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Distribution of Carbofuran in a Rice-Paddy-Fish Microecosystem

Allan R. Isensee[1] and Nuansri Tayaputch[2]

[1]Pesticide Degradation Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705 and [2]Division of Agricultural Toxic Substances, Department of Agriculture, Bangkok 109001, Thailand

Culture of fish in rice paddies has long been a common practice in Asian countries. Fish normally migrate into the paddies during flooding, and when the rice matures the fish are harvested to provide additional income or food for the farmer. Use of pesticides in rice (Oryza sativa L.) production has recently become a common practice, even on small farms. Fish mortality has resulted, primarily from the use of carbofuran (2,3-dihydro-2,2dimethyl-7-benzofuranyl methylcarbamate) and endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a, 6,9-9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) (FAO/IAEA, 1983). Better understanding of pesticide degradation patterns and behavior in rice paddy environments is needed for safe fish production. Carbofuran is widely used for rice production and its metabolism and persistence under aerobic and flooded soil conditions has been studied (Caro et al. 1979, Siddaramappa and Seiber, 1979, Siddaramappa et al. 1978, Venkateswarlu and Sethunathan 1979). However, the fate and behavior of carbofuran in soil and water under rice-paddy conditions is not well known. This investigation was undertaken to determine (1) the usefulness of a rice-paddy-fish microecosystem to study the fate and behavior of carbofuran in a simulated rice-paddy-fish environment and (2) potential effects of carbofuran residues on fish.

METHODS AND MATERIALS

A microecosystem chamber (Figure 1) was designed to evaluate the fate of carbofuran in soil, water, and fish in a simulated rice-paddy environment. Glass chambers (40 x 45 x 25 cm) were constructed with an 8-cm high glass partition located 15 cm from one end. The experiments were set up as follows: 3 kg untreated Crowley silt loam (Typic Albaqualfs) (pH 6.3, organic matter content 7.1%, sand, silt, and clay contents 9.6, 70.4, and 20.0%, respectively) were uniformly added to the large chamber (30 x 40 cm). Eleven soil sampling tubes (made by cutting the bottom off glass liquid scintillation vials and packing glass wool into the vial neck) were placed neck end down on the soil. Sampling tubes were installed at the start of the experiment to insure the retrieval of undisturbed soil samples from flooded soil. An additional 5 kg of untreated soil were uniformly added to the chamber and sampling tubes. A thin layer of silica sand was placed on top

