CARBOFURAN AND 3-HYDROXYCARBOFURAN RESIDUES IN RICE PLANTS AND GRAINS[1]

M. Iman and B. Morallo-Rejesus[2]

Carbofuran and 3-OH carbofuran residues in rice plant and its grains were determined. Carbofuran was accumulated mainly in the leaves. Accumulation in the plant was proportional to dosage. The maximum accumulation of carbofuran occured at 45 DAT for the 2 kg a.i./ha insecticide level. The metabolic product, 3-OH carbofuran started to accumulate at 65 DAT; and occurred in stems, grains and mainly in the leaves. The 3-OH carbofuran residues found in the leaves, stems and grains at different dosages and sampling dates were not significantly different.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a broad spectrum insecticide-nematocide effective as contact and systemic toxicant for insect control on a wide variety of crops (Dominick 1967; Hofmaster et al. 1967; Knaak et al. 1970; IRRI 1971, 1972, 1973; van Middelem and Peplow 1973; Halteren et al. 1974; Pathak et al. 1974). When applied to the soil, carbofuran is usually absorbed and translocated to aerial portions of the growing plant. The persistence of carbofuran translocated in cotton plants grown under greenhouse conditions seemed to be controlled by both the organic matter and clay content of the soils (Abdellatif et al. 1967).

It has been reported that the biological efficacy of carbofuran on the rice plant against stem borers, green leafhoppers, and brown planthoppers was considerably reduced when the treated rice seedlings were transplanted in the field (IRRI 1970, 1971). Huynh and Morallo-Rejesus (1975) noted that the rapid decline in the toxicity of carbofuran to brown planthoppers was correlated to temperature and sunlight. Cook et al. (1969) reported that 80-90 percent of the carbofuran residues found in weathered corn were either 3-hy-

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[2] Pest and Diseases, Central Research Institute for Agriculture (CRIA), Jl. Merdeka 99, Bogor, Indonesia and Associate Professor, Department of Entomology, University of the Philippines at Los Baños, College, Laguna, respectively.

droxycarbofuran or 3-hydroxycarbofuran glycoside. Ashworth and Sheets (1972) found that the major metabolites in tobacco by root uptake studies were 3-hydroxycarbofuran and its glycosides. These glycosides were found to be quite persistent and often exhibited anticholinesterase activity following hydrolysis by B glycosidase. These compounds were also found in cabbage leaves (van Middelem and Peplow 1973), in small fruits (Williams and Brown 1973) and in rice plant (Huynh and Morallo-Rejesus 1975). Studies by Cook et al. (1969) indicated that the mechanism of carbofuran metabolism in field corn involves oxidation followed by conjugation at the 3-position of the benzofuran ring, and/or hydrolysis followed by conjugation at the 7-position of the benzofuran ring. Metcalf et al. (1968) and Huynh and Morallo-Rejesus (1975) reported the 3-keto-carbofuran to be hydrolytically unstable and rapidly converted to 2,3-dihydro-7-hydroxy-2,2-dimethyl-3-oxobenzofuran which in turn could be conjugated by forming glycosides.

This study was carried out to determine the residue of carbofuran and its metabolites in the rice plants and its grains, arising from root-zone application of encapsulated carbofuran.

MATERIALS AND METHODS

Insecticide application. The amount of either 104, 208, or 417 mg of three percent carbofuran granules which were equivalent to 0.5, one and two kg active ingredients per hectare (a.i./ha), respectively, were placed inside gelatin capsules and applied into the soil by hand about 2.5 cm away from IR32 rice plants. The experiment was conducted in the greenhouse and replicated three times.

Sampling. The rice plants were uprooted at five, 25, 65, 85, 100 and 140 days after treatment. Grains, leaves, stems, and roots were kept separately in labelled polyethylene bags in the freezer at -10°C until analyzed.

Extraction. Ten to fifteen g of the chopped samples were placed in a 1000 ml round-bottom flask containing a magnetic stirring bar and 150 ml of 0.25 N hydrochloric acid was added. The mixture was refluxed for one h using a heating mantle with continuous stirring as described by Cook et al. (1969). After heating, an additional 10 ml of 0.25 N hydrochloric acid was used to wash the flask and condenser. The sample was cooled for one h at -10°C prior to filtering. The cooled sample was filtered through a wad of glass wool and transferred into a 500 ml separatory funnel. Ten percent sodium lauryl sulfate in water was added to break any emulsion that occuurred and the aqueous phase was extracted three times with 50 ml of distilled methylene chloride. The methylene chloride layers were combined, dried with anhydrous sodium sulfate and concentrated to about one ml by using a rotary evaporator.

To extract the metabolic product 3-OH carbofuran, the concentrated sample of the parent compound was again washed with 20 ml of propanol, three to four drops of concentrated HCl and 100 ml of 0.25N HCl, and refluxed for 30 min. After refluxing, the same procedure as for the parent compound was followed.

