Effects of Simulated Rain on the Transport of Fonofos and Carbofuran from Agricultural Soils in a Three-Part Environmental Microcosm

E. Paul Lichtenstein* and Tung T. Liang

The effects of simulated rain on the transport and metabolism of [^{14}C]fonofos and [^{14}C]carbofuran from insecticide-treated soil through deeper insecticide-free soil layers into surface waters were studied under laboratory conditions in a three-component microcosm. Radiocarbon derived from both [^{14}C]fonofos and [^{14}C]carbofuran was transported with soil runoff water from the place of the insecticide soil application through previously insecticide-free deeper soil layers into aquaria water and its sediments. Due to the lower water solubility of fonofos, less of this chemical was transported with water than did occur with carbofuran. Water, after its percolation through insecticide-free soil, contained 1.3% and 15.3% of the originally applied fonofos- and carbofuran-derived radiocarbon and <0.1% and 9.1% of the originally applied insecticides in the form of fonofos and carbofuran, respectively. Exposure of mosquito larvae to runoff water, percolated water, and aquaria water from [^{14}C]fonofos-treated soils resulted in lower insect mortalities than did exposure of larvae to comparable water samples from [^{14}C]carbofuran-treated soils. Thirty-six days after the start of the experiments and 21 days after the last rain application, aquaria water plus sediments from [^{14}C]fonofos- or [^{14}C]carbofuran-treated soils contained a total of 1.3% and 6.0% of the originally applied radiocarbon, respectively, but no fonofos and less than 0.1% of the originally applied carbofuran.

Since the introduction and widespread use of synthetic pesticide chemicals after World War II, the environmental fate of these chemicals has been of concern to both scientists and the public at large. As early as 1951 a group of entomologists from the midwestern states established a northcentral regional project entitled "Hazards Resulting from the Use and Misuse of Pesticides and Means for Their Elimination" with the objective "to isolate, define, and minimize or eliminate certain specific hazards associated with the use of insecticides, fungicides, herbicides, and other pesticides". Minutes of the November 3, 1951, committee meeting also state that "This project is a pioneering effort and that it is being proposed for the first time and therefore has no history as a regional project."

Since that time, various aspects of environmental behavior and fate of pesticide chemicals, in particular of insecticides, have been investigated. Studies pertaining to the transport of pesticides in the environment with water, however, were conducted during later decades. Thus, Haan (1971) reported on experiments in which "the movement of aldrin, dieldrin and DDT by runoff and erosion was studied under controlled conditions. It was found that the concentrations of the pesticides in the eroded soil was on the order of 10 to 30 ppm while that in the runoff water was only 1 to 70 ppb." One year later, Lichtenstein et al. (1972) studied the movement and fate of Dyfonate in various soil types under leaching and nonleaching conditions. Spalding et al. (1978) described field experiments with different pesticides in ground water beneath irrigated farmland in Nebraska, while the effects of percolating water on the translocation and metabolism of [^{14}C]phorate in a soil–plant ecosystem were described by Lichtenstein et al. (1974) and those on [^{14}C]carbofuran by Koeppe and Lichtenstein (1982). During the last decade, the fate of aldicarb in soils has been extensively studied. Leistra et al. (1976) reported that this insecticide as well as its oxidation products was very mobile in soil. Chesters et al. (1982) studied the occurrence and movement of aldicarb in ground water in the central sand plain of Wisconsin. The authors reported that the "highest concentrations of aldicarb were detected in the shallow wells, that no aldicarb was detected in any of the deep monitoring wells, that aldicarb seems to be concentrated in a 5- to 10-foot layer near the water table, and that marked seasonal fluctuations in aldicarb concentrations occurred in several wells". The fate of six insecticides of increasing water solubilities ([^{14}C]DDT, [^{14}C]lindane, [^{14}C]fonofos, [^{14}C]parathion, [^{14}C]phorate, [^{14}C]carbofuran) was studied under irrigation conditions relative to the movement and metabolism of these chemicals in two soils in which oat plants were grown (Fuhremann and Lichtenstein, 1980). The investigators reported that the more water-soluble insecticides, [^{14}C]phorate and [^{14}C]-carbofuran, were more mobile in soils and plants and were metabolized to a greater extent than insecticides of lower water solubilities. They emphasized in particular the importance of chemical structure, water solubility, and soil type in predicting the comparative environmental behavior of pesticides.

Studies described in this paper represent a continuation and expansion of previously described investigations (Lichtenstein et al., 1978; Fuhremann and Lichtenstein, 1980). Experiments reported here were designed to investigate the movement and metabolic fate of two widely used insecticides of different water solubilities. Utilizing an environmental microcosm, experiments were conducted under both soil–water runoff and soil-percolating conditions. The insecticides used were [^{14}C]fonofos and [^{14}C]-carbofuran with water solubilities of 15.7 and 320 ppm, respectively. We state, however, that these types of laboratory model experiments should be extended and confirmed by appropriate field investigations.

