(hydantoin) and 3-(2-imidazolin-2-yl)-2-imidazolidinethione (Jaffe's base) seem to be present in several extracts, but further confirmation is necessary. At least seven and possibly 10 distinct radioactive areas have been observed on TLC plates from methanol extracts of ETU- and EBDC-fungicide treatments. The TLC pattern was similar, regardless whether ETU and EBDC fungicides were sprayed onto the soybean leaves or injected into the soil. The products were probably a mixture of those obtained by simply exposing the fungicides to air (Czegledi-Janko, 1967), metabolic degradation (Sijpesteijn and Kaslander, 1964; Vonk and Sijpesteijn, 1971), and possibly photodegradation (Cruickshank and Jarrow, 1973; Ross and Crosby, 1973). Engst and Schnaak (1974) have proposed a degradation pathway that results in five end products: 2-imidazoline, ethylenebis(isothiocyanate) sulfide (EBIS), ethylenebis(thiocyanate), EU, and ED. The 2-imidazoline is an end product from both the EBDC, with ETU as an intermediary, and ETU. Recently, Newsome et al. (1975) have found *N,N'*-dimethylene-5-imino-1,2-dithia-4-azolidine-3-thione (DIDAT) or 5,6-dihydro-3*H*-imidazo-[2,1-*c*]-1,2,4-dithiazole-3-thione (DIDT), formerly known as ethylenethiuram monosulfide (ETM), to be an important degradation product in addition to smaller amounts of ED.

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Fate of *O*-Ethyl *O*-[4-(Methylthio)phenyl] *S*-Propyl Phosphorodithioate (BAY NTN 9306) in Cotton Plants and Soil

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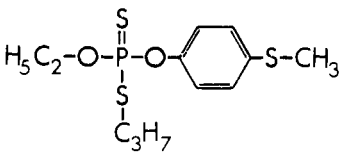
About half the dose of ^{14}C- or ^{32}P-labeled BAY NTN 9306 (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate) applied to the leaves of cotton plants was absorbed during the first 24 h; surface and internal residues of the insecticide and its toxic derivatives diminished to insignificant levels after 8 and 32 days, respectively. The principal alteration products of BAY NTN 9306 found in different tests with plants, soil, and water were the toxic sulfoxide and sulfone derivatives formed by oxidation of the ethereal sulfur and the respective substituted phenols, both free and conjugated, produced by hydrolysis of the organophosphorus esters.

The experimental insecticide BAY NTN 9306 (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate)



is being developed for use in controlling phytophagous lepidopteran insects, especially *Heliothis* sp., that attack cotton. Previous reports by Bull and Ivie (1976) and Ivie et al. (1976) described in detail the metabolic fate of BAY NTN 9306 in white rats and a lactating dairy cow. The present paper reports additional studies of the fate of the

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chemical after application to cotton plants, soil, and aqueous media.

EXPERIMENTAL SECTION

Chemicals. Samples of BAY NTN 9306 (hereafter designated 9306) labeled with ^{32}P (initial specific activity 2.18 mCi/mmol) or uniformly labeled with ^{14}C in the phenyl ring of the molecule (specific activity 7.06 mCi/mmol) were provided by Chemagro Agricultural Division, Mobay Chemical Corp., Kansas City, Mo. The radiochemical purity of samples was >95%, as determined by thin-layer chromatography (TLC). Also, nonradioactive, theoretical metabolites were provided for use as analytical standards; these chemicals were the same as those described by Bull and Ivie (1976).

Enzyme Inhibition Study. A colorimetric procedure (Simpson et al., 1964) was used to assess the in vitro inhibitory activity of 9306 and its potentially toxic oxidation products against mammalian (bovine erythrocyte; Sigma Chemical Co., St. Louis, Mo.) and insect (homogenates of heads of the house fly, *Musca domestica* L.) acetylcholinesterase (AChE). Concentrated stock solutions of the toxicants were made with absolute ethanol and stored at −10 °C; these were diluted to the desired concentration range with buffer just before each analysis. The AChE solutions were preincubated with the inhibitors for 30 min, then the substrate solution (acetylcholine bromide) was added and the remaining enzyme activity was measured during an additional 30-min incubation period. All measurements were made at 37 °C, and each treatment was replicated three times.

