Systemic Activity and Metabolism of Dimethyl *p*-(Methlythio)phenyl Phosphate in Cotton

Lloyd E. Wendel and Don L. Bull

Studies were made of the fate of ^{32}P-labeled dimethyl *p*-(methylthio)phenyl phosphate (Allied Chemical Co. compound GC-6506) in field-grown cotton after different methods of treatment. GC-6506 was oxidized very rapidly in plants to the sulfoxide form and more slowly to the sulfone. The oxidative derivatives were shown to be more active than GC-6506 as anticholinesterase agents, but were slightly less toxic to adult boll weevils, *Anthonomus grandis* Boheman, after topical application. GC-6506 applied to the main stems of cotton plants was absorbed readily and translocated to all parts of the plant except the fruit. The compound also was absorbed rapidly after application to the surfaces of the leaves. The biological half-lives of GC-6506 and its oxidative derivatives inside the plant did not exceed two days, and all toxicants were usually depleted after four weeks. The degradation of toxic compounds involved cleavage of the *O*-methyl and *P*-*O*-phenyl linkages.

The experimental insecticide dimethyl *p*-(methylthio)-phenyl phosphate (Allied Chemical Co. compound GC-6506) has shown promising contact and systemic activity against a broad spectrum of phytophagous pests. Certain aspects of the systemic insecticidal action of this compound and its metabolism in plants have been reported (Metcalf *et al.*, 1964; Reynolds *et al.*, 1966). In addition, similar studies have been made with closely related compounds such as Dasanit, *O*,*O*-diethyl *O*-*p*-[(methylsulfinyl)phenyl] phosphorothioate (Benjamini *et al.*, 1959a,b) and fenthion (Metcalf *et al.*, 1963). The previous work was done either in the laboratory or greenhouse; this report represents a detailed account of the fate of GC-6506 after treatment of cotton in the field under normal conditions.

EXPERIMENTAL

Chemicals. Two batches of ^{32}P-labeled GC-6506, each with an initial specific activity of 10 mc per mmole and better than 99% pure, were purchased from Amersham/Searle Corp., Des Plaines, Ill., and used for all absorption and metabolism studies. Technical GC-6506 (95%) was supplied by Allied Chemical Co., New York, N.Y., and purified by partitioning between water and hexane. The sulfoxide [dimethyl *p*-(methylsulfinyl)phenyl phosphate] and the sulfone [dimethyl *p*-(methylsulfonyl)phenyl phosphate] derivatives of GC-6506 were prepared as follows with a modification of the procedure of Zincke and Ebel (1914). Samples of pure GC-6506 were combined with 1 or 2 molar equivalents of hydrogen peroxide in glacial acetic acid and heated in a boiling water bath for about 1 hr; then the mixture was diluted with distilled water and extracted with chloroform. The chloroform fraction was neutralized with three successive washes of 5% sodium bicarbonate followed by three washes with distilled water. After removal of chloroform under vacuum, the residual oily liquid was taken up in ethyl ether and partitioned three times with water. Any GC-6506 that remained was removed with the ether layer; the water phase contained the major portion of the oxidative derivatives and they subsequently were removed by extraction with chloroform. Following removal of solvent under vacuum, GC-6506-sulfoxide was purified with thin layer chromatography (tlc), and the final product, a light amber liquid, was analyzed. Calcd: C, 40.91; H, 4.96%. Found: C, 40.75; H, 5.10%. The GC-6506-sulfone was recrystallized from chloroform and *n*-hexane, m.p. 68.5–69.5° C. Calcd: C, 38.57; H, 4.68%. Found: C, 38.52; H, 4.49%. The *O*-demethyl derivatives of GC-6506 [methyl *p*-(methylthio)phenyl phosphate], GC-6506-sulfoxide [methyl *p*-(methylsulfinyl)phenyl phosphate], and GC-6506-sulfone [methyl *p*-(methylsulfonyl)phenyl phosphate] were prepared by refluxing the appropriate parent compound in dry acetone for 5 min with an equimolar amount of sodium iodide. The acetone was removed under vacuum, and any residual parent material was removed by partitioning the product between chloroform and water. The sodium salts of *O*-demethyl derivatives were recovered by lyophilizing the aqueous fractions. *O*-demethyl GC-6506 was recrystallized from chloroform, acetone, and hexane, m.p. 213–214° C. Calcd: C, 37.52; H, 3.93%. Found: C, 37.28; H, 4.15%. *O*-demethyl GC-6506-sulfoxide was a highly hygroscopic yellow solid that was purified with tlc, then lyophilized. Calcd: C, 33.11; H, 4.18%. Found: C, 32.63; H, 4.42%. *O*-demethyl GC-6506-sulfone was recrystallized from chloroform, acetone, and hexane, m.p. 233–237° C. Calcd: C, 33.34; H, 3.50%. Found: C, 33.36; H, 3.68%. In addition to these theoretical metabolites, samples of dimethyl and methyl phosphate were obtained from the Shell Development Co., Modesto, Calif. Radioactive derivatives of GC-6506 were prepared as described and purified by solvent partitioning and, if necessary, with tlc.

