Bull. Environm. Contam. Toxicol. 23,544-551 (1979)

Evidence for Vapor Loss of ^{14}C-carbofuran from Rice Plants

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Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate) has been widely used for control of common insect pests of rice. Studies conducted to determine the cause(s) for decreased effectiveness of this insecticide in controlling brown planthopper (Nilaparvata lugens) revealed a rapid degradation of carbofuran by chemical hydrolysis, especially in the paddy water (SIDDARAMAPPA et at. 1978). In soil, however, the degradation was due mainly to microbial activity.

Placement of carbofuran at the root zone of rice has extended the period of insecticide availability because of increased persistence in the soil (SIDDARAMAPPA et al. 1978).

Radioautographic studies have indicated a rapid absorption and translocation of paddy-water-applied carbofuran in rice plants, resulting in accumulation of residues in the leaf tips (Fig. 1). Furthermore, the radioactivity observed in guttation fluids collected on leaf blades of treated plants suggested the possibility of vapor loss of systemically applied carbofuran from rice plants. More detailed studies conducted to determine the vapor loss of carbofuran from rice plants under greenhouse conditions are reported in this paper.

MATERIALS AND METHODS

Labeled compounds. Uniformly ring or carbonyl-labeled ^{14}C-carbofuran was donated by the FMC Corporation, Middleport, New York. 0.01 mCi of carbofuran-ring ^{14}C (specific activity, 2.2 mCi/mM) and 0.02 mCi of carbofuran-carbamate-carbonyl ^{14}C (specific activity, 3.54 mCi/mM) were used in experiment 1 and 2, respectively. In experiments 3 and 4, 0.01 mCi of carbofuran-ring ^{14}C (specific activity, 26.73 mCi/mM) was used.

The radioactive samples of carbofuran were dissolved separately in 100 ml of acetone and stored in a refrigerator until used. Suitable aliquots of this stock solution were evaporated off to remove acetone and equilibrated with distilled water (a rate of 10 ml distilled water to 1 ml of stock solution was followed) for 6 h. The solution was passed through a millipore filter of 0.45 µ pore size and was used in the following experiments.

0007-4861/79/0023-0544 $01.60

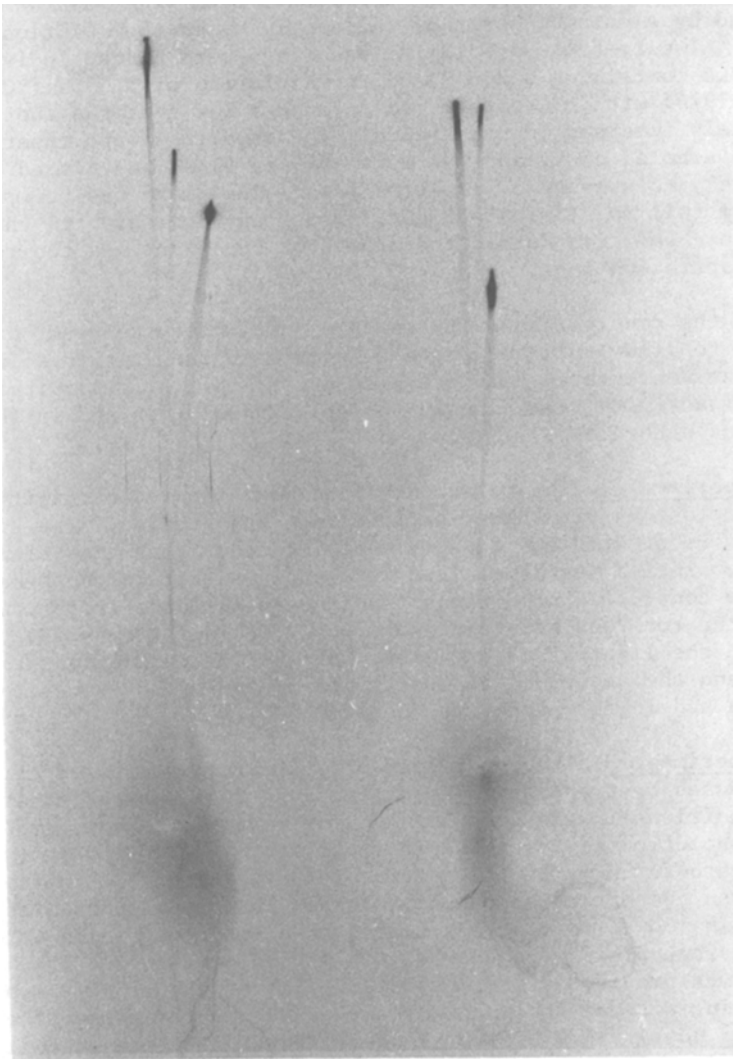

Fig. 1. Autoradiograph showing the movement and accumulation of carbofuran residues in rice plant (^{14}C-carbofuran was applied to paddy water).