Plant Studies. Cotton plants of the Stoneville 213 variety were grown in the field with the customary procedures, except that no insecticides were applied.

Fully expanded, individual leaves were treated in situ with 100 μg of ^{32}P- or ^{14}C-labeled 9306 in 100 μl of ethanol. These solutions were applied manually with a micropipet and spread as uniformly as possible over the upper surface of the leaf. At the specified times, triplicate samples of treated leaves were collected, and the unabsorbed radioactivity (external rinse) was recovered by washing the leaves thoroughly with methanol. Radioactivity within the rinsed leaves (internal extract) was extracted by homogenization (1 g fresh weight per 25 ml) with a solution of acetone and water (9:1, v/v) in a semimicroblender (Eberbach Corp., Ann Arbor, Mich.). Solids were removed by centrifugation and then extracted similarly two more times. Extracted plant material was dried 24 h at 40 °C and ground to a fine powder with a mortar and pestle; then triplicate subsamples were analyzed for unextractable radiocarbon by oxygen combustion as described by Bull and Ivie (1976) or for radiophosphorus after digestion in nitric acid (Wendel and Bull, 1970). Samples of external rinses and internal extracts were radioassayed and then the methanol or acetone was removed under vacuum (10 ml of water was added to external rinses prior to evaporation). The remaining aqueous portions of these samples were extracted three times with chloroform (1:2.5, v/v); centrifugation was used to break emulsions and separate the fractions. Organic extracts were combined and dried over anhydrous sodium sulfate; then both fractions were radioassayed, evaporated to a convenient volume, and analyzed by TLC.

A special study was conducted in the greenhouse to determine whether absorbed radioactivity was translocated from leaves after foliar application of ^{14}C-labeled 9306. For this, two potted cotton plants were allowed to develop until the bolls were just beginning to form; then each leaf was treated with 100 μg of 9306 as described. After 32 days, the plants were harvested and divided into subsamples including: new foliar growth, stem and root, individual treated leaves, bract and calyx of bolls, and hulls and meat of seeds. These samples were dried thoroughly at 40 °C, weighed, ground in a Wiley mill (A. H. Thomas Co., Philadelphia, Pa.), and then analyzed for radioactivity by oxygen combustion.

Soil Studies. Soils used for the tests included Lufkin fine sandy loam and construction sand; some of their characteristics were reported by Bull et al. (1970). Radiocarbon-labeled 9306 was mixed with each soil at a level of 2.5 ppm. Weighed samples (20 g each) were held in glass containers at different conditions; moisture levels of some samples were adjusted to 5% and, if held in open containers, maintained with periodic additions of water. Prior to treatment with ^{14}C-labeled 9306, a portion of the loam was sterilized by heat (100 °C) for 72 h and then by repeated autoclave treatments. Also, certain of the treated samples of sand were sealed under nitrogen in glass ampules.

At specified times, triplicate samples were collected and extracted twice with a mixture of acetone and methanol (1:1, v/v) and a third time with a mixture of chloroform and acetonitrile (1:1, v/v). For each extraction, the soil was first wet with 5 ml of water and then combined with the solvent mixture (1:5, w/v) in a glass-stoppered flask and agitated vigorously for 1 h with a wrist-action shaker (Burrell Corp., Pittsburg, Pa.). The three extracts were combined, radioassayed, and filtered, and then organic solvents were removed under vacuum and the remaining aqueous portion was extracted with chloroform. Unextractable radiocarbon was determined by combusting triplicate samples (0.5 g) of air-dried soil in an oxygen atmosphere at 1000 °C as described by Bull et al. (1970).

Buffer Study. Buffered solutions (0.05 M) were prepared to provide a pH sequence of 3 (citrate), 7 (phosphate), and 11 (carbonate). ^{14}C-Labeled 9306 (50 μg each) was added to glass ampules, and the organic solvent was evaporated with a gentle stream of dry nitrogen. The respective buffers (5 ml each) were added to the ampules, and they were sealed with an oxygen torch and held at 40 °C. Samples were agitated daily, and triplicate samples of each buffer treatment were collected at specified times, chilled to near freezing, and opened, and then solutions were transferred quantitatively to glass-stoppered flasks with 25 ml of ethyl acetate. These mixtures were shaken vigorously for 30 min, and then the organic and aqueous phases were separated, radioassayed, and analyzed by TLC as described.

