Rice Plant Uptake of Fresh and Aged Residues of Carbofuran from Soil

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The uptake of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) residues, fresh and aged, by rice plants was investigated by growing them for 42 days in soils containing freshly treated (T-1), 3-month-aged (T-2), and 6-month-aged residues (T-3). Mineralization of [^{14}C]carbofuran in soil to $^{14}CO_2$ during the period was 4.4 (T-1), 11.0 (T-2), and 15.7% (T-3). 3-Ketocarbofuran phenol (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol) was the major metabolite of the aged soils, whereas 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl *N*-methylcarbamate) was the major metabolite in the shoots. The ^{14}C radioactivity present in rice plant tissues after harvest was 26.8 (T-1), 21.4 (T-2), and 10.3% (T-3). The nonextractable soil-bound residues were 8.3 (T-1), 37.9 (T-2), and 54.6% (T-3) of the originally applied carbofuran. The small upward translocation of ^{14}C radioactivity in T-3 suggests that the major metabolite, 3-ketocarbofuran phenol, is conjugated in roots and the low recovery in T-1 indicates the loss of carbofuran from the shoots.

INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate), which was developed by FMC as a contact and systemic insecticide in 1969, is also effective as an acaricide and a nematocide and has been widely used for the control of brown plant hoppers, green rice leaf hoppers, and rice stem borers in rice paddies in Korea since 1975. Carbofuran has a high acute oral toxicity, but a rather low dermal toxicity. Hence, it is effective when applied to rice paddies or in the rhizosphere in soil as a granular formulation (IRRI, 1975).

The carbofuran residue that was absorbed by plant roots from soil was rapidly translocated into the shoots and metabolized to various metabolites (Metcalf et al., 1968; Knaak, 1970; Ashworth and Sheets, 1972; Fuhremann and Lichtenstein, 1980). The reactions undergone by carbofuran remaining in plants and animals include mainly hydrolysis, hydroxylation, oxidation, and conjugation (Metcalf et al., 1968; Dorough, 1968a,b; Ivie and Dorough, 1968). Investigations on the degradation of carbofuran in soil include the effect of the pH and organic matter content of soil on the persistence and degradation of carbofuran and its major metabolite, carbofuran phenol (Getzin, 1973), and the degradation of carbofuran in flooded soil (Venkateswarlu et al., 1977; Venkateswarlu and Sethunathan, 1978). Fuhremann and Lichtenstein (1980) applied six insecticides, including carbofuran, to two soils and grew oat plants to investigate their persistence, movement, and metabolism in soil and in oat plants.

The metabolism and behavior of carbofuran in rice plants grown in rice paddies have been investigated in and outside the Republic of Korea in recent years (Rajagopal and Sethunathan, 1984; Brahmaprakash and Sethunathan, 1985; Park and Oh, 1986; Lee et al., 1987).

As seen above, most of the investigators reported the absorption, translocation, and degradation of pesticides by crops that were treated with them immediately before growing or during cultivation. The present investigation was aimed at clarifying plant uptake of the aged residues of carbofuran and its metabolites versus the uptake of freshly applied carbofuran.

For the formation of aged residues of carbofuran, the soils treated with carbofuran were incubated for 3 and 6 months. Rice plants were grown in soils where carbofuran was applied immediately before plant growth and in treated soils aged for 3 and 6 months, respectively, to assess the bioavailability of the different aged residues to rice plants.

MATERIALS AND METHODS

Soil Used. The soil sample was collected from a rice paddy in Kakyung-dong, Cheong Ju, Korea, air-dried, and passed through a 2-mm sieve. The physicochemical properties are as follows: pH (KCl, 1:5), 5.4; organic matter, 1.3%; CEC (m.e./100 g of soil), 10.2; sand, 38.1%; silt, 37.6%; clay, 24.3%; texture, loam.

[^{14}C]Carbofuran. The labeled position in the structural formula of carbofuran is the 3-carbon. The specific activity was 3.48 MBq/mg. Since the labeled carbofuran proved to contain some degradation products, it was purified before use with a silicic acid column using petroleum ether–chloroform–absolute ethanol (7:2:1 v/v/v) as the eluting solvent. The radiochemical purity was confirmed to be greater than 99.5% by autoradiography.

Thin-Layer Chromatography (TLC) and Autoradiography. For the confirmation of the radiochemical purity and the identification of the metabolites of [^{14}C]carbofuran, TLC followed by autoradiography was used. The film was Fuji X-ray Film, Medical (FUJI Photo Film Co., Ltd.). The developing solution was the X-ray film developer (Poohung Photo-chemical Co., Ltd.). X-FIX (for X-ray film, Poohung Photo-chemical) was used as the fixer. For TLC, precoated plates (Art. 5554, DC-Alufolien, silica gel 60 F_{254} 20 × 20 cm, 0.2 mm, E. Merck) were used. The developing solvent was petroleum ether–chloroform–absolute ethanol (7:2:1 v/v/v). The metabolites were identified by comparing the R_f of each spot with that of authentic compounds. The relative amounts of the metabolites were determined by scraping off the areas on the thin-layer plates corresponding to the black spots on the autoradiogram, putting them into 15-mL vials, and measuring the radioactivity by using a toluene cocktail (PPO, 4 g, and POPOP, 0.5 g, were dissolved in toluene to 1 L) in a liquid scintillation counter (LSC, PW 4700, Philips).