MATERIALS AND METHODS

Chemicals. [U-*phenyl*-^{14}C]Fonofos (Dyfonate; sp act. 22.16 mCi/mM), nonradioactive fonofos, and its potential metabolities [oxygen analogue of fonofos, thiophenol, diphenyl disulfide, methyl phenyl sulfone, and 2-, 3-, or 4-hydroxy phenyl methyl sulfone] were obtained from the Stauffer Chemical Co., Mountain View, CA. [*carbonyl*-

Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.

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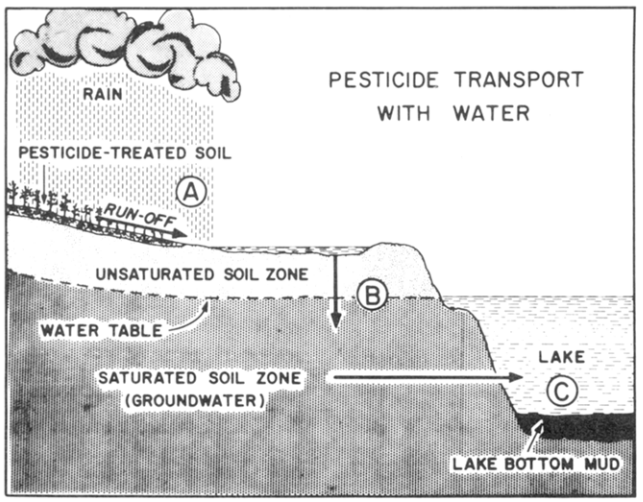

Figure 1. Schematic drawing depicting the potential transport of pesticides with water from pesticide-treated soils (A), through pesticide-free soil into deeper soil layers (B), and finally into lakes or rivers (C).

^{14}C]Carbofuran (sp act. 2.56 mCi/mM) and nonradioactive carbofuran were obtained from FMC Corp., Middleport, NY. [^{14}C]Fonofos or [^{14}C]carbofuran was diluted with the respective nonradioactive insecticide before its addition to soil.

Solvents. Acetone, benzene, chloroform, ethyl acetate, hexane, pentane, dioxane, methyl Cellosolve, and dichloromethane were redistilled before use. Methanol, toluene, diethyl ether, and ethanolamine were of analytical grade.

Soils. A Plano silt loam soil (4.7% organic matter, 5% sand, 71% silt, 24% clay; pH 6.0), free of insecticide residues, was collected at the University of Wisconsin Experimental Farm near Madison. A Plainfield sand (0.6% organic matter, 93.4% sand, 3.6% silt, 3% clay; pH 5.6), free of insecticide residues, was collected in Adams County, WI. These soils were stored in a moist condition at 22 ± 2 °C prior to use. Lake mud (12.5% organic matter, 37% sand, 57% silt, 6% clay; pH 7.4), collected from Lake Mendota, Madison, WI, at a depth of 9 m, was drained of excess water and stored under refrigeration.

Fish and Mosquito Larvae. Guppy fish (*Peocilia sp.*) were purchased from a local fish supply store. Mosquito eggs (*Aedes aegypti* L.) were obtained from the Department of Veterinary Science, University of Wisconsin, Madison, WI.

Soil Treatment. Moist loam soil was screened and treated as described by Lichtenstein et al. (1978) with [^{14}C]fonofos (6.36 μCi) or with [^{14}C]carbofuran (4.19 μCi) to yield concentrations of 6 ppm. Aliquots of the freshly treated soil were removed and extracted to determine the actual soil application level. This level was then used as the initial dose from which all later data were calculated.

Experimental Procedures. Procedures utilized in this study were designed to simulate under laboratory conditions the potential transport with water of pesticide chemicals in the environment. As shown in Figure 1, the fate of these chemicals is to a large extent determined by three phenomena: soil–water runoff after rainfall (A, Figure 1), percolation of pesticide-contaminated water through deeper soil layers (B, Figure 1), and finally, movement of this water into surface water such as lakes or rivers (C, Figure 1). To investigate these steps under laboratory conditions, a compartmentalized microcosm was used as described by Lichtenstein et al. (1978), except that soil–water runoff was not added directly to the aquaria but

