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LITERATURE CITED

Becker, G., Plüghan, A. *Pflanzenschutz-Nachr.* **22**, 405 (1969).
Cheng, H. H., Führ, F., *J. Agric. Food Chem.* **24**, 421 (1976).
Cheng, H. H., Führ, F., Mittelstaedt, W., *Environ. Qual. Saf., Suppl.* **3**, 271 (1975).
Führ, F., Mittelstaedt, W., *Landwirtsch. Forsch. Sonderh.* **32**, 286 (1976).
Geissbühler, H., Martin, H., Voss, G., in "Herbicides: Chemistry, Degradation, and Mode of Action", 2nd ed, Kearney, P. C., Kaufman, D. D., Ed., Marcel Dekker, New York, N.Y., 1975, p 209.
Hack, H., *Pflanzenschutz-Nachr.* **22**, 331 (1969).
Jarczyk, H. J., *Pflanzenschutz-Nachr.* **25**, 21 (1972).
Mittelstaedt, W., Still, G. G., Dürbeck, H., Führ, F., *J. Agric. Food Chem.* **25**, 908 (1977).
Pont, V., Jarczyk, H. J., Collet, G. F., Thomas, R., *Phytochemistry* **13**, 785 (1974).
Wagner, G. H., in "Soil Biochemistry", Vol. 3, Paul, E. A., McLaren, A. D., Ed., Marcel Dekker, New York, N.Y., 1975, p 269.
Wallnöfer, P., Tillmanns, G., Thomas, R., Wünsche, C., Kurz, J., Jarczyk, H. J., *Chemosphere* **5**, 377 (1976).

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Movement and Metabolism of [^{14}C]Phorate in a Flooded Soil System

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Experiments were conducted to study the effects of soil flooding on the fate and metabolism of [^{14}C]phorate in an agricultural loam soil, on the movement and metabolism of the insecticide in a soil–water–plant system, and factors affecting these phenomena. [^{14}C]Phorate residues were readily released from submerged soils into water, amounting to 45% of applied radiocarbon during the first 3 days after flooding. After a 2-week incubation period as much as one-half of the radiocarbon applied to the soil was recovered from the water. Phorate was much more persistent under flooded then under nonflooded conditions. It was the principle compound recovered from submerged soils where it accounted for approximately 70% of the total residues recovered. Phorate sulfoxide was the major metabolite present in the water. While in nonflooded soils phorate sulfone was the major metabolite, only traces of it were detected in the flooded system. However, when *Elodea* plants were introduced into the system, phorate sulfone amounted after 14 days to 30% of all benzene-extractable ^{14}C residues recovered, phorate sulfoxide to 44%, and phorate to 27%. At that time soils, water, and plants contained 32, 39, and 17% of the applied radiocarbon, respectively. While more lipid-soluble volatile metabolites were recovered from nonflooded soils, more $^{14}CO_2$ was evolved from the flooded soil. The production of $^{14}CO_2$ was a function of microbiological activity. When [^{14}C]phorate-treated soil was flooded with increasing amounts of water, the amounts of radiocarbon residues in the water increased. However, amounts of ^{14}C residues in the water decreased when increasing amounts of soil were used.

Insecticides in soils can be transported via erosion and runoff from agricultural fields into aquatic systems where they are subjected to different environmental conditions. These, in turn, will most probably affect the persistence, metabolism, and ultimate fate of the pesticide. The metabolism of phorate, a soil applied systemic insecticide, has been studied in aerobic soils under both laboratory and field conditions (Bache and Lisk, 1966; Getzin and Chapman, 1960; Getzin and Shanks, 1970; Lichtenstein, 1966; Lichtenstein et al., 1973). However, relatively little information is available about its fate in aquatic and possibly anaerobic environments. Sievers et al. (1970) demonstrated that phorate can be removed from experimental field plots by both runoff water and runoff sediments. Walter-Echols and Lichtenstein (1977) recently showed that phorate sulfoxide, an oxidative derivative of phorate, was microbiologically reduced to the more toxic parent compound phorate in flooded loam soils and particularly in soils which had been deposited as a sediment on lake bottom mud. Flooded soils are characteristically different from nonflooded soils in their physical, chemical, and microbiological properties (Ponnamperuma, 1972). Such systems, therefore, have generally been employed to study the rate and mechanism of degradation of insecticides under environmental conditions simulating those found in river and lake sediments. The microbial degradation of some insecticides in flooded soils has been reviewed by Sethunathan (1973). Takase and Nakamura (1974) reported that disulfoton (disyston), an insecticide closely related to phorate, was rapidly oxidized to the corresponding sulfoxide and sulfone derivatives and that disulfoton sulfoxide was reduced to disulfoton in a flooded silt loam soil. Our study was undertaken to investigate the effects of flooding and the fate and metabolism of [^{14}C]phorate in an agricultural loam soil, the movement and metabolism of [^{14}C]phorate in a soil–water–plant system, and factors affecting these phenomena.