Plants and Their Treatment. The cotton plants used were Deltapine Smoothleaf variety grown in the field according to customary commercial procedures, except that no insecticides were applied.

Petiole Injections. Individual leaves were injected *in situ* with 10 μl of a solution containing 100 μg of ^{32}P-labeled GC-6506, or one of its derivatives. The solvent for GC-6506 was 4 to 1 water-ethanol with a trace of Triton X-100 (American Cyanamid Co., New York, N.Y.); all other compounds were dissolved in water. For the treatments, finely pointed capillary tubes (1 × 30 mm) were charged with the desired volume of solution, then inserted into the petiole at a point 1.5 cm from the leaf blade. Uptake was rapid and quantitative. After treatment, triplicate samples were harvested and processed at specified intervals through 32 days; tests were replicated three times.

Department of Entomology, Texas A&M University and Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas 77840

Table I. Properties of GC-6506 and Its Derivatives

Compound	Toxicity: LD_{50} (μg/ins)	Toxicity: pI_{50}	Partitioning (Org. Solv./Water): $CHCl_3$	Partitioning: Ethyl Ether	Partitioning: Hexane	R_f Values[a]: A	B	C	D
GC-6506	0.10	6.00	250.00	14.29	5.55	0.88	0.50		
GC-6506-sulfoxide	0.25	6.13	25.00	0.07	0.02	0.10	0.18		
GC-6506-sulfone	0.25	6.44	50.00	0.32	0.01	0.75	0.32		
O-demethyl GC-6506								0.56	0.79
O-demethyl GC-6506-sulfoxide								0.26	0.44
O-demethyl GC-6506-sulfone								0.37	0.61
Dimethyl phosphate								0.16	0.26
Methyl phosphate								0.04	0.03
H_3PO_4								0.00	0.00

[a] Solvent mixtures: A, 4:1 ethyl ether and acetonitrile; B, 90:10:3 benzene, ethanol, and ethyl ether. C and D, 12:8:6 butanol, pyridine, and water. Mixtures A and B were used for tlc on plates coated with silica gel, C for tlc on plates coated with cellulose, and D for paper chromatography.

Foliar Treatments. Each leaf was treated *in situ* with 50 μl of a 50% ethanolic solution containing 100 μg of the appropriate radiolabeled compound. The solution was applied with a micropipette and spread as uniformly as possible over the upper surface of the leaf. Sampling and repetition of tests were the same as described.

Stem Treatments. Young, intact cotton plants were treated just prior to fruiting when they had about eight fully-expanded leaves. A 5-cm band of ^{32}P-labeled GC-6506 (4 mg in 100 μl acetone) was applied topically near the base of the main stem of each plant and the treated area was enclosed immediately with an inverted styrofoam cup to minimize the contamination of foliage by volatilized insecticide. Duplicate treated plants were harvested at definite intervals through 28 days, divided arbitrarily into different parts, and analyzed.

Excised Leaf Treatments. Young mature leaves of uniform size were cut from field-grown cotton plants, and the petioles were immersed immedately in distilled water. The excised leaves were taken to the laboratory and held there 24 hr before they were treated. Each leaf was allowed to imbibe 200 μl of an aqueous solution containing 100 μg of radiolabeled compound from a small test tube (1 × 7.5 cm), then held in distilled water until analyzed at specified intervals through 8 days.

