Korean J. Environ. Agric.
Vol.8, No.2, December 1989

Uptake of the Fresh and Aged Residues of Carbofuran by Rice Plants from Soil

Jae Koo Lee and Kee Sung Kyung *

W. B. Wheeler **

浸透性 殺蟲劑 Carbofuran 土壤 殘留物의 벼에 依한 吸收

李載球·慶箕性*·W. B. Wheeler**

Abstract

In order to investigate the uptake of the systemic insecticide, carbofuran, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methyl(arbamate) residues, fresh and aged, by rice plants, they were grown for 42 days in soils containing freshly treated (T-1), 3-month-aged (T-2), and 6-month-aged residues (T-3). The amounts of $^{14}CO_2$ evolved from ^{14}C-carbofuran during the 3-and 6-month aging in soil (temp. 22±1℃ ; moisture, 50% of the maximum water-holding capacity) were 8.9 and 26.7% of the original radioactivity applied, respectively. Mineralization of ^{14}C-carbofuran in soil to $^{14}CO_2$ during 42 days of rice growing was 4.4% (T-1), 11.0% (T-2), and 15.7% (T-3). The methanol extract of the 3-and 6-month-aged soils revealed that 3-keto carbofuran phenol (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol) was the major metabolite, where as 3-hydroxy carbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl-N-methylcarbamate) turned out to be the major metabolite in the shoots by the enzymatic cleavage of the possible conjugate present in the methanol extract. Volatilization of ^{14}C-carbofuran in soil during 3-and 6-month-aging, and 42 days of rice growing was 0.026, 0.05, and 0.012-0.018% of the applied radioactivity, respectively. The ^{14}C-radioactivity which was absorbed from the soils by rice plants during 42 days of the growing period and persisted in rice plant tissues was 26.8, 21.4, and 10.3% in T-1, T-2, and T-3, respectively. The non-extractable bound residues were 8.3, 37.9, and 54.6% of the originally applied carbofuran in T-1, T-2, and T-3, respectively. The small translocation of ^{14}C-radioactivity in T-3 upwards suggests that major metabolite 3-keto carbofuran phenol is conjugated in roots and the low recovery in T-1 indicates the loss of carbofuran from the shoots.

This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1987.

* 忠北大學校 農科大學 農化學科
Department of Agricultural Chemistry, Chung Buk National University, Cheong Ju, Korea
** 美國 Florida 大學校
University of Florida, IFAS-Pesticide Research Laboratory, Gainesville, Florida 32611 U. S. A.

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.[1] Carbofuran belongs to a carbamate and while its acute oral toxicity is very high, its acute dermal toxicity is rather low. Hence, it is effective when applied to rice paddies or in the rhizosphere in soil as a granular form.[2]

The carbofuran residue which was absorbed by plant roots from soil is rapidly translocated into the shoots and metabolized to form various metabolites.[3,4,5,6] The reactions undergone by carbofuran remaining in plants and animals include mainly hydrolysis, hydroxylation, oxidation, and conjugation.[3,7,8,9] The investigations on the degradation of carbofuran in soil include the effect of the pH and organic matter contents of soil on the persistence and degradation of carbofuran and its major metabolite, carbofuran phenol[10] and the degradation of carbofuran in flooded soil.[11,12]

Fuhremann and Lichtenstein[6] treated 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 behaviour of carbofuran in rice plants grown in rice paddies have been investigated in and outside the country in recent years.[13,14,15,16]

As seen above, most of the investigators reported the absorption, translocation, and degradation of pesticides by crops which were treated with them immediately before the growing or during cultivation. The present investigation was aimed at clarifying the plant uptake of the aged residues of carbofuran and its metabolites which were expected to be adsorbed or bound to the soil organic matter as a result of the continued application in relation to the fresh residue.

For the formation of aged residues of carbofuran, the soils treated with carbofuran were incubated for three and six months. Rice plants were grown in soils where carbofuran was applied immediately before the growing, aged for three and six months, respectively, to assess the bioavailability of the different residues to crops as a function of aging periods.

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 physico-chemical properties are as follows : pH(KCl, 1 : 5), 5.4 ; organic matter, 1.3% ; C.E.C. (me/100g 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 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 99.5% by autoradiography as seen in Fig. 1.

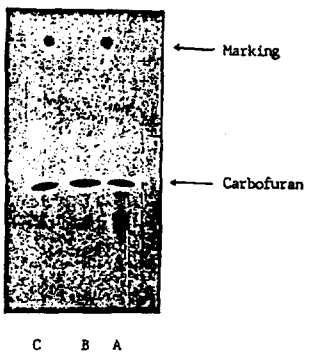

Fig. 1. Autoradiogram of ^{14}C-carbofuran purified.
TLC developing solvent : Petroleum ether-Chloroform-Ethanol
absolute(7 : 2 : 1, v/v)
A : Before purification
B, C : After purification

Thin-layer chromatography and autoradiography

For the confirmation of the radiochemical purity and the identification of the metabolites of ^{14}C-carbofuran, autoradiography was used. The film used was FUJI X-Ray Film, Medical (FUJI Photo Film Co., LTD, Japan). The developing solution was the X-ray film developer (Poohung Photo-chemical Co., LTD, Korea). X-FIX (For X-ray film, Poohung Photo-chemical Co., LTD, Korea) was used as the fixer.

For TLC, the precoated plates (Art. 5554, DC-Alufolien, Silica gel 60F 254, 20x20cm, 0.2mm, E. Merck, West Germany) were used. The developing solvent was petroleum ether-chloroform-absolute ethanol (7 : 2 : 1,v/v/v). The metabolites were identified by comparing each spot with that of the authentic compound in the chromatogram which was obtained by developing with the above solvent.

The relative amounts of the metabolites were determined by scraping off the spots on the thin-layer plates corresponding to the black spots on the autoradiogram, putting them into 15ml-vials, and measuring the radioactivity, using the toluene cocktail (PPO 4g and POPOP 0.5g were dissolved in toluene to 1 l).