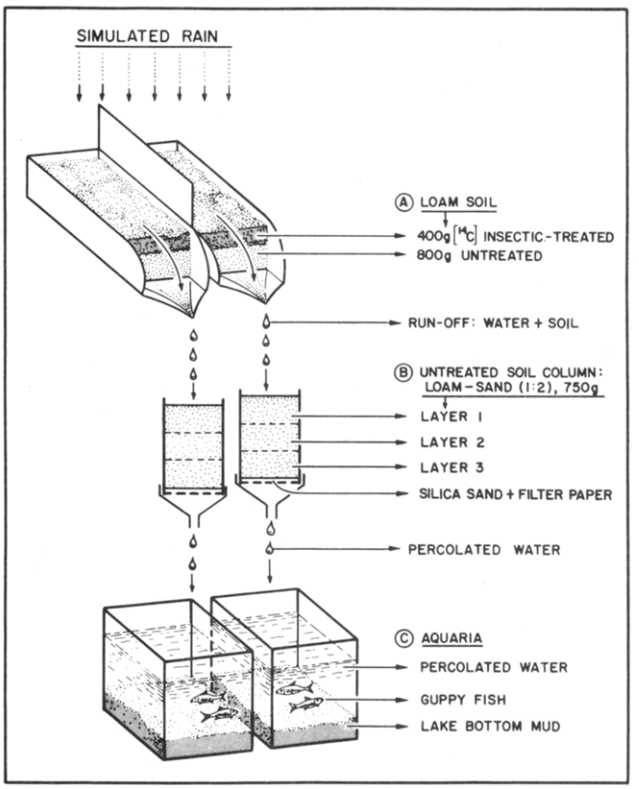

Figure 2. Schematic drawing of the model ecosystem, depicting its three components: the insecticide-treated soil (A), the untreated soil columns through which soil runoff water was percolated (B), and the aquatic portion (C).

was placed on top of insecticide-free soil columns (B, Figure 2). Water, after its percolation through these columns, was added to aquaria that contained a bottom layer of lake mud (C, Figure 2).

The experimental procedure during a 36-day period is described in a flow sheet (Figure 3). Experiments were conducted in duplicate with either [^{14}C]fonofos or [^{14}C]-carbofuran. These insecticides were applied to the upper 400-g (dry-weight basis) soil layer (A, Figure 2), since under field conditions this is the area where most of pesticide residues are located. These 400 g of insecticide-treated soils was then placed on top of 800 g (dry-weight basis) of untreated soils (Figure 2). Rain was applied for 9 min as described (Lichtenstein et al., 1978) on days 1, 8, and 15 after soil treatment. Each time a total of 1000 mL of water covered, in cone form, the two soil containers (A, Figure 2). This resulted in a runoff of approximately 500 mL from each container. Each 500 mL of water contained approximately 25 g (dry-weight basis) of soil, which represented about 2% of the soil placed into the container. This runoff water and the suspended soil were initially collected in beakers and mixed. An aliquot of 30 mL was then 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 below. Each of the remaining runoff water plus soil samples collected from each container (approximately 470 mL) was then carefully transferred onto the top of each of two insecticide-free soil columns kept within 1-L cartons (8.5-cm diameter × 16.5-cm height). Each soil column consisted of an insecticide-free 750-g Plano silt loam–Plainfield sand mixture (1:2) and represented the percolating portion of the system (B, Figure 2).

The amount of soil-free water collected each time after its percolation through one of the soil columns was approximately 450 mL. After thorough mixing of this per-

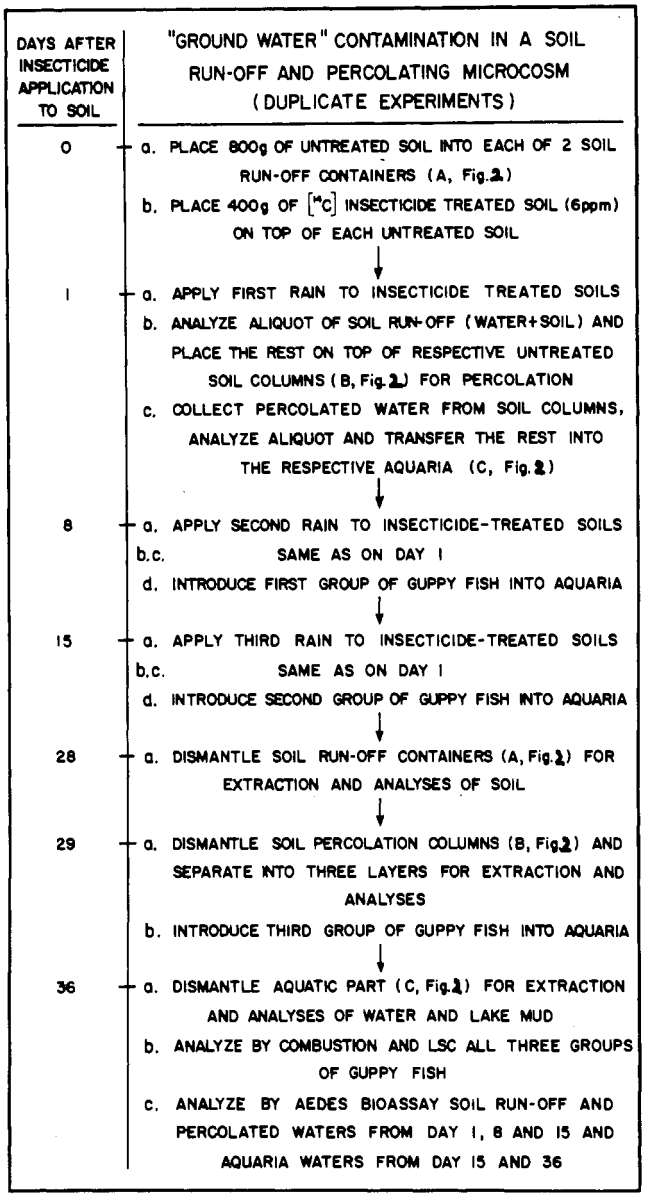

Figure 3. Flow sheet depicting experimental procedures.

colated water, a 150-mL aliquot was removed for bioassays with third instar mosquito larvae (20 mL), analyses by liquid scintillation counting (LSC; 2 × 1 mL), and extraction (100 mL) as described below. The remainder of the water samples collected on days 1, 8, and 15 was then transferred into the respective aquaria (C, Figure 2), each containing a previously deposited 220-g layer of lake bottom mud. As indicated in Figure 3, all components of the microcosm were extracted and analyzed.

