three soils were weighed into 20-mL glass vials and then treated with 100 μL of a methylene chloride solution containing 25 μg of [$^{14}$C]RH-

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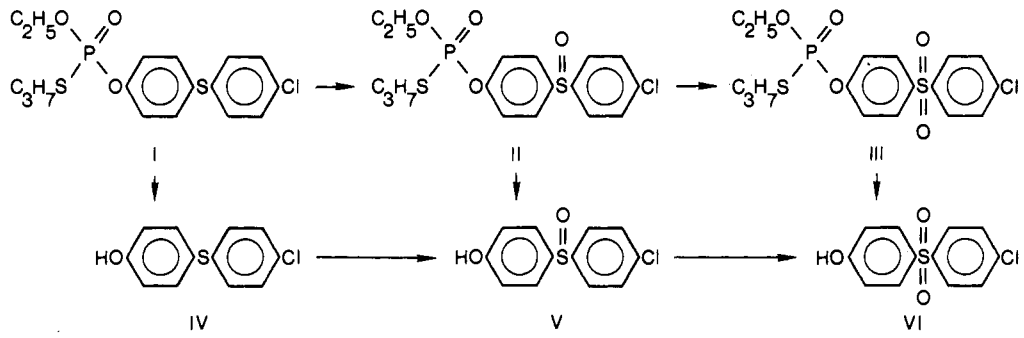

**Figure 1.** Pathway for the formation of degradation products of RH-0994 detected in soil.

Table I. Properties of Test Soils

| sample | pH | org matter, % | cation exch cap., mequiv/100 g | bulk $d$, g/cm$^3$ | $H_2O$ retention, % at $^1/_3$ atm | mechanical analysis, % | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | sand | loam | clay |
| sand | 8.0 | 0.6 | 0.39 | 1.54 | 0.8 | 98.4 | 0.8 | 0.8 |
| loam | 6.8 | 1.1 | 9.31 | 1.19 | 14.4 | 60.7 | 28.3 | 11.0 |
| clay | 6.8 | 1.3 | 33.12 | 1.08 | 38.6 | 12.6 | 38.6 | 48.8 |

0994. After the methylene chloride evaporated, vials were capped and their contents were mixed thoroughly. The vials were then opened and a sufficient volume of water was added to wet each soil sample to ca. 75% of the 0.33-bar moisture retention level. Vials were capped for ca. 8 h to facilitate distribution of moisture throughout the samples, and then they were opened and placed in a ventilated glass chamber that was partially filled with water to maintain a high relative humidity. This chamber was held at room temperature (25 ± 2 °C) in a fume hood. After 1 month in aerobic conditions, sufficient samples of treated loam were purged thoroughly with nitrogen, capped tightly, and held submerged in water for evaluations of effects under anaerobic conditions after an additional 1 and 2 months. Three replicates were prepared for each soil and collection time.

At the specified times posttreatment, samples of each soil were transferred quantitatively to glass-stoppered flasks (250 mL) with 50 mL of a mixture (9:1 v/v) of acetone and distilled water. Each sample was acidified with 0.5 mL of 6 N hydrochloric acid and then agitated vigorously for 30 min on a wrist-action shaker. After the mixture was allowed to stand overnight, solvent extracts were decanted and the samples were extracted once more with 50-mL portions of the aqueous acetone solution. The second extracts were decanted, and samples were centrifuged to effect complete separation of the solvent and soil.

Pooled extracts of each sample were radioassayed and the acetone was removed under vacuum. The volume of the remaining aqueous solution was adjusted to 10 mL with water and then partitioned twice against 50-mL portions of methylene chloride. Both fractions were radioassayed, and then the methylene chloride fraction was evaporated to a convenient volume and analyzed via TLC/autoradiography. If aqueous fractions of partitioned samples contained appreciable levels of radioactive material, they were lyophilized and then dissolved in 1 N HCl and heated for 4 h at 100 °C in sealed glass ampules. The samples were then adjusted to ca. pH 1 and extracted with methylene chloride, and the organic phase was analyzed by TLC.

Extracted soil was dried at room temperature and then combusted and radioassayed as described to determine levels of unextracted $^{14}$C-labeled residues.