Formation of soil-aged carbofuran residues

Based on the degradation rate of ^{14}C-carbofuran in soil which had been obtained from a preliminary experiment, the soils treated with a mixture of ^{14}C-labeled and non-labeled carbofuran were aged at 21±1℃ for 3 and 6 months, respectively.

The final radioactivity and concentration at the completion of the aging were intended to be 185 KBq/1.5kg soil and 5mg/kg soil, respectively. Throughout the aging in the dark, the moisture contents were kept at 50% of the maximum water-holding capacity of the soil.

The $^{14}CO_2$ evolved during the aging period was absorbed in 1N-NaOH and the radioactivity was measured at an interval of one week with a Liquid Scintillation Counter (PW 4700, Philips).

The volatile substances were adsorbed on Amberlite XAD-2(20-50 mesh, Sigma, U.S.A) and the radioactivity was measured after the extraction with acetone. Table 1 shows the original treatment levels of ^{14}C-carbofuran for the aging in soil.

Table 1. Treatment levels of ^{14}C-carbofuran in the aging in soil

Aging period (month)	Radioactivity dpm(KBq)/6.5 kg soil	Total concentration (mg/6.5kg soil)
3	55,683,300 (928.1)	37.4
6	64,721,740 (1078.1)	43.2

Growing of rice plants

After the aging periods of 3 and 6 months, respectively, the soils were air-dried and their radioactivities were measured by combustion with a Biological Oxidizer (R.J.Harvey Instrument Corporation, U.S.A). For the growing of rice plants, the soils were fertilized with N-P-K at the ratio of 15-9-11 kg/10 a, respectivelty. Three types of soil samples containing carbofuran residues which were freshly applied, 3-month aged, and 6-month aged, respectively, were put into specially devised pots made of stainless steel (I.D.17cm×H.10cm) and the 50-day-grown seedlings of rice plants were transplanted.

In each pot 8 seedlings were grown with 2 seedlings per hill. Rice plants were grown in a vinyl house with good ventillation for 42 days. The moisture loss was supplied once per day at the early stage and twice at the middle and late stage. The radioactivities in soil before the growing of rice plants were shown in Table 2.

Mineralization to $^{14}CO_2$ and volatilization of ^{14}C-carbofuran and its metabolites during the growing period of rice plants

The $^{14}CO_2$ and volatile substances evolved from ^{14}C-carbofuran and its metabolites during the growing period of rice plants were absorbed in 1N-NaOH and 0.1N-H_2SO_4, respectively. The radioactivities were measured at weekly intervals.

Table 2. Radioactivities of the soils where ^{14}C-carbofuran was freshly treated, 3-month-aged, and 6-month-aged, respectively, for the growing of rice plants

Pot No.	Treatment method	^{14}C-Radioactivity remaining in soil, dpm(KBq)	Remarks
1	Control	0	
2	(T-0)	0	
3	Fresh	10,943,262(182.4)	
4	(T-1)	〃	
5		〃	
6	3-Month-aged	10,424,547(173.7)	3-keto
7	(T-2)	〃	carbofuran
8		〃	phenol formed
9	6-Month-aged	9,888,597(164.8)	
10	(T-3)	〃	
11		〃	

Harvest of rice plants and autoradiography

At the completion of 42 days of the growing period, the shoots and roots of rice plants were separately harvested. The roots were rinsed thoroughly with tap water to remove soil. After the fresh weights of the shoots and roots were separately measured, they were freeze-dried for 4 days (Chem Lab Instruments LTD., England, Model SB 4).

The freeze-dried weights were also measured. Autoradiography was performed by using the fresh rice plants harvested from pot No. 3(fresh), 6(3-month-aged), and 9(6-month-aged). The exposure period was 3 weeks.

Measurement of radioactivity

The radioactivity of soil was measured by combusting 0.3g of each sample which was air-dried and ground homogeneously, with the Biological Oxidizer, while the radioactivity of rice plants was measured by combusting 0.2g of each sample which was freeze-dried and pulverized.

The $^{14}CO_2$ evolved was absorbed in the ^{14}C-cocktail (For Harvey Biological Oxidizer, R. J. Harvey Instrument Corporation, New Jersey, U. S. A) and the radioactivity was measured with the Liquid Scintillation Counter. The flow rate of oxygen and nitrogen in the Biological Oxidizer was 300 ml/min.. The temperatures of catalyst zone and combustion zone were 700℃ and 900℃, respectively, and the combustion time was 4 minutes.

As for the cocktails, the toluene cocktail was used for the samples dissolved in organic solvents which were ordinarily evaporated before adding the cocktail. For the measurement of radioactivity of $^{14}CO_2$ absorbed in 1N-NaOH and volatile substances absorbed in 0.1N-H_2SO_4, the Aquasol (Du Pont, NEN Research Products, U.S.A). (U.S.A.) was used.

The radioactivity was measured with the Liquid Scintillation Counter after the samples added with scintillation cocktails were homogeneously mixed and stabilized at 4℃ in the refrigerator for 24 hours.

Extraction of soil and rice plants

15g of sample soils added with 50 ml of methanol was shaken for 4 hours and centrifuged at 27,000g for 10 minutes.

As for rice plants, 0.8 g of the shoots and roots, respectively, was extracted with methanol, 0.25N-HCl, acetone, and BF_3 MeOH (ca. 14% BF_3), respectively, for 2 hours in a sonicator, for comparison of the extractability.

After extracted, the samples were centrifuged at 36,000g for 15min. The same procedure was repeated

until the radioactivity of the extracts showed the background level. Meanwhile, for autoradiography more amounts of samples of soil and rice plants were extracted separately.

For the elucidation of conjugated metabolites, 50g of soil samples was extracted with three 70ml-aliquots of methanol on a shaker.150ml of 0.25N-HCl was subsequently added to the soil samples and incubated at 60℃ for 5 hours. At the completion of the incubation, the incubation mixture was centrifuged at 36,000g for 15 minutes.

