Abate Insecticide. The Fate of *O,O,O',O'*-Tetramethyl *O,O'*-Thiodi-*p*-phenylene Phosphorothioate on Bean Leaves

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A study of the physicochemical and metabolic fate of tritium-labeled *O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate on bean leaves indicates that this insecticide is relatively resistant to degradation. Intact Abate insecticide was by far the principal constituent of the residue. The major metabolic product was the sulfoxide derivative which accounted for less than 5% of the dose. Trace amounts of the sulfone derivative and the oxygen analog were found also. Increasing amounts of glucosidic conjugates of the phenolic hydrolysis products from Abate insecticide and its sulfoxide and sulfone derivatives were found with increased biotic exposure; after 28 days of exposure, these very polar conjugates accounted for about 17% of the applied dose.

Abate insecticide, the active ingredient of which is the *O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene ester of phosphorothioic acid (also referred to as Compound CL 52,160), is registered for control of mosquito and midge larvae. Its use is also being studied for the control of mosquito larvae in potable water because of its low mammalian toxicity (Gaines *et al.*, 1967; Laws *et al.*, 1967). Before Abate insecticide could be used commercially, data concerning its residual behavior in natural waters and adjacent mud were acquired for registration purposes (Bowman and Orloski, 1966). The residue analytical procedure used for this study was designed to respond only to the parent compound since a preliminary metabolic study of this material in natural waters, mud, and bean plants using nonradiotracer techniques showed little evidence of any postulated metabolic degradation products (Blinn and Pasarela, 1966). The desirability of using Abate insecticide to control mosquito larvae in food-producing areas and to control several insect pests of food crops has required additional residue studies and the detailed evaluation of the metabolic fate of Abate insecticide in a typical plant environment. Bean plants were chosen for convenience and greenhouse conditions were utilized to minimize physical dissipation.

EXPERIMENTAL

Radiotracer. *O,O,O',O'*-Tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate (hereafter designated Compound I), labeled with tritium as shown in Figure 1 (Wagner, 1966), was used throughout this study. The specific activity of this preparation was 288 mc. per mmole. (0.6178 μc./μg.). Its radiopurity was established by chromatography in Systems 1 through 5 as listed in Table I, no areas of radioactivity being found on the chromatograms other than those corresponding to authentic Compound I. Its infrared spectrum was in good agreement with that of authentic, highly purified reference material.

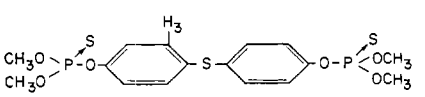

Figure 1. Abate insecticide (I)

Plant Treatment and Extraction Procedures. Three-week-old Sieva lima bean plants, growing in waxed cups (3.5-inch diameter × 2 inches high), were thinned to one plant per cup and the primary leaves marked with paper-covered wire. An aliquot of 100 μl. of a solution containing 44.5 μg. (27.5 μc.) of tritium-labeled Compound I in acetone-water (60 to 40) was spread on the top surface of each of these leaves by means of a micropipet.

The treated plants were placed in a greenhouse fitted with acrylic windows which transmit radiation of wavelengths from 370 to 1620 mμ and were watered by subirrigation to prevent washing the deposits from the leaf surfaces. The plants were held under these conditions for 10 to 28 days after treatment before being removed for processing.

At each sampling period, the treated leaves were cut from about 20 plants with as little petiole as possible, combined, and extracted repeatedly with methanol in a blender. The remaining aerial portions were also combined and extracted with methanol. Roots and soil were not analyzed because preliminary experiments showed essentially no translocation of radioactivity throughout the plant.

After determination of gross radioactivity levels in the methanol extracts by liquid scintillation counting as described below, the methanol was evaporated from the extract of the treated leaves and the residue was partitioned between equal volumes of chloroform and water. These two fractions were subjected to chromatographic, enzymatic, and chemical tests as described below. The radioactivity in the extracts of the untreated portions of the plants was so low as to preclude any further characterization.