**Biometer Flask Study.** The biometer flask procedure of Bartha and Pramer (1965), as modified by Mansager et al. (1979), was used to determine if the radiolabeled phenyl moiety of the RH-0994 molecule was degraded in soil to $^{14}CO_2$. Three samples of sand (100 g) were each mixed with 250 μg of [$^{14}$C]RH-0994 and then placed in glass biometer flasks (Bellco Glass, Inc., Vineland, NJ) and moistened with 10 mL of distilled water. Fifteen milliliters of 1 N sodium hydroxide was added to the side arm of each flask, and then flasks were sealed and held at room temperature in an area protected from direct exposure to light. At the specified times posttreatment, the trapping solution was removed for analysis and replaced.

Radiocarbon in the trapping solution was radioassayed directly by LSC. The presence of $^{14}CO_2$ was verified by adding a saturated solution of barium chloride, and radioassaying precipitated barium [$^{14}$C]carbonate as described by Mansager et al. (1979). Also, some of the trapping solution was acidified and radioassayed again. In this case, a reduction in radiocarbon content of the solution would indicate that $^{14}CO_2$ was released as a result of the reaction with sodium [$^{14}$C]carbonate. This acidified solution was also extracted with methylene chloride and analyzed as described to determine the presence of any other radioactive products.

**Leaching of [$^{14}$C]RH-0994 in Soil.** Tests of the leaching of [$^{14}$C]RH-0994 in loam and clay were done in the laboratory with TLC procedure described by Helling et al. (1971). For this, 20 × 20 cm glass TLC plates were coated (500–750-μm layer) with each soil, air-dried, and then spotted with 2 μg of [$^{14}$C]RH-0994. For purposes of comparison, similar amounts of a highly water soluble (485 mg/mL) compound [$^{14}$C]TD-1123 (potassium 3,4-dichloro-5-isothiazolecarboxylate) and a water-insoluble (0.0002 mg/mL) compound [$^{14}$C]diflubenzuron were spotted on the same plate. Treated plates were developed in water until the solvent front migrated 10 cm from the point of sample application, and then they were air-dried and exposed to X-ray film to allow detection of any movement of the radioactive areas. Leaching tests were replicated 3 times.

**Fate of [$^{14}$C]RH-0994 in Field Soil.** Samples of Lufkin fine sandy loam (20 g each) were treated directly by mixing the soil thoroughly with 50 μg of [$^{14}$C]RH-0994 or treated indirectly by mixing the soil with sufficient finely ground radioactive plant material to produce an initial radiocarbon level of 2.5 ppm. This plant material was obtained from a plot of cotton that had been sprayed with [$^{14}$C]RH-0994

Table II. Fate of [$^{14}$C]RH-0994 in Different Soils in the Laboratory[a]