Experiment 1. The loss of carbofuran from rice was indirectly assessed by a balance sheet of radioactivity in the solution-culture system. Twenty-five-day-old IR8 seedlings were placed individually in flasks containing a 200 ml culture solution of pH 5.5 (YOSHIDA et al. 1976) with ^{14}C-carbofuran in such a way that the roots were completely immersed in the liquid. The seedlings were inserted through a holed cork, and the mouth of the flask was closed completely to prevent evaporation loss. The flask was covered with aluminum foil to eliminate light. Thus, any decrease in the volume of culture solution during the growth of the plant was the result of transpiration loss.

In the control, however, the top portion of the plant was removed to allow only the roots to remain in the culture solution. The flask was covered completely as described. At periodic intervals the radioactivity remaining in the culture solution and in the rice plant was determined.

Experiment 2. The relationship between the radioactivity loss to water loss by transpiration from rice was studied. The rice plants were treated as in experiment 1 (except that the volume of culture solution was 25 ml) and were grown either in the greenhouse or under controlled conditions. In the latter, the temperature inside the room was maintained at 27 $\pm$ 1^{o}C; relative humidity, 60-70%; and the light at 12 h cycles. After four days of growth the volume and the amount of radioactivity remaining in the culture solution and in the plants was determined.

Experiment 3. The vapor loss of systemically applied carbofuran was assessed by trapping the escaping insecticide under constant air-flow conditions in the greenhouse. The rice plants were grown in culture solution as described in experiment 1. Two levels (x, 2x) of ^{14}C-carbofuran were included to study the effect of initial carbofuran concentration on vapor loss. The vessel with plants growing in ^{14}C-carbofuran was placed in a tray containing water and a transparent plexiglass chamber was placed over on the tray. The chamber was provided a continuous air-flow of about 5 lit/min. The circulating air was continuously sucked through a series of ethylene glycol (3 nos.) and 1 N NaOH (2 nos.) traps. The ethylene glycol absorbed mostly the carbamates and the NaOH absorbed $^{14}CO_2$. The radioactivity in the traps was assayed daily.

Ethylene glycol was pooled, diluted with distilled water, acidified and, extracted with methylene chloride for radioactivity determination and thin-layer chromatography analysis. The volume of NaOH was made up to 100 ml and aliquots of 0.5 ml were mixed with 10 ml Bray's liquid scintillator (PPO, 4 g; POPOP, 0.2 g; napthalene, 60 g; methanol, 100 ml; ethylene glycol, 20 ml; and 1, 4-dioxane to make up to 1 ℓ) to measure the radioactivity in a Tri-Carb Liquid Scintillation Counting System (Packard Instruments, U. S. A.).

Both at the beginning and after 10 days of growth, the radioactivity present in the culture solution and in the plants was determined. The volume of culture solution remaining after 10 days was also measured.

Experiment 4. Seedlings were grown in flooded Maahas soil (pH, 5.9; O.M. 2.24%) collected from 0-15 cm depth, air dried, and passed through a 2-mm sieve. The soil (200 g) in plastic bottles (50 x 100 mm) was flooded with 230 ml of distilled water and two seedlings of 25-day-old IR8 rice were planted in each bottle. After 1 day, 10 ml of ^{14}C-carbofuran solution was added, either directly to the flood water or injected at the root zone at about 3 cm below the surface of the flooded soil. This was followed by addition of 10 ml of non-labeled carbofuran solution (20 ppm) to serve as carrier. The growing plants were transferred to continuous air-flow chambers in the greenhouse. Vapor loss was determined as described in experiment 3, but at 5-day intervals.