Extraction and Analyses. Soils were extracted and analyzed as described by Fuhremann and Lichtenstein (1980). Lake bottom mud obtained from experiments with fonofos was handled as described by Liang and Lichtenstein (1980) and lake bottom mud from carbofuran-treated soils as described by Koeppe and Lichtenstein (1982). Runoff water, percolated water, and aquaria water from experiments with fonofos were extracted and analyzed as described by Liang and Lichtenstein (1980) and those from experiments with carbofuran as described by Koeppe and Lichtenstein (1982). To determine the total [^{14}C] content of some materials, aliquots were combusted as described by Flashinsky and Lichtenstein (1974). To test potential water toxicities, both insects and fish were utilized. Bioassays with mosquito larvae (*A. aegypti* L.) were conducted as described by Lichtenstein et al. (1974) and with guppy fish as described by Koeppe and Lichtenstein (1982). To better interpret results obtained from bioassay tests with mosquito larvae, the toxicity of the insecticide was tested by exposing the larvae to insecticide-treated water. It was found that LD_{50} values for fonofos or carbofuran were obtained during a 19-h exposure period at concentrations of 0.58 and 0.28 ppm, respectively.

Table I. Movement of Insecticides and ^{14}C with Water in a Soil Runoff Percolating "Lake" Water Microcosm

material		upper layers of treated soil (A, Figures 1 and 2)[a,j] [^{14}C]fonofos[j] fonofos[b]	[^{14}C]fonofos[j] ^{14}C	[^{14}C]carbofuran[j] carbofuran[b]	[^{14}C]carbofuran[j] ^{14}C
A. treated soil[a]	U[c]	26.2	50.0 ± 0.4	42.0	51.8 ± 0.5[i]
	L	3.9	8.1 ± 0.5	5.2	7.2 ± 0.1
	T	30.1	58.1 ± 0.1	47.2	59.0 ± 0.4
run-off[d]					
water		1.7	7.0	12.3	14.1
soil		*d*	14.4 ± 0.7[d]	*d*	8.7 ± 0.3[d,h]
B. percolated	U[c]	10.0	19.1 ± 0.2	0.3	5.9 ± 0.5[g]
soil	M	0.7	1.6 ± 0.2	0.1	1.6 ± 0.3
	L	0.2	0.8 ± 0.1	<0.1	1.3 ± <0.1[i]
	T	10.9	21.5 ± 0.4	0.4	8.8 ± 0.2[g]
percolated water[d]		<0.1	1.3	9.1	15.3[g]
C. aquaria[e]					
water		0.0	0.7 ± 0.1	<0.1	3.2 ± 0.3[h]
guppies		*e*	0.1 ± 0.0[e]	*e*	0.2 ± 0.0[e,h]
sediment[f]		0.0	0.6 ± 0.1	0.0	2.8 ± 0.2[h]
total		0.0	1.4 ± 0.2	<0.1	6.2 ± 0.6[h]
total (A + B + C)		41.0	81.0 ± 9.6	47.6	74.0 ± 0.4[h]

[a] Upper soil layer was treated at 6 ppm with either [^{14}C]fonofos (6.36 μCi) or [^{14}C]carbofuran (4.19 μCi) (Figures 2 and 3, day 0). [b] Extracts of organic-soluble compounds were separated by TLC and analyzed by LSC. Presence and amounts of fonofos and carbofuran were also confirmed and measured by GLC. [c] Key: U, M, L = upper, middle or lower soil layers; T = total. [d] Results for both runoff and percolated water were determined with pooled samples. They, therefore, represent the total of the data obtained on days 1, 8, and 15. Soils from runoff water samples were also pooled, followed by combustion to determine the total ^{14}C content. [e] Aquaria were dismantled and combusted 36 days after the start of the experiment (Figure 3, day 36). Guppies (eight for each duplicate) were analyzed, thus determining total ^{14}C content only. [f] Lake bottommud. [g–i] Data showing ^{14}C recoveries from systems treated with [^{14}C]carbofuran were significantly different from comparable data obtained with [^{14}C]fonofos at the 0.1% (*g*), 1% (*h*), and 5% (*i*) levels (Student's t-test). [j] Recovered in percent of applied.

