Degradation of Carbofuran in Rice Soils as Influenced by Repeated Applications and Exposure to Aerobic Conditions following Anaerobiosis

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The persistence of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) in flooded rice soils was studied after its repeated application. Repeated applications of carbofuran to flooded rice soils at rates close to field applications do not seem to favor rapid buildup of the microorganisms capable of decomposing carbofuran. An isotope study showed that degradation of carbofuran in flooded soils was more rapid under undisturbed conditions than under aerobic conditions provided by shaking. Under continued anaerobiosis of undisturbed flooded soils, the hydrolysis products, carbofuran phenol (2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran) in particular, accumulated; but when the undisturbed soil was returned to aerobic conditions, the hydrolysis products decreased rapidly.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) was reported to be the most effective insecticide in controlling the major rice pest, brown planthopper (*Nilaparvata lugens* Stal) when broadcast to the flooded rice fields as granules or when incorporated to the root zone in paper or gelatin capsules (IRRI, 1975). However, recent reports from the International Rice Research Institute (IRRI), Philippines show that carbofuran is no longer effective against rice brown planthoppers after 2 or 3 years of its continuous use in IRRI farms (IRRI, 1977). Interestingly, rice crop in carbofuran-treated plots developed more serious hopperburn, a damage characteristic of brown planthopper attack (PANS, 1970), than the plants in untreated plots (Siddaramappa et al., 1978).

According to an earlier report (Sethunathan and Pathak, 1972), the decreased efficiency of another insecticide, diazinon, in controlling brown planthoppers after 3.5 years of its use was attributed, in part, to the development of diazinon-degrading bacteria in rice fields upon its repeated applications. Microorganisms have been implicated in the degradation of carbofuran in flooded soils after an initial lag of about 20 days (Venkateswarlu et al., 1977). The present study was aimed to find out whether the decreased efficiency of carbofuran was in part due to the buildup of carbofuran-degrading microorganisms upon its repeated applications. Also, an attempt was made to determine the relative persistence of [^{14}C]carbofuran in flooded soils under static and intermittent shaking conditions.

MATERIALS AND METHODS

Degradation in Samples from Treated and Untreated Fields. a. *Paddy Water and Soil.* Degradation of carbofuran in soil and paddy-water samples from the carbofuran-treated fields was studied. The soil and water samples were collected from untreated and carbofuran-treated field plots planted with the rice variety Pankaj. Furadan granules containing 3% carbofuran were broadcast at 1 kg and 2 kg of active ingredient (AI)/ha every 15 days. The samples were collected 34 days after the fourth application of the insecticide.

Five milliliters of paddy water was directly incubated with 5 mL of 240 μg/mL of aqueous solution of technical carbofuran at room temperature. The soil samples were first air-dried and ground to pass a 2-mm sieve. Twenty-gram portions of soil contained in test tubes (25 × 200 mm) were treated with 10 mL of 106 μg/mL of aqueous carbofuran solution and 15 mL of distilled water. At desired intervals, carbofuran residues were assayed in two replicate samples.

b. *Rhizosphere and Nonrhizosphere.* The fields planted with the rice variety Jaya received Furadan granules at the rate of 1.5 kg of AI/ha at 20-day intervals starting from 10 days after transplanting. Rhizosphere and nonrhizosphere soils from both untreated and carbofuran-treated plots were collected 12 days after the fourth application of the insecticide. The large soil aggregates were removed by gentle tapping of the roots. The soil still adhering to the roots represented the rhizosphere sample and was brought to suspension by shaking the roots with 100 mL of sterile distilled water. Ten milliliters of this suspension was incubated with 4 mL of 260 μg/mL of aqueous carbofuran solution. Soil collected from between the hills served as the nonrhizosphere sample. Two replicates of each sample were taken for residue analysis at desired intervals. Also, bacterial population in rhizosphere and nonrhizosphere soil samples from untreated and carbofuran-treated rice fields was examined after conventional dilution and plating on soil extract–dextrose–agar medium as described earlier (Sethunathan and Pathak, 1972).

