A Compartmentalized Microcosm for Studying the Fate of Chemicals in the Environment

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A microcosm apparatus is described for studying the fate, metabolism, and movement of synthetic chemicals in the environment. This microcosm is compartmentalized and consists of terrestrial and aquatic components which can be held separately under a variety of environmental conditions. Simulated rain delivered occasionally to the terrestrial portion results in soil run-off which is channeled into the aquatic component with its layer of lake bottom mud and its animal and plant inhabitants. The microcosm can be used to study the effects of rainfall and other environmental conditions on the fate, movement, the potential bioaccumulation, and interaction of one or several test compounds after their application to soils and/or crops. Problems related to a particular test chemical can be studied in fallow or plant-covered soil, in crop plants grown in this soil, in run-off water containing soil particles, in lake mud deposits, and in various organisms within the aquatic part of the microcosm. Because of relatively small size and cost of the stainless steel apparatus, experiments can be easily replicated. Model experiments were conducted with freshly deposited [^{14}C]phorate soil residues in comparison to 1-month-aged residues. Corn plants grown in these soils were twice irrigated. The metabolism and movement of [^{14}C]phorate residues from soils into crops and the contamination of the aquatic system with its guppy fish and water ferns are reported. With a soil slope of only 5° and a "rainfall" applied twice for 15 min, a transport of 3% of the applied radiocarbon into the components of the aquatic system was noticed.

Research pertaining to the environmental fate and behavior of synthetic chemicals, such as pesticides, has been conducted under both field and laboratory conditions. In the latter case, the number of experimental variables can be restricted to such an extent that reliable answers are obtained to a particular problem. In most cases these types of studies have been restricted to the investigation of the effects of single environmental factors, such as a particular soil type, temperature, water movement, light, etc. on the fate of a particular pesticide. More recently, the fate of insecticides has been studied in multicomponent systems involving a variety of environmental factors (Metcalf et al., 1971). In some of these studies, the behavior and deposition of pesticides were investigated by assembling isolated components of the environment into a more unified laboratory "microcosm". Thus, larger terrestrial laboratory systems have been described to simulate field conditions for monitoring the fate of pesticides (Gillett et al., 1974; Gillett and Gile, 1975, 1976; Beall et al., 1976). In our laboratory, movement and metabolism of aldrin, parathion, and [^{14}C]fonofos were studied in treated and untreated layers of several soil types relative to the effects of percolating water (Lichtenstein et al., 1967, 1972). Subsequently, a more complete microcosm was employed in which corn was planted in soil freshly treated with [^{14}C]phorate and through which water was percolated (Lichtenstein et al., 1974). Utilization of more complex microcosms, however, makes it possible to simulate more environmental conditions which may affect the fate of a specific chemical in the environment. Such a microcosm, however, should be manageable in such a way that data collected would give specific answers to specific questions, that the experiment can be replicated and that the cost of such a system would be reasonable.

In this paper we describe a combined terrestrial and aquatic microcosm which facilitates the study of the fate, metabolism, movement, and possible bioaccumulation of a given test chemical under specific environmental conditions. In this study the movement of the test compound, [^{14}C]phorate, from soils into crops and the contamination of an aquatic system by soil run-off due to crop irrigation is investigated.

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MATERIALS AND METHODS

Description of the Microcosm. The apparatus utilized (Figure 1) is constructed from stainless steel. Its overall dimensions are 49 cm high, 39 cm deep, and 26 cm wide. Individual components, shown in Figure 2, consist of two soil containers (Figure 2D), each 10 cm high, 22 cm deep, and 10 cm wide which are used for plant growth and can be individually placed under different environmental conditions in growth chambers. When rainfall or irrigation are being simulated, the two soil containers are placed into the "run-off container" (Figures 1 and 2C, 10 cm high, 32 cm deep, 21 cm wide). It has a divider of 20 cm height. This container in turn fits into another one (Figures 1 and 2B, 18 cm high, 31 cm deep, and 25.5 cm wide) which by means of an adjustable wingnut screw attached to container C makes it possible to lift container C at one end. In this way, different slopes are obtained, thus affecting the amounts of run-off during irrigation. Finally, containers B, C, and D with soil and plants are placed into the holding stand (Figure 1A, 32 cm high, 31.5 cm deep, and 27 cm wide) and coarse wire screens (6-mm mesh) are placed in front of the soil (Figure 2C).