Simulated Pond Study. Six liters of water (pH 6.8) contained in a glass battery jar (15 × 45 cm) was treated with ^{14}C-labeled 9306 at a level of 0.1 ppm. After the solution was thoroughly mixed, a number of 10-ml beakers, each containing 5 g of moist Lufkin fine sandy loam, were placed at the bottom of the container. The "pond" was then exposed to field conditions by burying the container at a depth that positioned the surface of the water even with that of the surrounding soil. At the specified times, triplicate samples (20 ml each) of the water were collected, radioassayed, extracted with chloroform, and analyzed by TLC. At the same time, beakers of silt were recovered and analyzed as described for soil studies. The container was covered during periods of rainfall and losses of water by evaporation were determined at each sample time.

Analytical Procedures. Quantitative and qualitative analyses of different extracts prepared in all studies were done with TLC by using glass plates that were coated (0.25 mm thick) manually or that were obtained precoated with

Table I. Anticholinesterase Activity of 9306 and Its Toxic Metabolites

Compound	Concn (M) for 50% inhibition of AChE[a]	
	Bovine erythrocyte	House fly head
9306	$>4.4 \times 10^{-4}$[b]	3.5×10^{-4}
9306 sulfoxide	7.4×10^{-5}	9.7×10^{-7}
9306 sulfone	1.9×10^{-4}	2.2×10^{-5}
O-Analogue	1.1×10^{-4}	7.5×10^{-7}
O-Analogue sulfoxide	8.0×10^{-6}	2.3×10^{-8}
O-Analogue sulfone	8.8×10^{-7}	2.5×10^{-9}

[a] AChE concentration: bovine (18 μg/ml), house fly (0.4 head/ml). [b] No inhibition at indicated concentration.

silica gel (Silplate F-22, 0.25 mm thick; Brinkmann Instruments, Westbury, N.Y.). Samples were chromatographed in two dimensions with different combinations of solvent mixtures: (A) 9:4:1 heptane, chloroform, and methanol; (B) 6:3:2 chloroform, hexane, and glacial acetic acid; (C) 6:3:2 chloroform, hexane, and acetone; and (D) 12:8:6 butanol, pyridine, and water. Identifications were based on the coincidence of radioactive areas, located by exposing plates to x-ray films, with authentic standards that were detected under ultraviolet light.

In some cases, water-soluble radioactivity associated with the plant studies was lyophilized, and subsamples were incubated with β-glucosidase as described by Bull and Stokes (1970) or hydrolyzed with 2 N hydrochloric acid for 1 h in a boiling water bath and then extracted with chloroform. Acid treatments were also used in attempts to characterize radioactivity bound by soils.

Radioactive measurements of different extracts and radioactive areas scraped from TLC plates were made by liquid scintillation counting. Appropriate corrections for radiophosphorus decay were made, and quenching was corrected by external or internal standardization techniques.

Plant and soil samples designated O in the following discussion were handled the same as all samples, and processing was initiated within 1 h after treatment.

RESULTS AND DISCUSSION

The relative R_f values for 9306 and its derivatives can be found in a previous report (Bull and Ivie, 1976). However, it should be mentioned that the appropriate use of the different solvent systems allowed definite resolution on TLC of all the compounds studied.

Enzyme Inhibition Study. The molar concentrations of 9306 and certain of its metabolites required to effect 50% inhibition of mammalian and insect AChE are shown in Table I. There was no inhibition of mammalian AChE by 9306 even at a very high concentration. This result was anticipated because the pure form of this type of organophosphorus insecticide is typically a poor inhibitor (O'Brien, 1960). That there was some inhibition of house fly AChE by 9306 suggests there may have been some oxidative activation during the incubation period by other enzymes present in the crude homogenates. All the oxidation products of 9306 proved to be inhibitors of AChE and as expected the O-analogue sulfone was most potent. In all comparisons, the insect AChE was substantially more susceptible to inhibition than the mammalian enzyme. This evidence, coupled with that which indicated 9306 and its toxic metabolites were rapidly detoxified in mammals (Bull and Ivie, 1976; Ivie et al., 1976), helps explain the observed selectivity of the insecticide.

Plant Study. Although conducted at different times of the growing season with plants of different maturity, there was good agreement between the studies with ^{32}P- and ^{14}C-labeled 9306.