Degradation in Soils after Repeated Applications. Two soils, viz., alluvial, from the experimental farm, CRRI, and an acid sulfate saline soil from Kerala, locally known as *Pokkali*, were chosen for this study. Twenty grams of each soil was placed in test tubes (25 × 200 mm) and then flooded with 20 mL of distilled water. Soils were pretreated with 20 μg of an aqueous solution of carbofuran (close to field application rate) at 10-day intervals in such a way that soil samples received zero, one, two, and three additions on the 30th day after flooding. On the 40th day, all the soil samples were treated with 6 mL of an aqueous solution of carbofuran (180 μg/mL for alluvial soil and 260 μg/mL for Pokkali soil). Carbofuran residues were estimated in two replicates at desired intervals.

Degradation in Flooded Soils under Anaerobic and Aerobic Conditions. The relative persistence of carbofuran in a flooded alluvial soil was studied under static ("anaerobic") and shaking conditions. In a preliminary experiment, 6 mL of 220 μg/mL of aqueous carbofuran solution was added to 20 g of alluvial soil placed in 25 × 200 mm test tubes (for anaerobic conditions) or 250-mL Erlenmeyer flasks (for aerobic conditions). The soils were then flooded with 19 mL of distilled water. The soils in test tubes were left undisturbed and the soils in flasks were shaken in a Gallenkamp orbital shaker throughout the experiment (40 days). Carbofuran in two duplicate soil

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samples were extracted and analyzed at the end of 20 and 40 days.

In the subsequent experiment, [^{14}C]carbofuran was used for further confirmation and insight into its metabolic pathway in flooded soil under static (anaerobic), shaking (aerobic), and static followed by shaking conditions. Uniformly ring-labeled [^{14}C]carbofuran (sp act. 2.20 mCi/mmol, 100 μCi) was dissolved in 100 mL of acetone. After evaporating the acetone in 6 mL of stock solution, the residues were equilibrated in 100 mL of distilled water for 8 h. Five milliliters of this aqueous solution was added to 20 g of alluvial soil placed in test tubes or 250-mL Erlenmeyer flasks together with 3 mL of an aqueous solution of 240 μg/mL of nonlabeled technical carbofuran as carrier and 17 mL of distilled water to provide a flooded condition. The soil samples in test tubes were left undisturbed to simulate anaerobic conditions. The soils in the flasks were either shaken continuously for 40 days or left undisturbed for 20 days followed by a shaking period of 10 or 20 days. The residues in duplicate soil samples were analyzed at desired intervals.

Extraction and Residue Analysis. The residues from soils or water were extracted three times using chloroform–diethyl ether (1:1) as described earlier (Venkateswarlu et al., 1977) and the solvent fractions pooled. After evaporating the solvent, the residues were dissolved in methanol and then analyzed after separation by thin-layer chromatography (TLC).

The residues in methanol were spotted on chromatoplates coated with silica gel G, 300 μm thick, along with the standards. The plates were developed with ether–hexane (3:1) for a distance of 15 cm and air-dried. In studies using nonlabeled carbofuran, the insecticide in the samples was eluted from the chromatoplate and then converted to phenol by alkaline hydrolysis prior to colorimetric determination after diazotization as described earlier (Venkateswarlu et al., 1977). In studies using labeled carbofuran, the silica gel areas opposite to the authentic compounds, carbofuran, and its predicted metabolites, carbofuran phenol (2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran) and 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl *N*-methylcarbamate), were transferred to 5 mL of liquid scintillator NE 213 (Nuclear Enterprises Limited, Sighthill, Edinburgh, Scotland) which consisted of PPO (3 g), POPOP (0.3 g), and toluene (1000 mL), and the radioactivity was determined in Liquid Scintillation System Model LSS 20 (Electronics Corporation of India Ltd., Hyderabad). Total radioactivity in the methanol extract and water phase remaining after solvent extraction was counted after mixing aliquots of the respective fractions with 5 mL of liquid scintillator. With the complex extraction and analytical procedures used, the recovery of carbofuran immediately after its application ranged from 70 to 75% from the alluvial and *Pokkali* soils and from 83 to 95% from water samples. Experimental values were not corrected to account for the recovery loss.