Preparation and Analysis of Treated Materials. The unabsorbed radioactivity on leaves that received the foliar treatments was removed by washing the surfaces thoroughly with methanol. Radioactivity inside treated plant tissue was extracted by homogenization with 80% methanol (25 ml per g) in a blender. Samples were centrifuged, and the precipitated solids extracted two more times in a similar manner with acetone. The supernatants were combined and radioassayed. After removal of organic solvents under vacuum, the remaining aqueous portion was partitioned three times with chloroform, and these latter fractions were combined and dried with anhydrous sodium sulfate. After radioassay, the aqueous and organic fractions were evaporated under vacuum to a convenient volume and analyzed by chromatography. Aqueous extracts were chromatographed on uncoated Whatman 3 mm paper (PC) or on cellulose-coated tlc plates (0.25 mm) by using a solvent mixture of 12:8:6 butanol, pyridine, and water. Organosoluble products were analyzed primarily on tlc plates coated (0.25 mm) with silica gel G (Brinkmann Instruments, Inc., Westbury, N.Y.) by using solvent mixtures of 4 to 1 ether and acetonitrile or 90:10:3 benzene, ethanol, and ethyl ether. Radioactive areas were located by autoradiography, tentatively identified by cochromatography with authentic compounds, and radioassayed. The authentic chloroform-soluble materials (GC-6506, GC-6506-sulfoxide, and GC-6506-sulfone) were located by exposing plates to iodine vapor; water-soluble compounds (all of the other theoretical metabolites prepared) were located with the phosphorus detection reagent of Hanes and Isherwood (1949).

Radioactivity which could not be extracted from treated plant tissue and that in certain plant parts which were not extracted with solvents was determined by using a digestion procedure. Samples were dried in an oven for 24 hours at 50° C, digested in boiling nitric acid (10 ml per g), and radioassayed.

All radioassays were made at ambient temperatures with a liquid scintillator. Appropriate corrections were made for quenching and radioactive decay.

Volatilization From Inert Surface. Cover glasses (2.2 × 2.2 cm) were coated with 20 μl of an acetone solution containing 100 μg of radiolabeled compound and held in a greenhouse. At specified intervals through 8 days, triplicate samples were collected, rinsed with 80% methanol, and radioassayed as described.

Toxicity Studies. The topical toxicities of acetone solutions of GC-6506 and its oxidative derivatives to 5- to 7-day old adult boll weevils, *Anthonomus grandis* Boheman, were determined, and the LD_{50}'s at 72 hr were calculated. The anticholinesterase activity of the same compounds against bovine acetylcholinesterase was determined by the assay procedure of Simpson *et al.* (1964). An inhibition period of 30 min was used with each compound.

RESULTS

Properties of GC-6506 and Its Derivatives. The results in Table I compare the relative toxicities and the partitioning properties of GC-6506, GC-6506-sulfoxide, and GC-6506-sulfone. Also shown are the R_f values for GC-6506 and several of its theoretical phosphorus-containing derivatives. All three aforementioned compounds proved to be potent anticholinesterase agents and also were highly toxic to adult boll weevils treated topically. The partitioning properties of GC-6506 and its oxidative derivatives between water and chloroform, ethyl ether, or hexane were used to great advantage for the initial purification, and later separation of toxic compounds from plant extracts.

Petiole Injection. A comparison of the results of studies of the metabolism of GC-6506 and its two oxidized derivatives is shown in Table II. The GC-6506 was oxidized readily to the sulfoxide form and was essentially depleted in two days. Even in the 0-hr samples, which were usually processed within 15 min of the initiation of treatments, more than half of the recovered radioactivity was in the sulfoxide

Table II. Metabolism of GC-6506 and Its Oxidation Products After Petiole Injection of Individual Cotton Leaves