Formation of Soil-Aged Carbofuran Residues. On the basis of the degradation rate of [^{14}C]carbofuran in soil which had

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Table I. Treatment Levels of [^{14}C]Carbofuran in the Aging in Soil

aging period, months	radioactivity, dpm (kBq)/6.5 kg of soil	total concn, mg/6.5 kg of soil
3	55 683 300 (928.1)	37.4
6	64 721 740 (1078.1)	43.2

Table II. Radioactivities of the Soils where [^{14}C]Carbofuran Was Freshly Treated, 3-Month-Aged, and 6-Month-Aged for the Growing of Rice Plants

pot	treatment method	^{14}C radioactivity remaining in soil, dpm (kBq)	remarks
1	control	0	
2	(T-0)		
3	fresh	10 943 262 (182.4)	
4	(T-1)		
5			
6	3-month-aged	10 424 547 (173.7)	3-ketocarbofuran phenol formed
7	(T-2)		
8			
9	6-month-aged	9 888 597 (164.8)	3-ketocarbofuran phenol formed
10	(T-3)		
11			

been obtained from a preliminary experiment, the soils treated with a mixture of ^{14}C-labeled and nonlabeled carbofuran were aged at 21 ± 1 °C for 3 and 6 months, respectively. Table I shows the initial soil treatment levels of [^{14}C]carbofuran to prepare the aged residues. The final radioactivity and concentration at the completion of the aging were intended to be 185 kBq/1.5 kg of soil and 5 mg/kg soil, respectively. Throughout the aging in the dark, the moisture content was kept at 50% of the maximum water-holding capacity of the soil. The $^{14}CO_2$ evolved during the aging period was absorbed in 1 N NaOH, and the radioactivity was measured at weekly intervals with the LSC. The volatile substances were adsorbed on Amberlite XAD-2 (Sigma), and the radioactivity was measured after extraction with acetone.

Growing of Rice Plants. After the aging periods of 3 and 6 months, the soils were air-dried and their radioactivities measured by combustion with a biological oxidizer (R. J. Harvey Instrument Corp.). For growing rice plants, the soils were fertilized with N-P-K at the ratio 15:9:11 kg/10 ares, respectively. The three soil treatments containing freshly applied carbofuran and 3-month-aged and 6-month-aged carbofuran residue were put into specially devised pots made of stainless steel (i.d. 17 cm × h 10 cm); 50-day-grown rice plant seedlings were transplanted into the soils. Eight seedlings were grown with two seedlings per hill. Rice plants were grown in a vinyl house with good ventilation for 42 days. Moisture was supplied once per day at the early stage and twice at the middle and late stage. The radioactivities in the soil before the growth of rice plants are shown in Table II.

Mineralization to $^{14}CO_2$ and Volatilization of [^{14}C]-Carbofuran and Its Metabolites during the Growth of Rice Plants. The $^{14}CO_2$ and volatile substances evolved from [^{14}C]-carbofuran and its metabolites in soil during the growth of rice plants were absorbed in 1 N NaOH and 0.1 N H_2SO_4, respectively. The radioactivities were measured at weekly intervals.

Harvest of Rice Plants and Autoradiography. At the completion of the 42-day growing period, the shoots and roots of rice plants were harvested separately. The roots were rinsed thoroughly with tap water to remove soil. After the fresh weights of the shoots and roots were measured, they were freeze-dried for 4 days (Chem Lab Instruments Ltd., Model SB 4) and then weighed again. Autoradiography was performed by using the fresh rice plants harvested from pots 3 (fresh), 6 (3-month-aged), 9 (6-month-aged).

Measurement of Radioactivity. After plant harvest, the soils were air-dried and then ground homogeneously. The

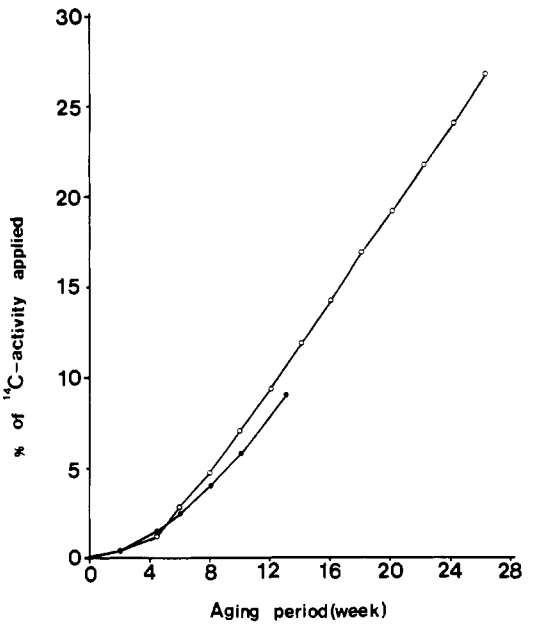

Figure 1. Mineralization of [^{14}C]carbofuran to $^{14}CO_2$ in soil during 3- and 6-month aging. (●) 3-month aging; (○) 6-month aging.