MATERIALS AND METHODS

Chemicals. Phorate, [*S-methylene*-^{14}C]phorate (sp act. 9.7 mCi/mmol), phorate sulfoxide, phorate sulfone, phoratoxon, phoratoxon sulfoxide, and phoratoxon sulfone were obtained through the courtesy of the American Cyanamid Company. Radioactive phorate was diluted with nonradioactive phorate prior to use. Solvents used were anhydrous methanol and redistilled acetone, benzene,

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chloroform, hexane, and toluene.

Soil. The agricultural soil was an insecticide-free Plano silt loam (4.7% organic matter; 5% sand, 71% silt, 24% clay; pH 6.0) which was stored at room temperature in a moist condition prior to use.

Soil Treatment. The moist loam soil was screened through a 2-mm sieve and then treated with acetone solutions of [^{14}C]phorate to yield dry weight concentrations of 2, 4, or 8 ppm. After removal of the acetone vapors and a thorough mixing of the insecticide-treated soil, portions were extracted for analyses to determine the initial insecticide concentration.

Plants. *Elodea nuttallii* (Plach.) St. John were obtained from the Department of Botany, University of Wisconsin, Madison. They were grown in a 38-L aquarium containing tap water and a 2-cm bottom deposit of insecticide-free loam soil.

Extraction. Soils and macerated plants were extracted twice with acetone–methanol (1:1) followed by a third extraction with acetone–methanol–benzene (1:1:1) and a cleanup as described by Lichtenstein et al. (1973). Water and KOH (utilized to trap $^{14}CO_2$) were partitioned three times with benzene. Corn oil-coated extraction thimbles, utilized as vapor traps in some of these experiments, were placed in a 250-mL jar and shaken with 100 mL of hexane. After decanting the hexane, the thimbles were soaked for 10 min in 100 mL of water and then shredded on a Lourdes Homogenizer. The water was filtered off under vacuum and the pulp was rinsed with 50 mL of water and then with 50 mL of hexane. In other experiments oil-coated glass wool was used as a vapor trap. The glass wool was extracted three times with 20-mL portions of hexane and then twice with 20-mL portions of water. All extraction liquids from either the thimble or the glass wool were combined in a separatory funnel and partitioned into hexane and water. Hexane extraction phases were dried over anhydrous sodium sulfate. Both hexane and water phases were then adjusted to volume for analyses. Complete removal of all radiocarbon from the thimbles was achieved since combustion of extracted trap material to $^{14}CO_2$ did not reveal detectable amounts of radiocarbon.

Analyses. Liquid scintillation analyses (LSC) of all benzene, hexane, and water extraction phases were performed as described by Lichtenstein et al. (1972). Unextractable or bound ^{14}C products in soil, plants, or vapor traps were determined by their oxidation to $^{14}CO_2$ and subsequent liquid-scintillation analyses as described by Flashinski and Lichtenstein (1974). Aliquots of KOH were counted in a dioxane scintillation solvent containing 3.5% Cab-O-Sil to prevent precipitation. Gas–liquid (GLC) and thin-layer chromatography (TLC) of benzene extracts were performed as described by Walter-Echols and Lichtenstein (1977). Minimum detectable levels (GLC) of phorate were 0.5 ng and 2 ng of phorate sulfoxide or sulfone. Autoradiography of thin-layer plates was conducted as described by Lichtenstein et al. (1972).

To verify the presence of $^{14}CO_2$ in the KOH traps, a procedure modified from Wang and Willis (1965) was utilized. Five milliliters of a solution containing 1 M $BaCl_2$ and 1 M NH_4Cl were added to precipitate carbon dioxide in the form of $BaCO_3$. The solution was mixed and warmed to 70 °C. After cooling to room temperature, the precipitate was collected on a Whatman No. 50 filter paper by vacuum filtration. The filter paper with the $Ba^{14}CO_3$ was placed in the sidearm of a biometer flask (Bartha and Pramer, 1965), and 20 mL of 0.1 N KOH was pipetted into the main Erlenmeyer flask of the apparatus. Dropwise addition of 20 mL of 1 N HCl to the $Ba^{14}CO_3$ in the sidearm resulted in a release of $^{14}CO_2$, which was again trapped in the KOH. After a 24-h equilibration of the system, two 1-mL aliquots of the KOH were analyzed by LSC. Radiocarbon determined in this way was considered to originate from $^{14}CO_2$.

Bioassay. Unextracted water was assayed for insecticidal activity by introducing 10 third-instar mosquito larvae (*Aedes aegypti*) into triplicate 10-mL aliquots. Toxicities of soil samples were determined with a *Drosophila melanogaster* Meigan bioassay (Edwards et al., 1957) whereby the insects were exposed in duplicate tests to dry residues of benzene extraction phases representing 3.0 g of soil. Mortality counts were conducted over a 72-h period.

EXPERIMENTAL SECTION

Three experimental series were conducted to study the movement and metabolism of [^{14}C]phorate in a flooded soil system. For this purpose, the effects of soil flooding on the fate, movement, and metabolism of the insecticide, the effects of increasing soil-insecticide concentrations, and the effects of different amounts of water flooding the soil were investigated.