Radioassay Procedures. The tritium concentrations in all aqueous and methanolic samples were determined by mixing 100 to 200 μl. of the sample solutions with 15 ml. of scintillator solution [6.0 grams of 2,5-diphenyloxazole (POP) and 200 mg. of 1,4-bis-2-(5-phenyloxazolyl) benzene dissolved in a mixture of 600 ml. of dioxane, 100 ml. of anisole, and 100 ml. of dimethoxyethane] containing 5%

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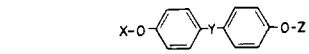
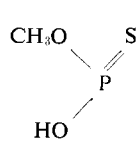
Table I. Typical R_f Values for Various Compounds Using Several Silica Gel Thin-Layer Chromatographic Systems



X	Y	Z	$R_f \times 100$ Solvent Systems[a] 1	2	3	4	5	6	7	8	9
$(CH_3O)_2P(=S)$	S	$(CH_3O)_2P(=S)$	25	67	69	63	33	67	71	78	100
$(CH_3O)_2P(=S)$	SO	$(CH_3O)_2P(=S)$	0	50	55	27	4	50	53	75	100
$(CH_3O)_2P(=S)$	SO_2	$(CH_3O)_2P(=S)$	3	60	64	47	11	61	64	79	100
$(CH_3O)_2P(=O)$	S	$(CH_3O)_2P(=O)$	0	23	17	0	0	13	11	60	98
$(CH_3O)_2P(=O)$	SO_2	$(CH_3O)_2P(=O)$	0	16	14	0	0	11	5	56	96
$(CH_3O)_2P(=O)$	S	$(CH_3O)_2P(=S)$	0	45	46	13	2	42	40	73	...
$(CH_3O)_2P(=O)$	SO_2	$(CH_3O)_2P(=S)$	0	36	40	6	0	35	25	72	...
H	S	H	0	13	47	43	12	45	60	40	...
H	SO	H	0	2	7	1	0	8	21	24	...
H	SO_2	H	0	5	31	15	0	29	40	34	...
H	S	$(CH_3O)_2P(=S)$	4	40	60	18	18	58	66	70	...
H	SO_2	$(CH_3O)_2P(=S)$	0	23	49	23	2	48	52	66	...
	S	$(CH_3O)_2P(=S)$	...	...	...	...	...	...	...	...	0.07stk

[a] Solvent systems: 1, toluene; 2, methanol, chloroform, toluene (10, 95, 95); 3, nitromethane, acetonitrile, toluene (37, 98, 165); 4, ethyl ether; 5, ethyl ether, hexane (1,1); 6, nitromethane, acetic acid, acetonitrile, toluene (11, 1, 28, 60); 7, methanol, ethyl ether (5, 95); 8, methanol, methylene chloride (10, 90); 9, methanol, acetone (5, 95).

Cab-O-Sil thixotropic gelling agent and counting in a Packard Tri-Carb scintillation spectrometer. Because chloroform is a strong scintillation quencher, chloroform solutions were radioassayed by evaporating the solvent and taking the residue up in methanol before mixing it with scintillator solution. Correction for quenching was determined by internal standardization using an aliquot of commercial tritiated toluene.

Solid samples, such as dried marc from extracted tissue, were radioassayed by a modification of the oxygen-flask method of Kelly *et al.* (1961). Samples were burned in an oxygen atmosphere and the tritiated water was frozen out by immersing the flask in a dry ice–2-propanol mixture. The excess oxygen in the flask was displaced with nitrogen and scintillator solution was added. After warming to room temperature, aliquots were taken for liquid scintillation counting as described above.

Radioactive areas on thin-layer chromatograms were located by means of a Vanguard Autoscanner (Model 880) with accessory glass plate scanner (Model 885) or by autoradiography using Kodak Royal Pan sheet film and Baumann's Diafine two-bath film developer as previously described (Blinn, 1967). Using the autoradiogram as a guide, the radioactive zones were marked on the chromatograms and the silica gel was scraped from each zone into a scintillation vial, suspended in scintillator solution containing gelling agent, and radioassayed. The other areas of the plates which contained little or no radioactivity were also radioassayed as a control in this manner. The per cent of the total radioactivity on the chromatogram which was present in each zone was calculated.

Chromatographic Systems. The chromatographic systems used and R_f values found for Compound I and some of its postulated metabolic products are presented in Table I. The 0.5-mm. thin-layer plates were prepared using pH 6.0 buffer solution and a mixture of silica gel G and silica gel HF, prepared as previously described (Blinn, 1964). The plates were prewashed with acetone and acti-

vated at 100° C. for an hour before use. Chromatograms were usually run as streaks on either 2 × 8 inch or 8 × 8 inch thin-layer plates. Solvents were of reagent grade quality and used without further purification.