The pH of the supernatant was adjusted to 6-7 with 1N-NaOH solution and concentrated on a rotary evaporator for TLC and autoradiography. In addition, in order to examine the possible hydrolysis of the conjugated metabolites by cellulase, the methanol extract of the shoots was concentrated at 40℃ and redissolved in 25ml of citrate-phosphate buffer (pH 5.0). 10 units of the cellulase (Produced by *Trichoderma viride*, Sigma) were added to it and the mixture was incubated at 37℃ for 12 hours. After 12 hours, another 10 units were added to the mixture and the mixture was incubated again for another 12 hours.

At the completion of the incubation, the mixture was extracted with CH_2Cl_2 amd the extract was concentrated to perform autoradiography for the elucidation of conjugate formation.

Autoradiography of the extracts of soil and rice plants

50g of soil was extracted with five 70ml-portions of methanol by the same method as above.

All the extracts collected were concentrated at 45 ℃ and the residue was reextracted with methanol. After evaporating methanol, 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. As for the samplesof rice plants, 1.5g of the pulverized shoots was extracted with six 15ml-portions of methanol by the same method. Autoradiography was done in like manner as in soil extracts. The extraction of roots was left out due to the small amount of radioactivity. In addition, 2g of the shoots added with 50ml of 0.25N-HCl was incubated at 60℃ for 5 hours.

Autoradiography was performed in the same manner as in soil extracted with 0.25N-HCl.

Distribution of radioactivity of soil extracts between aqueous and organic phases

5 ml of methanol extracts of soil was taken into a screw-capped test tube and the methanol was evaporated by bubbling air stream. 5ml of distilled water was then added to the tube and mixed homogeneously. To this water sample was added 5ml of dichloromethane and shaken vigorously. After standing, 3ml each out of aqueous and organic phases was taken out.

The radioactivity in organic phase was measured with toluene cocktail and that in aqueous phase measured with Aquasol.

Analysis of non-extractable soil-bound ^{14}C

2g 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 conc. HCl to the point where no more precipitate was formed. This mixture was centrifuged at 16,000g for 10min. The supernatant corresponds to fulvic acid and the precipitate to humic acid. The radioactivities of fulvic acid and the precipitate to humic acid. The radioactivities of fulvic acid and humic acid which was dissolved with 0.1N-NaOH were measured with Aquasol as the acintillation cocktail. The extracted soils were combusted to determine humin.

Desorption experiment

In order to compare the uptake of carbofuran and its metabolites by rice plants with their desorption from soil, 50g of soil samples of fresh treatment, 3-month, and 6-month aging were extracted with 70ml of distilled water for 4 hours on a shaker and centrifuged at 27,000g for 10min.. The same procedure was repeated until the radioactivity of the extract became almost the background level.

Results and discussion

Transformation 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 degradation rates per week being 0.69 and 1.03%, respectively, as show in Fig. 2. The mineralization of carbofuran to $^{14}CO_2$ is believed to result from chemical and microbial degradation.[10,11,17] Getzin[10] reported that carbofuran phenol was mainly formed as a result of the hydrolysis of the carbamate moiety in carbofuran structure in the weakly alkaline soil of pH 7.8 and it was rapidly bound to soil constituents. However, in our study using the soil of pH 5.4, it was found in the analysis of the soil extract that the oxidation of the position 3 carbon in the benzofuran occurred as the main degradation pathway in addition to the hydrolysis of the carbamate linkage. Meanwhile, the total amounts of $^{14}CO_2$ evolved during 42 days of rice plant growing are presented in Table 3. 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 treated, during the same growing period. Judging from the ever-increasing amounts of $^{14}CO_2$ during the aging periods, it is believed that some of carbofuran was already transformed to the metabolites in T-2 and T-3 which were so vulnerable to the degradation to $^{14}CO_2$ that the amount of $^{14}CO_2$ evolved during the rice plant growing also increased much more than in T-1. In addition, the amount of $^{14}CO_2$ evolved during the same period in the aging as 42 days of the growing of rice plants was in the range of 2.5-2.8 % of the originally applied radioactivity, being a good contrast with the $^{14}CO_2$ amount of 4.4-15.7% in the growing of rice plants. Based on this result, it is believed that the growing of crops such as rice plants increased the mineralization of carbofuran and its metabolites to $^{14}CO_2$, as indicated in the previous investigation by Lee et al.[18] that when bean and radish were grown, respectively, in soils containing non-extractable bound residues of ^{14}C-bentazon, they enhanced the degradation of the soil-bound residues. According to Venkateswarlu and Sethunathan,[12] there were nearly four times as many bacteria in the rhizosphere of rice plants in the carbofuran-treated soil as those in the control without carbofuran. Accordingly, it is believed that these microorganisms were involved in the degradation of carbofuran. Besides, the possible action of various enzymes exuded from the rice roots can not be ruled out.

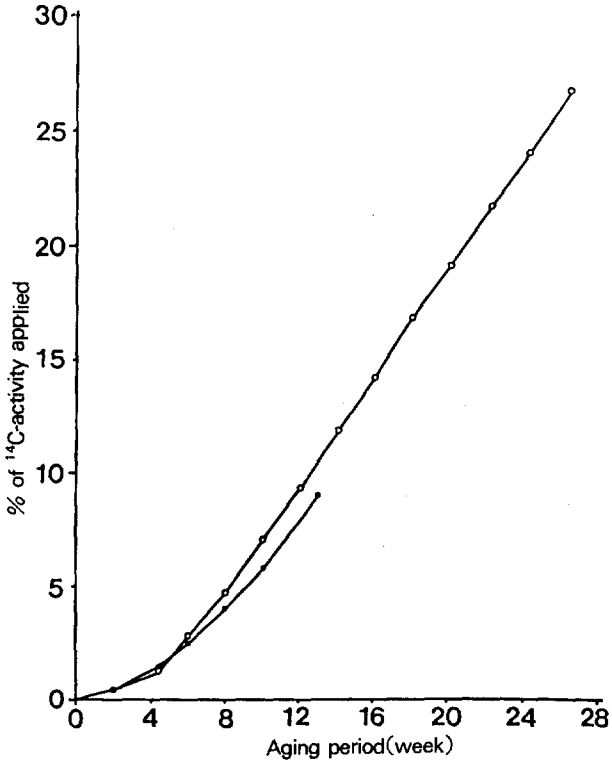

Fig. 2. Mineralization of ^{14}C-carbofuran to $^{14}CO_2$ in soil during 3-and 6-month aging.
●—● : 3-Month aging
○—○ : 6-Month aging