| days post-treatment | % of dose as indicated product or fraction[b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | unidentified compd | | | identified compd | | | | | | water soluble | unextractable | recovery |
| | A | B | C | I[c] | II | III | IV | V | VI | | | |
| | | | | | | Construction Sand[b] | | | | | | |
| 0 | | | | 96.8 | 3.0 | | | | | | 0.2 | 100.0 |
| 3 | | | | 88.9 | 4.1 | | | | | 0.7 | 0.8 | 94.5 |
| 7 | | | | 77.3 | 8.5 | | 3.1 | 2.0 | | 1.3 | 1.2 | 93.4 |
| 14 | | | | 65.3 | 12.5 | | 3.1 | 8.1 | | 0.8 | 1.9 | 91.7 |
| 28 | | 3.1 | | 48.2 | 11.6 | | 1.6 | 10.9 | 2.2 | 3.0 | 6.9 | 87.5 |
| 56 | | 5.4 | | 20.4 | 6.5 | | | 21.0 | 4.5 | 2.7 | 13.0 | 73.5 |
| 84 | 0.9 | 6.0 | | 11.9 | 2.3 | | | 15.9 | 7.7 | 4.2 | 17.6 | 66.5 |
| 176 | 2.3 | 8.1 | | 3.4 | 2.4 | | 2.3 | 3.6 | 6.9 | 1.9 | 29.6 | 60.5 |
| 268 | 5.8 | 3.3 | 10.4 | 3.2 | | | | 2.9 | | 1.9 | 25.0 | 52.5 |
| 364 | | | 26.6 | | | | | | | 1.8 | 24.2 | 52.6 |
| | | | | | | Lufkin Fine Sandy Loam | | | | | | |
| 0 | | | | 96.4 | 3.4 | | | | | | 0.2 | 100.0 |
| 3 | | | | 82.9 | 11.5 | | | | | 0.3 | 0.8 | 95.5 |
| 7 | | | | 78.2 | 11.6 | | | 3.0 | | 1.6 | 1.0 | 95.4 |
| 14 | | | | 67.9 | 17.6 | | | 6.0 | | 2.7 | 0.9 | 95.1 |
| 28 | | | | 54.0 | 22.0 | | 0.1 | 12.6 | 1.4 | 2.3 | 2.1 | 94.5 |
| 56 | | | | 34.4 | 20.7 | | | 23.5 | 6.8 | 2.3 | 2.7 | 90.4 |
| 84 | 1.2 | | | 28.0 | 20.3 | | | 18.8 | 7.9 | 2.7 | 6.1 | 85.0 |
| 176 | 2.4 | | | 13.3 | 15.8 | | 1.2 | 25.7 | 12.7 | 4.7 | 10.2 | 86.0 |
| 268 | 1.8 | | | 8.2 | 7.6 | | 1.4 | 28.2 | 22.3 | 3.5 | 8.2 | 81.2 |
| 364 | 1.5 | | | 9.4 | 5.9 | | | 29.4 | 17.2 | 4.2 | 13.7 | 81.3 |
| | | | | | | Houston Clay | | | | | | |
| 0 | | | | 91.8 | 7.5 | | | | | 0.1 | 0.6 | 100.0 |
| 1 | | | | 87.5 | 8.2 | | | | | 0.1 | 1.6 | 97.4 |
| 3 | | | | 84.8 | 7.9 | | | | | 0.3 | 2.6 | 95.6 |
| 7 | | | | 80.7 | 7.6 | | 2.7 | 1.3 | | 0.0 | 2.2 | 94.5 |
| 14 | | | | 75.0 | 4.2 | | 5.3 | 2.2 | | 1.6 | 1.3 | 89.6 |
| 28 | | | | 65.9 | 8.2 | | 6.9 | 5.3 | 0.7 | 0.0 | 3.9 | 90.9 |
| 56 | | | | 52.8 | 11.2 | 1.5 | 4.2 | 12.8 | 3.4 | 0.4 | 3.1 | 89.4 |
| 84 | 0.6 | | | 43.2 | 13.1 | | 2.1 | 11.2 | 14.2 | 0.4 | 4.7 | 89.5 |
| 176 | 1.1 | | | 32.9 | 8.8 | | 2.4 | 13.4 | 18.8 | 0.8 | 7.6 | 85.8 |
| 268 | 2.2 | | | 23.9 | 8.1 | | 1.9 | 10.4 | 22.2 | 2.0 | 13.6 | 84.3 |
| 364 | 2.7 | | | 22.7 | 8.5 | | 0.0 | 10.6 | 28.3 | 1.9 | 13.2 | 87.9 |

[a] Samples held under aerobic conditions. [b] Unknown(s) A is (are) TLC base-line radioactivity, unknown(s) B migrated above parent compound with streaking, and unknown(s) C represents (represent) streaking of radioactivity above and below the parent compound. [c] RH-0994.

(Bull and Ivie, 1981). Each sample of treated soil was contained in a 200-mesh stainless steel screen packet (ca. 8 × 8 cm) and then buried in the field in the same kind of soil to a depth of ca. 10 cm. (There is no significant loss of soil from these packets but they are readily penetrated by moisture.)

At the specified times posttreatment, triplicate samples of the packets were collected, and the treated soil was extracted and analyzed as described above for the laboratory study. Additional samples of untreated soil surrounding the packets were collected for determinations of moisture content.

**Persistence of [$^{14}$C]RH-0994 Residues in Field Soil.** For this test, which was a continuation of the study reported by Bull and Ivie (1981), we used an isolated plot of Stoneville 213 cotton (1.7 m$^2$) that had been sprayed 10 times at ca. 5-day intervals with an aqueous emulsion of the EC formulation of [$^{14}$C]RH-0994 at a rate equivalent to 1.12 kg of active ingredient in 93.5 L of water per ha per application. These treatments were initiated on July 24 and terminated Sept 5, 1979. Treated plants were removed from the plot on Nov 2 and then dried 48 h at 50 °C and milled to pass a 20-mesh screen. This plant material was analyzed, and then on Dec 28, 1979, it was manually cultivated into the soil of the same plot (ca. 0–7.5 cm deep).