RESULTS AND DISCUSSION

Degradation after Repeated Applications. Carbofuran was incubated with flood water, and soil samples were collected from untreated and carbofuran-treated rice fields. After an initial lag of about 20 days, degradation of carbofuran proceeded rapidly in water (Table I) and soil (Table II) samples from both untreated and carbofuran-treated rice fields. Interestingly, applications of carbofuran led to almost a fourfold increase over untreated controls in the bacterial population in the rhizosphere of rice plants grown in carbofuran-treated fields. Bacterial population increased from 25.6×10^{10}/g of soil in control rhizosphere to 108×10^{10}/g in carbofuran-treated rhizosphere soil. Despite this intense microbial activity, the rhizosphere soil samples from treated fields were not very effective in accelerating the degradation of carbofuran. Thus, no appreciable differences in the degradation rates occurred when carbofuran was incubated with the rhizosphere and nonrhizosphere soil samples of rice plants from untreated and carbofuran-treated fields (Table III).

In a laboratory study, carbofuran showed almost similar trends in persistence in Pokkali soil previously subjected to zero, one, two, and three preapplications of the insecticide (Table IV). But in alluvial soil, carbofuran, after 40 days of incubation, decreased to 49, 28, 33, and 22% of its original level with soil receiving zero, one, two, and three preapplications of the insecticide, respectively. Although carbofuran disappeared somewhat faster from the alluvial soil subjected to preapplications of the insecticide, the initial lag of about 20 days in its degradation was not appreciably reduced after successive applications of the insecticide to the soil. These results suggested that carbofuran-degrading microorganisms do not proliferate upon its repeated applications. This is expected if, as reported in many instances, microorganisms could attack carbofuran, not as an energy source, without proliferating by a process known as cometabolism. Degradation of carbofuran in soil or aquatic environments essentially

Table I. Degradation of Carbofuran in Paddy Water from Untreated and Carbofuran-Treated[b] Rice Soils

incubation, days	μg of carbofuran recovered/5 mL of water[a]	
	untreated	treated[b]
0	1170	1145
10	1130	1035
20	1085	830
30	435	385

[a] Carbofuran added to 5 mL of water, 1200 μg. [b] Carbofuran was applied at 2 kg of AI/ha.

Table II. Persistence of Carbofuran in Soils from Untreated and Carbofuran-Treated Rice Fields

incubation, days	μg of carbofuran recovered/20 g of soil[a]		
		treated	
	untreated	1 kg of AI/ha	2 kg of AI/ha
0	740	730	705
10	685	610	550
20	433	420	393
40	305	270	260

[a] Carbofuran added to 20 g of soil, 1060 μg.

Table III. Carbofuran Loss from Nonrhizosphere (NR) and Rhizosphere (R) Soil Samples of Untreated and Carbofuran-Treated[b] Rice Fields

incubation, days	μg of carbofuran recovered/10 mL of soil suspension[a]			
	untreated		carbofuran-treated[b]	
	NR	R	NR	R
0	835	830	955	935
10	500	485	530	560
20	380	325	410	515
30	345	215	395	280

[a] Carbofuran added to 10 mL of soil suspension, 1040 μg. [b] Carbofuran was applied at 1.5 kg of AI/ha.

Table IV. Degradation of Carbofuran in Flooded Soils after Its Repeated Applications

incubation, days[c]	μg of carbofuran recovered/20 g of soil			
	no applic.	one applic.	two applic.	three applic.
	Alluvial[a]			
0	715 ± 35	755 ± 5	775 ± 15	735 ± 5
10	655 ± 65	640 ± 55	705 ± 30	615 ± 55
20	530 ± 70	550 ± 30	540 ± 40	515 ± 15
40	350 ± 10	213 ± 27	253 ± 17	158 ± 2
	Pokkali[b]			
0	1070 ± 50	990 ± 70	1040 ± 20	1060 ± 0
20	740 ± 10	690 ± 10	675 ± 5	555 ± 5
30	475 ± 15	495 ± 15	455 ± 25	440 ± 10
40	260 ± 30	260 ± 0	250 ± 20	225 ± 15

[a] Carbofuran (1080 μg) was applied to alluvial soil pretreated with zero, one, two, and three applications of carbofuran. [b] Carbofuran (1560 μg) was applied to Pokkali soil pretreated with zero, one, two, and three applications of carbofuran. [c] Incubation (days) after receiving 1080 μg in alluvial and 1560 μg in Pokkali soil.

involved hydrolysis, perhaps a cometabolic reaction when microorganisms are involved. In fact, cometabolism has been implicated in hydrolytic reactions of several pesticides, as for example diazinon and parathion (Sethunathan et al., 1977), mediated by microorganisms. This would explain, at least in part, the failure of carbofuran-degrading microorganisms to proliferate in rice fields upon repeated applications of carbofuran. Thus, decreased effectiveness of carbofuran after intensive use in rice fields could not be attributed to the development of carbofuran-degrading microorganisms. It may be mentioned that buildup of bacteria, capable of hydrolyzing diazinon and then metabolizing its hydrolysis product as energy source, in rice fields following diazinon applications was an important factor in the detoxication and resulting decreased efficiency of diazinon against rice brown planthoppers (Sethunathan and Pathak, 1972).

