Effects of Percolating Water, Captafol, and EPTC on the Movement and Metabolism of Soil-Applied [^{14}C]Carbofuran in an Agromicrocosm

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The effects of water percolating through [*ring*-^{14}C]carbofuran-treated soils (3.6 ppm; 2.9 μCi) and of the fungicide captafol and the herbicide EPTC on the persistence, movement, and metabolism of the insecticide in an agromicrocosm were investigated. After a 3-week incubation period, 49% of the soil-applied radiocarbon had been removed with percolating water from soils, while 37% was recovered from soils and corn. In nonpercolated soils, however, 80% of the applied ^{14}C was still associated with soils and corn. The aquatic components (water, lake mud, *Elodea* plants, and guppy fish) contained 25% of the soil-applied radioacrbon at the end of the experiment, even though 49% had been initially added to the aquariums via the percolated water. This loss of 24% was partially accounted for by degradation of ^{14}C-labeled compounds to $^{14}CO_2$. About $^3/_4$ of all the radiocarbon found in the aquatic system was associated with the lake bottom mud, most of it as unextractable ^{14}C-labeled compounds. Carbofuran was the major compound recovered from control and percolated soils, amounting to 39% and 15% of applied radiocarbon, respectively, while 3-ketocarbofuran and 3-hydroxycarbofuran were identified as the major metabolites. The aquatic component, however, contained only 0.3% of the initially applied [^{14}C]carbofuran, and most of this was associated with the bottom mud layer. While the addition of captafol to [^{14}C]carbofuran-treated soils resulted in a more rapid disappearance of the insecticide from terrestrial soils and a reduced uptake of ^{14}C-labeled compounds by corn plants, EPTC had no effects. In the aquatic components, however, captafol and EPTC caused increased recoveries of ^{14}C-labeled residues from lake bottom mud.

Carbofuran (Furadan), a soil systemic insecticide, is widely used for the control of several insect pests. Because of its relatively high water solubility of 320 ppm (Bowman and Sans, 1979), carbofuran is more mobile in soils and is metabolized to a greater extent than insecticides of lower water solubilities. This was demonstrated by Fuhremann and Lichtenstein (1980), who grew oats in soils treated with six insecticides of different water solubilities.

In this study, a microcosm was used to examine the effects of water percolating through [^{14}C]carbofuran-treated soils on the movement and metabolism of the insecticide in both the terrestrial and aquatic components of the system. Since agricultural soils usually contain a variety of pesticides, we also investigated potential effects of the fungicide captafol (Difolatan) and the herbicide EPTC (Eptam) on the fate and movement of carbofuran in these systems.

MATERIALS AND METHODS

Chemicals. [^{14}C]Carbofuran [[*benzene*-^{14}C(U)]-2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate] (sp act. 2.2 mCi/mM), carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran phenol, 3-ketocarbofuran phenol, and carbofuran phenol were obtained through the courtesy of FMC Corp. (Middleport, NY). [^{14}C]Carbofuran was diluted with nonradioactive insecticide to 0.38 mCi/mM before its addition to soil. Captafol [*N*-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-carboximide], and EPTC (*S*-ethyl *N*,*N*-dipropylthiocarbamate) plus its antidote (*N*,*N*-diallyl-2,2-dichloroacetamide) were obtained through the courtesy of Ortho Chevron Chemical Co. (Richmond, CA) and Stauffer Chemical Co. (Mountain View, CA), respectively. Solvents used were redistilled acetone, benzene, dichloromethane, methyl cellosolve, and dioxane, as well as toluene, ethyl ether, and methanol.

Soils. A Plano silt loam (4.7% organic matter, 5% sand, 71% silt, and 24% clay with a pH of 6.0), free of insecticide residues, was collected at the University of Wisconsin Experimental Farm near Madison and stored at 22 ± 2 °C in a moist condition prior to use. A Plainfield sand (0.6% organic matter, 94% sand, 3.0% silt, and 3.0% clay and a pH of 5.6), free of insecticide residues, was collected in Adams County, WI, and stored as described above. Lake mud (12.5% organic matter, 37% sand, 57% silt, and 6% clay and a pH of 7.4), collected from Lake Mendota, Madison, WI, at a depth of 9 m, was drained of excess water and stored under refrigeration.

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

***Elodea*, Guppies, and Mosquito Larvae.** *Elodea nuttallii* (Plach.) St. John, obtained from the Department of Botany, University of Wisconsin, Madison, were grown in a 38-L aquarium containing tap water and a 2-cm bottom deposit of insecticide-free loam soil. Guppy fish (*Peocilia* sp.) were purchased from a local fish supply store. Mosquito eggs (*Aedes aegypti* L.) were obtained from Raltech Scientific Services, Inc., Madison, WI, and the larvae were reared in our laboratory as needed.