Nature of Radioactivity	% of Dose at Indicated Days After Treatment						
	0	1	2	4	8	16	32
GC-6506							
GC-6506	41.8	6.3	0.1	0.0	0.0	0.0	0.0
GC-6506-sulfoxide	52.9	46.3	33.0	11.0	3.3	0.9	0.3
GC-6506-sulfone	0.2	1.9	3.7	3.7	1.0	0.2	0.1
H_2O soluble	3.1	14.3	12.4	13.2	12.7	9.6	8.4
Unextractable	2.0	19.1	26.7	29.9	26.2	20.3	15.3
Lost	0.0	12.1	24.1	42.2	56.8	69.0	75.9
GC-6506-Sulfoxide							
GC-6506	0.7	0.4	0.0	0.0	0.0	0.0	0.0
GC-6506-sulfoxide	92.2	65.2	47.6	22.1	2.8	0.8	0.0
GC-6506-sulfone	2.8	4.3	5.6	5.0	2.4	0.2	0.0
H_2O soluble	2.3	7.9	10.1	13.3	12.4	9.0	6.6
Unextractable	2.0	18.0	20.8	29.9	25.8	24.0	10.2
Lost	0.0	4.2	15.9	29.7	56.6	66.0	83.2
GC-6506-Sulfone							
GC-6506	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GC-6506-sulfoxide	4.5	2.4	0.5	0.1	0.0	0.0	0.0
GC-6506-sulfone	92.3	54.6	22.9	5.0	0.4	0.2	0.0
H_2O soluble	1.4	11.1	18.0	16.1	15.1	11.6	8.8
Unextractable	1.8	21.1	29.3	33.3	24.2	20.7	12.6
Lost	0.0	10.8	29.3	45.5	60.3	67.5	78.6

Table III. Metabolism and Translocation of GC-6506 and Its Metabolites After Treatment of Individual Leaves by Petiole Injection

Plant Part	% of Dose as Indicated Product at Two Days After Treatment				
	GC-6506	GC-6506 Sulfoxide	GC-6506 Sulfone	H_2O Soluble	Unextractable
Treated leaves	0.3	40.2	3.4	10.1	23.7
Untreated leaves	0.0	1.8	0.1	0.3	13.3
Terminal growth	0.0	0.0	0.0	0.1	0.1
Fruit	0.0	0.0	0.0	0.1	0.2
Stem	...	...	...	...	3.2
Root	...	...	...	...	1.0

Table IV. Metabolism of GC-6506 in Excised Cotton Leaves

Nature of Radioactivity	% of Dose at Indicated Days After Treatment				
	0	1	2	4	8
GC-6506	67.8	9.6	0.5	0.0	0.0
GC-6506-sulfoxide	30.0	56.9	42.9	29.3	15.8
GC-6506-sulfone	0.0	2.0	2.4	2.5	1.0
H_2O soluble	2.2	11.8	12.3	16.6	23.0
Unextractable	0.0	18.2	38.2	45.4	54.5
Holding container	0.0	1.5	2.8	2.8	2.3
Lost	0.0	0.0	0.9	3.4	3.4

Table V. Absorption and Metabolism of GC-6506 and Its Oxidation Products After Foliar Treatment of Individual Leaves

Nature of Radioactivity	% of Dose at Indicated Days After Treatment						
	0	1	2	4	8	16	32
GC-6506							
External							
GC-6506	83.6	0.2	0.0	0.0	0.0	0.0	0.0
GC-6506-sulfoxide	15.9	2.1	2.0	0.5	0.2	0.0	0.0
GC-6506-sulfone	0.0	0.6	0.8	0.2	0.1	0.0	0.0
H_2O soluble	0.0	0.6	0.3	0.1	0.2	0.4	0.0
Internal							
GC-6506	0.0	0.2	0.0	0.0	0.0	0.0	0.0
GC-6506-sulfoxide	0.4	30.8	12.8	9.4	2.4	0.1	0.2
GC-6506-sulfone	0.0	2.6	2.5	1.3	0.2	0.0	0.0
H_2O soluble	0.0	6.2	6.8	7.3	6.9	5.9	4.7
Unextractable	0.1	13.2	19.1	19.6	20.4	14.5	7.4
Lost	0.0	43.5	55.7	61.6	69.6	79.1	87.7
GC-6506-Sulfoxide							
External							
GC-6506-sulfoxide	95.3	4.0	3.2	0.6	0.2	0.0	0.0
GC-6506-sulfone	3.1	0.5	0.6	0.2	0.1	0.0	0.0
H_2O soluble	0.0	0.2	0.2	0.1	0.2	0.0	0.0
Internal							
GC-6506-sulfoxide	0.3	35.8	30.2	8.2	2.1	0.4	...
GC-6506-sulfone	0.0	3.0	2.9	1.0	0.0	0.0	...
H_2O soluble	1.1	6.4	6.2	12.8	9.5	8.8	...
Unextractable	0.2	12.8	14.5	24.5	17.2	18.1	...
Lost	0.0	37.3	42.2	52.6	70.7	72.7	...
GC-6506-Sulfone							
External							
GC-6506-sulfone	98.5	17.3	14.1	3.9	1.1	0.2	...
H_2O soluble	0.0	0.2	0.5	0.2	0.1	0.3	...
Internal							
GC-6506-sulfone	0.9	30.1	11.4	7.3	1.0	0.2	...
H_2O soluble	0.2	10.1	12.3	15.9	12.9	10.1	...
Unextractable	0.4	11.3	29.4	26.8	23.7	13.6	...
Lost	0.0	31.0	32.3	45.9	61.2	75.6	...