RESULTS AND DISCUSSION

Data obtained after analyses of the above described systems clearly indicate that a movement of the insecticides or their metabolites with water had occurred. It was also obvious that the degree of water solubility of the test chemicals played an important role in the environmental behavior of the two insecticides (water solubilities of fonofos and carbofuran are 15.7 and 320 ppm, respectively, and vapor pressures are 2.0×10^{-4} and 8.3×10^{-6} mmHg at 25 °C, respectively; Fuhremann and Lichtenstein, 1980). An overall picture of the movement of [^{14}C]fonofos and [^{14}C]carbofuran with rainwater from upper insecticide-treated soil layers (A, Figures 1 and 2), followed by percolation through insecticide-free soil layers (B, Figures 1 and 2) into surface water (C, Figures 1 and 2), is presented in Table I. In insecticide-treated soils (A, Table I) the total amounts of [^{14}C] recovered at the end of the experiments were similar in both [^{14}C]fonofos- and [^{14}C]carbofuran-treated soils (58% and 59% of applied, respectively). The total amounts of radiocarbon recovered from the

Table II. Mortalities of *A. aegypti* Larvae Exposed at Different Days to Runoff, Percolating, and Aquaria Waters

		mortalities in waters from treated soil[a]			
		% mortality/24 h		LD_{50}, h	
material	day[b]	fonofos	carbofuran	fonofos	carbofuran
runoff, water only	1	48	100	24	0.2
	8	53	100	24	0.2
	15	13	100	48	0.2
percolated water	1	0	84	*c*	4
	8	0	94	*c*	4
	15	0	100	*c*	4
aquaria water	8	ND[d]	ND	ND	ND
	15	0	40	*c*	29
	36	0	0	*c*	*e*
controls[f]		0	0	0	0

[a] See footnote *a* in Table I. [b] Waters were tested 1, 8, and 15 days after the start of the experiment (day 0, Figure 2). [c] No mortalities were observed within 96 h of exposure. [d] ND = not determined. [e] 32% mortality was observed within 72 h of exposure. [f] Samples for controls were taken at days 1, 8, 15, and 36, 4 days of exposure.

percolated soil columns (B, Table I), however, were 21.5% of the originally applied [^{14}C]fonofos as opposed to only 8.8% of the applied dose of [^{14}C]carbofuran. This was related to the reduced removal with water of [^{14}C]fonofos-derived radiocarbon from the soil columns, since the percolated water (B, Table I) contained only 1.3% of the applied radiocarbon as opposed to 15.3% in water that had percolated through [^{14}C]carbofuran-contaminated soil columns. Consequently, this increased removal of [^{14}C]-carbofuran-derived radiocarbon was reflected in the presence of larger amounts of [^{14}C] (6.2% of applied) in aquaria components containing [^{14}C]carbofuran residues, as opposed to only 1.4% in experiments with [^{14}C]fonofos (C, Table I). However, the distribution of [^{14}C] within the aquaria between water, sediments, and fish was nearly identical for both insecticides. Thus, 50–52% of the total radiocarbon in the aquaria was associated with the water, 43–45% with the sediments, and 3–7% with the guppy fish.

In comparison to [^{14}C]fonofos, more [^{14}C]carbofuran-derived radiocarbon was found in runoff water, less in runoff soil and percolated soil, but 11.8 times more in percolated water and 4.6 times more in aquaria water and sediments. These facts were undoubtedly related to the higher water solubility of carbofuran.

As shown in Table II, exposure of mosquito larvae to runoff water, percolated water, and aquaria water from [^{14}C]carbofuran-treated soils also resulted in higher insect mortalities than did exposure of larvae to the water samples from [^{14}C]fonofos-treated soils. Guppy fish were exposed to aquaria water on days 8, 15, and 36 of the experiments. Results (Table III) show that basically no mortalities were observed in experiments with [^{14}C]fonofos, while exposure of guppy fish to the water from experiments with [^{14}C]carbofuran did result in significant fish mortalities, in particular on day 15. At that time 69%, 88%, and 100% of the fish had died after 24-, 76-, and 96-h exposure periods, respectively. However, 21 days later, this same water was no longer toxic to the fish.

Components of the whole system were extracted and analyzed as described. Data as summarized in Table IV represent the amounts of [^{14}C] recovered from organic-soluble and water-soluble extraction phases as well as the amounts of unextractable, bound [^{14}C] residues. Since the organic-soluble fractions were further analyzed, only differences in the amounts of bound [^{14}C] residues will be discussed at this point. The largest amounts of bound

Table III. Mortalities of Guppy Fish Exposed at Different Days to Aquaria Water

		mortalities (%) after time (h) of exposure to aquaria water from treated soil[a]					
		fonofos			carbofuran		
	day[b]	24	72	96	24	76	96
water	8	0	0	13	20	63	63
	15	0	0	0	69	88	100
	36	0	0	0	0	0	0
controls[c]		0	0	0	0	0	0

[a] See footnote *a* in Table I. [b] Waters were tested 8, 15, and 36 days after the start of the experiment (day 0, Figure 2). [c] Samples for controls were taken at days 8, 15, and 36.

radiocarbon were located in the originally treated soil (A, Table IV), where they represented 15.8% of the applied dose of [^{14}C]fonofos, but only 2.8% of the applied dose of [^{14}C]carbofuran. In percolated soils (B, Table IV), amounts of bound residues were primarily located in the upper soil layers and represented only 6.9% and 0.2% of applied [^{14}C]fonofos and [^{14}C]carbofuran, respectively. Aquaria sediments from experiments with [^{14}C]fonofos, however, contained significantly smaller amounts of bound radiocarbon (0.5% of applied) then sediments from experiments with [^{14}C]carbofuran (1.5% of applied recovered). This was probably related to the removal of 15.3% of the [^{14}C]carbofuran-derived radiocarbon with the percolating water from the soil columns (B, Tables I and IV).