Soil Treatment. A total of 8000 g of a moist 2:1 mixture of Plainfield sand and Plano silt loam was treated as described by Lichtenstein and Schulz (1959) in five 1600-g portions (1494 g dry), each with 40 mL of an acetone solution that contained 5.4 mg (9.3 μCi) of [^{14}C]carbofuran. This resulted in an insecticide concentration of 3.6 ppm on a dry weight soil basis. Following soil treatment, 2 × 50 g portions were removed and extracted to determine the actual concentration of the insecticide in the soil. This level was later used as the data basis for recovery calculations. In addition, three 1600-g portions of [^{14}C]carbofuran-treated soils were removed and treated with an acetone solution of either captafol at 10 ppm, EPTC at 5 ppm plus its antidote at 1 ppm, or captafol (10 ppm) plus EPTC (5 ppm) plus antidote (1 ppm).

Description of the Agromicrocosm. A modified version of the system described by Lichtenstein et al.

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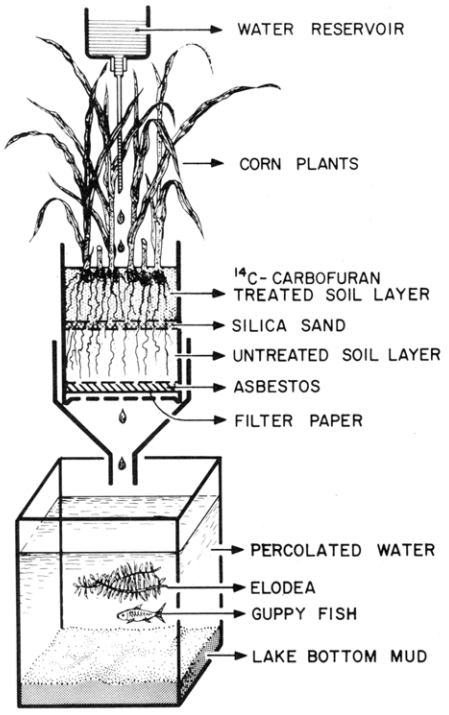

Figure 1. Schematic drawing of the agromicrocosm.

(1974) was utilized as shown in Figure 1. It consisted of a terrestrial component with seven corn plants and an aquatic component. The latter one consisted of a glass container with a bottom layer of 150 g of moist lake mud and contained up to 300 mL of percolated water into which *Elodea* and guppy fish were introduced as specified.

EXPERIMENTAL PROCEDURES

Two experimental series were conducted to study (a) the effects of percolating water on the transport and fate of soil-applied [^{14}C]carbofuran and (b) the potential effects of captafol and EPTC on the fate of the insecticide.

Effects of Percolating Water on the Transport and Fate of Soil-Applied [^{14}C]Carbofuran in a Soil–Corn–Water Microcosm. Procedures used in triplicate experiments are described in the flow sheet shown in Figure 2. Five-hundred grams of the soil mixture treated as described with [^{14}C]carbofuran at 3.6 ppm (2.9 μCi) was placed on top of a 500-g untreated soil mixture (Figure 1) in each of six containers, and seven corn seedlings were planted in each of the containers. They were then placed in a growth chamber for 21 days at 24 °C during the daytime (16 h) and 19 °C in the dark. The initially determined weight of each container was maintained by adding water or Hoagland's nutrient solution as often as necessary. During the 21-day incubation period, soils in the three control containers (I, II, and III in Figure 2) were kept moist while 340 mL of distilled water was percolated through the soil in each of containers IV, V, and VI (Figure 2) on days 9 and 20 in order to collect each time 150 mL of water. The percolation procedure (Lichtenstein et al., 1974) yielded a rate of 1 drop of water/s, and the total amount of water percolated through the soils was equivalent to 2.3 in. (5.85 cm) of rainfall.