Meanwhile, the volatilization of ^{14}C-carbofuran and its metabolites during the aging and the growing of rice plants was less than 0.05% of the originally applied amount as seen in Table 4. This will be partly due to the fact that carbofuran was mixed homogeneously with soil, not treated on the surface.

Table 3. Mineralization of ^{14}C-carbofuran and its metabolites in soil to $^{14}CO_2$ during 42 days of rice plant growing

Pot No.	^{14}C-Radioactivity in 1.5 kg soil dpm (KBq)	^{14}C-Radioactivity mineralized (%)	Mean (%)
3		4.98	
4(T-1)	10,943,262 (182.39)	4.14	4.37 ± 0.53
5		4.00	
6		11.20	
7(T-2)	10,424,547 (173.74)	13.07	10.95 ± 2.26
8		8.58	
9		16.04	
10(T-3)	9,888,597 (164.81)	15.35	15.71 ± 0.35
11		15.74	

Table 4. Volatilization of ^{14}C-carbofuran in soil during the aging periods of 3-and 6-months and the rice growing period of 42 days

(%)

Pot No.	Treatment method	Aging period	Rice growing period	Mean
3	Fresh (T-1)		0.030	0.018 ± 0.011
4			0.012	
5			0.011	
6	3-Month-aged (T-2)	0.026	0.014	0.018 ± 0.008
7			0.027	
8			0.014	
9	6-Month-aged (T-3)	0.050	0.010	0.012 ± 0.007
10			0.006	
11			0.019	

Harvest of rice plants and their freeze-drying

The fresh and dried weights of rice plants after the 42 days' growing are shown in Table 5. Compared with the control, growth of rice plants in the carbofuran-treated soil is believed to have been normal.

^{14}C-Radioactivity remaining in rice plants

The ^{14}C-radioactivity of carbofuran and its metabolites remaining in rice plants as a result of the degradation through the metabolism in the tissues after the uptake by roots and the translocation[3,4,5,6,7,19,20,21] or of

Table 5. Fresh and dry weights of the rice plants harvested

(g)

Pot No.	Treatment method	Root		Shoot	
		Fresh	Dry	Fresh	Dry
1	Control	38.96	7.06	41.79	13.67
2	(T-0)	38.99	7.51	51.64	13.75
3	Fresh	35.61	6.84	45.62	11.77
4	(T-1)	34.93	6.25	40.35	10.90
5		35.74	6.51	43.56	13.14
6	3-Month-aged	29.24	7.14	43.12	12.80
7	(T-2)	40.59	7.82	46.15	13.98
8		42.82	7.51	52.38	15.67
9	6-Month-aged	31.46	7.03	46.88	14.71
10	(T-3)	44.47	8.06	50.09	14.40
11		29.10	6.49	47.06	13.83

Table 6. Uptake of ^{14}C-carbofuran and its metabolites from the soils containing different residues by rice plants during the growth period of 42 days

Pot No	Treatment method	Uptake (%)					
		Root	Mean	Shoot	Mean	Total	Mean
3	Fresh	5.89	6.52 ±0.58	19.93	20.45 ±3.73	25.82	26.83 ±3.81
4	(T-1)	6.62		17.01		23.63	
5		7.04		24.41		31.05	
6	3-Month-aged	4.75	4.21 ±0.57	17.89	17.14 ±0.66	22.64	21.35 ±1.21
7	(T-2)	4.26		16.92		21.18	
8		3.62		16.62		20.24	
9	6-Month-aged	3.19	3.00 ±0.55	7.71	7.30 ±0.44	10.90	10.29 ±0.59
10	(T-3)	3.42		6.84		10.26	
11		2.38		7.34		9.72	

the volatilization from the surface of rice plants[20,22,23] during the 42 days' growing period is shown in Table 6. As seen in T-1 of this table, the ^{14}C-radioactivity translocated to shoots is more than three times that in roots, whereas in T-3, the ^{14}C-radioactivity present in shoots is about twice as much as that in roots, its amount being only one third of that in T-1. The explanation for this will be that in T-1 carbofuran as such was absorbed by roots and translocated to shoots rapidly, whereas in T-3 during the aging period of 6 months, only 56.1% of the originally applied ^{14}C-carbofuran remained intact, 39.7% being transformed to the main metabolite 3-keto carbofuran phenol (Refer to Table 13). Accordingly, it is believed that in T-3, a lot of 3-keto carbofuran in addition to carbofuran was absorbed by roots and thereafter adsorbed on and/or bound to roots and hence almost none or 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-keto carbofuran phenol (Refer to Table 13). In consequence, the pattern of absorption and translocation of ^{14}C-radioactivity in T-2 lay between T-1 and T-3. Summarizing these results, when carbofuran was treated in soil immediately before transplanting rice plants (T-1), 26.8% of the originally applied ^{14}C-