The various isolated radioactive fractions were identified by thin-layer cochromatography techniques in which the radioactive fractions were applied to the chromatographic plate over those nonradiolabeled compounds which were postulated to be present in the fractions. Identical R_f values for the radioactive material and for the nonradiolabeled material in at least three chromatographic systems was taken as evidence of identity.

Infrared Characterization. When sufficient material was present in a radioactive chromatographic zone to allow infrared characterization, it was eluted with acetone and rechromatographed several times using different developing systems until purified from interferences from the substrate. The radioactive area of the final chromatogram was eluted with redistilled acetone. A portion of this eluate was spread on both sides of a 50 × 20 × 1 mm. KRS-5 Wilks multiple internal reflectance plate and the acetone was evaporated in a stream of warm air. The plate was then placed in a Wilks Model 9 single-beam internal reflection attachment mounted on a Perkin-Elmer Model 421 infrared spectrophotometer and the infrared characteristics of the residue were recorded.

RESULTS AND DISCUSSION

Distribution of Radioactivity in Plant Parts. The amounts of extractable and unextractable radioactivity

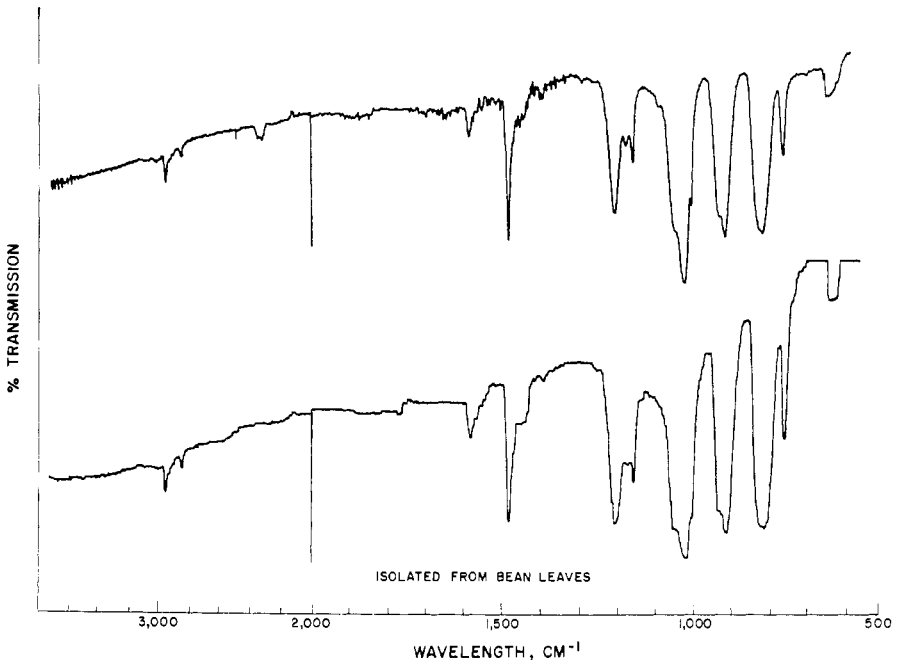


Figure 2. Infrared characteristics for Abate insecticide (I, upper) and for a radioactive component isolated from bean leaves (lower)