Whenever needed, we produced artificial rain with a "Tee Jet" spray nozzle (No. TG1, full-size cone tip, Spraying Systems Co., Wheaton, Ill.). This nozzle, mounted 100 cm above the soil surface, delivered in cone form 125-mL of water/min, covering two soil containers (Figure 2D) within one "run-off container" (Figures 1 and 2C). Before water entered the nozzle, it passed a pressure regulator and a "Bantam Meter" (Kontes, Vineland, N.J., K 627980, Model 1039) which permitted controlled delivery of a specific amount of water per time unit. Run-off water containing soil was channelled into the two aquaria shown in Figure 1. Based on the size of these aquaria and the slope of the soil, the amount of "rain" was adjusted accordingly. To create more realistic conditions, a layer of lake bottom mud was placed into the aquaria before run-off water with soil entered them. When the water appeared clear after settling of the soil, organisms such as water plants, fish or insect larvae were introduced into the aquatic part of this microcosm. Both the soil containers

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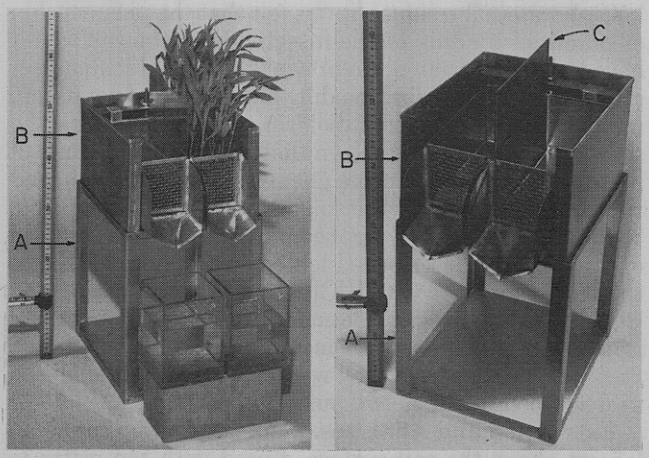

Figure 1. Set up of the total microcosm: A, holding stand for B; B, slope adjustable container for C; C, soil run-off containers.

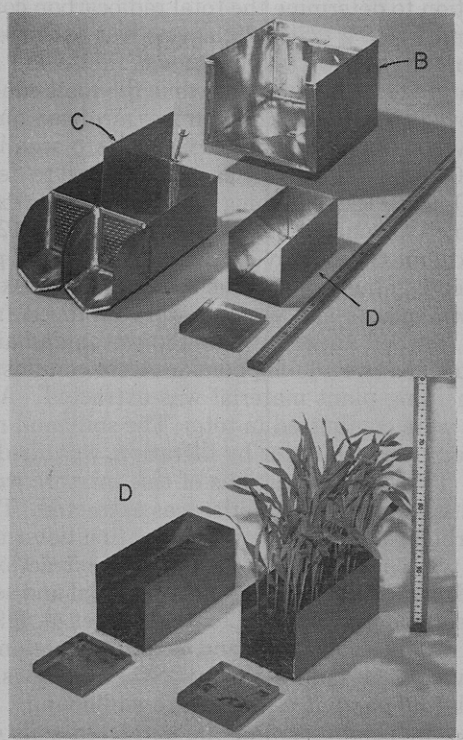

Figure 2. Components of the terrestrial part of the microcosm: B, slope adjustable container for C; C, soil run-off containers for D; D, soil containers.

of the terrestrial part and the aquaria can be placed into climatic chambers with different environmental conditions.

This microcosm can be used to study the effects of rainfall and other environmental conditions on the fate, movement, the potential bioaccumulation, and interaction of one or several test compounds after their application to soils and crops. Thus problems related to a particular test chemical can be studied in fallow or plant-covered soil, in crop plants grown in this soil, in run-off water containing soil particles, in lake mud deposits, and in various organisms within the aquatic part of the microcosm. There are several advantages in using this microcosm. Between irrigations the terrestrial and/or aquatic compartments can be placed into chambers with different environmental conditions. Experiments can easily be replicated because of the relatively small size, cost, and space requirements of the apparatus. This, in turn, will assure more meaningful results. All components can be easily separated for analyses. It should be realized, however, that only portions of the many problems which face us today can be investigated. It is doubtful that a manageable system could be designed which would answer a multitude of questions in a meaningful way.