Table III. Mineralization of [^{14}C]Carbofuran and Its Metabolites in Soil to $^{14}CO_2$ during 42 Days of Rice Plant Growth

pot		^{14}C radioactivity in 1.5 kg of soil, dpm (kBq)	^{14}C radioactivity mineralized, %	mean, %
3	T-1	10 943 262	4.98	
4		(182.39)	4.14	4.37 ± 0.53
5			4.00	
6	T-2	10 424 547	11.20	
7		(173.74)	13.07	10.95 ± 2.26
8			8.58	
9	T-3	9 888 597	16.04	
10		(164.81)	15.35	15.71 ± 0.35
11			15.74	

radioactivity of soil was measured by combusting 0.3 g of each sample, using the biological oxidizer. The radioactivity of rice plants was measured by combusting 0.2 g of each sample that had been freeze-dried and pulverized. The $^{14}CO_2$ evolved was absorbed in the ^{14}C cocktail (for Harvey biological oxidizer), and the radioactivity was measured with the LSC. The flow rate of oxygen and nitrogen in the biological oxidizer was 300 mL/min. The temperatures of the catalyst zone and combustion zone were 700 and 900 °C, respectively, and the combustion time was 4 min. The toluene cocktail was used for the samples dissolved in organic solvents that were ordinarily evaporated before the cocktail was added. For the measurement of radioactivity of $^{14}CO_2$ absorbed in 1 N NaOH and volatile substances absorbed in 0.1 N H_2SO_4, Aquasol (Du Pont, NEN Research Products) was used. Radioactivity was measured with the LSC after the samples had stabilized at 4 °C in the dark for 24 h.

Extraction of Soil and Rice Plants. Fifteen grams of air-dried soils was shaken for 4 h with 50 mL of methanol and then centrifuged at 13 000 rpm for 10 min. Samples of 0.8 g of freeze-dried rice plant shoots and roots were extracted with different solvents for 2 h in a sonicator (Type 6442 AE, Ultrasonic Ltd.) to compare extractability. After extraction, the samples were centrifuged at 15 000 rpm for 15 min. The same procedure was repeated until the radioactivity of the extracts showed the background level.

For the elucidation of conjugated metabolites, 50 g of air-dried soil samples was extracted with three 70-mL aliquots of methanol on a shaker. For acid hydrolysis, the same amount of the soil samples was incubated at 60 °C for 5 h after being added with 150 mL of 0.25 N HCl. At the completion of the incubation,

Table IV. Uptake of [^{14}C]Carbofuran and Its Metabolites from the Soils Containing Different Residues by Rice Plants during the Growth Period of 42 Days

		uptake, %					
pot	treatment method	root	mean	shoot	mean	total	mean
3	fresh	5.89		19.93		25.82	
4	(T-1)	6.62	6.52 ± 0.58	17.01	20.45 ± 3.73	23.63	26.83 ± 3.81
5		7.04		24.41		31.05	
6	3-month-aged	4.75		17.89		22.64	
7	(T-2)	4.26	4.21 ± 0.57	16.92	17.14 ± 0.66	21.18	21.35 ± 1.21
8		3.62		16.62		20.24	
9	6-month-aged	3.19		7.71		10.90	
10	(T-3)	3.42	3.00 ± 0.55	6.84	7.30 ± 0.44	10.26	10.29 ± 0.59
11		2.38		7.34		9.72	

Table V. Behavior of [^{14}C]Carbofuran in Soil

			volatilization						
pot	[^{14}C]carbofuran in 1.5 kg of soil, dpm (kBq)	$^{14}CO_2$ evolved (aging period), %	aging period, %	rice growing period, %	$^{14}CO_2$ evolved (rice growing period), %	^{14}C radioactivity in rice plant, %	^{14}C radioactivity remaining in soil, %	recovery, %	unaccountable ^{14}C radioactivity, %
3	10 943 262			0.030	4.98	25.82	24.68	55.51	44.49
4	(182.39)			0.012	4.14	23.63	29.52	57.30	42.70
5				0.011	4.00	31.05	20.09	55.55	44.45
6	10 424 547			0.014	11.20	22.64	44.97	78.82	21.18
7	(173.74)	8.91	0.026	0.027	13.07	21.18	44.62	78.90	21.10
8				0.014	8.58	20.24	42.61	71.44	28.56
9	9 888 597			0.010	16.04	10.90	62.98	89.93	10.07
10	(164.81)	26.72	0.050	0.006	15.35	10.26	60.21	85.83	14.17
11				0.019	15.74	9.72	60.47	85.94	14.06