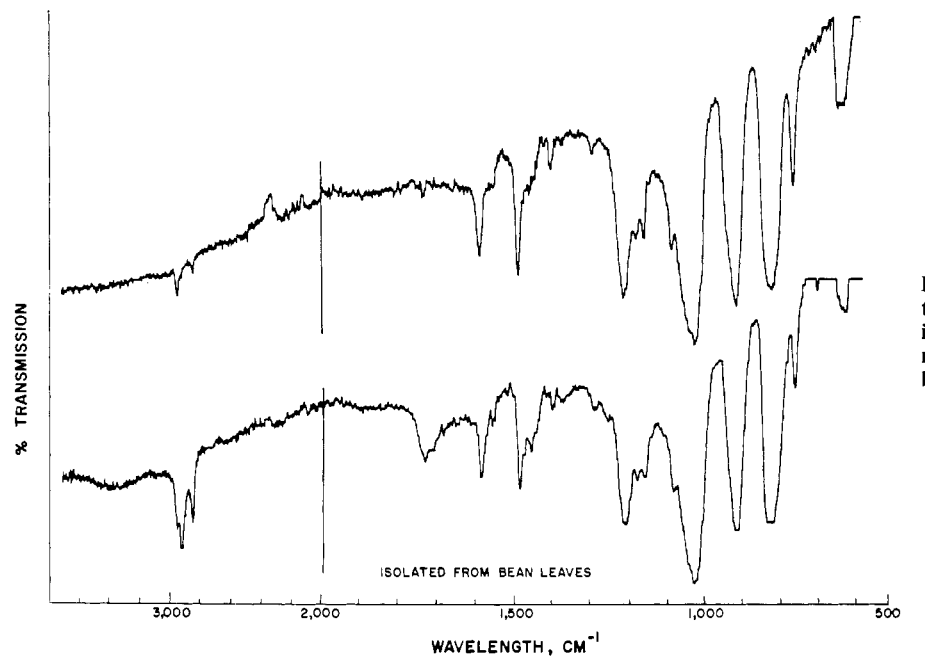


Figure 3. Infrared characteristics for the sulfoxide derivative of Abate insecticide (II, upper) and for a radioactive component isolated from bean leaves (lower)

Table II. Distribution of Radioactivity in Treated Bean Leaves and in Remainder of Bean Plant

Plant Part	% Distribution of Radioactivity, Days after Treatment 10	21	28
Treated leaf			
Methanol extract	94.1	81.6	93.1
Marc	0.8	2.6	3.4
Remainder of plant parts			
Methanol extract	0.002	0.08	...
Marc	0.008	0.08	...
Totals	94.9	84.4	96.5

found at 10, 21, and 28 days post-treatment in the treated leaves and in the remaining portions of the plant are presented in Table II. Certainly there is little, if any, migration of Abate insecticide from the site of application under the conditions that existed for this study. The root and soil portions of the biosystem were not investigated because of this lack of evidence of migration.

Identification of Chloroform-Soluble Metabolites. Chromatography in System 1 of the chloroform-soluble material from the extract of the treated leaves yielded only two radioactive zones, one at the origin and the other at R_f 0.25.

The latter zone proved to be radiochemically pure, since only one radioactive band was found on each chromatogram when this zone was chromatographed sequentially in Systems 2, 3, and 4, and eluted at each step with acetone. After elution from the final chromatogram with acetone, this zone was identified by infrared characterization (Figure 2) and cochromatography as Compound I.

The origin zone, upon elution with acetone and chromatography in System 3, yielded four radioactive bands, the principal one at R_f 0.55. This band was shown to be radiochemically pure by sequential chromatography as before and was identified by infrared characterization (Figure 3) and cochromatography as the sulfoxide derivative (II) of Compound I. The remaining three radioactive zones were eluted together with acetone and identified by cochromatography as the sulfone derivative (III), its unsymmetrical mono-oxono analog (VIII), and the symmetrical dioxono analog (V) of Compound I. There were no additional radiometabolites found in the chloroform-soluble fraction which remained unidentified.

Characterization of Water-Soluble Extractives. The radiometabolites present in the water-soluble fraction were present in such low concentration and were so badly contaminated with tissue extractives that chromatographic isolation for infrared characterization was not possible. However, chromatography in the relatively polar System 9 yielded five to eight ill-defined radioactive zones, none of which corresponded to any of the compounds listed in Table I. Hydrolysis of this fraction in 1*N* HCl at 100° C. for 1 hour, or incubation with β-glucosidase (almond emulsion, Caliochem β-grade) in pH 5.25 acetate buffer for 40 hours at 37° C., resulted in the release of a large part of the radioactivity in a form extractable with chloroform and ether. These radioactive hydrolysis products were shown by cochromatography to be the three phenolic compounds 4,4′-thiodiphenol (VI), 4,4′-sulfinyldiphenol (VII), and 4,4′-sulfonyldiphenol (IV), suggesting that the water-soluble metabolites are glucosidic conjugates of phenols resulting from the partial or complete hydrolysis of Compound I and its sulfoxide and sulfone derivatives.

Degradation Pathways for Abate Insecticide Residues on Bean Leaves. Table III shows the relative amounts of the various metabolites found in the treated leaves 10, 21, or 28 days after treatment, expressed in the percentage of the applied dose due to each metabolite. Absolute separation of certain of the compounds was difficult, so combined percentages are given in the table for these materials.