Results obtained after analyses by TLC, LSC, and GLC of the organic-soluble extraction phases are summarized in Tables V and VI. It is important to realize that data for treated soils, percolated soils, and aquaria components were obtained 28, 29, and 36 days, respectively, after the application of the insecticides (Figure 3). In the experiments with [^{14}C]fonofos-treated soils, a total of 41% of the applied insecticidal dose was recovered in the form of fonofos (Table V). Of this amount, 73% was still associated with the insecticide-treated soil layers, 27% with the percolated soil columns, but none with the aquaria components. The major fonofos metabolites identified in the total microcosm were fonofos-oxon, methyl phenyl sulfone, diphenyl disulfide, and thiophenol, all in relatively small amounts of 1.4–1.6% of the applied insecticidal dose (Table V).

With [^{14}C]carbofuran-treated soils (Table VI), a total of 47.6% of the applied dose was recovered as carbofuran, of which, however, 99% was associated with the treated soil, close to 1% with the percolated soil, and less than 0.1% with the aquaria water. Since on day 36 this water was not any more toxic to guppy fish and only slightly toxic to mosquito larvae (32% mortality after 72 h of exposure; Table II), the higher mortalities observed with water 3 weeks earlier (15 days after the start of the experiment) indicate that on day 15 more carbofuran was present in the aquaria water, but had been degraded and/or patially volatilized during the additional 21-day incubation period between days 15 and 36. The major carbofuran-derived metabolites identified in the total microcosm (Table VI) were 3-hydroxycarbofuran (4.1% of the applied insecticide) and 3-ketocarbofuran (2.9% of the applied insecticide).

In summary, data presented above indicate that radiocarbon derived from both [^{14}C]fonofos and [^{14}C]carbofuran was transported with water from the place of the insecticide soil application through previously insecticide-free deeper soil layers into surface water and its sediments. Due to the higher water solubility of carbofuran, more of this chemical was transported with water than was the case with fonofos. This was especially apparent with water

Table IV. Recoveries of Organic- and Water-Soluble and Bound ^{14}C Residues from the Soil Runoff Percolating "Lake" Water Microcosm

material		upper layers of treated soil (A, Figures 1 and 2)[j]							
		[^{14}C]fonofos				[^{14}C]carbofuran			
		organic-sol	water-sol	bound[b]	total	organic-sol	water-sol	bound[b]	total
A. treated soil[a]	U[c]	31.3 ± 1.3	5.2 ± 0.0	13.4 ± 0.9	50.0 ± 0.4	48.8 ± 0.4[h]	0.6 ± 0[g]	2.4 ± 0.1[h]	51.8 ± 0.5[i]
	L	4.7 ± 0.2	1.1 ± 0.1	2.4 ± 0.2	8.1 ± 0.5	6.6 ± 0[h]	0.2 ± 0[h]	0.4 ± 0[h]	7.2 ± 0.1
	T	36.0 ± 1.1	6.3 ± 0.1	15.8 ± 1.1	58.1 ± 0.0	55.4 ± 0.4[h]	0.8 ± 0[g]	2.8 ± 0.1[h]	59.0 ± 0.4
runoff water[d]		4.9	2.1		7.0	13.7	0.4		14.1
runoff soil[d]					14.4 ± 0.7				8.7 ± 0.3[h]
B. percolated soil	U[c]	11.3 ± 0.6	1.8 ± 0.3	6.0 ± 0.1	19.1 ± 0.2	5.5 ± 0.5[h]	0.2 ± 0[i]	0.2 ± 0[g]	5.9 ± 0.5[g]
	M	0.8 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	1.6 ± 0.2	1.4 ± 0.2[i]	0.2 ± 0	<0.1[h]	1.6 ± 0.3
	L	0.3 ± 0	0.2 ± 0	0.3 ± 0	0.8 ± 0.1	1.2 ± 0[h]	0.1 ± 0[h]	<0.1[h]	1.3 ± 0.1[i]
	T	12.4 ± 0.7	2.2 ± 0.4	6.9 ± 0	21.5 ± 0.4	8.1 ± 0.3[i]	0.5 ± 0.1[i]	0.2 ± 0[g]	8.8 ± 0.2[g]
percolated water[d]		0.4	0.9		1.3	15.0	0.3		15.3
C. aquaria[e]									
water		<0.1	0.7 ± 0.1		0.7 ± 0.1	0.4 ± 0.1[h]	2.8 ± 0.4[h]		3.2 ± 0.3[h]
guppies					0.1 ± 0.0[e]				0.2 ± 0.0[e,h]
sediment[f]		<0.1	0.1 ± 0	0.5 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	1.2 ± 0.2[h]	1.5 ± 0[h]	2.8 ± 0.2[h]
total		<0.1	0.8 ± 0.1	0.5 ± 0.1	1.4 ± 0.2	0.5 ± 0.1	4.0 ± 0.2[h]	1.5 ± 0[h]	6.2 ± 0.6[h]
total (A + B + C)		48.4	9.3	23.2	81.0	64.0	5.3	4.5	74.0