UTILIZATION OF THE MICROCOSM

A model experiment was conducted with the described microcosm utilizing [^{14}C]phorate as the test compound. This insecticide was used because of our previous experience with phorate in a "model soil–plant ecosystem" (Lichtenstein et al., 1974).

Chemicals. Phorate, [*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 Co. [^{14}C]Phorate was diluted with nonradioactive insecticide before its addition to soils. The solvents used were redistilled benzene, acetone, and acetonitrile, toluene, methanol, and nitromethane.

Soils. 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. Lake mud collected from Lake Mendota, Madison, Wis., at a depth of 9 m, was drained of excess water and stored under refrigeration. Its composition was 12.5% organic matter, 37% sand, 57% silt, and 6% clay, and the mud had a pH of 7.4.

Soil Treatment. Only loam soil was treated with the insecticide. This moist soil was screened through a 2-mm sieve and then treated with acetone solutions of [^{14}C]-phorate to yield dry weight concentrations of 4 ppm. After evaporation of the acetone vapors and thoroughly mixing the treated soil, portions were removed and extracted for analyses to determine the actual soil application level. This level was then used as the actually applied dose, to which later data were referred.

Corn Plants. Corn seeds free of pesticides (Funk Hybrid G-4444 blight resistant) were obtained through the courtesy of Funk Seeds International, Bloomington, Ill. They were pregerminated between wet paper towels in glass dishes before planting in the insecticide-treated soils.

Water ferns (*Salvinia* sp.) were obtained from the Department of Botany at the University of Wisconsin and **guppy fish** (*Peocilia* sp.) were purchased from a local fish supply store.

Experimental Design. Because phorate is rapidly metabolized in this loam soil (Lichtenstein et al., 1973), potential differences in the behavior of freshly deposited and aged [^{14}C]phorate residues were studied. Figure 3 outlines the experimental design and shows how freshly deposited (I, II in Figure 3) and 28-day-aged (III, IV in Figure 3) insecticide soil residues were obtained. For brevity purposes these two soils will be referred to as "fresh" and "aged". Initially (day 0), 450 g of soil treated with the insecticide at 4 ppm (9.83 μCi) was placed into each of two soil containers (III and IV) and kept for 28 days in growth chambers with 16 h of daylight at 25 °C and 8 h of darkness at 21 °C. To keep this soil in a moist condition, approximately 50 mL of water had to be added to it on each day. On day 28, two additional 450-g portions were treated with [^{14}C]phorate at 4 ppm and were placed in two other containers (I and II). Thirty-six corn seedlings were then planted in each of the four containers and were grown in the above-mentioned growth chambers for a period of 22 days (to day 50 in Figure 3). Water was added as necessary. Two and three weeks after corn planting (days 42 and 49 in Figure 3) when the plants had grown to a height of 14–22 and 24–32 cm, respectively, rain was applied to the system for 15 min as described above. In

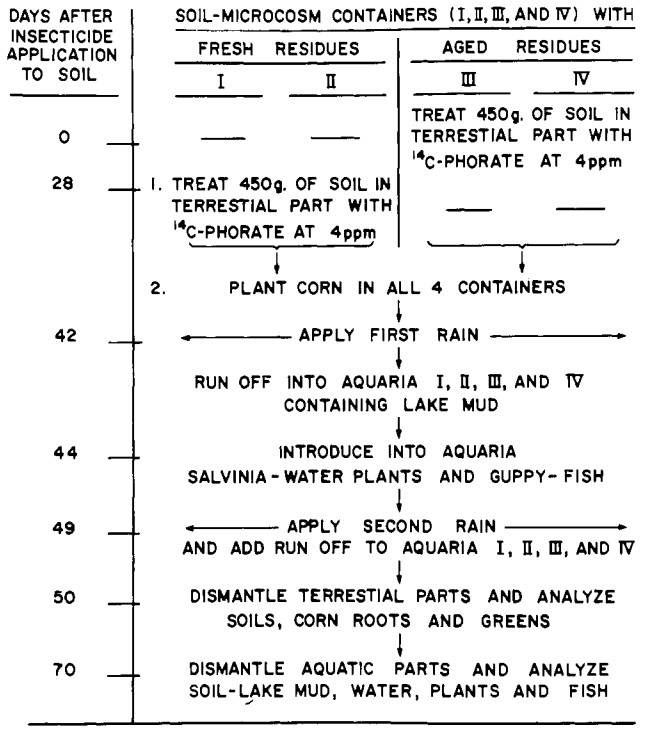

Figure 3. Flow sheet depicting experiments conducted with the microcosm.