Beginning on Aug 3, 1979, and continuing at approximately monthly intervals until Feb 27, 1981, three or more cores of soil (22.5 cm deep) were collected from the plot, divided into three equal portions according to depth, and analyzed by oxygen combustion procedures to determine the levels of radiocarbon in the soil.

**Residues in Rotation Crops.** In the spring of 1980, onions, beans, broccoli, and grain sorghum were planted in the aforementioned plot that was used for treatments of cotton with [$^{14}$C]RH-0994. These plants, as well as some volunteer weeds and grass, were collected at appropriate stages of development and divided into subsamples. These samples were dried 24 h at 50 °C, milled to pass a 20-mesh screen, and then analyzed by combustion to determine levels of possible radioactive residues. In all cases, tests were replicated at least 3 times, and samples of the same kinds of plants grown in untreated soil were analyzed and used to correct for natural background radioactivity.

## RESULTS AND DISCUSSION

**Fate of [$^{14}$C]RH-0994 in Soil in the Laboratory.** Results of studies of the fate of [$^{14}$C]RH-0994 in sand, loam, and clay in the laboratory are shown in Table II. The insecticide was oxidized to the intact ester sulfoxide derivative (II) in all three soils; however, that product tended to accumulate more in treated loam. Evidence of further oxidation of II to the intact ester sulfone derivative (III) was found only in extracts of clay, which at 56 days posttreatment apparently contained minor amounts (1.5% of dose) of that product. The lack of accumulation of III in soil might be attributable to a very rapid degradation of the product as it was formed or to the fact that it was

not formed under the test conditions used.

Three phenolic metabolites (IV, V, and VI) were formed during the degradation of RH-0994 in the three treated soils. The phenol sulfoxide (V) tended to predominate during the first few months of the study but concentrations of the phenol sulfone (VI) increased progressively, and especially in clay, VI was a major degradation product in the latter part of the test. Concentrations of the unoxidized phenol (IV) did not tend to accumulate much in sand or in loam but did reach a level of 7% of the dose in clay at 28 days posttreatment.

Only minor concentrations of unknown(s) A and unidentified water-soluble radioactive material were detected. In sand it was obvious that RH-0994 and its derivatives were being degraded more extensively than in loam and clay. Extracts of treated sand after ca. 1 month contained unidentified radioactive material [unknown(s) B] that migrated on TLC as a streak above the parent compound, and samples collected after 9 and 12 months contained appreciable amounts of radioactive material that migrated as a streak along much of the migration route of the radioactive products. On the basis of the results of the biometer study described below, it appears likely that unknowns B and C were derived from rupture of the phenolic ring structure.

Unextractable residues tended to accumulate progressively, especially in sand. The overall rate of degradation of RH-0994 and its derivatives proceeded in the order of sand > loam > clay. Although precise reasons for the observed differences in rates of degradation were not established, it seems likely that the relative compaction of the soil samples was a major factor. A general recovery of lesser concentrations of oxidation products from treated clay suggests that the rather dense nature of moist samples of that soil afforded greater protection of the chemical from atmospheric oxygen. The faster degradation of RH-0994 in sand can probably be attributed to greater exposure of the chemical to atmospheric oxygen as a result of the looser compaction of soil particles. In addition, the slightly alkaline pH of sand may have influenced degradation because Ivie et al. (1981) found that RH-0994 was highly unstable (half-life $\ll$ 1 day) in buffer solution at pH 10. Since the percentage of organic matter was higher in loam and clay (Table I), it is possible that adsorption by organic matter was also a factor in lowering the disappearance rate of RH-0994 in those two soils.

The transfer of some of the samples of loam and clay to anaerobic conditions at 28 days posttreatment did not result in a change in the radioactive products detected or in a drastic change in the rate of degradation of [$^{14}$C]RH-0994 (Table III). However, there was some apparent reduction in the rate at which certain oxidation products were formed.