After each percolation the 150 mL of water collected was well mixed, and a 32-mL aliquot was removed for bioassays with mosquito larvae (20 mL), analyses by liquid scintillation counting (LSC) (2 × 1 mL), and extraction (10 mL) as described. The remaining water obtained on day 9 and again on day 20 was poured into the aquariums, each containing a previously deposited 150-g layer of lake

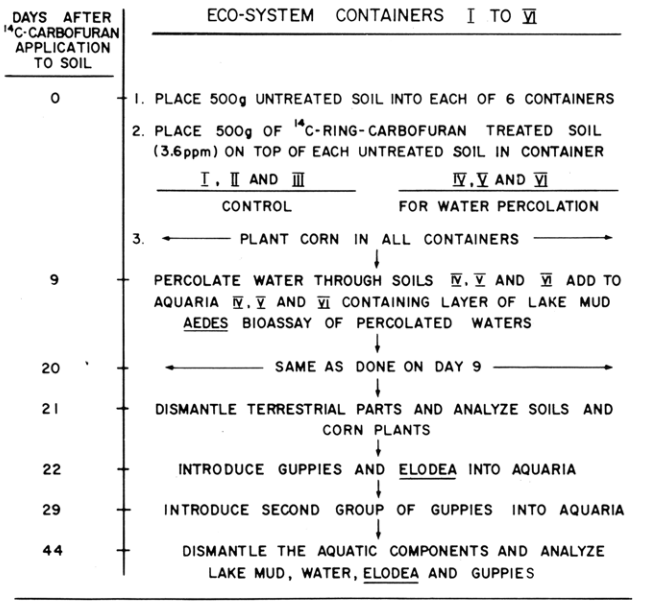

Figure 2. Flow sheet depicting experimental procedures.

bottom mud. On day 21, the terrestrial part of the system was dismantled and the various components (soil layers, corn roots, and leaves) were extracted and analyzed. One day later (day 22), when soil particles in the water had settled, four guppy fish (two male and two female) and eight 2.5-cm pieces of *Elodea* plants were added to the water in each of the three aquariums. A second group of four fish was added to each of the aquariums 1 week later (day 29). The aquatic systems were then incubated for a total of 3 weeks (days 22–44) at 24 ± 2 °C under a bank of Gro-Lux lamps on a 16-h photoperiod. On day 44 the aquatic systems were dismantled, and water, *Elodea*, lake bottom mud, and guppies were analyzed as described below.

To obtain a total ^{14}C balance of the aquatic components, a complementary study to the one described above was conducted with a closed aquatic system similar to that described by Ferris and Lichtenstein (1980). To that effect, [^{14}C]carbofuran was added to water at concentrations similar to those found previously in the percolated water samples collected on day 9 (3.2 ppm) and on day 20 (2.0 ppm). Thus, on day 0 of these additional experiments (corresponding to day 9 in Figure 2), three glass cylinders (18 × 6 cm i.d.), each containing 150 g of lake mud and 135 mL of distilled water previously treated with [^{14}C]-carbofuran at 3.3 ppm (0.77 μCi), were closed with rubber stoppers which contained tubes for air inlet and outlet. Polyurethane and KOH traps were placed as described (Ferris and Lichtenstein, 1980). The system was flushed with air (15 mL/min) continuously while the KOH traps were renewed daily for analyses by LSC. So that a second percolation on day 11 (corresponding to day 20 in Figure 2) could be simulated, 135 mL of distilled water treated with [^{14}C]carbofuran at 2.2 ppm (0.51 μCi) was again added to each aquarium. On day 13 (corresponding to day 22 in Figure 2), *Elodea* plants were introduced. Finally, on day 35 (corresponding to day 44 in Figure 2), the aquatic components were separated. The polyurethane plugs, lake mud, water, and *Elodea* plants were then extracted and analyzed by LSC as described.

Effects of Captafol and EPTC on the Fate of [^{14}C]Carbofuran in a Soil–Corn–Water Microcosm under Percolating Conditions. To study the potential effects of captafol and EPTC plus its antidote on the fate of [^{14}C]carbofuran in soils under percolating conditions,

we conducted experiments as described in Figure 2. In addition to control soils treated only with [^{14}C]carbofuran, experiments were conducted in triplicate with soils treated with [^{14}C]carbofuran plus captafol, [^{14}C]carbofuran plus EPTC and its antidote, or [^{14}C]carbofuran, captafol, and Eptam plus its antidote. It was felt that the presence of captafol in the loam soil might affect the degradation of carbofuran within the soil as shown by Ferris and Lichtenstein (1980) with parathion in cranberry soils and that EPTC plus its antidote might alter the uptake and translocation of carbofuran into corn as shown by Schulz et al. (1976) with phorate. Also, the combined effects of those pesticides on the fate of carbofuran in the soil–corn–water system might be different than those from the individual chemicals.

After incubation of the terrestrial and aquatic components (Figure 2), the 12 systems representing the 4 experimental variations in triplicate were dismantled. Soils, corn tops, water, *Elodea* plants, and lake mud were extracted and analyzed by LSC. Corn roots and guppies were combusted and analyzed by LSC, as described.