that way a total of 1875 mL of water covered, in cone form, the two soil containers. This resulted in a run-off of approximately 650 mL from each container. Each 650 mL of water contained approximately 14 g (dry weight basis) of soil which represented about 3% of the soil placed into the container. This run-off water with suspended soil was initially collected in beakers and mixed, and 100 mL was passed under suction through Whatman No. 1 filter paper to separate the soil from the water. Soil and water were then analyzed separately as described. Each of the remaining 550 mL volumes of run-off water and soil from each container were then carefully poured into one of four aquaria, into which previously a layer of 150 g of lake bottom mud had been deposited. Two days later (day 44 in Figure 3), after soil particles had settled, three male and three female guppy fish were added to the water and ten free floating, water ferns (*Salvinia* sp.) were deposited onto the water surface. Roots of these plants hung in the water to a depth of 3–4 cm and did not contact the soil.

On day 49 the second rain was applied, and 100-mL run-off aliquots were processed as described above. Before the remaining run-off mixtures were added to each aquarium, guppies were removed with some water. After 2 days the run-off soil had settled, and the guppies were reintroduced into the appropriate aquarium. These containers were then held for an additional 21 days (to day 70 in Figure 3) at a temperature of 25 ± 2 °C under a 16 h/day Gro-Lux illumination. Tap water was added as needed to compensate for water loss through evaporation.

On day 50, twenty-two days after corn planting, the terrestrial parts of the microcosm were dismantled, separated into soils, corn roots, and leaves, and prepared for extraction and analyses as described. On day 70, four weeks after establishment of the aquatic components of the microcosm, the aquaria were dismantled by separating their contents into the lake mud–soil mixture, water, guppies, and *Salvinia* plants. These components were then prepared for extraction and analyses.

Binding of [^{14}C]Phorate to the Loam Soil. To better evaluate data from freshly deposited and from 4-week-aged [^{14}C]phorate soil residues, it was felt that the phenomenon of potential binding of the insecticide to the soil would have to be investigated. In previous studies (Lichtenstein et al., 1977) we described binding phenomena of parathion, methyl parathion, fonofos, dieldrin, and DDT utilizing the same loam soil. It was shown that increasing amounts of insecticide residues became unextractable with time, and the amounts bound to the soil after 28 days ranged from 7 to 42% of the initially applied insecticide dose. Identical experiments as described (Lichtenstein et al., 1977) were therefore conducted with [^{14}C]phorate, except that the soil was treated at an insecticide concentration of 4 ppm or 0.23 μCi/10 g of soil. Soils were incubated for 0, 7, 14, 21, and 28 days at which time they were extracted and analyzed as described in the above publication.

Extraction and Analyses. Corn leaves were cut about 0.5 cm above the soil surface, rinsed with tap water to remove adhering soil particles, and dried for 2 days at 28 °C. Two grams (seven to nine leaves) were used for combustion to determine the total radiocarbon content as $^{14}CO_2$ and 7 to 8 g (27 to 29 leaves) was extracted.

The muddy soil with its roots was first dried at room temperature for 3 days. At that time the roots could easily be removed. They were then further air-dried for 3 days. The dry root material was then cut into small pieces. Three 100-mg portions were each combusted to determine the $^{14}CO_2$ while the remaining root material was extracted. Soils which now had a moisture content of 10–12% were screened and thoroughly mixed. Two 500-mg portions were then combusted, and the remainder was extracted.

Salvinia plants and guppies were removed from the aquaria, washed under running tap water, and air-dried. With *Salvinia* two 100-mg portions were combusted, and the remaining plant material was extracted. Air-dried guppies were combusted in toto. The soil–mud mixture, separated from the water by filtration, was air-dried for 5 days. Two 500-mg portions of this mixture were then combusted, and the remainder was extracted. The total radiocarbon content of the water was first determined by LSC of two 1-mL portions, followed by extraction of the remainder with benzene. All plant material and soils were extracted as described by Lichtenstein et al. (1973), resulting finally in benzene and water extraction phases which were analyzed for ^{14}C by LSC techniques. Unextractable radioactivity, referred to as "bound", was determined by combustion of the extracted soils or plant material to $^{14}CO_2$ and analyses by LSC as described by Flashinski and Lichtenstein (1974).