In general, the following experimental set-up was employed. Two hundred milliliters of tap water was poured into glass cylinders (18 × 6 cm i.d., capacity 450 mL) and allowed to stand for 24 h. After that, [^{14}C]phorate-treated loam soil was added in small portions to the water and allowed to settle. To reduce evaporation of water, the containers were closed with aluminum foil and Saran wrap secured by a rubber band. The samples were incubated at 20 ± 2 °C in the dark for different time periods. Following incubation the water was carefully siphoned off the soil with a pipet. Care was taken not to withdraw visible soil particles. The remaining water-saturated soil and the siphoned-off water were then extracted and analyzed by LSC and GLC as described. In particular, three series of triplicated experiments were conducted.

Effects of Flooding on the Fate and Metabolism of [^{14}C]Phorate in an Agricultural Loam Soil. The effect of flooding on the metabolism of [^{14}C]phorate was tested with 25 g of loam soil treated with [^{14}C]phorate at 2 ppm (0.25 μCi) and incubated for 14 days under nonflooded or flooded (200 mL of water) conditions. Volatile, apolar metabolites were collected in vapor traps. These traps consisted of Whatman extraction thimbles (43 × 60 mm), previously soaked in a 1% solution of corn oil in hexane. Two-centimeter wide absorbent cellulose (CEL-U-ROL) strips, also previously soaked in the corn oil solution, were wrapped around the thimbles in order to hold it in the glass container and also to serve as an additional trap for lipid-soluble volatile compounds. After the 2-week incubation period, the soil, vapor traps, and water were extracted and analyzed by LSC. The benzene extraction phases of soil and water were also analyzed by GLC and TLC. Bioassays of soil extracts and water were conducted as described.

To follow the fate of radiocarbon and to determine the effect of flooding on the potential evolution of $^{14}CO_2$ derived from [^{14}C]phorate, an additional experimental series was conducted in a closed system which facilitated the measurement of all volatile substances including $^{14}CO_2$. To that effect, 10 g of [^{14}C]phorate-treated (4 ppm, 0.20 μCi) soil was placed into the Erlenmeyer portion of a soil-biometer flask (Bartha and Pramer, 1965) and kept under both nonflooded and flooded (100 mL of water) conditions. To trap volatile, lipid-soluble compounds, oil impregnated glass wool was placed into the connecting tube. For the collection of $^{14}CO_2$, 20 mL of 0.1 N KOH

was placed into the sidearm vessel. Both the Erlenmeyer portion and the sidearm of the soil-biometer flasks were closed with rubber stoppers. The evolution of $^{14}CO_2$ was measured by removing and analyzing 100 μL each of KOH 1, 2, 3, 7, and 14 days after soil treatment. After 2 weeks of incubation, the system was dismantled, resulting in soil, water, oil trap, and the remaining KOH for separate analyses as described. To study the involvement of microorganisms in the evolution of $^{14}CO_2$, previously autoclaved soil (on two successive days for 1 h each time at 121 °C and 1.4 kg/cm^2) was treated with [^{14}C]phorate as described and transferred aseptically into identically prepared, sterile biometer flasks filled with 100 mL of sterile water. After 2 weeks of incubation, the sterility was confirmed by incubating aliquots of the soil in yeast extract-glucose medium. The KOH was analyzed as described.

Movement and Metabolism of [^{14}C]Phorate in a Soil–Water–Plant System. The rate of release of [^{14}C]phorate residues from insecticide-treated soil into water and the potential uptake of ^{14}C compounds by the water plant *Elodea* were investigated. For this purpose 12 glass cylinders were filled with 200 mL of tap water, to which 10 g each of [^{14}C]phorate-treated (4 ppm, 0.20 μCi) loam soil was added. After 1 h, soil particles had settled and the water appeared clear. At that time four 8 cm long pieces of *Elodea* were loosely tied together and placed into the water of each container. To ensure that insecticide residues would only be taken up from the water, plants were lifted with the string so that developing roots could be cut off before they might have come into contact with the insecticide-treated soil. The containers were closed with Saran wrap and held under a bank of Gro-Lux lamps (Sylvania Electric Products, Inc.) on a 16-h photoperiod. One hour and 3, 7, and 14 days later, triplicate containers were dismantled and soil, water, and plants were extracted and analyzed by LSC, GLC, and TLC as described.

Factors Affecting the Fate and Metabolism of [^{14}C]Phorate in a Flooded Agricultural Loam Soil. To test the effects of different amounts of water flooding the soil, [^{14}C]phorate was applied to soil at 2 ppm (0.25 μCi) as described. Twenty-five grams of this soil were then placed into glass cylinders (18 × 6 cm) containing either 50, 100, or 200 mL of water. Oil-coated extraction thimbles were fitted into the containers to collect volatile lipid-soluble metabolites. After an incubation period of 2 weeks, extraction and analyses of each of these components were conducted by LSC, GLC, and TLC. The insecticidal activity of unextracted water and of benzene extracts of soil was tested with bioassays utilizing *Aedes aegypti* larvae and *Drosophila melanogaster* flies, respectively, as described.