According to recent reports from the International Rice Research Institute, Philippines, the rice crop in carbofuran-treated fields develops severe hopperburn fairly in advance of the crop in untreated fields. Several factors may govern the decreased effectiveness of carbofuran against brown planthoppers. Our studies reported in this paper and independent studies by Siddaramappa et al. (1978) show that the decreased effectiveness of carbofuran cannot be attributed to the development of carbofuran-degrading microorganisms. Elimination of the predators by carbofuran, a broad-spectrum insecticide, can lead to a buildup of insects. In fact, carbofuran has been shown to be highly toxic to spiders, an important predator of brown planthoppers (Chiu and Cheng, 1976), although direct correlation linking the elimination of spiders by carbofuran and buildup of brown planthoppers is lacking. Also, carbofuran may alter the physiology of the rice plant and/or the reproductory phases of the brown planthoppers favoring a buildup; but no direct evidence is available yet on the mechanism of resurgence of brown planthoppers in carbofuran-treated fields.

Degradation in Flooded Soils under Anaerobic and Aerobic Conditions. In the preliminary experiment, nonlabeled carbofuran was incubated with flooded soils under static ("anaerobic") and shaking (aerobic) conditions. In anaerobic soils, carbofuran concentration decreased from 945 μg at the start to 622 μg at the end of 20 days and then to 317 μg after 40 days. In aerobic soil, carbofuran decreased from 945 to 595 μg after 20 days and to 545 μg after 40 days. Evidently, aerobic conditions retarded the degradation of carbofuran.

In a subsequent experiment to confirm the above finding and obtain additional information on the metabolic products of carbofuran, [^{14}C]carbofuran was incubated with flooded soil under static ("anaerobic"), shaking, and alternate static and shaking conditions. Until 20 days, no appreciable difference was noticed in the levels of carbofuran under anaerobic and aerobic conditions (Table V). Analysis by TLC revealed that carbofuran phenol, the major metabolite of carbofuran and 3-hydroxycarbofuran accumulated in anaerobic soils but not in aerobic soils, presumably due to the inhibition of their oxidation in predominantly anaerobic conditions existing in static soils. Between 20 and 30 days, no appreciable degradation of carbofuran occurred in aerobic soils; but in anaerobic soils, the insecticide declined further with a concomitant increase in the accumulation of carbofuran phenol. The same trend continued until 40 days when carbofuran levels reached less than 30% of the amount recovered at the start in anaerobic soils as compared to more than 60% recovery in aerobic soils. Both carbofuran phenol and, to some extent, 3-hydroxycarbofuran were recovered in large quantities from anaerobic soils even at the end of 40 days, indicating their resistance to degradation under anaerobic conditions. When the flooded soils were shaken for 10 and 20 days after 20 days incubation under anaerobic conditions, no further degradation of carbofuran occurred. Moreover, carbofuran phenol and 3-hydroxycarbofuran that accumulated under 20 days of anaerobic conditions

Table V. Degradation of Carbofuran in Flooded Soils under Anaerobic and Aerobic Conditions

treatment	incubation, days	radioactivity recovered, cpm × 10^4/20 g of soil[a]				
		solvent phase	residues in solvent phase			water phase
			carbofuran	3-hydroxy-carbofuran	carbofuran phenol	
anaerobic	20	31.14	22.56	0.39	3.03	0.49
	30	25.00	15.65	0.18	4.97	0.81
	40	20.91	9.61	0.24	5.39	0.61
aerobic	20	25.12	23.15	0.08	0.58	0.44
	30	22.71	22.41	0.18	0.40	0.97
	40	22.30	20.73	0.08	0.39	0.57
anaerobic (20 days)[b] + aerobic (10 days)	30	20.91	20.80	0.04	0.41	0.41
anaerobic (20 days)[c] + aerobic (20 days)	40	21.69	20.44	0.08	0.43	0.59