TLC of the benzene phases was performed using EM Laboratories (E. Merck) precoated silica gel "60" plates developed in nitromethane–acetonitrile–toluene (15:40:45). Visualization of the compounds was made by spraying the developed plate with 0.5% palladium chloride in 0.25 N HCl and then with 5 N NaOH. Detection of radioactive compounds in thin-layer chromatograms was also performed by autoradiography, using Kodak No-Screen X-ray film. Areas of silica gel containing ^{14}C compounds which had been isolated from chromatograms of the terrestrial components, the soil–lake mud mixture or the water, were removed and eluted with methanol for analyses by LSC. In this way the amounts of each isolated compound could be calculated and expressed in percent of all the benzene-soluble radiocarbon.

RESULTS AND DISCUSSION

Results of previously described field experiments (Lichtenstein et al., 1973) indicated that in a Plano silt loam, phorate was rapidly oxidized during the first week after soil treatment and that 1 month later only one-half of the

Table I. Fate and Movement of "Aged" and "Freshly" Deposited [^{14}C]Phorate Soil Residues in a Plant-Soil-Water Microcosm[a]

Recovered from	^{14}C recovered in percent of applied[b] to soil			
	"Fresh" residues		"Aged" residues	
	Total sample	Per g wt[c]	Total sample	Per g wt
Terrestrial part				
Soils (S)	65.6 ± 0.5	0.17	60.6 ± 0.4	0.15
Corn (C)				
Leaves	16.9 ± 0.6	3.12	9.8 ± 0.4	1.92
Roots	8.7 ± 0.1	0.80	9.0 ± 0.5	0.86
Total (C)	25.6		18.8	
Total (S + C)	91.2		79.4	
Run-off 1				
Water (W)	0.4 ± 0.1	0.0006	0.2 ± 0	0.0003
Soil (S)	1.1 ± 0.1	0.080	1.1 ± 0	0.080
Total (W + S) 1	1.5		1.3	
Run-off 2				
Water (W)	0.5 ± 0.1	0.0008	0.3 ± 0	0.0005
Soil (S)	1.0 ± 0.1	0.08	1.1 ± 0.1	0.08
Total (W + S) 2	1.5		1.4	
Total (1 + 2)	3.0		2.7	
Aquatic Part				
Soil and lake mud (S)	2.0 ± 0	0.03	1.5 ± 0.1	0.02
Water (W)	0.8 ± 0.1	0.0008	0.6 ± 0.1	0.0005
Guppies (G)	0.02 ± 0	0.08	0.01 ± 0	0.04
Salvinia (P)	0.2 ± 0.1	0.29	0.1 ± 0	0.18
Total (S, W, G, P)	3.02		2.21	
Total				
Terrestrial (T)	91.2		79.4	
Aquatic (A)	3.02		2.21	
T + A	94.22		81.61	

[a] Results determined by combustion to $^{14}CO_2$, except water, are averages of duplicated tests. [b] Applied [^{14}C]phorate at 4 ppm to 450 g of soil (9.83 μCi). [c] Per gram of dry weight or per milliliter of water.

Table II. Effects of "Freshly" Deposited (F) and "Aged" (A) Residues of [^{14}C]Phorate Applied to a Silt Loam at 4 ppm (9.83 μCi) on the Distribution of Radiocarbon in Terrestrial Soils and Corn Plants (Results Are Averages of Duplicated Tests)

		^{14}C recovered from extraction phases of terrestrial soils and plants						
		Benzene		Water		Bound[a]		Total (=100%)
		% applied[b]	% T[c]	% applied	% T	% applied	% T	% applied
Soils	F	47.6 ± 1.3	72	1.0 ± 0	1	17.7 ± 0.5	27	66.3 ± 0.8
	A	39.7 ± 1.5	64	0.5 ± 0	1	21.2 ± 1.6	35	61.4 ± 0.1
Corn[d]								
Leaves	F	1.6 ± 0.2	10	4.5 ± 0.1	28	9.8 ± 1.0	62	15.8 ± 1.1
	A	1.1 ± 0.2	12	3.3 ± 0.3	35	4.9 ± 0.8	53	9.3 ± 0.3
Roots	F	1.6 ± 0.1	20	1.7 ± 0	21	4.8 ± 0.2	59	8.1 ± 0.1
	A	1.2 ± 0.1	14	2.2 ± 0.1	26	5.0 ± 0.4	60	8.4 ± 0.5