form. Further oxidation to the sulfone occurred, but there was little accumulation of that product, due apparently to slow formation and perhaps rapid decomposition. The water-soluble radioactivity that could be extracted reached maximum concentration during the first week after treatment then declined; however, the amounts of these products recovered were relatively low. No less than 10 compounds, including the five theoretical degradative products, were detected in the aqueous fraction, but there was no tendency for a particular compound to predominate since the radioactivity was almost always fairly evenly distributed among the group. A rapid accumulation of unextractable radioactivity occurred during the first week, then it declined slowly at subsequent times. The buildup probably was the result of incorporation of ^{32}P into insoluble structural components of the plant and the decline at later times to normal turnover within the metabolic pool. Most of the radioactivity that is designated as lost was, in fact, translocated from treated leaves to other parts of the plant. This was demonstrated by a special test involving extraction of the whole plant after treatment of individual leaves by petiole injection. Almost all radioactivity (97.9%) was accounted for, and the bulk of that translocated was found in untreated leaves (Table III). Further, when translocation was circumvented by treating excised leaves with GC-6506, almost quantitative recoveries of the applied radioactivity were obtained (Table IV).

After petiole injections with GC-6506, the apparent bio-

Table VI. Absorption and Metabolism of GC-6506 in Cotton Plants After Surface Applications on the Main Stems of Individual Plants

Nature of Radioactivity	μg GC-6506-Equivalents at Indicated Days After Treatment				
	1	3	7	14	28
Old Leaves					
GC-6506-sulfoxide	342.9	452.2	160.9	12.2	0.0
GC-6506-sulfone	9.6	72.3	88.9	17.0	0.0
GC-6506	25.0	3.3	0.0	0.0	0.0
H_2O soluble	30.3	84.3	117.6	132.9	122.9
Unextractable	62.1	187.4	286.1	393.5	306.0
Total	469.9	799.5	653.5	555.6	428.9
Young Leaves					
GC-6506-sulfoxide	55.2	110.0	56.6	13.0	0.0
GC-6506-sulfone	1.9	13.3	12.8	9.0	0.0
GC-6506	1.4	0.4	0.0	0.0	0.0
H_2O soluble	4.7	40.6	40.3	99.2	67.6
Unextractable	8.6	78.4	128.2	469.2	230.3
Total	71.8	242.7	237.9	590.4	297.9
Terminal Growth					
GC-6506-sulfoxide	5.5	6.6	3.5	0.6	0.0
GC-6506-sulfone	0.3	0.5	0.3	0.0	0.0
GC-6506	0.0	0.0	0.0	0.0	0.0
H_2O soluble	1.1	11.1	19.1	23.8	18.1
Unextractable	1.5	13.5	35.1	58.4	67.6
Total	8.4	31.7	58.0	82.8	85.7
Fruit					
$CHCl_3$ soluble	0.0	0.0	0.0	0.0	0.0
H_2O soluble	0.0	0.0	0.0	9.4	18.8
Unextractable	0.0	0.0	0.0	16.2	73.3
Total	0.0	0.0	0.0	25.6	90.1
Stems (untreated portion)					
Uncharacterized	110.3	84.6	106.7	233.7	349.7
Treated Stem and Roots					
Uncharacterized	2932.7	1966.6	1622.9	879.6	988.6
Lost	406.9	874.9	1321.0	1632.3	1759.1

Table VII. Metabolism of *O*-Demethylated Derivatives of GC-6506, GC-6506-Sulfoxide, and GC-6506-Sulfone in Excised Leaves