Table VI. Extraction of [^{14}C]Carbofuran-Treated Soil Samples in the Absence and Presence of Rice Plants with MeOH and Partition of the Extracts between Aqueous Phase and Organic Phase

			distribution of ^{14}C after partitioning, %			
treatment method	rice plant	methanol-extractable, %	aqueous phase	organic phase (CH_2Cl_2)	bound, %	recovery,[a] %
fresh	no	93.56	1.00	92.56	8.33	101.89
(T-1)	yes	35.33	1.62	33.71	61.92[b]	97.25
3-month-aged	no	55.09	1.48	53.61	37.92	93.01
(T-2)	yes	12.17	7.62	4.55	83.53[b]	95.70
6-month-aged	no	41.26	2.61	38.65	54.63	95.89
(T-3)	yes	5.14	3.41	1.73	91.45[b]	96.59

[a] Methanol-extractable plus bound. [b] Much higher percentage of bound residues resulted because rice plants already absorbed the available or extractable ^{14}C radioactivity.

the incubation mixture was centrifuged at 15 000 rpm for 15 min. The pH of the supernatant was adjusted to 6–7 with 1 N NaOH solution and concentrated on a rotary evaporator for TLC and autoradiography. In addition, to examine the possible hydrolysis of the conjugated metabolites by cellulase, the methanol extract of the shoots was concentrated on a rotary evaporator at 40 °C and redissolved in 25 mL of citrate–phosphate buffer (pH 5.0). Ten units of the cellulase (produced by *Trichoderma viride*, Sigma) were added to it, and the mixture was incubated at 37 °C for 12 h. After 12 h, another 10 units was added to the mixture, and the mixture was incubated again for another 12 h. At the completion of the incubation, the mixture was extracted with CH_2Cl_2, and the extract was concentrated to perform autoradiography for the elucidation of conjugate formation.

Autoradiography of the Extracts of Soil and Rice Plants. Fifty grams of soil was extracted with five 70-mL portions of methanol by the same method as above. All the extracts collected were concentrated at 40 °C, and the residue was reextracted with ethanol. After methanol was evaporated, the extract dissolved in a small amount of methanol was developed on TLC plates with petroleum ether–chloroform–absolute ethanol (7:2:1 v/v/v) as the developing solvent. The TLC plates were used for autoradiography. A total of 1.5 g of the pulverized shoots was extracted with six 15-mL portions of methanol by the same method. The extraction of roots was not done due to the small amount of radioactivity. In addition, 2 g of the shoots was incubated with 50 mL of 0.25 N HCl at 60 °C for 5 h.

Distribution of Radioactivity of Soil Extracts between Aqueous and Organic Phases. Five milliliters of methanol extracts of soil was evaporated by a bubbling air stream. Five milliliters of distilled water was added and mixed homogeneously. Five milliliters of dichloromethane was then shaken vigorously with the aqueous solution. The radioactivities in the organic phase and in the aqueous phase were measured.

Analysis of Nonextractable Soil-Bound ^{14}C. Two grams of the soil samples, which were exhaustively extracted with methanol, was extracted again with 0.1 M $Na_4P_2O_7$ to the degree that the radioactivity of the extract reached the background level. To the collected extracts was added concentrated HCl to the point where no more precipitate was formed. This mixture was centrifuged at 10 000 rpm for 10 min. The supernatant corresponds to fulvic acid and the precipitate to humic acid. The radioactivities of fulvic acid and humic acid which were dissolved with 0.1 N NaOH were measured with Aquasol as the scintillation cocktail. The extracted soils were combusted to determine the ^{14}C content of the humin.

RESULTS AND DISCUSSION

Translocation of [^{14}C]Carbofuran during the Aging Periods and the Growing of Rice Plants. The total amounts of $^{14}CO_2$ evolved during the aging periods of 3 and 6 months for the formation of aged residues of carbofuran in soil were 8.91 and 26.72% of the originally applied radioactivity of carbofuran, the average degra-

Table VII. Change in the Nonextractable ^{14}C after Applied to Soil Samples as [^{14}C]Carbofuran in the Absence and Presence of Rice Plants (Fulvic Acid + Humic Acid + Humin = 100%)

treatment method	rice plant	nonextractable bound residue	fulvic acid, %	humic acid, %	humin, %
fresh	no	8.33	5.10	1.68	1.55
(T-1)	yes	61.92	25.08	10.06	26.78
3-month-aged	no	37.92	20.14	6.98	10.80
(T-2)	yes	83.53	36.52	11.04	35.97
6-month-aged	no	54.63	26.52	6.95	21.16
(T-3)	yes	91.45	35.70	16.34	39.41