[a] Bound = unextractable radioactivity determined by combustion to $^{14}CO_2$. [b] ^{14}C recovered in percent of applied to the soil. [c] % T = ^{14}C in percent total radiocarbon recovered. [d] Corn seedlings were planted immediately following (F) or 28 days after (A) soil treatment and grown for 22 days at 25 °C (16-h day) and 21 °C (8-h night).

applied insecticide could be recovered in the form of phorate, its sulfoxide, and sulfone. The present laboratory experiments indicated that amounts of extractable radiocarbon derived from [^{14}C]phorate were 46.5 ± 0.9% of the applied dose, 28 days after soil treatment. However, the amounts of unextractable, soil-bound radiocarbon were at that time 26.4 ± 1.1% of the applied dose, thus resulting in a total recovery (extractable plus bound) of 72.9% of the applied insecticide (Figure 4). Contrary to results with [^{14}C]fonofos and [^{14}C]parathion (Lichtenstein et al., 1977), binding of ^{14}C compounds derived from [^{14}C]phorate did not increase after the first week of soil incubation. It is important to realize that corn seedlings planted in the two "aged" soils (III and IV in Figure 3) on day 28 were from the start exposed to [^{14}C]phorate residues of which approximately 26% were soil-bound and 47% extractable. With freshly deposited residues, however, 98% of the applied ^{14}C was extractable and only 1.8% had been determined as soil-bound when the corn was planted.

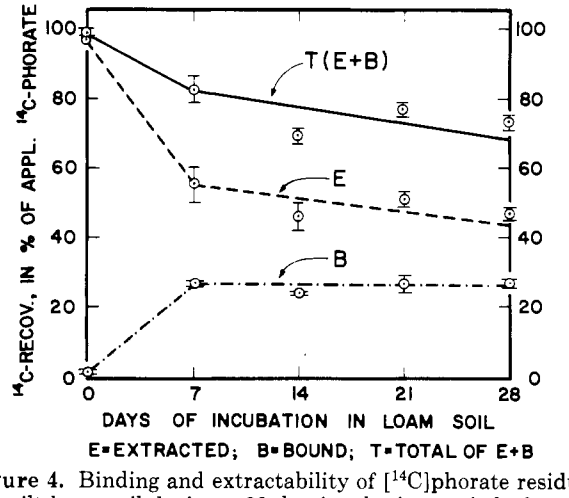

Figure 4. Binding and extractability of [^{14}C]phorate residues in a silt loam soil during a 28-day incubation period after soil treatment at 4 ppm.

Table III. [^{14}C]Phorate and Metabolites Recovered from the Benzene-Extraction Phases of Terrestrial Soil and Corn Plants as Determined by Thin-Layer Separation and LSC[a]

		Recovered in percent of all benzene soluble radiocarbon[c]							
		PS	PSO_2	PSO	PO	$POSO_2$	POSO	X_1	X_2
	$R_f \rightarrow$	0.94	0.86	0.46	0.72	0.56	0.11	0.61	0.30
Soil	F	2.8 ± 0	72.4 ± 0.7	23.9 ± 0.6	ND[e]	ND	ND	ND	ND
	A	1.9 ± 0.2	83.2 ± 1.0	14.0 ± 0.8	ND	ND	ND	ND	ND
Corn[d]									
Leaves	F	ND	68.2 ± 0.3	10.7 ± 0.8	5.1 ± 0.4	9.0 ± 0.3	3.8 ± 0	2.1 ± 0.2	0.8 ± 0.1
	A	ND	58.7 ± 2.0	7.3 ± 1.9	22.7 ± 5.0	6.3 ± 0.6	1.9 ± 0.5	1.8 ± 0.5	1.0 ± 0.1
Roots	F	1.8 ± 0.7	80.8 ± 2.0	13.6 ± 1.0	2.0 ± 0.3		1.8 ± 0.3	ND	ND
	A	1.8 ± 0.4	86.3 ± 0.7	7.3 ± 0.1	2.9 ± 0.3		1.6 ± 0.1	ND	ND

[a] Plants were grown in soils containing "freshly" deposited (F) or "aged" (A) residues of [^{14}C]phorate. Results are averages of duplicated tests. [b] PS = phorate; PSO_2 = PS sulfone; PSO = PS sulfoxide; PO = phoratoxon; $POSO_2$ = PO sulfone; POSO = PO sulfoxide; X = unknown. [c] Data for all the benzene-soluble radiocarbon recovered is presented in vertical column 1 of Table II. [d] Corn seedlings were planted immediately following (F) or 28 days after (A) soil treatment and grown for 22 days at 25 °C (16-h day) and 21 °C (8-h night). [e] ND = nondetectable.