Residue extraction and analysis

Culture solution. After washing down the root system the culture solution was transferred to a separatory funnel, acidified with con. HCl, and about 5 g NaCl was added before extracting with methylene chloride. The methylene chloride extraction was repeated three times, each with 40 ml of methylene chloride and the organic fraction was pooled, dried on anhydrous Na_2SO_4, and reduced to near dryness in a Rotavapor (Brinkman Instruments, N.Y.). The residues were dissolved in 2 ml of methanol, and 0.1-ml aliquots of this sample were mixed with 10 ml of liquid scintillator (PPO, 5 g; POPOP, 0.3 g and toluene 1 ℓ) for determining the radioactivity. In certain cases, 100 µl of methanol extract were spotted on silica gel G plates, separated to a distance of 15 cm in ether: hexane (3:1) solvent system. Silica gel areas opposite authentic carbofuran were scraped into scintillation vials and mixed with 10 ml of liquid scintillator for measurement of radioactivity.

The non-extractable radioactivity present, if any, in the water phase was determined by mixing 0.5 ml aliquots of the sample with 10 ml of Bray's scintillator.

Plant. The plants were separated into leaves, stem with leaf sheath, and roots. Separated portions were finely chopped and transferred separately into flasks with 100 ml of boiling 0.25 N HCl. The flasks were connected to a water-cooled system and heated with a mantle for 1 h. The contents were filtered through glass wool and washed with additional warm 0.25 N HCl and cooled. The filtrate was transferred to separatory funnels for extraction with methylene chloride. The organic fraction separated was passed through activated carbon (Darco) to remove color substances and washed with fresh methylene chloride and dried on anhydrous Na_2SO_4. The volume of the organic solvent was reduced to near dryness in a

Rotavapor, and the residues were redissolved in 2 ml of methanol. Suitable aliquots (0.5 ml) of methanol extract were mixed with 10 ml of liquid scintillator for measuring the radioactivity. Also, 100-200 µl samples were used for qualitative and quantitative analysis of residues by TLC (thin layer chromatography) and autoradiography. Fortification tests carried through the entire procedure for plant samples have shown a recovery of more than 88%.

RESULTS AND DISCUSSION

The balance sheet showing the radioactivity accounted for during 10 days of rice growth in culture solution containing ^{14}C-carbofuran is presented in Table 1. The difference between the radioactivity recovered from the culture solution of the control and that from the growing plant is attributed to absorption. Therefore, a comparison of the uptake value with the actual quantity recovered from plants after extraction was referred to as "unaccounted radioactivity."

After 10 days, the percentage of initial radioactivity recovered from the culture solution of the control and the growing plant were 54.7 and 32.8. The difference of 21.9% was attributable to plant uptake. But the radioactivity extracted from plants, was only 2.8% of what was initially added, showing that an appreciable quantity was not accounted for (Table 1). The radioactivity in the stem portion was consistently lower than in the leaf blade and root. The decrease in radioactivity in the control appears to have been the result of the unsterilized conditions employed. The unaccounted for radioactivity was found to increase with the growth of the rice plant.

TABLE 1

Balance sheet of total radioactivity in the solution-culture system.

Days	Control	Radioactivity recovered, 10^4 cpm			
		Growing plant			
		Culture solution	Leaf blade	Stem and leaf sheath	Root
0*	10.64	10.62	0.0	0.0	0.006 (0.014)
1	9.89	8.56	0.033	0.007	0.073 (1.217)
5	7.94	5.73	0.087	0.021	0.131 (1.971)
10	5.82	3.49	0.128	0.026	0.155 (2.021)

*After 1 hour.
Initial radioactivity added, 11.576×10^4 cpm.
Figures in parentheses represent unaccounted for radioactivity.

The relationship between radioactivity loss and water loss by transpiration is presented in Table 2. Greenhouse conditions characterized by higher temperatures than in the growth cabinet resulted in more transpiration loss during the four-day period (13.5 ml) as compared with controlled conditions (7.5 ml). Similarly, the uptake and the unaccounted for radioactivity was higher in the greenhouse than under controlled conditions. The results indicated that the total radioactivity loss increased with increase in water loss by transpiration. It was presumed that carbofuran and its breakdown products are carried to leaf surfaces and transpired by subsequent vaporization. Interestingly, the guttation fluid collected from the blades of treated plants contained detectable levels of radioactivity.