Table II. Absorption and Metabolism of ^{32}P-Labeled 9306 in Cotton Leaves after Foliar Treatments[a]

Nature of radioact.	% applied dose at indicated days posttreatment						
	0	1	2	4	8	16	32
External Rinse							
Unknown(s) 1[b]	0.0	0.6	0.5	0.0	0.0	0.0	0.0
Unknown(s) 2[c]	0.0	5.4	5.9	8.2	5.4	3.2	0.3
9306	81.5	12.3	0.9	0.0	0.0	0.0	0.0
9306 sulfoxide	2.6	13.5	12.5	10.2	0.0	0.0	0.0
9306 sulfone	2.5	7.6	8.3	2.3	5.2	2.9	0.0
Internal Extract							
Unknown 1[b]	0.0	1.1	1.4	1.3	1.5	1.2	0.4
Unknown 2[c]	0.1	0.8	1.6	3.6	2.6	3.0	2.3
9306	7.3	8.5	3.0	0.4	0.1	0.0	0.0
9306 sulfoxide	4.5	10.0	11.1	6.3	4.0	3.9	3.9
9306 sulfone	0.0	1.3	3.8	3.3	2.4	2.5	1.7
O-Analogue sulfone	0.5	0.8	1.1	0.4	0.0	0.3	0.3
Water soluble	1.0	3.1	5.9	9.8	10.0	7.2	6.9
Unextractable	0.0	4.3	9.3	13.6	14.0	14.2	10.1
Lost	0.0	30.7	34.7	40.6	54.8	61.6	74.1

[a] Averages of six replicates; tests initiated mid-June, 1974. [b] Unidentified organosoluble radioactivity. [c] Radioactivity that remained near origin of TLC plate.

Table III. Absorption and Metabolism of ^{14}C-Labeled 9306 in Cotton Leaves after Foliar Treatment[a]

Nature of radioact.	% of applied dose at indicated days posttreatment					
	0	1	2	4	8	16
External Rinse						
Unknown(s) 1[b]	0.1	3.9	2.8	2.8	0.7	0.1
9306	93.1	2.2	1.7	0.4	0.0	0.0
9306 sulfoxide	3.7	13.7	7.7	2.2	0.2	0.2
9306 sulfone	0.0	4.1	2.8	1.2	0.2	0.1
O-Analogue sulfone	0.0	0.2	0.3	0.2	0.0	0.0
Phenol sulfoxide	0.4	0.8	0.5	0.1	0.0	0.0
Phenol sulfone	0.0	0.7	0.4	0.2	0.1	0.0
Internal Extract						
Unknown(s) 1[b]	0.0	1.6	3.6	4.7	3.9	2.9
9306	1.6	17.7	10.8	1.2	0.1	0.0
9306 sulfoxide	0.7	15.6	16.2	12.1	7.4	6.1
9306 sulfone	0.2	5.4	2.9	3.4	2.1	2.2
O-Analogue sulfone	0.0	0.3	1.0	1.6	1.2	0.4
Phenol sulfoxide	0.0	0.7	0.6	0.4	1.1	0.7
Phenol sulfone	0.0	1.2	1.5	1.6	0.4	0.2
Water soluble	0.0	8.0	13.9	25.9	31.6	33.9
Unextractable	0.2	2.3	3.9	5.6	6.2	7.2
Lost	0.0	21.6	29.4	36.4	44.8	46.0

[a] Averages of six replicates; tests initiated mid-July, 1974. [b] Radioactivity that remained near origin of TLC plate.

After foliar treatment of individual leaves, about half the dose of radiolabeled 9306 was absorbed, mostly during the first day (Tables II and III). Radioactive residues were recovered from surfaces of treated leaves throughout the 16- or 32-day studies, but toxic compounds were essentially depleted in 8 days. Compounds recovered from leaf surfaces and tentatively identified included for the most part 9306, 9306 sulfoxide (*O*-ethyl *O*-[4-(methylsulfinyl)phenyl] *S*-propyl phosphorodithioate), and 9306 sulfone (*O*-ethyl *O*-[4-(methylsulfonyl)phenyl] *S*-propyl phosphorodithioate). Minor concentrations of the O-analogue sulfone (*O*-ethyl *O*-[4-(methylsulfonyl)phenyl] *S*-propyl phosphorothioate), phenol sulfoxide [*p*-(methylsulfinyl)phenol], and phenol sulfone [*p*-(methylsulfonyl)phenol] were also detected. In both studies, radioactivity designated as unknown(s) 1 included that which remained or streaked a short distance from the

origin of TLC plates. (A separate and more detailed study of the photodecomposition of 9306 on foliar and inert surfaces will be reported later.) Radiophosphorus designated unknown(s) 2 moved with the solvent front of TLC.