**Biometer Flask Study.** Results of tests with biometer flasks provided definite evidence that some $^{14}CO_2$ evolved during the degradation of [$^{14}$C]RH-0994 in sand. For example, at 14, 28, 42, 56, 72, and 90 days posttreatment, the accumulative radioactivity attributable to the evolution of $^{14}CO_2$ was 0.6, 2.2, 4.2, 6.2, 9.1, and 10.4% of the applied radiocarbon. Verification tests demonstrated that >95% of the radioactive material in the trapping solution of each sample time was accounted for by $^{14}CO_2$. Trace amounts (0.3% of dose) of the phenol sulfide (IV) were detected in the trapping solution at 14 days posttreatment, but at subsequent times, only $^{14}CO_2$ was found.

**Leaching of [$^{14}$C]RH-0994 on Soil-TLC Plates.** Results of the study with soil-TLC plates indicated that RH-0994 did not leach in either loam or clay under the conditions used. The standards demonstrated the same mobility characteristics previously reported by Bull and Shaver (1980); that is, diflubenzuron did not move from the point of application but TD-1123 moved with the solvent front in loam and about half the distance of the solvent front in clay.

Table III. Fate of [$^{14}$C]RH-0994 in Soil after Establishment of Anaerobic Conditions[a]

| nature of radioact material | % of dose as indicated product at different days posttreatment | | | |
|---|---|---|---|---|
| | loam | | clay | |
| | 56 | 84 | 56 | 84 |
| unknown(s) A | 1.6 | 1.1 | 0.4 | 0.9 |
| I | 39.9 | 40.4 | 52.7 | 48.9 |
| II | 22.8 | 10.8 | 11.9 | 10.6 |
| III | 0.0 | 0.0 | 0.9 | 0.0 |
| IV | 0.0 | 6.5 | 9.4 | 11.8 |
| V | 17.3 | 24.3 | 15.9 | 18.0 |
| VI | 1.9 | 4.1 | 2.5 | 3.7 |
| water soluble | 2.4 | 1.7 | 0.3 | 0.1 |
| unextractable | 5.7 | 10.8 | 3.7 | 3.2 |

[a] Samples were held under aerobic conditions for 28 days (Table II) and then changed to anaerobic conditions.

**Fate of [$^{14}$C]RH-0994 in Field Soil.** Results of studies of the fate of [$^{14}$C]RH-0994 in Lufkin fine sandy loam that was treated and buried in the field in stainless steel screen packets are shown in Table IV. The radiocarbon content in soil, treated directly with technical [$^{14}$C]RH-0994 or indirectly with plant material containing radioactive residues, declined steadily at approximately the same rate during the course of the study. After 8 weeks, <40% of the applied dose remained. The same products found in the laboratory tests were also detected in extracts of the field samples. Since soil-TLC tests indicated that RH-0994 did not leach in loam, the rapid decline in concentrations of the insecticide (<5% after 8 weeks) in packets of treated soil obviously must be attributed to its degradation to water-soluble products which did leach.

Unextractable radioactive material in samples of the soil that was treated indirectly with contaminated plant material was appreciably higher than in soil treated directly with technical [$^{14}$C]RH-0994. Since these unextractable residues were also high in "0-h" soil samples, as well as in the dried plant materials per se (Bull and Ivie, 1981), they most likely can be attributed to either a firm binding of certain radioactive products by the plant material or earlier incorporation of radioactive fragments into structural constituents of the treated plants.

**Persistence of [$^{14}$C]RH-0994 Residues in Field Soil.** The results of combustion analyses of core samples of soil from the treated field plot are shown in Table V. Throughout the course of the 2-year study, the highest levels of radiocarbon were always found in the upper 7.5-cm portion of soil cores, and only insignificant amounts were found at the 15–22.5-cm depth. Residues were also generally low in the 7.5–15-cm depth but did appear to be increasing slightly in that layer during the last 3 months of the test period.