Table VIII. Comparison of Extractability of Rice Plants with Different Solvents

	solvent	extracted, %
root	acetone	9.85
	methanol	10.66
	0.25 N HCl	24.16
	BF_3–MeOH	39.17
shoot	acetone	25.41
	methanol	59.26
	0.25 N HCl	52.22
	BF_3–MeOH	78.27

dation rates per week being 0.69 and 1.03%, respectively, as shown in Figure 1. The mineralization of carbofuran to $^{14}CO_2$ is believed to result from chemical and microbial degradation (Getzin, 1973; Venkateswarlu et al., 1977; Williams et al., 1976). The reason a somewhat larger amount of $^{14}CO_2$ per unit time was evolved for the 6-month-aged soil than for 3-month-aged soil may be attributed to the larger amount of carbofuran added (43.2 mg/6.5 kg of soil) for the 6-month-aged treatment than for the 3-month aged treatment (37.4 mg/6.5 kg of soil). It is also possible that the 3-month-aged soil contains tightly adsorbed and/or bound components that were not readily available to microorganisms in soil, whereas in the 6-month-aged soil more free carbofuran residue versus bound forms would be available. Getzin (1973) reported that carbofuran phenol was mainly formed as a result of hydrolysis in weakly alkaline soil (pH 7.8) and it was rapidly bound to soil constituents. However, in our study using soil of pH 5.4, it was found by analysis of the soil extract that the oxidation of the position 3 carbon in the benzofuran ring occurred as the main degradation pathway, in addition to the hydrolysis of the carbamate linkage. The total amounts of $^{14}CO_2$ evolved during 42 days of rice plant growing are presented in Table III. As seen in this table, the amounts of $^{14}CO_2$ released from T-2 and T-3, where carbofuran had been aged for 3 and 6 months, respectively, were larger than those from T-1, where carbofuran had been freshly applied. Judging from the ever-increasing amounts of $^{14}CO_2$ evolved during the aging periods, it is believed that some of the carbofuran was already transformed to the metabolites in T-2 and T-3 which were so vulnerable to the degradation to $^{14}CO_2$. In addition, the amount of $^{14}CO_2$ evolved during the aging period was in the range 2.5–2.8% of the originally applied radioactivity, in contrast to 4.4–15.7% evolved during the growing of rice plants. On the basis of this result, it is believed that the growing of rice plants increased the mineralization of carbofuran and its metabolites. This is consistent with research by Lee et al. (1988), who reported that when bean and radish were grown in soils containing nonextractable bound residues of [^{14}C]bentazon, they enhanced the degradation of soil-bound residues. According to Venkateswarlu and Sethunathan (1978), there were nearly 4 times as many bacteria in the rhizosphere of rice plants in carbofuran-treated soil as those in the control without carbofuran. Accordingly, it is believed that microorganisms were involved in the degradation of carbofuran. In addition, the possible action of various enzymes exuded from the rice roots cannot be ruled out.

The volatilization of [^{14}C]carbofuran and its metabolites during the aging and growth of rice plants was less than 0.05% of the originally applied amount in all three treatments. This is partly due to the fact that carbofuran was mixed homogeneously with soil, not applied on the surface.

^{14}C Radioactivity Remaining in Rice Plants. The ^{14}C present in rice plants after 42 days of growth is presented in Table IV. For T-1, the ^{14}C radioactivity translocated to shoots is more than 3 times that in roots, whereas in T-3 the ^{14}C radioactivity present in shoots is about twice that in roots, its amount being only one-third of that in T-1. The explanation for this may be that in T-1 intact carbofuran was absorbed by roots and translocated to shoots rapidly, whereas in T-3 only 56.1% of the originally applied [^{14}C]carbofuran remained intact, 39.7% being transformed to the main metabolite 3-ketocarbofuran phenol (refer to Table IX). Accordingly, it is believed that in T-3 3-ketocarbofuran, in addition to carbofuran, was absorbed by roots and adsorbed on and/or "bound" to roots, and hence very little of the ^{14}C radioactivity translocated to shoots.

In the case of T-2, only 74.1% of the originally applied [^{14}C]carbofuran remained intact, 21.9% being transformed to 3-ketocarbofuran phenol (refer to Table IX). As a result, the pattern of absorption and translocation of ^{14}C radioactivity in T-2 lay between those of T-1 and T-3. Summarizing these results, when carbofuran was treated in soil immediately before rice plants were transplanted (T-1), 26.8% of the originally applied ^{14}C radioactivity was detected in plant tissues after the growing period.

In the cases of the aging for 3 (T-2) and 6 months (T-3), 21.4 and 10.3% of the originally applied ^{14}C radioactivity were found in the tissues, respectively. Undoubtedly, the nature and quantities of the ^{14}C compounds will be different.

Fate of [^{14}C]Carbofuran in Soil. The fate of [^{14}C]-carbofuran applied to soil is summarized in Table V. As can be seen, the amount of $^{14}CO_2$ evolved during the growth period of 42 days in T-1 was 4–5% of the originally applied ^{14}C radioactivity, whereas the amounts in T-2 and T-3 were 9–13 and 15–16%, respectively. The reason much larger amounts of $^{14}CO_2$ were evolved in T-2 and T-3 than in T-1 is believed to be due to the possibility that some carbofuran was already transformed to labile metabolites, leading to the formation of CO_2 during the aging periods of 3 and 6 months. The 3-ketocarbofuran phenol formed amounted to 21.92% in T-2 and 39.72% in T-3 of the originally applied ^{14}C radioactivity (Table IX).