Nature of Radioactivity	% of Dose at Indicated Hours After Treatment					
	O-Demethyl GC-6506		*O*-Demethyl GC-6506 Sulfoxide		*O*-Demethyl GC-6506 Sulfone	
	0	24	0	24	0	24
H_3PO_4	4.4	8.8	5.8	11.5	6.9	13.1
Methyl phosphate	0.0	0.5	0.0	0.9	0.8	1.2
O-demethyl GC-6506-sulfoxide	42.5	3.4	35.8	1.1	0.0	0.0
O-dimethyl GC-6506-sulfone	0.0	0.0	0.0	0.4	33.4	0.9
O-dimethyl GC-6506	13.7	1.5	0.0	0.0	0.0	0.0
Unknown 2	0.0	0.0	0.0	1.1	0.0	0.0
Unknown 3	0.0	0.0	0.0	2.2	0.0	1.0
Unextractable	39.4	80.9	58.4	79.1	58.9	78.3
Lost	0.0	4.9	0.0	3.7	0.0	5.5

Table VIII. Volatilization of GC-6506, GC-6506-Sulfoxide, and GC-6506-Sulfone From a Glass Surface

Nature of Radioactivity	% of Dose at Indicated Days After Treatment					
	0	1/3	1	2	4	8
GC-6506						
$CHCl_3$	93.6	58.6	47.5	34.5	26.9	19.8
Water soluble	6.4	8.5	9.6	14.3	20.2	21.1
Lost	0.0	32.9	42.9	51.2	52.9	59.1
GC-6506-Sulfoxide						
$CHCl_3$ soluble	99.9	87.4	84.5	82.3	71.4	59.9
Water soluble	0.1	5.8	7.4	9.4	18.1	24.8
Lost	0.0	6.8	8.1	8.3	10.5	15.3
GC-6506-Sulfone						
$CHCl_3$ soluble	96.7	87.1	83.7	82.3	80.4	77.3
Water soluble	3.3	12.9	13.0	14.2	15.9	18.7
Lost	0.0	0.0	3.3	3.5	3.7	4.0

logical half-life for it and its toxic metabolites was slightly longer than 1 day. In leaves treated with GC-6506-sulfoxide, the metabolism was somewhat similar to that described (Table II). There was very little further oxidation and subsequent accumulation of the sulfone, and the half-life for toxicants was about two days. Similarly with GC-6506-sulfone treatments, the half-life was between 1 and 2 days (Table II).

Foliar Treatments. The absorption, chemical changes on leaf surfaces, and metabolism of absorbed GC-6506, GC-6506-sulfoxide, and GC-6506-sulfone after foliar treatments are compared in Table V. Concentrations of GC-6506 and GC-6506-sulfoxide remaining on the surfaces of leaves were reduced to less than 5% of the dose after 1 day and were essentially depleted after 2 days. The GC-6506-sulfone was somewhat more persistent on leaf surfaces, requiring about 2 weeks for depletion. The amounts of each compound absorbed by the leaves appeared to be somewhat similar, but were difficult to estimate with accuracy owing to the continual translocation of absorbed radioactivity. Once in the leaf, the metabolism was similar to that reported for petiole injections. On leaf surfaces, there was appreciable oxidative conversion of GC-6506 to the sulfoxide form but very little further oxidation of the sulfoxide to the sulfone. The degradation of all compounds to nontoxic products on leaf surfaces was minimal.

Stem Treatments. As much as 37.2% (after 14 days) of the applied dose penetrated the stem and was translocated above the site of treatment (Table VI). The highest concentrations of radioactivity always were found in foliage that was present at the time plants were treated; however, substantial amounts (including toxic products) also were found in leaves that developed after treatment. Toxic compounds were never found in the fruit and were absent from all foliage after 28 days.

The detection of small concentrations of *O*-demethyl derivatives of GC-6506, GC-6506-sulfoxide, and GC-6506-sulfone in most extracts prompted a study of their metabolic stability in order to assess their importance to the overall metabolic pathway. Results of studies of the fate of these compounds in excised leaves are shown in Table VII. All three materials were extremely unstable in leaves; even in the 0-hr samples, usually processed within 30 min of the initiation of treatments, more than half of the parent material was decomposed to simpler fragments; after 24 hr, all were essentially depleted. Complete degradation to inorganic phosphate was suggested by the very rapid buildup of unextractable radioactivity.

Volatilization from Inert Surface. Results shown in Table VIII compare the rates at which GC-6506 and its two oxidative derivatives were lost from a glass surface in the greenhouse. The GC-6506 volatilized much more rapidly than either of the other compounds, and only negligible concentrations of the sulfone were lost. All three com-

pounds were degraded about equivalent rates, to the extent of approximately 20% of the dose after 8 days.