Table 7. Behaviour of ^{14}C-carfbofuran in soil

Pot No.	^{14}C-carbofuran in 1.5kg soil dpm (KBq)	$^{14}CO_2$ evolved (Aging period)	Volatilization		$^{14}CO_2$ evolution (rice growing period)	^{14}C-Radioactivity absorbed by rice plant	^{14}C-Radioactivity remaining in soil	Recovery	Losses through rice plant
			Aging period	Rice growing period					
3				0.030	4.98	25.82	24.68	55.51	44.49
4	10,943,262			0.012	4.14	23.63	29.52	57.30	42.70
5	(182.39)			0.011	4.00	31.05	20.09	55.55	44.45
6				0.014	11.20	22.64	44.97	78.82	21.18
7	10,424,547	8.91	0.026	0.027	13.07	21.18	44.62	78.90	21.10
8	(173.74)			0.014	8.58	20.24	42.61	71.44	28.56
9				0.010	16.04	10.90	62.98	89.93	10.07
10	9,888,597	26.72	0.050	0.006	15.35	10.26	60.21	85.83	14.17
11	(164.81)			0.019	15.74	9.72	60.47	85.94	14.06

radioactivity remained in the tissues of rice plants after the growing period of 42 days.

Meanwhile, 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 remained in the tissues, respectively. Undoubtedly, the nature of the compounds corresponding to the ^{14}C-radioactivity will be different from one another.

Fate of ^{14}C-carbofuran in soil

The fate of ^{14}C-carbofuran applied to soil is summarized in table 7. As can be seen in this table, 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 why 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 $^{14}CO_2$ during the aging periods of 3 and 6 months.

For one thing, 3-keto carbofuran phenol formed amounted to 21.92% in T-2 and 39.72% in T-3, of the originally applied ^{14}C-radioactivity (Table 13). Meanwhile, the loss of ^{14}C-carbofuran applied to soil by volatilization during the same growth period of rice plants was a negligible amount of 0.01-0.03% of the original ^{14}C-radioactivity. However, in case carbofuran was applied on the soil surface, the possible loss by volatilization will increase on account of its relatively high vapor pressure (8.3×10^{-6}mm Hg/25℃)[6]. In contrast, very little amount of carbofuran was lost by volatilization in this investigation where carbofuran had been homogeneously mixed with soil. In the meantime, the soil-bound residues of ^{14}C-radioactivity of different variants 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. That is, the fact that 20-30% of the origianl ^{14}C-radioactivity persisted in the soil of T-1 means that carbofuran and its metabolites were adsorbed or bound onto soil constituents during the growth period of 42 days and hence were not available to rice plants. On the other hand, much larger amounts of ^{14}C-radioactivity persisted in the soils of T-2 and T-3.

This will be due to the fact that much of carbofuran was already degraded to 3-keto carbofuran 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 gro-

wth period of rice plants for 42 days.

In addition, the relatively lower recovery of carbofuran in rice experiment than other pesticides is believed to be due to the fact that great portions of carbofuran and its mitabolites which had been translocated to the shoots were lost through volatilizations[6,21,22]. However, the little loss of ^{14}C-radioactivity by volatilization in T-3 will result from the fact that much of the ^{14}C-radioactivity absorbed and translocated by rice plants corresponded to the carbofuran metabolites as well as carbofuran as such and they are readily adsorbed or bound to the tissues of rice plants, leading to the reduced loss by volatilization.

Extraction of soils

Soil samples were extracted with MeOH[4] instead of 0.25N-HCl, because the former turned out to be better than the latter in extractability (Table 8) and the results are presented in Table 9. In this table, it can be seen that the non-extractable bound residues increased with the aging period. The reason why much higher percentage of bound residues in the soils where rice plants were grown in T-1, T-2, and T-3 resulted is that rice plants already absorbed the available and/or extractable ^{14}C-radioactivity, giving the relatively high ratios of non-extractable bound residues relative to the original soil radioactivity.

Table 8. Extraction of the 3-month-aged soil with MeOH and 0.25N-HCl

Solvent	Extracted (%)	Bound (%)	Recovery
MeOH	48.90	54.17	103.07
0.25N-HCl	29.31	72.63	101.94

Partition of the radioactivity of soil extracts between aqueous and organic phases

In order to examine how much of carbofuran applied to soil was transformed to polar metabolites, the partition of the radioactivity of soil extracts between aqueous and organic phases was carried out and the result is shown in Table 10. 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 corresponding metabolites, however, could not be identified on TLC and autoradiography.

Non-extractable soil-bound residues of ^{14}C-carbofuran and its metabolites

The feature of the non-extractable soil-bound residues of ^{14}C-carbofuran and its metabolites is shown in Table 11. In this table, it is noticeable that the radioactivities in fulvic acid, humic acid, and humin increased

Table 9. Extraction of soil samplis with MeOH

(%)

Treatment method	Rice plant	Methanol-extractable	Bound	Recovery
Fresh	No	93.56	8.33	101.89
(T-1)	Yes	35.33	61.92*	97.25
3-Month-aged	No	55.09	37.92	93.01
(T-2)	Yes	12.17	83.53*	95.70
6-Month-aged	No	41.26	54.63	95.89
(T-3)	Yes	5.14	91.45*	96.59

* Much higher percentage of bound residues resulted, because rice plants already absorbed the available or extractable ^{14}C-radioactivity.

Table 10. Partition of the methanol-extracts from soil samples treated with ^{14}C-carbofuran in the absence and presence of rice plants between aqueous phase and organic phase

(%)

Treatment method	Rice plant	Methanol-extractable	Distribution of ^{14}C after partitioning	
			Aqueous phase	Organic phase(CH_2Cl_2)
Fresh	No	93.56	1.00	92.56
(T-1)	Yes	35.33	1.62	33.71
3-Month-aged	No	55.09	1.48	53.61
(T-2)	Yes	12.17	7.62	4.55
6-Month-aged	No	41.26	2.61	38.65
(T-3)	Yes	5.14	3.41	1.73

Table 11. Change in the non-extractable ^{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%, in radioactivity)

(%)

Treatment method	Rice plant	Non-extractable bound residue	Radioactivities in		
			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

all in proportion to the aging period in the soils in the absence of rice plants. Meanwhile, in the soils after aging and the growing of rice plants, the amounts of radioactivity in fulvic acid and humin were almost the same in 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 radioactivity in humic acid and humin increased to some degree, even if that of fulvic acid decreased a little bit.