The loss of [^{14}C]carbofuran applied to soil by volatilization during plant growth was negligible (0.01–0.03% of the original ^{14}C radioactivity). However, when carbofuran was applied on the soil surface in the usual application, the loss by volatilization would very likely increase owing to its relatively high vapor pressure (8.3×10^{-6} mmHg/25 °C) (Fuhremann and Lichtenstein, 1980). In contrast, very little carbofuran was lost by volatilization in this investigation, where carbofuran had been homogeneously mixed with soil. Soil-bound residues of ^{14}C radioactivity of different treatments after the growth period of 42 days were 20–30, 43–45, and 60–63% of the original ^{14}C radioactivity in T-1, T-2, and T-3, respectively. Thus,

Table IX. Relative Amounts (Percent) of Carbofuran and Its Metabolites in the MeOH and the 0.25 N HCl Extracts of Soils

solvent	soil sample	carbofuran phenol	carbofuran	3-keto-carbofuran phenol	3-hydroxy-carbofuran	3-hydroxy-carbofuran phenol	others
MeOH	3-month-aged	0.86	74.08	21.92	1.74	0.93	0.47
	6-month-aged	0.80	56.12	39.72	1.33	1.08	0.95
	pot 3	0.50	73.08	23.60	1.09	0.97	0.76
	pot 6	0.48	76.40	18.61	1.99	0.97	1.55
	pot 9	0.67	69.17	25.52	2.00	1.28	1.36
0.25 N HCl	3-month-aged	0.87	73.01	22.41	2.01	1.00	0.70
	6-month-aged	0.92	72.38	23.61	1.25	1.11	0.73

since 20–30% of the original ^{14}C radioactivity became bound to the soil of T-1, carbofuran and its metabolites were not available to rice plants. Even less ^{14}C radioactivity was available to plants in the soils of T-2 and T-3. This is due to the fact that much of the carbofuran was already degraded to 3-ketocarbofuran phenol, which could readily be adsorbed and/or bound to soil organic matter during the aging periods of 3 and 6 months, and the same process continued during the growth period of rice plants.

Extraction of Soils. In Table VI, it can be seen that the nonextractable bound residues increased with the aging period. The reason there was a much higher percentage of bound residues in the soils where rice plants were grown in T-1, T-2, and T-3 is probably that rice plants already absorbed the available and/or extractable ^{14}C radioactivity, giving the relatively high ratios of nonextractable bound residues relative to the original soil radioactivity.

Partition of the Radioactivity of Soil Extracts between Aqueous and Organic Phases. To examine how much of the carbofuran applied to soil was transformed to polar metabolites, the radioactivity in the soil extracts was partitioned between aqueous and organic phases; the results are shown in Table VI. The polarity increased in T-2 and T-3, where carbofuran had been aged for 3 and 6 months, respectively, and rice plants were grown. The polar metabolites, however, were not consistently different from those of T-1 as verified by TLC and autoradiography (Table IX).

Nonextractable Soil-Bound Residues of [^{14}C]-Carbofuran and Its Metabolites. The distribution of the nonextractable, soil-bound residues of [^{14}C]carbofuran and its metabolites is shown in Table VII. ^{14}C in fulvic acid, humic acid, and humin increased in proportion to the aging period in the soils in the absence of rice plants. In the soils after aging and the growing of rice plants, the amounts of ^{14}C in fulvic acid and humin were almost the same for both T-1 and T-2, even if their amounts increased with aging and the presence of rice plants. Between T-2 and T-3, the amounts of ^{14}C in humic acid and humin increased to some degree, while that of fulvic acid decreased.

Extraction of Rice Plants. The extractability of the shoots and roots of rice plants with various solvents is presented in Table VIII. As can be seen in this table, the extractability of the roots is lower than that of the shoots for all solvents used. Khan et al. (1984) reported that when [^{14}C]carbofuran was applied to radishes and when edible portions were sampled 21 days after application and were exhaustively extracted with solvents, the amount of nonextractable (bound) ^{14}C residues was 92.6%. They indicated that the formation of bound residues in radishes may be related to a process involving chemical entrapment of the pesticide or its metabolites by lignin and/or other plant macromolecules.

Looking through the table, even in the case of BF_3–MeOH, which turned out to be the best of all solvents tested, about 61% of the ^{14}C radioactivity present in the roots was not extractable, being adsorbed and/or bound to the root constituents. Even if BF_3–MeOH proved to be excellent for the extraction of both shoots and roots, the extraction with 0.25 N HCl and/or MeOH was believed to be the most suitable for the subsequent analysis including autoradiography.

Table X. Distribution (Percent) of Carbofuran and Its Metabolites in the MeOH Extracts of Rice Shoots

sample	carbofuran phenol	carbofuran	3-keto-carbofuran phenol	3-hydroxy-carbofuran	3-hydroxy-carbofuran phenol	others
pot 4 (T-1)	0.10	64.24	4.05	24.27	4.84	2.50
pot 6 (T-2)	0.24	66.96	2.96	23.46	2.74	3.64
pot 9 (T-3)	0.40	73.00	1.34	23.20	1.38	0.68

Autoradiography of Rice Plants. The ^{14}C radioactivity which had been absorbed and translocated by the plants (T-1) was detected somewhat in the roots and the lower parts of the shoots. However, much larger amounts of ^{14}C radioactivity were present in the upper parts of the shoots. Almost the same distributions were also observed in T-2 and T-3. Quite a few investigators (Lee et al., 1987; Archer et al., 1977; Arunachalam and Lakshmanan, 1982) reported that carbofuran applied to soil was rapidly absorbed, translocated, and accumulated at the tip of the plants. In this investigation, however, as the growing period was extended, the ^{14}C radioactivity was distributed broadly above the middle parts of the shoots, as well as at the tip.