Because the volume of a [^{14}C]phorate-treated soil layer could have an effect on the fate and movement of the insecticide, different amounts of soil containing identical amounts of the insecticide and therefore different insecticide concentrations, were tested in the above described system. To that effect 5, 10, and 20 g of soil were treated with [^{14}C]phorate (0.20 μCi), at 8, 4, and 2 ppm, and triplicates were placed into 200 mL of water as described. Extraction thimble vapor traps were fitted into all containers. After the 14-day incubation period the nine systems were dismantled, and the soil, water, and vapor traps were separately analyzed by LSC and GLC as described.

Because under actual environmental conditions, some movement of water standing over a soil layer is likely to occur, the water in three glass cylinders of another experimental series was exchanged weekly over a 4-week period. In this way we intended to simulate conditions of moving water, while the original water in three control cylinders remained unchanged for the total period. Exchange of water was done by placing a filter paper on top of the soil while the water was carefully siphoned off with a pipet. This water was extracted and analyzed. New water was then pipetted into the containers onto the filter paper in order not to disturb the soil sediment. The filter paper was then removed. At the end of the fourth week soils and water were separated, extracted, and analyzed by LSC and GLC.

RESULTS AND DISCUSSION

Effects of Flooding on the Fate and Metabolism of [^{14}C]Phorate in an Agricultural Loam Soil. Two weeks after soil treatment and incubation, differences in the persistence and metabolism of [^{14}C]phorate due to flooding were obvious. Analyses of the various extraction phases for radiocarbon indicated higher recoveries of ^{14}C compounds under nonflooded conditions (controls), where 90.0% of the applied radiocarbon was still associated with the soil and 5.2% had volatilized. Under flooded conditions, however, 43.3% of the applied radiocarbon was associated with the soil, 32.1% with the water standing above the soil and only 2.4% had volatilized. Slightly more ^{14}C was bound to and unextractable from soils under nonflooded conditions. The amounts of water-soluble ^{14}C produced were relatively small under both conditions, indicating that the major metabolism of phorate ended apparently with the production of its sulfoxide and sulfone. Because, especially under flooded condition, only portions (77.7%) of the applied radiocarbon were recovered, the possibility of the production of $^{14}CO_2$ was tested in another experimental series.

Analyses of benzene extraction phases indicated that phorate itself was least persistent under aerobic, nonflooded conditions; only 1.8% of the applied dose could be recovered. However, 24.2 and 51.7% of applied phorate were recovered as its sulfoxide and sulfone, respectively. Under anaerobic, flooded conditions, however, 19.4% of the applied insecticides were still present as phorate and 40.1% as its sulfoxide; only traces of phorate sulfone could be detected. Though under flooded conditions most of the phorate was recovered from soil, most of the phorate sulfoxide was associated with the water.

In the experiments conducted with soil biometer flasks, the evolution of $^{14}CO_2$ from [^{14}C]phorate was studied under flooded and nonflooded conditions. Soils, water, and oil-impregnated vapor traps were also analyzed. Radiolabeled carbon dioxide was produced in both flooded and nonflooded soil. Of the radiocarbon trapped in the KOH, 94% was identified as $^{14}CO_2$. In flooded soils more $^{14}CO_2$ had been produced than in nonflooded soils. Thus 1.7 ± 0.2, 6.0 ± 0.6, and 9.9 ± 0.5% of the applied [^{14}C]phorate had been evolved as $^{14}CO_2$ after 3, 7, and 14 days of incubation in flooded soils, respectively, while with nonflooded soils 1.3 ± 0.2, 2.3 ± 0.2, and 3.3 ± 0.2% had been evolved after identical incubation periods. Because only traces of radiocarbon (0.6 ± 0.1% of applied) were found in KOH traps from autoclave-sterilized flooded soils, microorganisms must have been responsible for the metabolism of [^{14}C]phorate to $^{14}CO_2$. This metabolism of phorate to carbon dioxide by microorganisms may be an important pathway of breakdown, especially in an aquatic environment. While the amounts of $^{14}CO_2$ trapped in KOH was greater under flooded conditions, the ^{14}C metabolites trapped on oil-coated glass wool were greater under nonflooded (6.2 ± 0.4% of applied) than under