DISCUSSION

The observation that the anticholinesterase activity of GC-6506 was increased appreciably upon oxidation to the sulfoxide form, and even more after further oxidation to the sulfone, was not unexpected since similar results were reported for the diethyl-substituted analog of GC-6506 (Fukuto and Metcalf, 1956). That the topical toxicity of GC-6506 to boll weevils was greater than either the sulfoxide or sulfone can probably be attributed to the differences in the rates of penetration of the insect cuticle and/or degradation of absorbed toxicant. As pointed out by Metcalf *et al.* (1964), the increased reactivity of the sulfoxide and sulfone forms also should result in more rapid hydrolytic decomposition.

After topical treatments of the stems of cotton plants with GC-6506, substantial portions of the dose, including toxic materials, were absorbed and translocated throughout most of the plant; thus, the compound apparently can function as a true systemic insecticide. However, since toxic materials did not accumulate in the fruit, the insects that feed primarily on squares and bolls might not be controlled through systemic action. After foliar application, GC-6506 was absorbed rapidly through the leaf cuticle; therefore, this type of treatment would provide both contact and local systemic insecticidal activity. Owing to rapid absorption and volatilization, the residual life of GC-6506 on the surface of foliage is very short. The combined biological data indicate that GC-6506 and its sulfoxide and sulfone derivatives all are short-lived toxicants that probably would present no serious problems with respect to toxic residues, either internally or on the surface of cotton foliage.

The metabolism studies demonstrated that the oxidation of GC-6506 to the sulfoxide form in plants was an extremely rapid reaction much like that reported for other organophosphate (Bull, 1967; Metcalf *et al.*, 1957) or carbamate (Bull, 1968; Metcalf *et al.*, 1966) insecticides containing thioether groups. Some further oxidation to the sulfone derivative occurred, but at a greatly reduced rate. That GC-6506-sulfoxide is highly toxic and formed so rapidly suggests that it may be the primary toxicant associated with the GC-6506 treatments.

The initial decomposition of the toxic compounds involved cleavage at both the *O*-methyl and *P-O*-phenyl linkages. The failure of any nontoxic metabolite to accumulate and the rapid increase in unextractable radioactivity indicated that decomposition products also were somewhat unstable and readily degraded to simple fragments, including inorganic phosphate. The fate of phenolic products formed during the decomposition of GC-6506 and its derivatives is under investigation and will be reported later.

LITERATURE CITED

Benjamini, E., Metcalf, R. L., Fukuto, T. R., *J. Econ. Entomol.* **52,** 94 (1959a).
Benjamini, E., Metcalf, R. L., Fukuto, T. R., *J. Econ. Entomol.* **52,** 99 (1959b).
Bull, D. L., *J. Econ. Entomol.* **60,** 332 (1967).
Bull, D. L., *J. Econ. Entomol.* **61,** 1598 (1968).
Fukuto, T. R., Metcalf, R. L., J. AGR. FOOD CHEM. **4,** 930 (1956).
Hanes, C. S., Isherwood, F. A., *Nature* **164,** 1107 (1949).
Metcalf, R. L., Fukuto, T. R., March, R. B., *J. Econ. Entomol.* **50,** 338 (1957).
Metcalf, R. L., Fukuto, T. R., Winton, M. Y., *Bull. World Health Organ.* **29,** 219 (1963).
Metcalf, R. L., Reynolds, H. T., Fukuto, T. R., Collins, C., *J. Econ. Entomol.* **57,** 531 (1964).
Metcalf, R. L., Fukuto, T. R., Collins, C., Borck, K., Burk, J., Reynolds, H. T., Osman, M. F., J. AGR. FOOD CHEM. **14,** 579 (1966).
Reynolds, H. T., Metcalf, R. L., Fukuto, T. R., *J. Econ. Entomol.* **59,** 293 (1966).
Simpson, D. R., Bull, D. L., Lindquist, D. A., *Ann. Entomol. Soc. Amer.* **57,** 367 (1964).
Zincke, T., Ebel, C., *Ber.* **47,** 1100 (1914).

Received for review November 24, 1969. Accepted January 23, 1970. This work was done in cooperation with Texas A&M University, Texas Agricultural Experiment Station, College Station, Texas, and supported in part by Allied Chemical Co., New York, N.Y. Mention of a pesticide or a proprietary product does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.