Internal extracts of treated leaves were found to include the same radioactive compounds detected in external rinses (Tables II and III). The principal metabolites of 9306 were the toxic oxidation products 9306 sulfoxide and (to a lesser extent) 9306 sulfone; concentrations of these built up early after treatment and then declined slowly with time. Small concentrations of O-analogue sulfone were detected, but these did not tend to accumulate. No O-analogue (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorothioate) or O-analogue sulfoxide (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorothioate) was detected in treated leaves. This was anticipated in the case of the former because it is well established with organophosphorus compounds that oxidation of the thioether sulfur occurs more readily than desulfuration of the thiono sulfur (Bull, 1972a).

The water-soluble radioactivity in extracts of plants treated with ^{14}C-labeled 9306 tended to accumulate throughout the test period and was the major radioactive fraction after the fourth day (Table III). None of this radioactive material corresponded on TLC plates with the phenols or with any available phosphorus-containing analytical standards, including the deethylated derivatives of 9306 or the O-analogue, but some did cochromatograph with β-[*p*-(methylthio)phenyl] D-glucoside (prepared as described by Bull and Stokes, 1970). Furthermore, when water-soluble fractions were pooled, lyophilized, and then heated in acid, there was a near quantitative (>95%) conversion of the radioactivity to a form that partitioned into chloroform. Analysis of this fraction with TLC revealed that it consisted entirely of the three free substituted phenols in the proportions 23% phenol sulfide [*p*-(methylthio)phenol], 41% phenol sulfoxide, and 36% phenol sulfone. Treatment of the fraction with β-glucosidase led to the conversion of as much as 42% to an organosoluble form; this too consisted entirely of the same three phenols. These results suggest that a major portion and perhaps all the water-soluble radioactivity is in the form of glycosidic conjugates of the substituted phenols that are liberated by cleavage of the molecules of 9306 or its phosphorus-containing oxidation products. However, it also is possible that small amounts of other water-soluble metabolites, such as deethylated derivatives of other oxidative derivatives of 9306, may have been present in these extracts and were not resolved with the TLC procedures used.

No attempt was made to characterize the radioactivity in the water-soluble fraction of leaves treated with ^{32}P-labeled 9306; this reached maximum relative concentration (10%) after 8 days and then declined at subsequent times. The results of these two studies are comparable to those found in reports of similar work (Bull, 1972b). That is, some of the phosphorus-containing products formed during metabolism apparently are of sufficient water solubility that they move with the sap stream, or perhaps are degraded to simple fragments that enter the metabolic pool; this could account for the observed lack of accumulation of radiophosphorus in the aqueous fraction. On the other hand, it is well established (Harborne, 1964) that phenolic compounds are readily incorporated into glycosidic conjugates in plants and that, once formed, there is very little translocation from the tissues in which they are synthesized (Miller, 1940). Such a result could account for the progressive accumulation of water-soluble radiocarbon

Table IV

Plant part	Av dry wt, g	Radioact., % of dose
Stem and root	2.14	0.10
Boll (bract and calyx)	1.72	0.10
Boll (seedhull)	1.11	0.03
Boll (seedmeat)	1.38	0.03
Treated leaves	3.12	72.90
New leaves	1.21	0.67

Table V. Fate of ^{14}C-Labeled 9306 after Treatment (2.5 ppm) of Lufkin Fine Sandy Loam and Construction Sand[a]