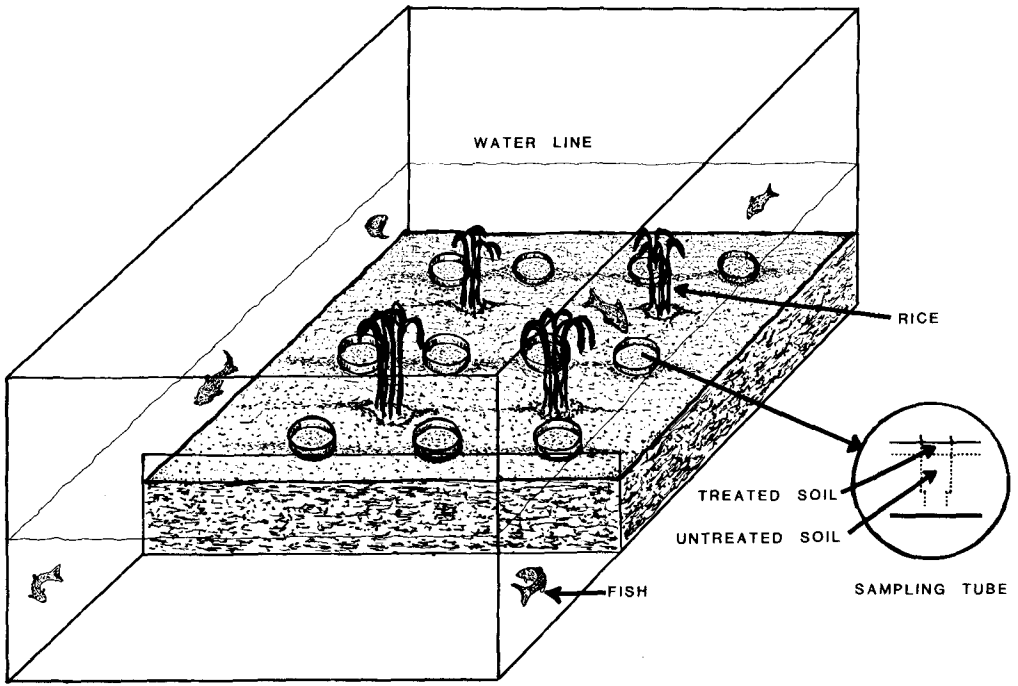

Figure 1. Rice-paddy-fish microecosystem. Chamber measures 45 x 40 x 25 cm and contains 14 L water.

of the soil in each sampling tube to help differentiate between treated and untreated soil at sampling time. The 8 kg of untreated soil was moistened to field capacity (24% w/w) and rice seeds were placed on the soil surface in four "hills" (4 seeds each) as shown in Figure 1. A final 1500-g layer of soil treated with [^{14}C-ring]carbofuran (97+% purity, 39.4 mCi mmole specific activity) at 6 and 12 ppm was added covering the rice seed to a depth of 1 cm.

The treated soil was moistened to 24% (w/w). Three replicates of each rate plus two controls were prepared and watered as needed for 18 days. At 18 days 14 L of water were added to each tank flooding the soil to a depth of about 6 cm. One day later 14 mosquito fish (Gambusia affinis) were added to each tank.

At 14 days duplicate soil sampling tubes were removed from each tank, wrapped in aluminum foil and stored at -10°C for later analysis. Empty liquid scintillation vials were placed in the holes left by the removal of the sampling tube. This soil sampling process was repeated at 25, 32, 46 and 60 days. Thus, one soil sample was taken before flooding and four after flooding. For analysis, the frozen soil cores (two for each microecosystem chamber) were pushed out of the tubes and then separated into treated and untreated sections. Like sections from each pair of tubes were combined, mixed and subsampled for moisture determination. The remaining soil was shake-extracted with acetonitrile-water (70:30 v/v) for 0.5 hr, allowed to stand overnight, and then shake-extracted for an additional 0.5 hr and filtered. Samples of the extract were analyzed by liquid scintillation (LS) for total ^{14}C, and the remaining extract was reduced in volume on a rotary evaporator. The remaining aqueous extract was then extracted with ether. The ether was spotted on thin-layer chromatography (TLC) plates (20 x 20 cm GF-254, E. Merck Darmstadt[1]) with unlabeled carbofuran and several metabolites and developed 15 cm, twice, using ether:hexane (5:1 v/v). Plates were scraped and total ^{14}C was determined by LS. Extracted soil was air-dried, mixed, and samples were oxidized to determine total residual ^{14}C. Water samples (triplicate 1-ml) were taken at 2-day intervals and analyzed by LS methods for total ^{14}C. Larger water samples (100 ml) were taken each week and extracted by using "C 18 Sep Paks" (Waters Associates, Inc.). Samples were passed through the Sep Paks at 2-4 ml/min. Recovery of ^{14}C-carbofuran plus metabolites from the Sep Paks was achieved by extracting with 10 ml methanol. Carbofuran extraction efficiency from water was 99%. Extracts were spotted on TLC plates and analyzed as described above. Two fish samples were taken 1, 3, 7, 15, and 30 days after they were added. Remaining fish (after 30 days) were placed in untreated water and sampled 4 and 10 days later. Whole fish were homogenized in acetonitrile and then filtered. The filtrate was analyzed directly by LS and TLC. The tissue was air-dried, then

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[1]Mention of a trade name or proprietary product does not constitute a guarantee or warranty of product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

oxidized to determine total residual ^{14}C. Unexpected fish mortality (control as well as treatment tanks) necessitated restocking after the 15-day sampling.