Cleanup and separation. Cleanup and separation of carbofuran and its metabolite from the phenols were carried out in a column containing 10 g of alumina by eluting with 100 ml of distilled chloroform as described by Bowman et al. (1967). The chloroform extract was evaporated in a rotary evaporator to almost dryness. The total carbofuran and 3-OH carbofuran content was then obtained by the reaction of the residue on a steam bath for 45 min to one h with 0.5 ml of one percent 1-fluoro, 2,4-dinitrobenzene in 25 ml of borax buffer solution at pH 9 and 50 ml of acetone. The 2,4-dinitrophenyl ether produced in this reaction was determined by gas chromatography after extracting with hexane (Cohen et al. 1970).

Detection by gas-liquid chromatography. The 2,4-dinitrophenyl ether of carbofuran or 3-OH carbofuran in hexane was analyzed by gas chromatography. The chromatograph, Varian aerograph model 1700, was equipped with Ni^{63} electron capture detector and operated under the following conditions.

Column	6' x 1" O.D. Borosilicate glass packed with 5% DC-200 on 80/100 Gas Chrom Q.	
Temperatures (°C)	Column	220
	Injector	240
	Detector	225-230
Nitrogen	67 ml/min.	

Under these conditions, the typical retention time taken from the base of the solvent peak to the base of the carbofuran or 3-OH carbofuran peak was two min and 17 sec for the parent compound and four min and 32 sec for the 3-OH carbofuran. The amount of carbofuran and 3-OH carbofuran in the sample was calculated by measuuring peak heights. The observed residue value was corrected by the ratio of the molecular weight of carbofuran and 3-OH carbofuran to that of the derivatized 2,4-dinitrophenyl ether.

Recovery studies. Ten g of the untreated rice plant was fortified with one ppm of carbofuran and two ppm of 3-OH carbofuran prior to digestion and carried through the entire procedure. The recoveries of the carbofuran and 3-OH carbofuran were 90 and 87 percent, respectively. The minimum detection limits were 0.005 mg/kg for the former and 0.01 mg/kg for the latter.

RESULTS AND DISCUSSION

Previous work has shown that the metabolic pathway of carbofuran in plants involves numerous carbamate and phenolic metabolic residues (Metcalf et al. 1967; Ashworth and Sheets, 1972; Huynh and Morallo-Rejesus 1975). However, for toxicological purposes, only the cholinesterase-inhibiting carbamate residues need to be considered.

The recovery of carbofuran residues in the whole plant at five DAT did not differ significantly at the three treatment levels used (Table 1). However, the higher the dosage, the higher the absorption of the carbofuran. The absorption was more pronounced at 25 and 45 DAT. The maximum residue was obtained at 45 DAT with the highest dosage applied and thereafter it decreased gradually with time. On the other hand, the concentration of the metabolite 3-OH carbofuran started to increase at 65 DAT for all insecticide levels (Table 2) and persisted at that level up to the harvest time.

TABLE 1. **Carbofuran residue, in mg/kg in the whole plant (fresh weight basis) of IR 32 as a result of rootzone application in capsule.**

Sampling Date (DAT)	Application Level .5	1	2
5	5.6 a A	9.0 ab A	10.2 bc A
25	4.9 a A	16.0 a B	16.2 b B
45	4.4 a A	7.7 ab A	25.8 a B
65	1.9 a A	2.0 b A	9.1 bc A
85	0.4 a A	.8 b A	2.8 c A
100[1]	1.4 a A	1.8 b A	4.3 c A
140[2]	0.9 a A	1.8 b A	6.6 c A

DAT — days after treatment.
[1] two weeks after flowering.
[2] at harvest time.

Means followed by the same small letter in each column and the same capital letter in each row are not significantly different at the 5% level by DMRT.

TABLE 2. **Residues of 3-OH carbofuran in the whole plant of IR 32.**

Sampling Date DAT	Residues (fresh weight basis) (mg/kg) .5	1	2
5	3.3 bc	3.4 b	6.2 bc
25	2.4 c	3.2 b	4.9 c
45	6.0 abc	6.2 b	10.6 bc
65	17.2 a	14.3 ab	15.8 abc
85	15.3 ab	8.3 b	14.2 abc
100	15.1 ab	25.4 a	18.6 ab
140	16.5 a	22.1 a	25.6 a

DAT — days after treatment.
Means followed by the same letter in each column are not significantly different at 5% level by DMRT.