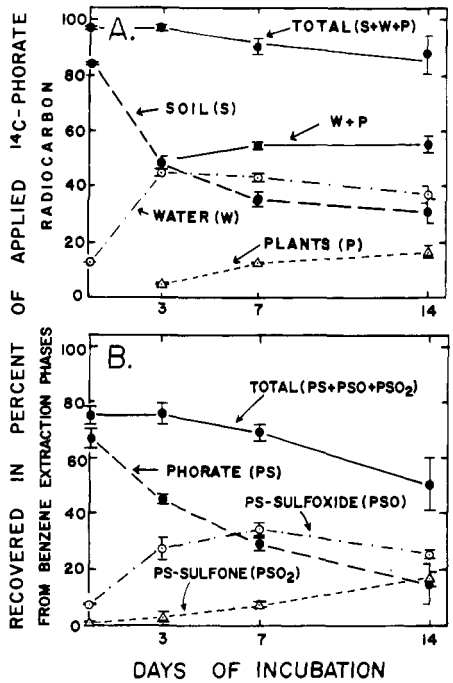

Figure 1. Metabolism of [^{14}C]phorate, applied to soil at 4 ppm, in a soil–water–plant system: (A) distribution of ^{14}C between soil, water, and plants; (B) amounts of phorate, phorate sulfoxide, and phorate sulfone in soil and water. Plants did not contain measurable amounts of insecticidal substances. Results are means ±SD of triplicate tests.

flooded conditions (3.8 ± 0.5%). Analyses by GLC of the hexane extracts from these traps showed that under both flooded and nonflooded conditions phorate amounted to 80–83% of the total recovered residues and phorate sulfoxide to 17–20%. Traces of phorate sulfone volatilized only from nonflooded soil. Total recoveries of radiocarbon in these closed systems were 96.7 ± 2.2% of applied with nonflooded and 93.8 ± 2.9% with flooded soils. Under flooded conditions 39.9 ± 5.9% of the applied ^{14}C were associated with the soil and 40.3 ± 3.7% with the water. In nonflooded containers, 87.1 ± 1.9% of the applied radiocarbon was recovered from the soil.

Movement and Metabolism of [^{14}C]Phorate in a Soil–Water–Plant System. The fate and metabolism of the insecticide were studied in a soil–water–plant system over a 2-week period. Results obtained are summarized in Figure 1, in which Figure 1A depicts the distribution of all the recovered ^{14}C between soil, water, and plants. It is apparent that after the addition of [^{14}C]phorate-treated soil to water, ^{14}C materials were rapidly released from the soil into the water. The total radiocarbon still associated with the soil declined from 83.9 ± 0.6% of the applied dose after 1 h to 31.5 ± 5.6% on day 14, with the most rapid decline during the first few days. With water, a rapid increase of its radiocarbon content occurred during the first 3 days, when nearly one-half of the total applied radiocarbon was recovered from it. After that the radiocarbon content of the water decreased (42.3 ± 1.0% of applied recovered on day 7 and 38.5 ± 3.2% on day 14), probably due to the uptake and accumulation of ^{14}C residues in *Elodea* plants. These plants contained 16.5 ± 2.9% of the applied radiocarbon after 14 days of incubation. Even though the radiocarbon in the water declined after the third day, the amounts released from the treated soil into the water and subsequently recovered from both water and plants increased slightly from day 3 to day 14.

Analyses for radiocarbon of the benzene and water extraction phases of soil, water, and plants after the 14-day incubation period indicated that the amounts of benzene-soluble radiocarbon were similar in soils (21.2 ± 6.0% of applied) and in water (26.5 ± 3.5% of applied), while only negligible amounts (0.2 ± 0.1% of applied) were found in plants. Water-soluble ^{14}C was primarily detected in water (11.9 ± 4.1% of applied) and in plants (2.0 ± 0.4% of applied), but only little (0.4 ± 0.1% of applied) in soil. Unextractable or bound ^{14}C residues were found in soil (9.9 ± 0.8% of applied) and primarily in plants (14.3 ± 2.9% of applied).

Analyses of benzene extraction phases of soil, water, and plants by GLC indicated that *Elodea* plants did not contain detectable insecticidal substances. However, water and soil did contain measurable amounts. These findings, depicted in Figure 1B, indicate that the total phorate recovered from soils and water declined continuously over the 2 weeks to 14.2 ± 7.3% of applied. Concurrently the levels of phorate sulfoxide and phorate sulfone increased. The amounts of phorate sulfoxide peaked at day 7 when a total of 34.0 ± 1.1% of the applied [^{14}C]phorate were recovered in the form of phorate sulfoxide, while 1 week later only 20.3 ± 0.3% of applied phorate were associated with its sulfoxide. This decline was apparently due to the oxidation of part of the sulfoxide to sulfone. Total phorate sulfone levels increased steadily amounting to 15.6 ± 3.7% of the applied [^{14}C]phorate after 2 weeks.

Most of the phorate was recovered from the soil (74–89% of the total recovered). This indicated that the increased persistence of phorate in an aquatic environment is mainly due to the stability or the reduced metabolism of this compound in soil sediments. Reduction of phorate sulfoxide to phorate under similar conditions was reported by Walter-Echols and Lichtenstein (1977). This may also contribute to the relatively large amounts of phorate recovered from the soil. Levels of phorate in water declined rapidly while the metabolites phorate sulfoxide and phorate sulfone appeared in it. From the third day on, phorate sulfoxide was the major metabolite recovered from water. From day 7 to day 14 the amounts of phorate sulfoxide decreased in the water from 30.8 ± 1.1% to 20.3 ± 0.3% of applied phorate, while the amounts of phorate sulfone increased from 5.8 ± 1.3% to 13.4 ± 3.3% which finally accounted for 39% of the total benzene-soluble residues recovered from the water. In previous experiments (Table I) in which [^{14}C]phorate-treated soil had been submerged under 200 mL of water, no phorate sulfone could be detected in the water. In the presence of *Elodea* plants, however, phorate sulfone was a major metabolite in the water. This could have been associated with the excretion of oxygen by the assimilating plants during the 16 h photoperiod.