Dismantling of the Terrestrial System. Twenty-one days after planting, corn tops were excised 1 cm above the soil surface, rinsed with cold tap water, cut into 0.5-cm pieces, and immersed in 40 mL of acetone–methanol–benzene (1:1:1) for extraction. Soils containing the roots were allowed to dry for an additional 48 h to facilitate easier separation of the roots from the soil. The upper soil layer was then separated from the lower one by cutting through the silica sand separation layer. Roots were removed from each soil layer, rinsed with cold tap water, and allowed to air dry, and two 25-mg portions were taken for combustion analysis as described below. The soils were mixed well, and 100 g each from the upper and lower soil layers was immersed in 300 mL of 0.25 N HCl for extraction.

Dismantling of the Aquatic System. On day 44 of the experiment (Figure 2), *Elodea* plants and guppy fish were removed from the water and rinsed with cold tap water. Guppies were analyzed by combustion in toto. The *Elodea* plants were cut into 0.5-cm pieces and immersed in 20 mL of acetone–methanol–benzene (1:1:1) for extraction. The water was carefully siphoned from the aquaria and extracted as described below. Finally, the lake mud was removed and immersed in 300 mL of 0.25 N HCl for extraction.

Extraction and Analyses. Soils and lake mud were extracted by refluxing for 1 h with 0.25 N HCl as described by Markus and Puma (1973). The reflux mixture was quantitatively filtered under vacuum and the filtrate was partitioned 3 times with dichloromethane, resulting in a dichloromethane and an (acid) water extraction phase, plus the remaining extracted soil. Plant material (corn tops or *Elodea*) was extracted as described by Fuhremann and Lichtenstein (1980), resulting among others in a benzene extraction phase, containing primarily nonconjugated compounds and in a dichloromethane phase, containing primarily previously conjugated compounds (Cook et al., 1969). Roots and guppy fish were analyzed for radiocarbon content only by combustion as described below. Percolated water and aquarium water samples were bioassayed with *Aedes aegypti* L. mosquito larvae (Lichtenstein et al., 1974). The remaining water samples were extracted by acidifying to 0.25 N HCl and partitioning 3 times with dichloromethane. Organic solvent and water extraction phases of all components were analyzed for radiocarbon content by LSC in a Packard Tri-Carb liquid scintillation spectrometer, Model 3255, as described by Fuhremann and Lichtenstein (1978). Acidic water extraction phases were first neutralized by using 25 μL of 5 N NaOH/1 mL of water before LSC analyses. Extracted soils and plant pulp were pelleted and combusted in a Packard Tri-Carb sample oxidizer, Model 305, to determine $^{14}CO_2$ derived from bound (unextractable) ^{14}C-labeled residues as described by Flashinski and Lichtenstein (1974). Thin-layer chromatography (TLC) of benzene and dichloromethane extraction phases was performed by using E. Merck precoated silica gel 60 plates developed in benzene–ether (3:1). Visualization of the compounds was performed as described by Metcalf et al. (1968). Dichloromethane phases of percolated water from day 9 and 20 were spotted separately on the TLC plate; however, data were reported (Figures 3 and 4) as the total radiocarbon recovered from TLC plates prepared from the day 9 and day 20 water samples. Radioactive compounds in thin-layer chromatograms were located by autoradiography, using Kodak No Screen X-ray film. Gas–liquid chromatography (GLC) was used to confirm the identity of organic solvent soluble compounds isolated by TLC (Fuhremann and Lichtenstein, 1980).

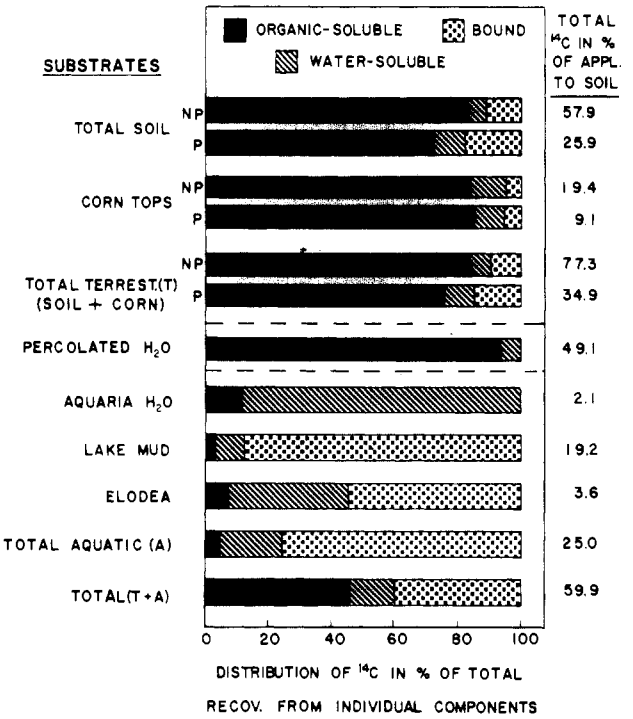

Figure 3. Degradation of soil-applied [*ring*-^{14}C]carbofuran in the various components of the agromicrocosm under nonpercolating (NP) and percolating (P) conditions.