The highest level (ca. 1.07 ppm) of radioactive residue in the soil was found in samples collected Sept 7, 1979, just after the last of the 10 spray applications of [$^{14}$C]RH-0994. Radioactive material observed in subsequent samples tended to decline progressively to ca. 0.27 ppm at the time (Dec 28, 1979) that the treated plant material was cultivated into the plot. Incorporation of treated plant material increased the radiocarbon content in the upper 7.5-cm layer of soil to a level of ca. 0.92 ppm. Thereafter, radioactive residues declined progressively but at an ap-

Table IV. Fate of [$^{14}$C]RH-0994 in Lufkin Fine Sandy Loam in the Field[a]

| nature of radioact | % of dose at indicated days posttreatment | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 7 | 14 | 28 | 56 |
| | | | Indirect Treatment (2.5 ppm) | | | |
| unknown(s) A | 4.1 | 3.7 | 0.0 | 0.0 | 0.0 | 1.4 |
| I | 11.5 | 9.5 | 9.1 | 7.1 | 5.3 | 4.4 |
| II | 24.6 | 20.2 | 11.8 | 7.9 | 5.9 | 4.3 |
| III | 3.3 | 3.1 | 3.6 | 1.5 | 0.0 | 0.6 |
| IV | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 |
| V | 25.6 | 24.9 | 17.5 | 15.6 | 13.0 | 9.1 |
| VI | 4.6 | 8.2 | 22.2 | 14.8 | 17.0 | 8.6 |
| water soluble | 7.3 | 5.2 | 4.2 | 2.9 | 3.6 | 2.8 |
| unextractable | 19.0 | 14.7 | 16.2 | 15.1 | 11.9 | 7.0 |
| total recovered | 100.0 | 89.5 | 84.6 | 64.9 | 56.7 | 38.4 |
| soil moisture, % | 11.0 | 11.2 | 11.0 | 13.3 | 14.5 | 6.1 |
| | | | Direct Treatment (2.5 ppm) | | | |
| unknown(s) A | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.9 |
| I | 92.4 | 84.2 | 73.4 | 59.0 | 24.4 | 7.5 |
| II | 4.7 | 5.5 | 11.9 | 10.6 | 13.8 | 9.1 |
| III | 0.8 | 0.5 | 0.0 | 0.0 | 0.0 | 0.4 |
| IV | 0.6 | 0.5 | 0.0 | 0.6 | 0.6 | 0.5 |
| V | 0.0 | 0.4 | 0.9 | 2.7 | 11.3 | 11.0 |
| VI | 0.0 | 0.0 | 0.0 | 0.0 | 3.1 | 4.0 |
| water soluble | 0.5 | 0.5 | 0.5 | 0.7 | 1.7 | 1.5 |
| unextractable | 1.0 | 1.7 | 1.0 | 1.3 | 1.7 | 1.4 |
| total recovered | 100.0 | 93.3 | 87.7 | 74.9 | 56.6 | 36.3 |
| soil moisture, % | 11.3 | 12.3 | 10.9 | 13.2 | 14.1 | 6.4 |

[a] Analyses of hydrolyzed water-soluble radioactive material in pooled samples from indirect treatments indicated the distribution (percent of total) as follows: unknown(s) A, 3.3; IV, 5.6; V, 71.3; VI, 19.8.

Table V. Radioactive Residues in the Soil of a Cotton Plot Previously Treated with [$^{14}$C]RH-0994

| sample date | ppm of [$^{14}$C]RH-0994 equiv ± SE at depth of | | |
|---|---|---|---|
| | 0-7.5 cm | 7.5-15 cm | 15-22.5 cm |
| 8/3/79 | 0.28 ± 0.03 | 0.02 ± 0.01 | <0.01 |
| 9/7/79 | 1.07 ± 0.05 | 0.02 ± 0.01 | 0.03 ± 0.01 |
| 11/2/79 | 0.74 ± 0.06 | 0.03 ± 0.00 | <0.01 |
| 12/4/79 | 0.32 ± 0.06 | 0.04 ± 0.01 | 0.02 ± 0.00 |
| 12/28/79 | 0.27 ± 0.01 | 0.01 ± 0.00 | <0.01 |
| 12/28/79[a] | 0.92 ± 0.04 | 0.07 ± 0.02 | 0.01 ± 0.00 |
| 2/6/80 | 0.59 ± 0.11 | 0.02 ± 0.00 | 0.01 ± 0.00 |
| 3/5/80 | 0.40 ± 0.04 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| 5/6/80 | 0.49 ± 0.05 | 0.05 ± 0.01 | 0.02 ± 0.00 |
| 6/2/80 | 0.24 ± 0.06 | 0.06 ± 0.03 | 0.01 ± 0.00 |
| 7/3/80 | 0.27 ± 0.03 | 0.14 ± 0.05 | 0.01 ± 0.00 |
| 8/13/80 | 0.32 ± 0.05 | 0.07 ± 0.02 | 0.01 ± 0.00 |
| 9/15/80 | 0.32 ± 0.01 | 0.04 ± 0.01 | 0.01 ± 0.00 |
| 10/9/80 | 0.20 ± 0.04 | 0.02 ± 0.00 | 0.01 ± 0.00 |
| 12/3/80 | 0.28 ± 0.03 | 0.13 ± 0.06 | 0.01 ± 0.00 |
| 1/15/80 | 0.22 ± 0.07 | 0.12 ± 0.06 | 0.01 ± 0.00 |
| 2/27/81 | 0.16 ± 0.04 | 0.10 ± 0.04 | 0.01 ± 0.00 |