Extraction of rice plants

The extractability of the shoots and roots of rice plants with various solvents is presented in Table 12. As can be seen in this table, the extractability of the roots is lower than that of the shoots in all solvents used. Khan et al.[24] reported that when radishes were applied with ^{14}C-carbofuran and the edible portions samples 21 days after application were exhaustively extracted with solvents, the amount of non-extractable (bound) ^{14}C residues was 92.6%. They indicated that the formation of bound residues in radishes is related with a process involving chemical entrapment of the pesticide or its metabolites by lignin and/or other plant

Table 12. Extraction of rice plants

	Solvent	Extracted(%)
Root (T-1)	Acetone	9.85
	Methanol	10.66
	0.25N-HCl	24.16
	BF_3 · MeOH	39.17
Shoot (T-1)	Acetone	25.41
	Methanol	59.26
	0.25N-HCl	52.22
	BF_3 · MeOH	78.27

macromolecules. Looking back 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 0.25N-HCl and/or MeOH was believed to be the most suitable for the subsequent analyses including autoradiography.

Autoradiography of rice plants

Fig. 3 shows the autoradiogram of rice plants which were grown for 42 days in T-1. The ^{14}C-radioactivity which had been absorbed and translocated by he plant 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 tendencies were also observed in T-2 and T-3. Quite a few investigators[16,20,25] 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 the tip.

Fig. 3. Autoradiogram of rice plants (Pot NO. 3, T-1) which were grown in the soil treated with ^{14}C-carbofuran for 42 days. The plant was divided into three parts.
A : Top, B : Middle, C : Bottom

Formation of carbofuran metabolites in soil

Table 13 shows the carbofuran metabolites formed in soil. It is noticeable that 3-keto carbofuran phenol was the major metabolite, whereas carbofuran phenol, 3-hydroxy carbofuran, and 3-hydroxy carbofuran phenol were the minor metabolites, under this rather aerobic condition different from the flooded one.

Table 13. Relative mounts of carbofuran and its metabolites in the MeOH and 0.25N-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 No. 3	0.50	73.08	23.60	1.09	0.97	0.76
	Pot No. 6	0.48	76.40	18.61	1.99	0.97	1.55
	Pot No. 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

* Successively followed the MeOH extraction.

There is a report[12] that carbofuran phenol and 3-hydroxy carbofuran were detected as the metabolites under the anaerobic flooded condition. Getzin[10] reported that carbofuran was hydrolyzed to carbofuran phenol in soil which was subsequently bound to soil constituents, followed by microbial degradation slowly.

Table 14 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-hydroxy carbofuran (23-24%) in contrast to the soil.

Sonobe et al.[26] reported angelic acid ester of 3-hydroxy carbofuran and 3-hydroxy carbofuran as the major metabolite of carbofuran in carrots, and 3-keto carbofuran phenol in potatoes. Besides this, there are a lot of investigations on the hydrolysis of the carbamate linkage of carbofuran structure and the metabolic pathways involving the oxidation of the position 3 carbon in the benzofuran ring in the metabolism of carbofuran in plants[4,5,21], or on the oxidation of the position 3 carbon of the benzofuran ring[6,19,27].

Autoradiography of the extracts of soil and rice plants

Fig. 4 shows the autoradiograms of the extracts of soils which were treated with carbofuran, aged for 3 and 6 months, respectively, and extracted with MeOH and 0.25N-HCl (A), and in which rice plants were grown for 42 days thereafter and extracted with MeOH (B). As can be confirmed by autoradiogram A, the intact carbofuran amounted to 74 and 56% of the total ^{14}C-radioactivity remaining in soil after the aging of 3 and 6 months, respectively, and the rest were degraded to the various metabolites. Among them, 3-keto carbofuran phenol was the major metabolite, amounting to 22 and 40% in the aging of 3 and 6 months, respectively. In addition, after having grown rice plants for 42 days following the aging of 3 and 6 months, the MeOH extract of the soils showed the intact carbofuran of the remaining ^{14}C-radioactivity to be 76 and 69%, the major metabolite , 3 keto carbofuran phenol being 19 and 26 %, respectively.

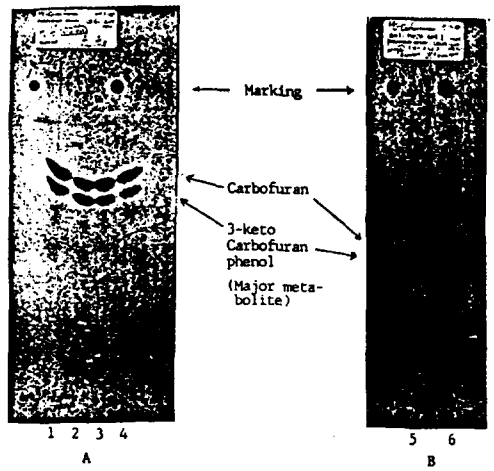

Fig. 4. Autoradiograms of the extracts of soils which were aged for 3 and 6 months (A) and where rice plants were grown for 42 days thereafter (B).
1 : MeOH extract, 3-month aged
2 : MeOH extract, 6-month aged
3 : 0.25N-HCl extract, 3-month aged
4 : 0.25N-HCl extract, 6-month aged
5 : MeOH extract (Pot No. 6, T-2)
6 : MeOH extract (Pot No. 9, T-3)

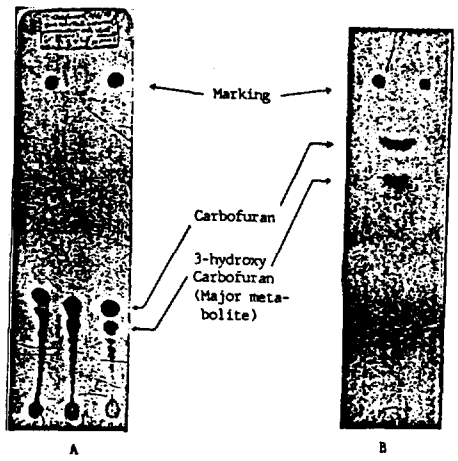

Fig. 5. Autoradiograms of the extracts of the shoots of rice plants in T-1, T-2, and T-3 (A) and of the extract which was hydrolyzed by cellulase (B).
The Rf's are greater in B, possibly because of the removal of hindering materials by enzymatic hydrolysis.