RESULTS AND DISCUSSION

Effects of Percolating Water on the Transport and Fate of Soil-Applied [^{14}C]Carbofuran in a Soil–Corn–Water Microcosm. Results of these experiments (Table I) indicate that appreciable amounts of ^{14}C-labeled compounds had been removed with percolating water from the upper insecticide-treated soil layer. Thus, only 37% of the applied ^{14}C [Table I, total (S + C)] remained in the terrestrial portion (soil plus corn) while a total of 49% were recovered from the water percolated through these soils. In nonpercolated soils, however, 80% of the applied radiocarbon was still associated with soils and corn. The majority (35%) of the ^{14}C-labeled residues in the control soil was associated with the originally uncontaminated lower soil layer (L.L. in Table I). This mobility was probably due to the relatively high water solubility (320 ppm) of carbofuran.

Table I. Effects of Water Percolation on the Transport of Radiocarbon Derived from Soil-Applied [*ring*-^{14}C]Carbofuran in a Soil-Corn-Water Ecosystem[a]

	^{14}C recovered, % of applied[b] to the upper soil layer			
	nonpercolated		percolated	
recovered from	total sample	per g wt[c]	total sample	per g wt[c]
terrestrial part (T)				
soils (S)				
U.L.[b]	22.95 ± 2.20	0.048	11.76 ± 1.00[d]	0.025[d]
L.L.[b]	34.95 ± 1.31	0.075	14.09 ± 0.59[e]	0.030[d]
total (S)	57.90 ± 1.19	0.061	25.85 ± 1.56[e]	0.028[d]
corn (C)				
leaves	19.38 ± 1.72	0.89	9.10 ± 0.82[e]	0.51[e]
roots	2.78 ± 0.33	1.28	1.68 ± 0.13	1.00
total (C)	22.16 ± 1.40		10.78 ± 0.94[e]	
total (S + C)	80.06 ± 0.59		36.63 ± 2.41[e]	
percolated water				
on day 9			30.06 ± 0.57	0.190
on day 20			19.07 ± 0.74	0.118
total			49.13 ± 1.01	0.154
aquatic part (A)				
water			2.14 ± 0.30	0.008
lake mud			19.17 ± 1.38	0.173
Elodea			3.65 ± 0.17	
guppies			0.19 ± 0.08	
total (A)			25.16 ± 1.24	
total terrestrial (T)	80.06 ± 0.59		36.63 ± 2.41[e]	
aquatic (A)			25.16 ± 1.24	
T + A	80.06 ± 0.59		61.79 ± 3.58[e]	

[a] Results, determined by analyses of extractable and bound residues, are means ± SD of triplicated tests. [b] [*ring*-^{14}C]Carbofuran applied at 3.6 ppm (2.92 μCi) to the upper (U.L.) 500-g sand-loam (1:1) soil layer which was then placed on top of the lower (L.L.) 500-g untreated soil layer. [c] Per gram weight of dry soils, dry roots, and fresh leaves or per milliliter of water. [d,e] Results are significantly different from those for nonpercolated controls at the 1% (*d*) and 0.1% (*e*) level (Student's *t* test).