Factors Affecting the Fate of [^{14}C]Phorate in a Flooded Agricultural Loam Soil. Experiments were conducted with 25 g of [^{14}C]phorate-treated soils which were kept flooded for 2 weeks with either 50, 100, or 200 mL of water. The amount of radiocarbon released from the soil into the water increased with the amount of water standing above the soil (Figure 2A). Thus, the radiocarbon recovered from the water amounted to 12.3 ± 0.3% of applied in 50 mL, 2.09 ± 0.1% in 100 mL, and 32.1 ± 0.2% in 200 mL of water. These values correspond to a concentration of phorate residues in the water of approximately 0.1 ppm, which is far below the water solubility of phorate or its oxidative metabolites. The amounts of radiocarbon or phorate residues recovered from the water appear not to have been determined by water solubility factors. The increase of radiocarbon with increasing amounts of water was mainly due to the appearance of

Table I. Effect of Soil Flooding on the Fate and Metabolism of [^{14}C]Phorate in an Agricultural Loam Soil

	Recovered from extraction phases in % of applied,[a] 14 days after soil treatment							
	Radiocarbon				Phorate (P)	P. sulfoxide	P. sulfone	Total
Substrates	Org. solvents[b]	Water	Bound[c]	Total	From benzene phases			
				Nonflooded (Control)				
Soil	75.9 ± 1.6	1.3 ± 0.2	12.8 ± 1.0	90.0 ± 2.2	1.8 ± 0.4	24.2 ± 0.5	51.7 ± 1.1	77.6 ± 1.5
Vapor trap	4.9 ± 0.2	0.3 ± 0.1		5.2 ± 0.1	NA[e]	NA	NA	NA
Total	80.8 ± 1.6	1.6 ± 0.2	12.8 ± 1.0	95.1 ± 2.3	1.8 ± 0.4	24.2 ± 0.5	51.7 ± 1.1	77.6 ± 1.5
				Flooded[d]				
Soil	33.0 ± 9.7	0.7 ± 0.1	9.6 ± 0.3	43.3 ± 10.0	18.0 ± 9.5	13.5 ± 1.7	Tr[e]	31.5 ± 11.1
Water	26.1 ± 0.4	6.0 ± 0.1		32.1 ± 0.5	1.4 ± 0.4	26.6 ± 5.4	Tr	28.0 ± 5.8
Vapor trap	2.3 ± 0.3	0.1 ± 0.1		2.4 ± 0.3	NA	NA	NA	NA
Total	61.3 ± 10.3	6.8 ± 0.3	9.6 ± 0.3	77.7 ± 10.5	19.4 ± 9.7	40.1 ± 6.6	Tr	59.5 ± 14.0

[a] Applied [^{14}C]phorate (0.25 μCi) at 2 ppm to 25 g of soil. Results are means ±SD of triplicate tests. [b] Benzene for soil and water extractions, hexane for vapor trap extractions. [c] Bound, unextractable residues determined by combustion to $^{14}CO_2$. [d] 200 mL of tap water on top of 25 g of soil. [e] NA not analyzed; Tr, trace.

Table II. Effects of Amounts of Soil and Insecticide Concentrations on the Fate and Metabolism of [^{14}C]Phorate in a Flooded Agricultural Loam Soil[a]