[a] See footnote *a* in Table I. [b] Bound = unextractable [^{14}C] residues. [c–f] See footnotes c–f in Table I. [g–i] Organic-soluble, water-soluble, bound, and total ^{14}C recovered from systems treated with [^{14}C]carbofuran were significantly different from comparable data obtained with [^{14}C]fonofos at the 0.1% (*g*), 1% (*h*), and 5% (*i*) levels (Student's t-test). [j] Recovered from extraction phases, in percent of applied.

Table V. Recoveries of [^{14}C]Fonofos and Metabolites from the Organic Extraction Phases (Table II) of the Microcosm Components

material		^{14}C recovered,[a,b] % of applied				
		F	F=O	$MPSO_2$	D, T	other
A. treated soil[a]	U[c]	26.2	0.9	0.9	1.2	2.1
	L	3.9	0.1	0.2	0.1	0.4
	T	30.1	1.0	1.1	1.3	2.5
runoff water[d]		1.7	0.4	0.4	0.1	2.4
B. percolated soil	U[c]	10.0	0.2	0.2	0.3	0.6
	M	0.7	<0.1	<0.1	<0.1	<0.1
	L	0.2	<0.1	<0.1	<0.1	<0.1
	T	10.9	0.4	0.2	0.3	0.6
percolated water[d]		<0.1	<0.1	0.1	<0.1	0.3
C. aquaria[e]						
water		0.0	<0.1	<0.1	0.0	<0.1
guppies		not analyzed				
sediment[f]		0.0	<0.1	<0.1	0.0	<0.1
total		0.0	<0.1	<0.1	0.0	<0.1
total (A + B + C)		41.0	1.4	1.3	1.6	3.1

[a] See footnote *a* in Table I. [b] Key: F, fonofos; F=O, fonofos-oxon; $MPSO_2$, methyl phenyl sulfone; D, diphenyl disulfide, T, thiophenol; other, trace amounts of 2-, 3-, and/or 4-hydroxyphenyl methyl sulfone plus unknowns. Data were obtained from aliquots of pooled extracts for TLC and LSC. [c–f] See footnotes c–f in Table I.

Table VI. Recoveries of [^{14}C]Carbofuran and Metabolites from the Organic Extraction Phases (Table II) of the Microcosm Components

material		^{14}C recovered,[a,b] % of applied			
		C	3-OH-C	3-keto-C	other
A. treated soil[a]	U[c]	42.0	2.9	2.5	1.5
	L	5.2	0.5	0.3	0.6
	T	47.2	3.4	2.8	2.1
runoff water[d]		12.3	0.3	0.1	1.0
B. percolated soil	U[c]	0.3	0.4	0.1	4.7
	M	0.1	0.1	<0.1	1.2
	L	<0.1	0.1	<0.1	1.0
	T	0.4	0.6	0.1	6.9
percolated water[d]		9.1	2.2	0.3	3.4
C. aquaria[e]					
water		<0.1	0.1	<0.1	0.1
guppies		not analyzed			
sediment[f]		0.0	<0.1	0.0	<0.1
total		<0.1	0.1	<0.1	0.1
total (A + B + C)		47.6	4.1	2.9	9.1

[a] See footnote *a* in Table I. [b] Key: C, carbofuran; 3-OH-C, 3-hydroxycarbofuran; 3-keto-C, 3-ketocarbofuran; other, unknowns. Data were obtained from aliquots of pooled extracts for TLC and LSC. [c–f] See footnotes c–f in Table I.

collected after percolation through previoulsly insecticide-free soil columns. In experiments with [^{14}C]fonofos, less than 0.1% of the applied insecticide was detected in the percolated water as fonofos, as opposed to 9.1% of the soil-applied [^{14}C]carbofuran, which was detected in the percolated water as carbofuran. Thirty-six days after the start of the experiments and 21 days after the last rain application, components of the aquaria from [^{14}C]fonofos- or [^{14}C]carbofuran-treated soils contained a total of 1.4% and 6.2% of radiocarbon, respectively. However, at the end of the experiments no fonofos could be detected within the water and sediments, and less than 0.1% could be detected as carbofuran in experiments with this insecticide. Also, aquaria water from both fonofos- or carbofuran-treated soils was at that time nontoxic to both mosquito larvae and guppy fish. It would be desirable if, in future experiments, results obtained with these laboratory model studies could be confirmed by appropriate field experiments.

Registry No. Fonofos, 944-22-9; carbofuran, 1563-66-2.