Table 14. Distribution 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 No. 4 (T-1)	0.10	64.24	4.05	24.27	4.84	2.50
Pot No. 6 (T-2)	0.24	66.96	2.96	23.46	2.74	3.64
Pot No. 9 (T-3)	0.40	73.00	1.34	23.20	1.38	0.68

Table 15. Dixtribution of carbofuran and its metabolites in the MeOH extract of shoots after enzymatic cleavage

(%)

Sample	Carbofuran phenol	Carbofuran	3-Keto carbofuran phenol	3-Hydroxy carbofuran	3-Hydroxy carbofuran phenol
Pot No. 6 (T-2)	2.97	58.67	2.93	28.53	6.90

Table 16. Extraction of the fresh, 3-month and 6-month-aged soils with distilled water

(%)

Sample	Total radioactivity	Extracted	Bound	Recovery
Fresh	100	87.68	10.52	98.20
3-Month-aged	100	63.44	38.25	101.69
6-Month-aged	100	44.37	54.74	99.11

Table 17. Comparison of the bioavailability of carbofuran to rice plants with the carbofuran extractability of distilled water

Carbofuran-treated soils	Extracted with distilled water (%)	Total uptake by rice plants(%) (Both remaining and lost)
Freshly treated (T-1)	87.68	71
3-Month-aged (T-2)	63.44	45
6-Month-aged (T-3)	44.37	23

The reason why the relative amount of the major metabolite, 3-keto carbofuran phenol decreased after the growing of rice plants in thought to be due to the uptake by rice plants, adsorption and/or binding to soil constituents, or easier mineralization to $^{14}CO_2$ than carbofuran as such during the growing period of rice plants (Table 13).

Fig. 5-A shows the autoradiogram of the MeOH extracts of the shoots of rice plants which were grown in T-1, T-2, and T-3. Since the tailing in Fig. 5-A 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 (Fig. 5-B). As can be seen in Fig.5 and Table 14, the major metabolite, 3-hydroxy carbofuran was formed as much as 23-24% in all treatments(Fig. 5-A), whereas after the hydrolysis by cellu-

lase the amount increased up to 29% (Table 15). Accordingly, it was recognized that the conjugate of 3-hydroxy carbofuran remaining in the tissues of rice plants, which was extractable with MeOH, was hydrolyzed up to some 5-6% by cellulase.

Relation of the uptake of carbofuran in soil by rice plants to the extractability with water

Table 16 shows the results obtained by extraction of freshly carbofuran-treated (T-1), 3-month-aged (T-2), and 6-month-aged(T-3)soils with distilled water. In T-1, 88% of the ^{14}C-radioactivity remaining in soil was extracted, whereas 63% and 44% were extracted in T-2 and T-3, respectively. Since the solubility of carbofuran in water (320 ppm) is relatively great, the absorption and translocation by plants are rather fast and hence rapidly degradable[6,22].

The ^{14}C-radioactivities remaining in the shoots and roots of rice plants in T-1, T-2, and T-3 following the absorption and translocation were 27%, 21%, and 10%, respectively (Table 6 and 7). If the losses from rice plants by volatilization and metabolism after the uptake by rice plants were considered, the average amounts absorbed by rice plants would amount to 71%, 45%, and 23% (Table 17). Accordingly, the bioavailability of carbofuran to rice plants during the growing period of 42 days falls within the limit of the carbofuran extractability of water.

요 약

침투성 살충제 Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate)의 신생 (Fresh) 및 숙성 (Aged) 잔류물의 벼에 의한 흡수를 구명하기 위하여 벼 재배 직전에 Carbofuran 을 처리한 토양(T-1). Carbofuran 을 처리하고 3개월간 숙성시킨 토양 (T-2) 및 Carbofuran 처리 후 6개월간 숙성시킨 토양 (T-3)에서 벼를 재배하였다. 토양(온도 22±1℃, 최대 용수량의 50% 수분함량) 중에서 3개월 및 6개월간 숙성시키는 동안 ^{14}C-Carbofuran 으로 부터 방출된 $^{14}CO_2$ 의 량은 최초 처리량의 각각 8.9% 와 26.7% 이었다. 42일간 벼를 재배하는 동안 방출된 $^{14}CO_2$의 량은 4.4% (T-1), 11.0% (T-2), 그리고 15.7% (T-3)이었다. 3개월과 6개월간 숙성시킨 토양을 MeOH로 추출하여 Autoradiography를 행한 결과 주대사 산물이 3-keto Carbofuran phenol(2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol)이었고 벼를 MeOH로 추출하고 Autoradiography를 행한 결과 3-hydroxy Carbofuran(2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl-N-methylcarbamate) 이 주 대사산물로 판명되었다. 3개월과 6개월의 숙성 기간과 42일간의 벼 재배 기간중 토양의 ^{14}C-Carbofuran 으로부터 휘발된 량은 최초 처리량의 각각 0.026, 0.05, 그리고 0.012-0.018 %이었다. 42일간의 벼 재배 기간중 벼에 의하여 토양으로 부터 흡수 이행되고 잔류해 있는 ^{14}C 방사능의 량은 최초 방사능의 각각 26.8% (T-1), 21.4% (T-2), 그리고 10.3% (T-3)이었다. T-1, T-2 및 T-3 에서의 추출 불가 토양 잔류물의 량은 최초 처리 Carbofuran의 8.3%, 37.9% 그리고 54.6% 이었다. T-3에서 ^{14}C 방사능이 지상으로 소량 이행된 것은 주 대사산물인 3-keto Carbofuran phenol이 뿌리에서 Conjugate를 형성했기 때문이며 T-1에서 특히 회수율이 낮은 것은 벼 조직의 표면으로 부터 Carbofuran 이 휘발되었기 때문이라 생각된다.