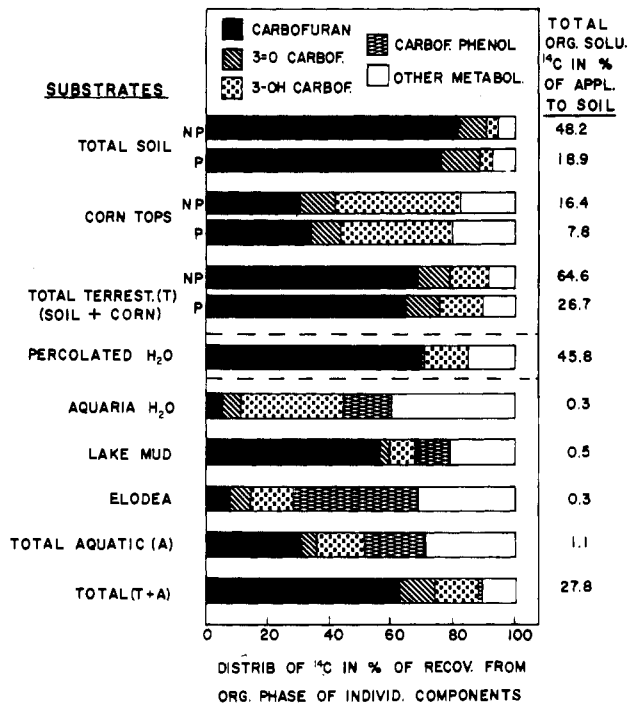

Figure 4. Metabolism of [*ring*-^{14}C]carbofuran in the various components of the soil–corn–water microcosm under percolating (P) and nonpercolating (NP) conditions. Data are based on TLC of the organic extraction phase, followed by autoradiography and LSC. 3=O CARBOF. = 3-ketocarbofuran; 3-OH CARBOF. = 3-hydroxycarbofuran; CARBOF. PHENOL = carbofuran phenol; OTHER METABOL. = 3-ketocarbofuran phenol, 3-hydroxycarbofuran phenol, and unknowns I, II, and III.

Amounts of ^{14}C-labeled residues recovered from corn plants were directly related to the amounts of radiocarbon recovered from these soils. Thus, 22% of the soil-applied ^{14}C was recovered from corn grown in control soils, but only 11% was recovered from that grown in soils through which water had been percolated. The major fractions (87% and 84%) of corn-associated radiocarbon were recovered from corn leaves.

The aquatic components contained 25% of the soil-applied radiocarbon at the end of the experiment, even though 49% had been initially added to the aquaria via the percolated water. This loss of 24% is partially accounted for by degradation of ^{14}C-labeled compounds to $^{14}CO_2$, as was shown by the complementary experiments discussed below. About $^3/_4$ of all the radiocarbon found in the aquatic system was associated with the lake bottom mud, most of it as unextractable (bound) ^{14}C-labeled compounds (Figure 3). Conversely, water in the aquarium and the *Elodea* plants contained only 2% and 3.7% of applied ^{14}C, respectively. Data, therefore, indicate that close to half of the radiocarbon originally added to the aquaria was lost via volatilization while most of the remainder was associated with the bottom mud layer, primarily as unextractable ^{14}C. Losses of ^{14}C from the aquatic system account for the overall lower recoveries (T + A, Table I) of 62% of soil-applied ^{14}C under percolating conditions as opposed to 80% with controls.

One good indication of the degradation of an insecticide is the formation of water-soluble and, to some extent, bound residues which are produced from the parent compound. These measurements were conducted with each of the components of the ecosystem. Results, expressed in percent of the total radiocarbon recovered from each individual component, are presented in Figure 3. In both soils and corn, 73–85% of the total radiocarbon recovered were organic soluble, the remainder being water soluble or bound to soil or corn. Although the absolute amounts of ^{14}C-labeled compounds recovered from soils and corn were very different under moist or percolated soil conditions (Figure 3, right column), the relative amounts of organic-soluble radiocarbon were nearly identical when

Table II. Pick Up of [*ring*-^{14}C]Carbofuran in Water by Lake Mud Bottom Deposits and Plants in a Closed System over a 35-Day Incubation Period[a]

recovered from	^{14}C recovered, % of applied to water[b]			
	bound	organic soluble	water soluble	total
aquatic components (A)				
water		1.31 ± 0.18	3.85 ± 0.58	5.15 ± 0.73
lake mud	52.06 ± 2.82	1.03 ± 0.20	2.86 ± 0.27	55.95 ± 3.22
Elodea	6.17 ± 1.22	0.59 ± 0.06	5.82 ± 0.82	12.57 ± 0.62
total (A)	58.23 ± 3.64	2.92 ± 0.12	12.53 ± 1.37	73.69 ± 4.07
vapor traps (V)				
KOH			11.35 ± 0.63	11.35 ± 0.63
polyurethane				0.00
total (V)			11.35 ± 0.63	11.35 ± 0.63
total (A + V)	58.23 ± 3.64	1.61 ± 0.16	27.68 ± 1.05	85.04 ± 3.56

[a] Results, determined by analyses of bound and extractable residues, are means ± SD of triplicated tests. [b] [^{14}C]Carbofuran was added to water on day 0 at 3.3 ppm (0.77 μCi) and again on the 11th day of incubation at 2.2 ppm (0.51 μCi).

expressed in percent of the ^{14}C recovered. Thus, corn tops from nonpercolated and percolated soils contained 19% and 9%, respectively, of the soil-applied radiocarbon, yet the amounts of organic-soluble ^{14}C were in both cases 85% of the total amount of radiocarbon recovered from corn tops.