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Synthesis and Characterization of Tissue-Retainable Methylsulfonyl Polychlorinated Biphenyl Isomers

Koichi Haraguchi,* Hiroaki Kuroki, and Yoshito Masuda

Eighty-six positional isomers of methylsulfonyl polychlorinated biphenyls (MSF-PCBs) have been synthesized by three synthetic routes: (1) the diazo coupling reaction of 3-(methylsulfonyl)chloroaniline with chlorobenzene; (2) nucleophilic substitution of PCB with methanethiolate and successive oxidation of the corresponding methyl sulfide; (3) the diazo coupling reaction of chloroaniline with chlorothioanisole and successive oxidation of the methyl sulfide. Pure isomers were characterized by their proton magnetic resonance and mass spectra and used to unambiguously identify the MSF metabolites retained in human tissues by using high-resolution capillary gas chromatography (GC). The GC analysis showed that 40 MSF derivatives were positively identified in the tissue of a patient with Yusho on the basis of comparisons of their GC retention data with those of the standard compounds.

Methylsulfonyl (MSF) derivatives of polychlorinated biphenyls (PCBs) have evoked great interest since they were found as metabolic products of PCBs in the excreta of experimental animals fed PCBs (Mio et al., 1976; Mizutani et al., 1978; Bergman et al., 1979) and wild animals (Jensen and Jansson, 1976) and human milk as well as adipose tissue (Yoshida and Nakamura, 1978; 1979). In our previous study (Haraguchi et al., 1984), several MSF-PCBs were also found to be accumulated at relatively high concentrations in the tissues of patients with Yusho, a PCB poisoning that occurred in Japan. The qualitative and quantitative analyses of these metabolites by gas chromatography (GC) are complicated by the complex composition of these MSF-PCBs and the unknown identities of the many individual components. Therefore, information concerning the precise composition of MSF-PCB mixtures is required for an understanding of the toxicity of the residual MSF-PCBs. In order to clarify the structures of these metabolites in human tissues, we synthesized various MSF-PCB congeners consisting of two to seven chlorine atoms and one or two hydrogen atoms at the lateral positions of the MSF group, because these metabolites were expected to be formed via arene oxide intermediates from PCBs that have at least two adjacent hydrogens in the phenyl ring of PCB (Preston et al., 1984). In this paper, we report the syntheses of 86 MSF-PCB isomers by three methods, their characterization by mass spectroscopy (MS) and proton magnetic resonance (^{1}H NMR) spectra, and the GC profiles of tissue-retainable MSF-PCB metabolites on three capillary columns.

EXPERIMENTAL SECTION

Chemicals. All the synthetic precursors used for this work were commercially available. 2,3-, 2,4-, 2,5-, 3,4-, and 3,5-dichloroaniline, 2,4,5-trichlorothioanisole, and methyl mercaptan sodium salt (ca. 15% in water) were purchased from Tokyo Chemical Industry Co. Ltd., Osaka, Japan. *o*-, *m*-, and *p*-dichlorobenzene, 1,2,3- and 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, and isoamyl nitrite were purchased from Wako Pure Chemical Industries Co. Ltd., Osaka, Japan. 2,3,4- and 2,4,5-trichloroaniline, 2,3,4,5- and 2,3,5,6-tetrachloroaniline and 1,3,5-trichlorobenzene were from Aldrich Chemical Co., Milwaukee, WI. Methanethiol (>98%) was obtained from Eastman Kodak Co., Rochester, NY. All the other chemicals employed were of reagent grade unless otherwise mentioned in the text.

Synthesis. The 3-MSF-PCB congeners were prepared by the Cadogan coupling (Cadogan, 1962) from chlorinated 3-(methylsulfonyl)aniline and chlorinated benzene with isoamyl nitrite, as indicated in the synthetic route of 3-MSF-4,5,3′,4′-tetra-CB (Scheme I). Chlorinated 3-(methylsulfonyl)aniline was prepared by a modified procedure of the method of Mizutani et al. (1978). Dichloroaniline (4.0 g) dissolved in H_2SO_4 (20 mL) was diazotized with $NaNO_2$ (5.0 g) at 4 °C. After neutralization by sodium acetate, a mixture of $NaSCH_3$ (20 mL), copper powder (3.5 g), NaOH (2.8 g), and water (50 mL) was added to the solutions with stirring over a period of 1 h. The hexane solution of the collected solid was filtered off, washed with water, and dried over Na_2SO_4 to yield 2,3-dichlorothioanisole. The product was oxidized with an excess of hydrogen peroxide in acetic acid to give the corresponding (methylsulfonyl)chlorobenzene, which was nitrated with KNO_3 (3.0 g) in concentrated H_2SO_4 (10 mL) at 80 °C for 2 h to yield 3-(methylsulfonyl)-4,5-dichloronitrobenzene. The crude nitro product was reduced by iron powder (2.0 g) in 70% acetic acid at 80 °C for 4 h to give the corresponding 3-(methylsulfonyl)chloroaniline. The substituted aniline (0.4 g) was subsequently converted to 3-MSF-PCB by Cadogan coupling with an excess of *o*-dichlorobenzene

Daiichi College of Pharmaceutical Sciences, Tamagawa-cho, Minami-ku, Fukuoka 815, Japan.

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