Results obtained with the terrestrial and aquatic portions of the microcosms are partially presented in Tables I, II, and III. The total amounts of radiocarbon in the various microcosm components were determined by combustion, except for water. These data are presented in Table I. With freshly deposited insecticide residues, a total of 94% of the applied radiocarbon could finally be accounted for. Of that, 91% were associated with the terrestrial part and 3% with the aquatic component. The aquatic insecticide residues resulted from two "rainfalls", each of which yielded a soil–water run-off containing 1.5% of the totally applied radiocarbon. Two-thirds of the ^{14}C recovered from the aquaria was associated with the soil–lake mud mixture. Within the terrestrial part, most of the ^{14}C residues were recovered from the soil (65% of applied) while 26% was recovered from the corn. These plants contained two-thirds of their total radiocarbon content in the leaves.

With "aged" residues (III and IV in Figure 3) only 82% of the originally applied radiocarbon were recovered as opposed to 94% with "freshly" insecticide-treated soil. This difference of 12% was noticed in the terrestrial portions. It is possible that during the first 28 days of the experiment some compounds were lost due to volatilization from the fallow soil. Corn plants from soils containing "aged" residues had considerably less radiocarbon (19% of applied) than plants from soils containing "fresh" residues (26% of applied). Expressed on a per gram dry weight basis, corn leaves from soil with "aged" residues contained 1.9% of the applied radiocarbon while leaves from soils with "fresh" residues contained 3.1%. Since roots from both soils contained similar amounts of ^{14}C, qualitative differences in ^{14}C compounds that penetrated into the roots could have been responsible for the quantitative differences observed with leaves of plants grown in the differently aged phorate-treated soils.

The amounts of radiocarbon in soil run-off were relatively small. This could have been related to the slight slope of the soil (5°) and the dense cover crop of corn plants. An increasing slope and a decreasing plant cover would undoubtedly have increased soil erosion and thereby would have increased the amounts of radiocarbon in the aquatic system. Under the conditions employed in this experiment, only 2–3% of the applied ^{14}C were transferred into the aquaria, of which 1.5–2% were still associated with the soil–lake mud mixture. *Salvinia* plants which grew and floated on the water surface and guppies from these aquaria contained relatively small amounts of radiocarbon.

The mobility of a chemical in the environment is to a large extent a function of its water solubility and vapor pressure. Compounds with substantially different physical–chemical properties than phorate (water solubility, 50 ppm and vapor pressure, 8.4×10^{-4} mmHg at 20 °C and 2.3×10^{-3} mmHg at 30 °C, Schrader, 1963) would most probably behave very differently in a system as described above.

Analytical results obtained by LSC after extraction of the terrestrial soil and the corn plants are shown in Table II. Data for the total radiocarbon recovered by extraction are shown in the last column of this table and are very similar to the ^{14}C recovered by combustion techniques (Table I). With soils, most of the recovered radiocarbon (64–72%) was benzene soluble, very little was water soluble, and the remainder (27–35%) was unextractable or bound. Corn leaves, however, translocated and metabolized ^{14}C residues. Contrary to soils, the smallest amounts (10–12%) of the total recovered radiocarbon from corn leaves were benzene soluble, while sizable amounts (28–35%) were associated with the water-extraction phase. The majority of the ^{14}C residues in leaves (53–62% of recovered), however, was unextractable and bound to the corn leaf tissue, mostly in plants grown in "fresh" soil residues. It is possible, therefore, that plants grown in freshly deposited [^{14}C]phorate soil residues were able to absorb more bindable compounds through their roots, thus making more ^{14}C compounds available for binding in the leaves. Results obtained with corn roots from both soils were similar.

Resolution of the benzene-extraction phases of soils and corn plants by TLC and autoradiography demonstrated the metabolism of [^{14}C]phorate in soils and the penetration, translocation, and metabolism of the insecticide in corn plants (Table III). In soils, only phorate, phorate sulfoxide, and phorate sulfone were present, 72–83% of which were in the form of the sulfone. "Freshly" treated soils contained relatively more phorate sulfoxide but less phorate sulfone than the soil with "aged" residues. In corn leaves, no phorate could be detected, but five of its metabolites were identified by cochromatography and two unknowns (X1 and X2) were detected. Most of the phorate-oxygen analogues were found in leaves from "aged" soil (30.9% of recovered) of which the majority (22.7% of recovered) was phoratoxon. In leaves from "freshly" treated soils, the major oxygen analogue was phoratoxon sulfone.