	Recovered from extraction phases in % of applied,[b] 14 days after soil treatment							
	Radiocarbon				Phorate (P)	P. sulfoxide	P. sulfone	Total
Substrates	Org. solvents[c]	Water	Bound[d]	Total	From benzene phases			
				5 g of Soil (8 ppm)				
Soil	15.6 ± 1.3	0.8 ± 0.3	3.7 ± 0.3	20.1 ± 1.8	8.8 ± 1.4	3.9 ± 0.5	Tr[e]	12.7 ± 1.8
Water	32.3 ± 0.5	16.5 ± 0.7		48.8 ± 0.6	3.2 ± 1.9	24.6 ± 1.9	Tr	27.8 ± 1.8
Vapor trap	14.0 ± 2.1	4.5 ± 1.4		18.5 ± 0.9	NA[e]	NA	NA	NA
Total	61.9 ± 3.0	21.8 ± 0.8	3.7 ± 0.3	87.4 ± 3.3	11.9 ± 1.5	28.4 ± 1.8	Tr	40.3 ± 0.4
				10 g of Soil (4 ppm)				
Soil	29.9 ± 1.4	1.8 ± 0.2	4.5 ± 0.2	36.2 ± 1.1	14.5 ± 2.6	7.7 ± 1.3	Tr	22.2 ± 1.6
Water	35.5 ± 1.9	9.9 ± 0.6		45.4 ± 1.3	2.9 ± 0.1	26.2 ± 1.7	Tr	29.1 ± 2.0
Vapor trap	9.7 ± 1.2	0.5 ± 0.2		10.2 ± 1.0	NA	NA	NA	NA
Total	75.1 ± 4.1	12.2 ± 0.7	4.5 ± 0.2	91.8 ± 3.2	17.4 ± 2.6	33.9 ± 0.4	Tr	51.3 ± 3.2
				20 g of Soil (2 ppm)				
Soil	49.2 ± 2.9	0.9 ± 0.5	7.2 ± 0.8	57.2 ± 1.8	31.3 ± 1.6	10.4 ± 1.5	2.0 ± 0.5	43.7 ± 3.2
Water	30.4 ± 0.5	5.1 ± 0.5		35.5 ± 0.5	1.6 ± 0.5	29.2 ± 4.1	Tr	30.8 ± 5.0
Vapor trap	4.6 ± 0.9	0.3 ± 0.1		4.9 ± 0.9	NA	NA	NA	NA
Total	84.1 ± 1.9	6.3 ± 0.6	7.2 ± 0.8	97.6 ± 0.8	32.9 ± 1.2	39.5 ± 2.7	2.0 ± 0.5	74.5 ± 3.0

[a] Soil was submerged under 200 mL of water. [b] [^{14}C]Phorate (0.20 μCi) applied to loam soil at 2, 4, or 8 ppm. Results are means ±SD of triplicate tests. [c] Benzene for soil and water extractions, hexane for vapor trap extractions. [d] Bound, unextractable ^{14}C residues determined by combustion to $^{14}CO_2$. [e] NA, not analyzed; Tr, trace.

more phorate sulfoxide in the water (Figure 2B). However, the total amount of phorate sulfoxide recovered from both soil and water under all three conditions ranged from 34 to 40% of the applied phorate. Therefore the increasing amounts of phorate sulfoxide in increasing volumes of water corresponded to decreasing amounts of phorate sulfoxide in the soil sediments. The amounts of phorate were not significantly different in any water volume. The different volumes of water only affected the distribution of [^{14}C]phorate residues in the system but not the production of phorate sulfoxide. As shown in Table I, water covering the soil caused a reduction of the amounts of the radiocarbon trapped in the vapor traps. Increasing the water volume from 50 to 100 and 200 mL also resulted in a decreased radiocarbon content of the vapor traps (6.0 ± 0.2, 4.1 ± 0.5, and 2.4 ± 0.3% of applied radiocarbon, respectively). Exposure of fruit flies to dry residues of these soils indicated a decrease in toxicity of soils with increasing amounts of water. Thus the time to obtain a 50% mortality was 4.7 h with soil flooded with 50 mL of water and 8.0 and 33 h with soil flooded with 100 and 200 mL of water, respectively. Unextracted water was nontoxic to *Aedes* larvae.

Interactions between bottom sediments and water would be expected to be greatest near the upper layer of the sediment and decline in deeper layers. The amount of soil and the thickness of the insecticide-treated soil layer could therefore affect the amount of insecticide residues released into the water. Experiments were therefore conducted with different amounts of [^{14}C]phorate-treated soil. In a preliminary test, loam soil was treated with 2 ppm [^{14}C]phorate and added in portions of 5, 10, 20, 40, or 80 g to 200 mL of water. After 2-weeks incubation close to 50% of the total radiocarbon applied to the 5- and 10-g soil portions were found in the water. This figure decreased, however, to 26, 14, and 8% with 20, 40, and 80 g of soil, respectively, although the absolute amounts of radiocarbon recovered from the water were very similar when expressed in percent of applied to the soil. As a continuation of these experiments, the amount of soil—flooded with 200 mL of water—was increased from 5 g to 10 and 20 g. However, [^{14}C]phorate concentrations were decreased, from 8 ppm to 4 and 2 ppm, respectively, thus resulting in identical amounts of the insecticide in the soil. Results showed (Table II) that with increasing amounts of soil, but decreasing insecticide concentrations, the total radiocarbon retained in the soil sediments increased from 20.1% to 36.2 and 57.2% of applied ^{14}C, respectively, while the radiocarbon content of the water declined from 48.8% to 45.4 and 35.5% of applied ^{14}C. Previous results had shown that various volumes of water had little effect on the amounts of radiocarbon that had been volatilized. Even though 200 mL of water had been used for the flooding of 5, 10, or 20 g of soil, the amounts