Formation of Carbofuran Metabolites in Soil and Rice Plants. Table IX shows the carbofuran metabolites detected in soil. It is noticeable that 3-ketocarbofuran phenol was the major metabolite, whereas carbofuran phenol, 3-hydroxycarbofuran, and 3-hydroxycarbofuran phenol were the minor metabolites, under this rather aerobic condition. There is a report (Venkateswarlu and Sethunathan, 1978) that carbofuran phenol and 3-hydroxycarbofuran were detected as the metabolites under anaerobic flooded conditions. Getzin (1973) reported that carbofuran was hydrolyzed to carbofuran phenol in soil which was subsequently bound to soil constituents, followed by microbial degradation slowly.

Table X represents the formation of carbofuran metabolites in the tissues of rice plants; 64–73% of the total radioactivity present in the shoots corresponded to the intact carbofuran, the major metabolite being 3-hydroxycarbofuran (23–24%), in contrast to the degradation pattern occurring in the soil. Sonobe et al. (1983) reported angelic acid ester of 3-hydroxycarbofuran and 3-hydroxycarbofuran as the major metabolite of carbofuran in carrots and 3-ketocarbofuran phenol in potatoes. In addition, there are many investigations on the hydrolysis of the carbamate linkage of carbofuran and the metabolic pathways involving the oxidation of the position 3 carbon in plants (Knaak et al., 1970; Ashworth and Sheets, 1972; Schlagbauer and Schlagbauer, 1972) or on the oxidation

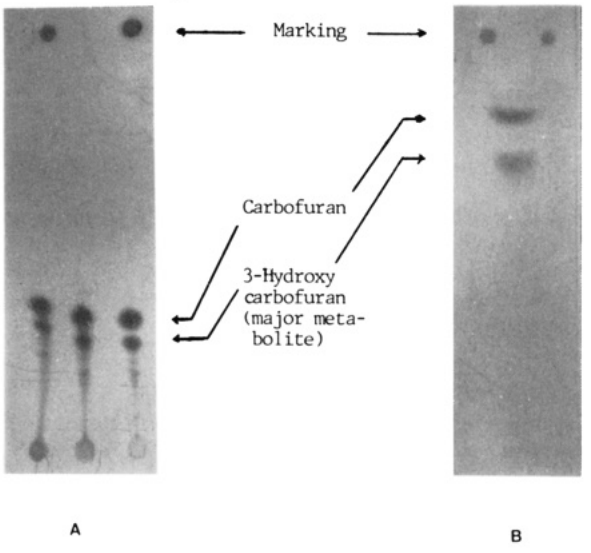

Figure 2. Autoradiograms of the extracts of the shoots of rice plants in T-1, T-2, and T-3 (A) and of the extract that was hydrolyzed by cellulase (B). The R_f values are greater in (B), possibly because of the removal of hindering materials by enzymatic hydrolysis.

Table XI. Distribution (Percent) of Carbofuran and Its Metabolites in the MeOH Extract of Shoots after Enzymatic Cleavage

sample	carbofuran phenol	carbofuran	3-ketocarbofuran phenol	3-hydroxycarbofuran	3-hydroxycarbofuran phenol
pot 6 (T-2)	2.97	58.67	2.93	28.53	6.90

of the position 3 carbon of the benzofuran ring (Fuhremann and Lichtenstein, 1980; Turner and Caro, 1973; Caro et al, 1973).

Autoradiography of the Extracts of Rice Plants. Figure 2A shows the autoradiogram of the MeOH extracts of the shoots of rice plants that were grown in T-1, T-2, and T-3. Since the tailing in the autoradiogram was believed to be due to the conjugation of carbofuran and/or its metabolites with some constituents of rice plants, the MeOH extract was hydrolyzed by cellulase prior to autoradiography (Figure 2B).

As can be seen in Figure 2 and Tables X and XI, the amount of carbofuran decreased after the hydrolysis by cellulase, whereas the amounts of other metabolites increased noticeably and the tailing disappeared.

Accordingly, it was recognized that the conjugates of carbofuran metabolites as well as carbofuran itself were hydrolyzed by the enzyme.

In the case of the acid hydrolysis, the tailing could not be removed to give any clear metabolites as expected.

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LITERATURE CITED

Archer, T. E.; Stokes, J. D.; Bringhurst, R. S. Fate of Carbofuran and Its Metabolites on Strawberries in the Environment. *J. Agric. Food Chem.* **1977**, *25* (3), 536–541.