RESULTS AND DISCUSSION

Two weeks after treatment (before flooding) about 50% of the ^{14}C in the treated soil had leached into the untreated soil (Table 1). After 25 days (7 days after flooding) less than 20% of the ^{14}C remained in the treated soil, and about 50% had leached out of the sampling tube completely. Little additional movement occurred with time, indicating that most of the leaching resulted from watering the rice seedlings and during the actual flooding process. The high water solubility (700 ppm) of carbofuran may account for the observed mobility in soil. The amount of ^{14}C extracted from both the treated and untreated soil decreased with time, and the unextractable ^{14}C (as determined by oxidation) generally increased with time in the flooded soil samples. Soil adsorption of carbofuran and/or its labeled products probably account for the increase in unextractable ^{14}C. These results agree well with a study conducted by Venkateswarlu and Sethunathan (1979) who found a significant increase in unextractable soil-bound residues when carbofuran-treated soils were incubated 10 or 20 days aerobically followed by 20 days anerobically. Incubation aerobically or anaerobically for 40 days did not result in as much soil adsorption. In the first soil extract (day 14) 70 to 76% of the recovered ^{14}C was chromatographically identical to carbofuran and 10 to 14% of the ^{14}C cochromatographed with authentic 3-ketocarbofuran (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl-N-methylcarbamate). In the flooded soil samples, carbofuran accounted for 85 to 90% of the recovered ^{14}C, but no 3-ketocarbofuran was detected. No other metabolites were found. Formation of 3-ketocarbofuran in aerobic soil has been established previously (Caro et al. 1973). The disappearance of 3-ketocarbofuran after 7 or more days of flooding was unexpected and may have been caused by rapid degradation under anaerobic conditions. Previous research has shown that these ecosystem soils become anaerobic in only 3 to 5 days after flooding (Isensee et al. 1979).

Total ^{14}C in water reached a maximum concentration of 12.2 and 13.1% (of the total ^{14}C applied to each tank at the start of the experiment) 9 days after flooding and decreased to 4.1 and 4.6% by day 44, 6 and 12 ppm rates, respectively (Table 2). Extractable ^{14}C equaled total ^{14}C on day 3, then decreased rapidly between day 9 and 17. TLC analysis of the water extracts indicated that 90% of the ^{14}C was carbofuran on day 3, 36% and 57% on day 9 and less than 10% by day 17 (6 and 12 ppm rates respectively). Thus, the concentration of carbofuran in water was 54 and 95 ppb (day 3), 11 and 56 ppb (day 9) and 1 and 2 ppb (day 17) for the 6 and 12 ppm rates, respectively. The acute toxicity of carbofuran to mosquito fish is not known. However, reported LC50 levels in water for 8 other species of fish ranged from 147 to 872 ug/L (Johnson and Finley, 1980). It thus seems likely that the concentrations in water for the 6 ppm treatment rate were not

Table 1. Recovery of ^{14}C from soil treated with ^{14}C-carbofuran in a fish-rice-paddy microecosystem.

Time after treatment	Treatment rate	Distribution of the total ^{14}C recovered[a]				
		Treated soil[b]		Untreated soil[b]		
		Extraction[c]	Oxidation[c]	Extraction[c]	Oxidation[c]	Total
(days)	(ppm)	(%)				
14	6	33.5±8.4[d]	12.9±3.1	38.9±10.8	12.7±4.7	98.0
	12	35.6±2.1	13.8±0.8	31.4±10.4	12.6±2.0	93.4
25	6	7.0±0.9	6.3±4.2	29.3±11.4	8.7±3.2	51.3
	12	6.8±0.6	7.7±0.8	25.5± 7.4	7.1±1.4	47.1
32	6	5.6±1.5	12.4±2.6	19.7± 6.1	10.2±2.8	47.9
	12	4.8±0.1	9.3±1.4	18.4± 5.6	7.1±1.0	39.6
46	6	3.4±0.5	15.5±3.4	7.5± 1.2	7.6±2.6	34.0
	12	4.1±0.7	13.3±1.8	12.8± 3.4	12.0±1.9	42.2
60	6	2.2±0.1	19.5±3.5	5.6±3.3	13.0±3.8	40.3
	12	3.1±0.0	21.8±2.1	7.4±3.4	15.4±1.5	47.7

[a] Total ^{14}C recovered from treated plus untreated soil expressed as a percent of the total ^{14}C applied to the soil at the beginning of the experiment.