Acknowledgnent

The authors acknowledge The Korea Research Foundation for the research grant and express their thanks to International Atomic Energy Agency for supplying ^{14}C-carbofuran.

References

1. Agricultural Chemicals Industrial Association (1976) : Agrochemical Year Book.
2. IRRI, International Rice Research Institute, Los Banos, Philippines, Annual Report for 1974 (1975).
3. Metcalf, R. L., Fukuto, T. R., Collins, C., Borck, K., El-Aziz, S. A., Munoz, R. and Cassil, C. C. (1968) : Metabolism of 2,2-dimethyl-2,3-dihydrobenzofurany-7-N-methylcarbamate (Furadan) in plants, insects and mamals, J. Agric. Food Chem., 16, 300.
4. Knaak, J. B., Munger, D. M. and McCarthy, J. F. (1970) : Metabolism of carbofuran in alfalfa and

bean plants, J. Agric. Food Chem., 18, 827.
5. Ashworth, R. J. and Sheets, T. J. (1972) : Metabolism of carbofuran in tobacco, J. Agric. Food Chem., 20, 407.
6. Fuhremann, T. W. and Lichtenstein, E. P. (1980) : A comparative study of the persistence, movement and metabolism of six carbon-14-insecticides in soils and plants, J. Agric. Food Chem., 28, 446.
7. Dorough, H. W. (1968) : Fate of furadan (Nia-10 242) in bean plants, Bull. Environ. Contam. Toxicol., 3, 164.
8. Dorough, H. W. (1968) : Metabolism of furadan (Nia-10242) in rats and house flies, J. Agric. Food Chem., 16, 319.
9. Ivie, G. W. and Dorough, H. W. (1968) : Furadan-^{14}C metabolism in a lactating cow, J. Agric. Food Chem., 16, 849.
10. Getzin, L. W. (1973) : Persistence and degradation of carbofuran in soil, Environmental Entomol., 2(3), 461.
11. Venkateswarlu, K., Gowda, T. K. S., Sethunathan, N. (1977) : Persistence and biodegradation of carbofuran in flooded soils, J. Agric. Food Chem., 25, 533.
12. Venkateswarlu, K. and Sethunathan, N. (1978) : Degradation of carbofuran in rice soils as influenced by repeated applications and exposure to aerobic conditions following anaerobiosis, J. Agric. Food Chem., 26, 1148.
13. Rajagopal, B. S. and Sethunathan, N. (1984) : Influence of nitrogen fertilizers on the persistence of carbaryl and carbofuran in flooded soils, Pestic. Sci., 15, 591.
14. Brahmaprakash, G. P. and Sethunathan, N. (1985) : Metabolism of carbaryl and carbofuran in soil planted to rice, Agric. Ecosys. Environ., 13, 33.
15. Park, C. K. and Oh, S. R. (1986) : Fate of C-14 labelled carbofuran in paddy plants and soil, Korean J. Environ. Agric., 5(2), 85.
16. Lee, Y. D., Lee, K. H, and Park, C. K. (1987) : Fate of ^{14}C-carbofuran in rice plant and paddy soil, Korean J. Environ. Agric., 6(1), 7.
17. Williams, I. H., Pepin, H. S., and Brown, M. J. (1976) : Degradation of carbofuran by soil microorganisms, Bull. Environ. Contam. Toxicol., 15, 244.
18. Lee, J. K., Cheon, S. Y., and Kyung, K. S. (1988) : Uptake of the residues of the herbicide bentazon in soil by soybean and radish, Korean J. Environ. Agric., 7(1), 1.
19. Turner, B. C. and Caro, J. H. (1973) : Uptake and distribution of carbofuran and its metabolites in field-grown corn plants, J. Environ. Quality, 2, 245.
20. Archer, T. E. Stokes, J. D. and Bringhurst, R. S. (1977) : Fate of carbofuran and its metabolites on strawberries in the environment, J. Agric. Food Chem., 25(3), 536.
21. Schlagbauer, B. G. L. and Schlagbauer, A. W. J. (1972) : The metabolism of carbamate pesticide-a literature analysis, Residue Reviews, 42, 1.
22. Siddaramappa, R. and Watanabe, I. (1979) : Evidence for vapor loss of ^{14}C-carbofuran from rice plants, Bull. Environ. Contam. Toxicol., 23, 544.
23. Ferreira, G. A. L. and Seiber, J. N. (1981) : Volatilization and exudation losses of three N-methylcarbamate insecticides applied systemically to rice, J. Agric. Food Chem., 29, 93.
24. Khan, S. U. Stratton, Jr. G. D. and Wheeler, W. B. (1984) : Characterization of bound (non-extractable) residues of dieldrin, permethrin, and carbofuran in radishes, J. Agric. Food Chem., 32, 1189.
25. Arunachalam, K. and Lakshmanan, M. (1982) : Translocation, accumulation and persistence of carbofuran in paddy, ground nut and cotton, Bull. Environ. Contam. Toxicol., 28, 230.
26. Sonobe, H., Carver, R. A., Krause, R. T., and Kamps, La Verne R. (1983) : Extraction of biologically incorporated ^{14}C-carbofuran residues from root crops, J. Agric. Food Chem., 31, 96.
27. Caro, J. H., Freeman, H. P., Glotfelty, D. E., Turner, B. C., and Edwards, W. M. (1973) : Dissipation of soil-incorporated carbofuran in the field, J. Agric. Food Chem., 21(6), 1010.