Arunachalam, K.; Lakshmanan, M. Translocation, Accumulation and Persistence of Carbofuran in Paddy, Ground Nut and Cotton. *Bull. Environ. Contam. Toxicol.* **1982**, *28*, 230–238.

Ashworth, R. J.; Sheets, T. J. Metabolism of Carbofuran in Tobacco. *J. Agric. Food Chem.* **1972**, *20*, 407–412.

Brahmaprakash, G. P.; Sethunathan, N. Metabolism of Carbaryl and Carbofuran in Soil Planted to Rice. *Agric. Ecosyst. Environ.* **1985**, *13*, 33–42.

Caro, J. H.; Freeman, H. P.; Glotfelty, D. E.; Turner, B. C.; Edwards, W. M. Dissipation of Soil-Incorporated Carbofuran in the Fields. *J. Agric. Food Chem.* **1973**, *21* (6), 1010–1015.

Dorough, H. W. Fate of Furadan(NIA-10242 in Bean Plants. *Bull. Environ. Contam. Toxicol.* **1986a**, *3*, 164–173.

Dorough, H. W. Metabolism of Furadan(NIA-10242) in Rats and Houseflies. *J. Agric. Food Chem.* **1968b**, *16*, 319–325.

Fuhremann, T. W.; Lichtenstein, E. P. A Comparative Study of the Persistence, Movement, and Metabolism of Six Carbon-14 Insecticides in Soils and Plants. *J. Agric. Food Chem.* **1980**, *28*, 446–452.

Getzin, L. W. Persistence and Degradation of Carbofuran in Soil. *Environ. Entomol.* **1973**, *2* (3), 461–467.

IRRI. Control and Management of Insects. *IRRI Annual Report for 1974*; International Rice Research Institute: Los Baños, Philippines, 1975.

Ivie, G. W.; Dorough, H. W. Furadan-C^{14} Metabolism in a Lactating Cow. *J. Agric. Food Chem.* **1968**, *16*, 849–855.

Khan, S. U.; Stratton, G. D., Jr.; Wheeler, W. B. Characterization of Bound (Nonextractable) Residues of Dieldrin, Permethrin, and Carbofuran in Radishes. *J. Agric. Food Chem.* **1984**, *32*, 1189–1191.

Knaak, J. B.; Munger, D. M.; McCarthy, J. F. Metabolism of Carbofuran in Alfalfa and Bean Plants. *J. Agric. Food Chem.* **1970**, *18*, 827–831.

Lee, J. K.; Cheon, S. Y.; Kyung, K. S. Uptake of the Residues of the Herbicide Bentazon in Soil by Soybean and Radish. *Korean J. Environ. Agric.* **1988**, *7* (1), 1–7.

Lee, Y. D.; Lee, K. H.; Park, C. K. Fate of ^{14}C-Carbofuran in Rice Plant and Paddy Soil. *Korean J. Environ. Agric.* **1987**, *6* (1), 7–16.

Metcalf, R. L.; Fukuto, T. R.; Collins, C.; Borck, K.; El-Aziz, S. A.; Munoz, R.; Cassil, C. C. Metabolism of 2,2-Dimethyl-2,3-Dihydrobenzofuranyl-7-N-Methylcarbamate (Furadan) in Plants, Insects, and Mammals. *J. Agric. Food Chem.* **1968**, *16* (2), 300–311.

Park, C. K.; Oh, S. R. Fate of C-14 Labelled Carbofuran in Paddy Plants and Soil. *Korean J. Environ. Agric.* **1986**, *5* (2), 85–94.

Rajagopal, B. S.; Sethunathan, N. Influence of Nitrogen Fertilizers on the Persistence of Carbaryl and Carbofuran in Flooded Soil. *Pestic. Sci.* **1984**, *15*, 591–599.

Schlagbauer, B. G. L.; Schlagbauer, A. W. J. The Metabolism of Carbamate Pesticides-A Literature Analysis. *Residue Rev.* **1972**, *42*, 1–90.

Sonobe, H.; Carver, R. A.; Krause, R. T.; Kamps, LaVerne R. Extraction of Biologically Incorporated [^{14}C]Carbofuran Residues from Root Crops. *J. Agric. Food Chem.* **1983**, *31*, 96–104.

Turner, B. C.; Caro, J. H. Uptake and Distribution of Carbofuran and Its Metabolites in Field-Grown Corn Plants. *J. Environ. Qual.* **1973**, *2* (2), 245–247.

Venkateswarlu, K.; Sethunathan, N. Degradation of Carbofuran in Rice Soils as Influenced by Repeated Applications and Exposure to Aerobic Conditions following Anaerobiosis. *J. Agric. Food Chem.* **1978**, *26*, 1148–1151.

Venkateswarlu, K.; Gowda, T. K. S.; Sethunathan, N. Persistence and Biodegradation of Carbofuran in Flooded Soils. *J. Agric. Food Chem.* **1977**, *25* (3), 533–536.

Williams, I. H.; Pepin, H. S.; Brown, M. J. Degradation of Carbofuran by Soil Microorganisms. *Bull. Environ. Contam. Toxicol.* **1976**, *15* (2), 244–249.

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