[b] Treated (^{14}C-carbofuran) and untreated soil recovered from sampling tubes.

[c] Soil was shake-extracted with acetonitrile:H_2O (70:30). Residual ^{14}C not removed by extraction was determined by oxidation.

[d] Means and standard deviation for three replications.

Table.2 Recovery of ^{14}C from water in a rice-paddy-fish microecosystem treated with ^{14}C-carbofuran.

Time after flooding	Treatment rate	Radioactivity recovered: Total in water[a]	Extracted from water[b]	Total recovered[c]
(days)	(ppm)	cpm/ml		%
3	6	314±56[d]	369±40	8.0
	12	298±42	322±85	7.6
9	6	480±41	181±139	12.2
	12	517±38	300±80	13.1
17	6	299±20	56±1	7.6
	12	347±15	63±4	8.8
23	6	279±25	57±6	7.1
	12	309±25	79±21	7.9
30	6	236±22	49±5	6.0
	12	252±11	64±11	6.4
37	6	182±20	36±5	4.6
	12	191±21	39±10	4.9
44	6	160±15	44±14	4.1
	12	180±7	32±11	4.6

[a] Direct count analysis of 1-ml water samples.
[b] Radioactivity extracted from 100-ml water samples expressed as cpm/ml.
[c] Total ^{14}C in water expressed as a percent of the total ^{14}C applied to soil at the beginning of the experiment.
[d] Means and standard deviation for three replications.

toxic to fish. However, concentrations in water from the 12 ppm treatment (twice the recommended rate) were close to known toxic levels, indicating that application rate is critical if fish are to be cultured in rice paddies. Polar metabolites in water (^{14}C remaining at the origin) increased proportionally as carbofuran decreased. Several studies have shown that carbofuran persistence in water is related to pH. Carbofuran was hydrolyzed to carbofuran-phenol (2,3-dihydro-2,2-dimethyl-7-benzofuranol) in 5 days under alkaline paddy water conditions (Siddaramappa et al. (1978). In another study, Siddaramappa and Seiber (1979) found that 70% of the carbofuran remained intact after 10 days at a pH of 6.0 or lower, 14% refmained at a pH of 7.1, and less than 5% remained after 5 days at pH 8. The water in our microecosystems was pH 6.8 one day after flooding and gradually increased to 8.2 by 25 days after flooding. Thus, the persistence of carbofuran in

our system (36 to 57% remaining after 9 days) agrees well with other studies.

Results from the analysis of the fish were of limited value due to unexplainable mortality. For example, by the 15 day sampling, 8 out of the 14 fish initially added had died in two of the three replications for the 6 ppm treatment (none died in the third replicate) while 2 to 4 fish died in each of the control and 12 ppm tanks. The higher mortality at the 6 ppm treatment rate compared to the 12 ppm treatment and the observed mortality in the control suggests that carbofuran may not have been the causative agent. However, even with the limited confidence in the fish analysis, some interesting trends were noted: (1) ^{14}C extracted from fish with acetonitrile accounted for about 60% of the total ^{14}C in fish on day 3 and then decreased to about 10% by 30 days. These results indicate that ^{14}C was being incorporated into the fish tissue. (2) The total ^{14}C levels increased continuously with time to 30 days, with little real loss of ^{14}C after placement in untreated water for 11 days. (3) The maximum concentration of carbofuran in the fish extracts was 88 ng/g for the 12 ppm treatment, day 1 sample (based on TLC analysis). Carbofuran accounted for 5 to 14% of the total ^{14}C in the fish extracts. The identity of the unextractable ^{14}C is unknown.

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