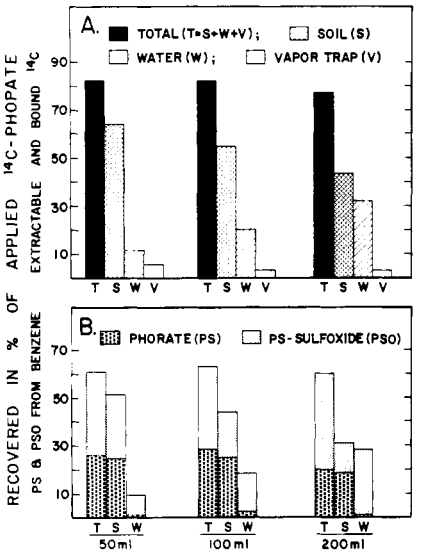

Figure 2. Effects of different amounts (50, 100, and 200 mL) of water standing for 2 weeks over a [^{14}C]phorate treated (2 ppm) soil on the movement and metabolism of the insecticide: (A) distribution of ^{14}C between soil (S), water (W), and vapor traps (V); (B) amounts of phorate and phorate sulfoxide in soil and water. Vapor traps were not analyzed. Results are means of triplicate tests.

of radiocarbon collected in the vapor traps were inversely proportional to the amounts of soil used. Thus, recoveries of volatile substances amounted to 18.5 ± 0.9, 10.2 ± 1.0, and 4.9 ± 0.9% of the applied ^{14}C with 5, 10, or 20 g of flooded soil, respectively. It was of interest to notice that with increasing amounts of soil, but with decreasing concentrations of the insecticide, the persistence of phorate, the total recovery of phorate and metabolites, and the amount of bound ^{14}C residues increased.

To study the effects of water movement over bottom sediments and therefore its replacement, water was exchanged weekly as described and compared with a system in which water stood over the [^{14}C]phorate-treated soil for the entire 4-week period (control). Data indicated that after 4-weeks incubation 12.2 ± 0.8% of the applied radiocarbon was recovered from the soil where water had been exchanged while 27.6 ± 3.0% was recovered from the control soil. Most of the radiocarbon (55.8 ± 0.3% of applied) had moved into water which had been removed after the first week while smaller amounts were removed with water after the second, third, and fourth week (25.8 ± 0.8, 12.1 ± 0.6, and 4.0 ± 0.4% of applied, respectively). In previous experiments in which flooded soil was incubated for 2 weeks (Table I and II), only traces of phorate sulfone were recovered. However, when water stood over the soil for the entire 4 weeks (control), 21.8 ± 10.9% of the applied [^{14}C]phorate was recovered as phorate sulfone from soil and water. Sixty-nine percent of that was located in the water. Total phorate levels had diminished to only 3.6 ± 1.5% of which most (85%) was in the soil.

The amounts of [^{14}C]phorate removed with the water during the water exchanges decreased because the insecticides had been metabolized. After the first week 22% of the total residues in the exchanged water were phorate, but only 2% were phorate after the second week. After the third and fourth week only traces of phorate could be detected. Simultaneously the portion of phorate sulfone in the exchanged water increased from 5.8% of the total recovered residues after 1 week to 20.7, 44.8, and 46.9% after 2, 3, and 4 weeks, respectively. These results demonstrate that during longer incubation periods phorate sulfone is being produced.

Results presented here indicate that phorate is more persistent in flooded, anaerobic soils than in nonflooded soils. The distribution and metabolism of this insecticide in a soil deposited in an aquatic environment is affected by water plants, depth of the bottom deposit, volume of water, and water movement.

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LITERATURE CITED

Bache, C. A. Lisk, D. J., *J. Assoc. Off. Agric, Chem.* **49**, 647 (1966).
Bartha, R., Pramer, D., *Soil Sci.* **100**, 68 (1965).
Edwards, C. A., Beck, S. D., Lichtenstein, E. P., *J. Econ. Entomol.* **50**, 622 (1957).
Flashinski, S., Lichtenstein, E. P., *Can. J. Microbiol.* **20**, 399 (1974).
Getzin, L. W., Chapman, R. K., *J. Econ. Entomol.* **53**, 47 (1960).
Getzin, L. W., Shanks, C. H., *J. Econ. Entomol.* **63**, 52 (1970).
Lichtenstein, E. P., *N.A.S.-N.R.C., Publ.* **No. 1402**, 221 (1966).
Lichtenstein, E. P., Fuhremann, T. W., Schulz, K. R., Liang, T. T., *J. Econ. Entomol.* **66**, 863 (1973).
Lichtenstein, E. P., Schulz, K. R., Fuhremann, T. W., *J. Agric. Food Chem.* **20**, 831 (1972).
Ponnamperuma, F. N., *Adv. Agron.* **24**, 29 (1972).
Sethunathan, N., *Res. Rev.* **47**, 143 (1973).
Sievers, D. M., Lentz, G. L., Beasley, R. P., *ASAE Trans.* **13**, 323 (1970).
Takase, I., Nakamura, H., *J. Agric. Chem. Soc. Jpn.* **48**, 27 (1974).
Wang, C. H., Willis, D. L., "Radiotracer Methodology in Biological Science", Prentice Hall, New York, N.Y., 1965, p 307.
Walter-Echols, G., Lichtenstein, E. P., *J. Econ. Entomol.* **70**, 505 (1977).

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