Analytical data obtained with the aquatic portion of the microcosm have been discussed as far as total ^{14}C recoveries are concerned (Table I). Of the total radiocarbon recovered from the soil–lake mud mixtures, 29–32% was benzene soluble, 63–71% was unextractable, and the rest was water soluble. Primarily phorate sulfone, but also small amounts of phorate sulfoxide, were recovered from

the benzene-extraction phase. Water standing above these soils contained only small amounts of radiocarbon (0.6 to 0.8% of applied) primarily in the form of phorate sulfone. Its amounts ranged from 39.4 ± 2.2% of recovered benzene-soluble ^{14}C from the water over "fresh" soils to 48.2 ± 1.7% of recovered from the water over "aged" soils. In addition, small amounts (12.6% of total recovered) of phoratoxon were in these waters.

Free-floating *Salvinia* plants contained relatively little radiocarbon (Table I). Of the total radiocarbon recovered, 86–89% was unextractable, 4–6% benzene soluble, and 7–8% water soluble. Compared to binding data obtained with corn plants, it seems that the appearance of unextractable compounds and their binding to plant constituents is dependent on the nature of a particular plant species. The amounts of radiocarbon in *Salvinia* were also calculated as dpm/g of dry weight. Thus, *Salvinia* tissues from "fresh" or "aged" residues contained 32.1 ± 0.5 and 23.7 ± 0.4 × 10^3 dpm bound radiocarbon, 2.3 ± 0.7 and 1.1 ± 0.3 × 10^3 dpm benzene-soluble and 3.1 ± 0.6 and 1.8 ± 0.3 × 10^3 dpm water-soluble radiocarbon, respectively.

No differences in fish mortalities were observed under the two experimental conditions described in Figure 3. In two aquaria (one each from "fresh" or "aged" residues) no fish mortality occurred, while in each of the remaining two, one female guppy died after 2 days and one male after 19 days of exposure. Based on these results it appears that the amounts of insecticidal substances in the water were at the lower end of their toxicity range. Since a control with untreated soil had not been included in these experiments, the fish mortality may not have been phorate related. The amounts of radiocarbon recovered from the guppies in these experiments were rather small (Table I). The differential uptake of ^{14}C by female and male fish under the experimental conditions was obvious when data were calculated as dpm/g of dry fish. Thus female guppies (average dry weight, 60 mg/fish) from aquaria with "fresh" or "aged" residues contained 12.4 ± 4 and 7.5 ± 0.2 dpm × 10^3/g of dry fish, respectively, and male guppies (average dry weight, 22 mg/fish) contained 16.4 ± 1 and 11.2 ± 0.04 dpm × 10^3/g of dry fish, respectively.

A separate exploratory experiment was also conducted in which six guppies (three males and three females) were placed into each of three aquaria. One of them contained insecticide-free water, one [^{14}C]phorate-treated water (55 μg/L), and one 200 g of lake mud plus a layer of 45 g of [^{14}C]phorate-treated (59 μg/45 g) soil. No mortalities occurred with the insecticide-free water. With insecticide-treated water, however, all six fish had died within 16 h and with insecticide-treated soil in the aquaria, three male guppies had died after 21 h and the three female fish after 2, 4, and 21 days, respectively. Fish from aquaria with insecticide-treated water contained a total of 17.8 × 10^3 dpm of radiocarbon and those from aquaria with insecticide treated soil 5 × 10^3 dpm.

In conclusion, we would like to reiterate that this terrestrial–aquatic microcosm lends itself to study the fate of various test chemicals under different environmental conditions. The amounts of test compound transported via soil run-off, undoubtedly, is a function of the physicochemical properties of the chemical itself, such as vapor pressure and volatilization, water solubility, and others. Moreover, factors such as soil type, slope of the soil, presence and kind of a particular cover crop, amounts, and duration and intensity of rain are probably all directly related to the mobility of the particular chemical. The fate and metabolism of a test compound and its potential interaction with other environmental chemicals can easily be studied under different conditions, such as various temperatures or light exposures. It would be of advantage in future experiments to place both terrestrial components and the aquaria into suitable devices which would make it possible to collect and measure the amounts of potential $^{14}CO_2$ produced. In this way it would be possible to achieve a more complete balance of the radiocarbon originally applied to the system.

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