These results suggest that the metabolic pathways in rice plants are similar to those reported in cotton, lettuce, beans, tobacco, and corn (Metcalf et al. 1968; Cook et al 1969; Knaak et al. 1970; van Middelem et al 1971; Ashworth and Sheets 1972). These metabolic pathways involve hydroxylation and hydrolysis and conjugation of the hydroxyl-containing metabolites. The major metabolite in rice plants was 3-OH carbofuran which was also reported to occur in cotton, corn, beans, and tobacco. It differs with the report of Huynh and Morallo-Rejesus (1975) where they found only trace amounts of 3-OH carbofuran and the major aglycones were unknowns X, Y, W, and Z. This is probably because in the present study the insecticide was applied in capsule form in the root zone that the release was slower than broadcasting and therefore the total amount of carbofuran applied was not absorbed and metabolized altogether to its aglycone and accumulated as 3-OH carbofuran.

It was known that the efficiency of the rice plant in taking up the insecticides increases when they were applied at the roots or root zone (Aquino 1973; Aquino and Malabuyoc 1975; IRRI 1973, 1974, 1975). Efficient absorption of the insecticides by root-zone application coupled with slow release of the compound from the capsule will increase the biological efficacy of the insecticides. This is confirmed by the present study that the highest concentration of carbofuran residues was found in the leaves of the rice plant at 45 DAT for all dosages and thereafter gradually declined to very low levels with time (Table 3). However, the concentration of carbofuran in stems, roots, and grains were low. This suggests that the absorbed carbofuran from the roots was mainly translocated and accumulated in the leaves rather than in the stems, roots or grains (Table 3). Although the concentration was low, it had been reported that this low dosage could still effectively control the sucking insects (IRRI 1973).

TABLE 3. **Carbofuran residues in the grain, leaf; stem and root of IR 32 by rootzone application in capsule.**

Treatment (kg ai/ha)	Residues (fresh weight basis) (mg/kg) 45 DAT	65 DAT	85 DAT	100 DAT	140 DAT
			Grain*		
.5				0.1	0.1
1				0.1	0.1
2				0.2	0.2
			Leaf		
.5	4.0 a A	1.7 a A	0.3 a A	1.2 a A	0.3 a A
1	6.9 b A	1.8 b B	0.6 a B	1.4 a B	1.1 a B
2	23.9 b A	8.3 b B	1.9 a BC	3.6 a BC	5.1 a C
			Stem		
.5	0.2 a A	0.1 a A	0.1 a A	0.1 a A	0.2 a A
1	0.4 a A	0.2 ab A	0.1 a A	0.1 a A	0.3 a A
2	0.9 b A	0.5 b AB	0.3 a B	0.2 a B	0.8 b A
			Root		
.5	0.2 a A	0.1 a A	0.1 a A	0.1 a A	0.2 a A
1	0.4 a A	0.1 a A	0.2 ab A	0.2 a A	0.3 a A
2	1.0 b A	0.3 a A	0.7 b A	0.3 a A	0.6 a A

DAT — days after treatment.
* There were no significant differences.
Means followed by the same small letter in each column and the same capital letter in each row are not significantly different at 5% level by DMRT.

TABLE 4. **Residues of 3-OH carbofuran in the grain, leaf stem and root of IR 32.**

Treatment (kg ai/ha)	Residues (fresh weight basis) (mg/kg) 45 DAT	65 DAT	85 DAT	100 DAT	140 DAT
			Grain*		
.5				0.6	1.2
1				4.3	1.6
2				3.5	1.2
			Leaf*		
.5	1.4	6.8	8.4	7.7	11.7
1	2.4	5.2	4.9	7.7	11.4
2	5.1	6.4	5.4	8.7	14.7
			Stem*		
.5	4.3	10.2	6.8	2.7	2.4
1	4.8	8.8	3.3	6.1	7.3
2	5.3	9.2	7.9	11.1	8.8
			Root		
.5	0.2 a A	0.2 a A	0.1 a A	0.8 a A	1.2 a A
1	0.2 a A	0.3 a A	0.2 a A	2.2 a A	1.7 a A
2	0.2 a A	0.3 a A	1.0 a A	3.6 a B	2.0 a A

DAT — days after treatment.
* There were no significant differences.
Means followed by the same small letter in each column and the same capital letter in each row are not significantly different at 5% level by DMRT.

Several official tolerance level ranging from 0.2 to 0.5 mg/kg have been established on grain, hay or forage of corn and rice. An evaluation of the observed total carbofuran residues found in the grains in this study indicated that carbofuran residues in the grains was below these tolerance levels (Table 3) even at rates as high as two kg a.i./ha.

The metabolic product, 3-OH carbofuran, started to increase at 65 DAT and accumulated mainly in the leaves, stems, and grains (Table 4). However, the residues of 3-OH carbofuran in the leaves, stems and grains at different dosages and at different sampling dates were not significantly different. This residue of 3-OH carbofuran on the plants may still be toxic to the insect pests and needs further investigation.

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