As shown in Figure 3, percolated water contained a total of 49% of the soil-applied radiocarbon, most of it (93%) in the organic-soluble form. However, after its incubation in the aquarium for over 3 weeks, 88% of the ^{14}C recovered from the water had been rendered water soluble. Conversely, lake bottom mud not only contained the majority of the ^{14}C-labeled residues recovered from all aquatic components but also 89% of these residues had become bound to the mud and were unextractable by the methods used.

So that further information could be obtained about the metabolism of [^{14}C]carbofuran, organic solvent extraction phases of components of the total system were analyzed by TLC, autoradiography, and LSC as described. Results (Figure 4) show that in both control and percolated soils carbofuran was the major component, amounting to 82% and 77% of the organic-soluble radiocarbon, respectively. Major metabolites detected were 3-ketocarbofuran (12% and 9% of organic-soluble ^{14}C) and 3-hydroxycarbofuran (4% of organic-soluble ^{14}C) from percolated and control soils, respectively. In addition, small amounts of 3-hydroxycarbofuran phenol and three unidentified metabolites (R_f 0.7, 0.4, and 0.1) were detected.

Since corn roots contained only small amounts of radioactivity, only the plant tops were qualitatively analyzed. As opposed to soils, the major compound detected was 3-hydroxycarbofuran (37% and 41% of organic-soluble ^{14}C) while the amounts or carbofuran constituted 33% and 30% of the organic-soluble ^{14}C in corn from percolated and control soils, respectively. As previously stated, the benzene extraction phases contain primarily nonconjugated components, while the dichloromethane extraction phases contain to a large extent previously conjugated material (Cook et al., 1969). On the basis of analyses by TLC and autoradiography of corn leaves from percolated soils, 2.5% of the soil-applied carbofuran was associated with the benzene extraction phase and trace amounts with the dichloromethane phase. Comparable figures for 3-hydroxycarbofuran were 1% and 1.9%, for carbofuran phenol 0% and 0.1%, for 3-ketocarbofuran phenol 0% and 0.8%, for 3-ketocarbofuran 0.5% and 0.3%, and for 3-hydroxycarbofuran phenol 0.2% and 0.25%, respectively.

Percolated water contained 69% of the organic-soluble radiocarbon in the form of carbofuran. This was not surprising, since the major residue in soils was also carbofuran. However, the aquatic system [total aquatic (A), Figure 4] contained only 31% of the organic-soluble ^{14}C as carbofuran, while the amounts of carbofuran phenol, 3-hydroxycarbofuran, and 3-ketocarbofuran accounted for 20%, 16%, and 5%, respectively, of the organic-soluble radiocarbon. Most of the carbofuran found in the aquatic system was associated with the bottom mud layer, but this amounted to only 0.3% of that which was initially applied to the terrestrial part of the system. Since the insecticide degrades more rapidly under anaerobic (Venkateswarlu and Sethunathan, 1978) and alkaline (Getzin, 1973) conditions, it was not surprising that the degradation of carbofuran occurred more rapidly in the aquatic than in the terrestrial part of our system. The pH of the water in the aquaria was not constant and was measured as pH 9.0, 7.6, and 8.5 on day 24, 30, and 44, respectively.

Toxicity tests were conducted with water after its percolation through soils and again with water from the aquarium. In studies conducted in our laboratory (Lichtenstein et al., 1979) with *A. aegypti* larvae, LD_{50} values of ca. 0.22, 2.5, 15, 30, 75, and 200 ppm were obtained with carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, carbofuran phenol, 3-hydroxycarbofuran phenol, and 3-ketocarbofuran phenol, respectively, indicating that an increase in the appearance of carbofuran metabolites would be associated with decreased toxic effects. Water, which had been percolated through the soil on both days 9 and 20 (Figure 2) contained substantial amounts of carbofuran and caused each time 100% mortality of mosquito larvae within a 30-min exposure. Guppy fish exposed to aquaria water on day 22 died within 1 h of exposure. Bioassays conducted 1 week later (day 29), however, indicated that the water had been detoxified, since no fish mortality occurred during the following 2 weeks of exposure and no insect mortality during a 48-h exposure period.