In all rapidly growing crop plants with adequate water, the net water movement is upward and outward. This causes a tendency for the systemic insecticide to accumulate in rapidly transpiring young but fully developed leaves. There may be no net movement down into the root, as suggested by the autoradiograph.

TABLE 2

Effect of growth conditions on the total radioactivity loss from rice plants grown in culture solution containing ^{14}C-carbofuran.

	Total radioactivity recovered, cpm x 10^4							
	Greenhouse conditions				Controlled conditions			
	Growing plant				Growing plant			
Days	Control	Culture soln.	Plant	Unaccounted	Control	Culture soln.	Plant	Unaccounted
0	5.58	5.58	0	-	5.58	5.58	0	-
4	5.34	2.77	1.06	1.51	5.41	4.60	0.43	0.38

More direct evidence for loss of systemically applied carbofuran was obtained by trapping the escaping radioactivity in ethylene glycol (Table 3). TLC analysis of residues revealed that the radioactivity trapped in ethylene glycol was mainly in the form of carbofuran. Carbon dioxide was trapped in the alkali trap. At two different levels of carbofuran addition, the loss of insecticide, as found in the ethylene glycol trap, appears to be related to the initial concentration, although the water loss by transpiration was nearly the same. Thus, during the 10-day period, about 8.7% (x) and 16.8% (2x) of the absorbed radioactivity was accounted for in the ethylene glycol trap, although the transpiration losses were nearly the same in both treatments (Table 3). It is evident from the data in Table 3

that ethylene glycol and NaOH traps fail to catch all of the lost ^{14}C. Apparently the air-flow rates and the trapping devices need further perfection.

TABLE 3

Vapor loss of radioactivity from rice plants grown in culture solution containing ^{14}C-carbofuran.

Days	Radioactivity, 10^4 cpm				
		Growing plant			
	Control	Culture solution	Plant	Ethylene glycol trap	NaOH trap
			X		
0	180.2	180.2	0	0	0
10	137.0	94.0	16.0	3.7	2.7
			2X		
0	360.4	360.4	0	0	0
10	276.1	174.7	34.2	17.1	4.8

X, 25 ml; 2X, 50 ml of aqueous ^{14}C-carbofuran in 250 ml culture solution. The volume of culture solution lost by transpiration during 10 days was 124.5 ml for X and 127 ml for 2X.

The effect of different methods of application on the rate of vapor loss of carbofuran and its breakdown products is presented in Table 4. In paddy water application the vapor loss of radioactivity increased rapidly up to 10 days as against a gradual increase up to 20 days in the root-zone application.

The rapid absorption associated with degradation of carbofuran under paddy water application, as compared with a gradually increasing uptake associated with root growth and an increased persistence under the root zone method of application, was observed in our related studies with carbofuran. Therefore, with these two methods of application the rate of loss of carbofuran depends largely on the uptake rate and also on persistence in the soil.

Root-zone application of systemic insecticides has been reported to increase the efficiency of these scarce inputs, apparently by reducing the surface losses. Root-zone application increases the insecticides activity due to increased persistence in the soil. We do not see how such increased activity could benefit rice, at least for carbofuran application.

TABLE 4

Vapor loss of radioactivity from rice plants grown in flooded soil treated with ^{14}C-carbofuran.

	Radioactivity recovered, 10^4 cpm					
Days	0-5	5-10	10-15	15-20	20-30	Total
Paddy water application						
Ethylene glycol trap	3.42	3.58	2.07	1.54	0.47	11.08
NaOH	1.39	3.05	0.86	0.74	0.79	6.83
Root zone application						
Ethylene glycol trap	0.51	2.44	3.42	4.95	3.71	15.03
NaOH trap	0	0.23	0.20	0.87	1.19	2.49

Initial radioactivity added to treatment, 151.37 x 10^4 cpm.

From the environmental protection point of view, the entry of toxic chemicals into atmosphere may create the problem of contamination.

Our findings are the first to report on vapor loss of systemically applied ^{14}C-carbofuran from rice.

ACKNOWLEDGMENTS

The authors wish to thank the FMC Corporation, Middleport, New York, for supplying samples of ^{14}C-labeled-carbofuran used in this study, to Dr. O. H. Fullmer, Sr. Research Chemist, FMC Corporation, Richmond, California, and to Dr. J. N. Seiber, Department of Environmental Toxicology, University of California, Davis, for useful suggestions.

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