Nature of radioact.	% of applied dose at indicated weeks posttreatment					
	0	0.5	1	2	4	8
	Sand					
Unknown(s) 1[b]	0.3	1.1	1.4	2.2	2.1	3.8
9306	92.0	76.3	73.2	58.5	52.9	23.4
9306 sulfoxide	6.7	19.6	19.6	20.0	21.6	35.7
9306 sulfone	0.2	1.1	0.9	1.8	1.6	1.3
O-Analogue sulfone	0.0	0.0	0.0	0.0	0.0	1.3
Phenol sulfoxide	0.7	1.8	3.1	4.6	6.9	11.5
Phenol sulfone	0.1	0.4	0.3	0.5	0.8	0.9
Unextractable	0.0	0.2	5.3	6.5	7.6	10.1
Lost	0.0	0.0	0.0	5.9	6.5	12.0
	Loam					
Unknown(s) 1[b]	0.4	0.7	0.8	0.7	0.5	0.5
9306	83.6	50.1	41.9	34.4	20.9	4.2
9306 sulfoxide	14.5	44.8	51.1	59.3	64.5	79.2
9306 sulfone	0.3	0.5	0.6	1.0	1.0	0.9
O-Analogue sulfone	0.0	0.0	0.0	0.0	0.0	1.6
Phenol sulfoxide	0.8	1.2	1.0	1.7	1.6	3.6
Phenol sulfone	0.0	0.7	0.5	0.6	0.7	0.4
Unextractable	0.4	2.6	3.0	4.6	5.4	0.8
Lost	0.0	0.0	1.1	0.0	5.4	8.8

[a] Averages of three replicates; soils held in open glass containers at 27 °C. [b] Radioactivity that remained near origin of TLC plate.

in studies with ^{14}C-labeled 9306, and it also would lend support to the contention that phenolic conjugates were the major constituents involved.

The results obtained in studies of the translocation of radioactivity from leaves treated with a foliar application of ^{14}C-labeled 9306 could be misleading because the growth and development of plants in the greenhouse are considerably slower than in the field. Nonetheless, the data shown in Table IV indicate that there was little translocation of radioactivity from treated leaves in these tests. Thus, the losses of radioactivity reported here and in Table III may be attributable primarily to volatilization unabsorbed materials from foliar surfaces. The most active plant growth subsequent to treatment was in the fruiting structures. Since this is totally unlike the growth pattern of cotton in the field, the detection of radioactive residues in seeds, and their levels, may not be representative of that which occurs in normal production.

Soil Studies. The ^{14}C-labeled 9306 applied to soils was degraded by oxidation and hydrolysis to the same radioactive products detected in the plant studies (Tables V and VI). Again the principal phosphorus-containing products were 9306 sulfoxide and 9306 sulfone; only minor concentrations of the O-analogue sulfone were found. The free phenol sulfoxide and phenol sulfone were found in all treatments as were small concentrations of unclassified polar materials [unknown(s) 1] that remained at or near the origins of TLC. For the most part, the recoveries of applied radioactivity were quite good, and there was little accumulation of unextractable radioactivity.

In tests with soils treated and held in open containers at room temperature (Table V), there was substantially

Table VI. Fate of ^{14}C-Labeled 9306 after Treatment (2.5 ppm) of Lufkin Fine Sandy Loam and Construction Sand and Held under Different Conditions[a]

Nature of radioact.	% of applied dose at indicated weeks posttreatment					
	1	2	4	1	2	4
	Sand, Anaerobic			Loam, Sterile		
Unknown(s) 1[b]	0.9	1.4	1.6	0.4	0.6	0.6
9306	82.6	81.0	73.2	58.8	48.1	40.5
9306 sulfoxide	9.2	9.2	15.4	37.2	49.0	53.5
9306 sulfone	1.2	1.2	1.3	1.0	0.8	0.7
O-Analogue sulfone	0.0	0.3	0.0	0.3	0.8	0.6
Phenol sulfoxide	1.7	2.5	3.2	0.4	0.3	0.7
Phenol sulfone	0.7	1.0	0.1	1.7	0.4	0.4
Unextractable	3.9	5.0	5.7	1.6	2.0	2.8

[a] Averages of three replicates at 27 °C. [b] Radioactivity that remained near origin of TLC plate.

more oxidative conversion of 9306 to 9306 sulfoxide in loam than in sand. This initial oxidation is a facile reaction, and the apparent faster rate probably is a function of the greater surface area for exposure offered by the more finely divided particles of loam. In either case, further oxidation in air proceeded very slowly as did degradation to nontoxic derivatives; in these conditions, substantial concentrations of 9306 and phosphorus-containing derivatives still remained after 8 weeks.