[a] Date treated plant material was incorporated into soil.

parently slower rate than was observed earlier in soil contaminated only by runoff of radiolabeled sprays. This slower rate in diminution of residual radiocarbon may be attributable to some protection afforded by the binding of the materials to contaminated plant material. Such binding was demonstrated in the aforementioned studies of soil treated indirectly with treated plant material and held in the field in screen packets. In addition, the prolonged drought of 1980 may have favored preservation of residues due to the absence of sufficient moisture to promote leaching.

**Residues in Rotation Crops.** Results of combustion analyses of different plants grown in the plot previously treated with [$^{14}$C]RH-0994 are shown in Table VI. Radioactive residues were detected in all the plants collected from the treated plot. In most cases these residues were highest in the root system, especially in beans (ca. 0.1 ppm)

Table VI. Residues of Radiocarbon in Rotation Crops

| plant material | harvest date | ppm of [$^{14}$C]RH-0994 equiv based on | |
|---|---|---|---|
| | | fresh wt | dry wt |
| broccoli | 5/14/80 | | |
| florets | | 0.013 ± <0.01 | 0.101 ± 0.01 |
| leaves | | 0.031 ± <0.01 | 0.160 ± 0.01 |
| edible stem | | 0.010 ± <0.01 | 0.110 ± 0.01 |
| inedible stem | | 0.034 ± 0.01 | 0.215 ± 0.03 |
| root | | 0.074 ± 0.01 | 0.289 ± 0.05 |
| sorghum | 7/7/80 | | |
| seed | | 0.005 ± <0.01 | 0.008 ± 0.01 |
| leaves | | 0.030 ± <0.01 | 0.084 ± 0.01 |
| stalk | | 0.008 ± <0.01 | 0.030 ± 0.01 |
| roots | | 0.082 ± 0.01 | 0.241 ± 0.03 |
| lima bean | 6/26/80 | | |
| seed | | 0.125 ± 0.01 | 0.130 ± 0.01 |
| shuck | | 0.094 ± 0.01 | 0.101 ± 0.01 |
| leaves | | 0.029 ± 0.01 | 0.151 ± 0.02 |
| stem | | 0.099 ± 0.02 | 0.444 ± 0.10 |
| roots | | 0.107 ± 0.02 | 0.410 ± 0.10 |
| onion | 7/7/80 | | |
| leaves | | 0.026 ± 0.01 | 0.225 ± 0.01 |
| bulb | | 0.029 ± <0.01 | 0.211 ± 0.02 |
| careless weed | 6/16/80 | | |
| head | | 0.010 ± <0.01 | 0.053 ± <0.01 |
| leaves | | 0.028 ± <0.01 | 0.152 ± 0.01 |
| stem | | 0.039 ± <0.01 | 0.310 ± 0.02 |
| root | | 0.044 ± <0.01 | 0.257 ± 0.03 |
| nut grass | 6/16/80 | | |
| head | | 0.050 ± <0.01 | 0.174 ± 0.01 |
| leaves | | 0.089 ± <0.01 | 0.474 ± 0.01 |
| root | | 0.196 ± <0.01 | 0.678 ± 0.02 |

and nut grass (ca. 0.2 ppm). Very little radiocarbon (based on fresh weight) was found in mature sorghum (<0.01 ppm), bean (ca. 0.1 ppm), or careless weed seeds (ca. 0.01 ppm) or in the edible parts of broccoli (ca. 0.01 ppm).

## CONCLUSION

The results of the present study indicate that RH-0994 and associated residues will decompose in representative agricultural soils. On the basis of our data, it is not possible

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

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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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