[a] The radioactivity (cpm × 10^4/20 g of soil) recovered at the start of the experiment was 39.02 in solvent phase, 34.50 as carbofuran, 0.20 as 3-hydroxycarbofuran, 0.72 as carbofuran phenol, and 0.07 in the water phase remaining after solvent extraction. [b] Twenty-day anaerobic cycle followed by 10 days of aerobic cycle. [c] Twenty-day anaerobic cycle followed by 20 days of aerobic cycle.

declined to low levels following 10 days of aerobic conditions. The instability of carbofuran phenol under aerobic conditions was confirmed in a repeat experiment when carbofuran phenol (5.8×10^4 cpm/20 g of soil) formed from carbofuran at the end of 20-day anaerobic cycle declined to negligible levels (0.1×10^4 cpm/20 g of soil) after 20 days of subsequent incubation under aerobic conditions as compared to a value of 9.0×10^4 cpm/20 g of soil under 40 days of continued anaerobiosis. These studies indicated that carbofuran was more rapidly hydrolyzed under anaerobic conditions than under aerobic conditions; but its hydrolysis products, carbofuran phenol and 3-hydroxycarbofuran, which resisted further degradation under continued anaerobiosis, were rapidly transformed, perhaps to carbon dioxide when the anaerobic system was returned to aerobic conditions. Alternate anaerobic and aerobic conditions generated by intermittent flooding and drying cycles in rice fields may thus assist in more intensive, but less extensive, degradation of carbofuran than in either system alone.

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Degradation of Pentachloronitrobenzene (PCNB) in Anaerobic Soils

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Anaerobic degradation of ^{14}C-labeled pentachloronitrobenzene (PCNB) was examined in flooded and moist Hagerstown silty clay loam, with and without cellulose amendments. All treatments were aerated with nitrogen in a continuous flow-through system which permitted trapping of CO_2 and volatilized products. PCNB enhanced soil respiration in all treatments. Total CO_2 production was greater in moist than in flooded soils. Essentially no $^{14}CO_2$ was evolved from any treatments. PCNB volatilized from all treatments, but volatilization was reduced by cellulose amendments. Although extractable radioactivity was the same (70%) from all treatments at the conclusion of the 40-day incubation period, differences were observed in the relative distribution of PCNB and its degradation products. Product identification was by thin-layer and gas–liquid chromatographic comparison with authentic standards. Pentachloroaniline (PCA) was the principal degradation product. Pentachlorothioanisole (PCTA) was more abundant in moist (5.8–8.2%) than in flooded soil (0.3–0.5%). Pentachlorophenol was also detected as a degradation product. Further degradation of PCA and PCTA were examined in similar anaerobic soils. PCTA did not significantly alter soil respiration, whereas PCA provided slight inhibition in cellulose amended soil.

Pentachloronitrobenzene (PCNB) is used as a seed dressing and as a soil treatment to control several soilborne plant pathogens. Application rates have sometimes been as high as 200 lb/acre (Sharvelle, 1961; Thomson, 1967). Considerably lower rates of application are now more commonly used, however. Its degradation in soil (Caseley, 1968; Bauser and Bosshardt, 1975; Ko and Farley, 1969; Wang and Broadbent, 1972, 1973; De Vos et al., 1974; Beck and Hansen, 1974; Nakanishi, 1972; Nakanishi and Oku, 1969; Chacko et al., 1966; U.S. EPA, 1976) has been examined. Generally, PCNB is degraded in soil more rapidly under anaerobic or flooded conditions than in moist aerobic soil. The principal degradation products, pentachloroaniline (PCA) and pentachlorothioanisole (PCTA), have been identified in both soil and microbial systems.

The significance of PCNB residues in the environment is not fully understood. Gorback and Wagner (1967) investigated PCNB residues in potatoes grown in PCNB-treated soil and detected PCNB, PCA, and one unidentified metabolite in the potato peel. Both PCA and the unidentified metabolite, but not PCNB, were also found in the inner potato tissue. The unidentified metabolite was later described by Kuchar et al. (1969) as having an identical gas chromatographic retention time as PCTA. Kuchar et al. (1969) found PCNB, PCA, and PCTA, in addition to several PCNB impurities (pentachlorobenzene, hexachlorobenzene, and 2,3,4,5-tetra-

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