As might be anticipated, the oxidative and other chemical changes of 9306 in sand held in anaerobic conditions were relatively small (Table VI). In loam sterilized prior to treatment (Table VI), the fate of 9306 was not too different from that observed in nonsterile loam (Table V). This latter result indicates that microbial degradation was not a significant factor under these conditions.

Most of the radioactivity that remained bound to soils after solvent extraction could be recovered with an acid treatment. For example, when some of the sand samples with the greatest residues were refluxed with 1 N HCl for 4 h or heated with 2 N HCl in a boiling water bath for 1 h, 95 and 90%, respectively, of the bound radioactivity was recovered. Analysis of a typical acid extract with TLC revealed the presence of 9306 (2.1%), 9306 sulfoxide (3.7%), phenol sulfide (0.7%), phenol sulfoxide (46.0%), and phenol sulfone (4.4%); the balance (43.1%) was unidentified material retained at the origins of the plates.

Buffer Study. The recovery of applied 9306 from glass ampules was quantitative. Test results (Table VII) indicated there were only minimum chemical changes of the 9306 held at pH 3 and 7, but substantial alteration occurred at pH 11. During the first 8 days in the alkaline buffer solution, there was a rapid decline in concentrations of 9306, with a concomitant accumulation of the hydrolysis products phenol sulfide and phenol sulfoxide. From 8 to 16 days, however, observed changes were negligible. Some 9306 sulfoxide was detected in all samples, but the compound did not tend to accumulate significantly.

Simulated Pond Study. With the conditions used, the radioactive residues in an artificial pond declined slowly; after 16 days, 80.4% of the applied radiocarbon still remained (Table VIII), and 50% depletion was reached only after 75 days. However, the 9306 was very rapidly oxidized to the sulfoxide derivative and hydrolyzed. After 2 h, only about half the radioactivity remained in the parent form. The principal terminal decomposition product at all times from 2 through 16 days was the phenol sulfoxide.

Obviously there was a much more rapid degradation of applied 9306 in the pond water than at a comparably neutral pH in buffer solution. However, the slow rate of hydrolysis in buffer is probably misleading and might be attributed to the fact that the concentration (10 ppm) of 9306 used in that study greatly exceeded the poor water solubility of 9306, whereas the level (0.1 ppm) used in the pond study did not.

Table VII. Stability of ^{14}C-Labeled 9306 in Buffer Solutions

Days post-treatment	pH	% of dose as indicated compound[a]			
		Phenol sulfoxide	Phenol sulfide	9306 sulfoxide	9306
0	3	0.0	0.0	3.5	96.5
4		0.1	0.0	4.4	95.5
8		0.3	0.0	5.5	94.2
16		0.5	0.0	4.8	94.7
0	7	0.0	0.0	3.6	96.4
4		0.6	0.0	5.6	93.8
8		0.7	0.0	6.8	92.5
16		0.8	0.0	7.2	92.0
0	11	0.0	0.0	3.6	96.4
4		9.7	19.3	3.9	67.1
8		35.8	32.0	3.6	28.6
16		37.8	35.3	3.2	23.7

[a] Data are averages of three replicates and represent the total radioactivity in both aqueous and organic fractions of samples.

Table VIII. Fate of ^{14}C-Labeled 9306 in the Water of a Simulated Pond Treated at a Rate of 0.1 ppm

Nature of radioact.[a]	% of dose at indicated h posttreatment					
	0	2	48	96	192	384
Unknown(s) 1[b]	0.0	0.0	3.5	9.4	12.3	12.1
9306	99.2	51.8	2.0	0.0	0.0	0.0
9306 sulfoxide	0.8	26.7	32.1	14.4	1.6	1.4
9306 sulfone	0.0	0.0	2.3	0.0	0.0	0.0
Phenol sulfoxide	0.0	21.5	48.4	54.7	72.8	66.9
Phenol sulfone	0.0	0.0	2.3	0.6	0.0	0.0
Recovered	100.0	100.0	90.6	89.1	86.7	80.4

[a] Radioactive residues in silt samples at 2, 48, 96, 192, and 384 h were 0.00, 0.01, 0.01, 0.02, and 0.02 ppm, respectively. The applied radioactivity was 50% depleted after 75 days. [b] Radioactivity that remained near origin of TLC plate.

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