Abate insecticide residues are resistant to both physicochemical and metabolic degradation; by far the greatest proportion of the radioactivity was found to be the parent compound at all time intervals. The principal organo-

Table III. Relative Amounts of Abate Insecticide and Its Metabolic Products Found on Bean Leaves

Text Designation	X	Y	Z	% of Applied Dosage, Days after Treatment 10	21	28
I	$(CH_3O)_2P(S)$	S	$(CH_3O)_2P(S)$	85.8	67.3	71.5
II	$(CH_3O)_2P(S)$	SO	$(CH_3O)_2P(S)$	8.3	4.9	4.3
III	$(CH_3O)_2P(S)$	SO_2	$(CH_3O)_2P(S)$	[a]	1.1	0.6
V	$(CH_3O)_2P(O)$	S	$(CH_3O)_2P(O)$	[a]		
VI	H	S	H	[a]		
VII	H	SO	H	[a]	8.2	16.7
IV	H	SO_2	H	[a]		
				Totals 94.1	81.5	93.1

[a] Not determined.
[b] Occurred as glucosidic conjugates.

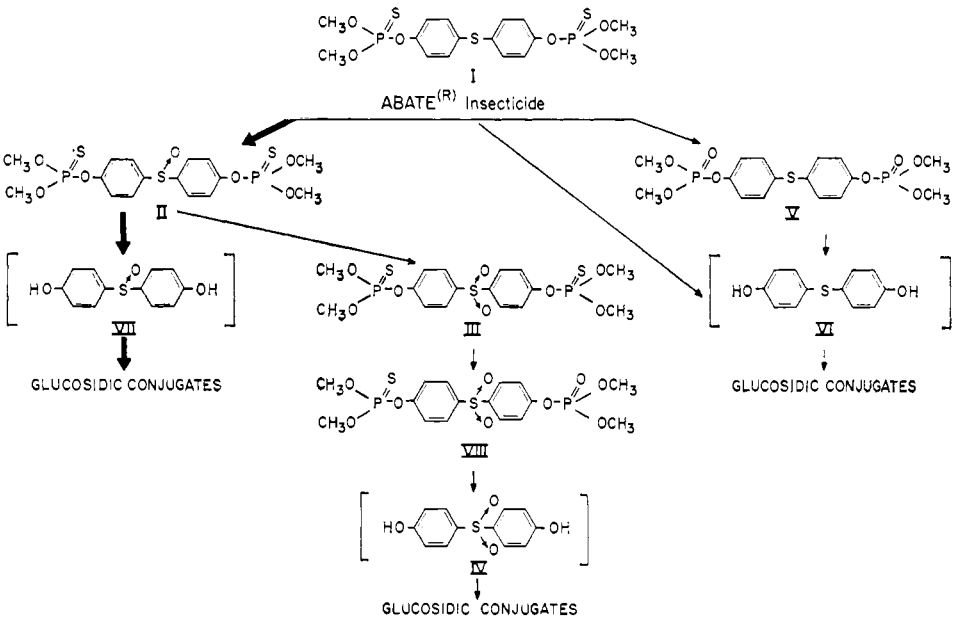


Figure 4. Postulated metabolic pathway for Abate insecticide on bean leaves

phosphorus metabolite was the sulfoxide derivative (II), although small amounts of the sulfone derivative (III) and the dioxono analog (V) were evident. The sulfone of the mono-oxono analog (VIII) was found in extremely small amounts. The water-soluble radioactivity, which appeared to be due primarily to glucosidic conjugates of the hydrolysis products of the parent compound and its sulfoxide and sulfone derivatives, increased gradually with time. However, since chromatography showed this fraction to consist of a number of different compounds, it is likely that no one of them is present in significant quantity, even in the later samples.

Because the plants were placed in a greenhouse with acrylic windows which allow transmission of ultraviolet radiation, degradation of Abate insecticide on and in the bean leaves could be caused by either physicochemical or biological processes. The principal route of degradation of Abate insecticide residues is through oxidation of the sulfide linkage to sulfoxide, followed by hydrolysis of the phosphate ester groups and glucosidic conjugation of the phenolic hydrolysis products. Further oxidation of the sulfoxide to the sulfone takes place to a limited extent before hydrolysis and conjugation. These compounds can also undergo oxidation to the mono or dioxono analogs, although these reactions are of minor importance. Therefore, the scheme presented in Figure 4 is proposed to show the major features of Abate insecticide physicochemical and metabolic degradation on bean leaves.

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