The complementary study conducted with the aquatic components in a closed system made it possible to obtain a better understanding as to what happened to radiocarbon derived from [^{14}C]carbofuran which had been directly added to the water. As shown in Table II, total amounts of radiocarbon recovered after the 35-day incubation period were 85% of those added to the water, of which 11.4% were trapped in the form of $^{14}CO_2$. Most of this $^{14}CO_2$ (7.8% of applied ^{14}C), however, had been released at a linear rate during the first 10 days (Figure 5). Although on day 11 (B in Figure 5) the water was again treated with [^{14}C]carbofuran (2.18 ppm), $^{14}CO_2$ evolution occurred at a slower rate, and only an additional 3.5% of the applied radiocarbon evolved as $^{14}CO_2$ during the next 24 days of incubation. The gradual decline in the rate of $^{14}CO_2$ evolution beginning at day 10 may have been due to the fact that a relatively large portion of [^{14}C]carbofuran residues became bound to the lake mud or *Elodea* plants. Poly-

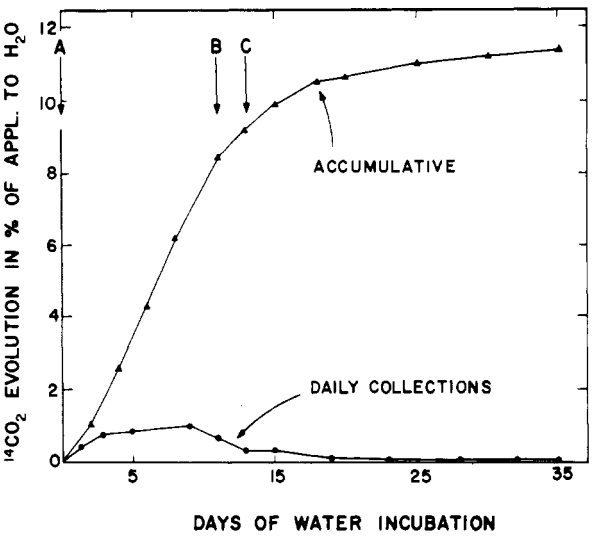

Figure 5. Evolution of $^{14}CO_2$ from a closed system with lake mud, *Elodea*, and water, after its treatment with [*ring*-^{14}C]carbofuran initially (A) and 11 days later (B). C = day 13, when *Elodea* plants were added to the water.

urethane plugs did not contain measurable amounts of radiocarbon.

As shown in Table II, the majority of the radiocarbon recovered from the closed system was associated with the lake bottom mud (56% of applied), most of which was in an unextractable, "bound" form.

Effects of Captafol and EPTC on the Fate of [^{14}C]Carbofuran in a Soil–Corn–Water Microcosm under Percolating Conditions. [^{14}C]Carbofuran-derived compounds disappeared most rapidly from captafol-treated soils since only 20.4 ± 1.5% of applied radiocarbon remained in fungicide-treated soils as opposed to 25.9 ± 1.6% in controls. Similarly, corn leaves grown in fungicide-treated soil also contained less radiocarbon (7.0 ± 0.7% of soil-applied ^{14}C) than those from control soils (9.1 ± 0.8%). However, the amounts of radiocarbon removed by percolating water from these soils were identical (50.8 ± 2.9% and 49.1 ± 1%). It appears that in captafol-treated soils an increased degradation of [^{14}C]carbofuran to $^{14}CO_2$ and/or other volatile compounds had occurred. Captafol inhibits the growth of soil fungi which could have resulted in an increased population of microorganisms capable of degrading carbofuran to volatile compounds. EPTC and its antidote had no effect in the terrestrial parts.

Contrary to results from the terrestrial components of the microcosm, the presence of captafol, EPTC plus antidote, or captafol plus EPTC and antidote resulted in an increased persistence of ^{14}C-labeled residues in the aquatic component. Total recoveries of ^{14}C-labeled residues amounted to 29.5 ± 1.5%, 31.7 ± 3.1%, and 32.0 ± 0.5% of soil-applied radiocarbon, respectively, as opposed to 27.3 ± 0.7% with controls. This increase in ^{14}C-labeled residue persistence, however, was associated with the bottom lake mud layers, from which 22.2 ± 0.4%, 23.4 ± 2.0%, and 23.4 ± 0.6%, rspectively, were recovered. The mud layer from controls contained only 19.2 ± 1.4% of the soil-applied radiocarbon. All the other components of the aquatic system contained ^{14}C-labeled residues similar to those of controls: water = 2.1 ± 0.3%, *Elodea* = 5.8 ± 0.5%, and guppy fish = 0.2 ± 0.1% of soil-applied radiocarbon.

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