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Accelerated Degradation of Carbaryl and Carbofuran in a Flooded Soil Pretreated with Hydrolysis Products, 1-Naphthol and Carbofuran Phenol

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There is considerable evidence implicating microorganisms in the degradation of chemically unstable carbaryl (1-naphthyl *N*-methylcarbamate) (Bollag 1979) and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) (Venkateswarlu et al. 1977; Felsot et al. 1981) in soils. In flooded soil the degradation of carbaryl and carbofuran was accelerated only to a small extent after their successive applications (Siddaramappa et al. 1978; Venkateswarlu and Sethunathan 1978; Rajagopal et al. 1983); but suspensions from retreated soils showed an exceptional capacity to degrade carbaryl and carbofuran in a nitrogen-free mineral salts medium (Rajagopal et al. 1983, 1984). The degradation of carbaryl and carbofuran in soil and water ecosystems proceeded essentially by hydrolysis, possibly a cometabolic reaction, wherein microorganisms in the soil degrade the parent molecule without proliferation. For instance, repeated additions of *p*-nitrophenol, a hydrolysis product of parathion, to a flooded soil led to accelerated hydrolysis of parathion with a concomitant increase in the population of parathion-hydrolyzing microorganisms that utilized *p*-nitrophenol as the energy source for their proliferation (Sudhakar-Barik et al. 1979). An attempt was therefore made to determine whether repeated additions of 1-naphthol and carbofuran phenol, the respective hydrolysis products of carbaryl and carbofuran, to a flooded soil would enhance the degradation of parent molecules.

MATERIALS AND METHODS

Technical formulations of carbaryl (99.5% purity) and carbofuran (99.5% purity) were obtained from Union Carbide (India) Ltd., Bombay, India and FMC Corporation, Middleport, New York, respectively. Naphthyl-1-^{14}C-carbaryl (specific activity 5.8 mCi/mmol; 98% purity) was obtained from Radiochemical Centre, Amersham, England while U-phenyl-^{14}C-carbofuran (specific activity 39.4 mCi/mmol; 96% purity) was gifted by FMC Corporation. Technical 1-naphthol was obtained from E. Merck, Bombay, India while carbofuran phenol was obtained from FMC Corporation.

A laterite soil (pH 7.2; organic matter 0.61%; total nitrogen 0.04%) from Sukinda, Orissa, India was used in this study. Soil

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*Present address: Dept. of Microbiology, Univ. Iowa, Iowa City, IA 52242 samples (20 g) contained in test tubes (25x200 mm) were flooded with 25 ml of sterile distilled water. After 10 days of flooding, 1 ml of an aqueous solution of 100 µg/ml of Millipore-filtered 1-naphthol or carbofuran phenol was added to the soil at 15-day intervals in such a way that at 40 days after flooding the soil samples received one, two or three applications of 1-naphthol or carbofuran phenol. Ten days (50 days after flooding) after each of the three applications of 1-naphthol, the soil samples were treated with naphthyl-1-^{14}C-carbaryl (25x 10^4 dpm/20 g soil) in 0.1 ml of acetone. ^{14}C-Carbaryl was also added to the soil not previously treated with 1-naphthol. Likewise, the soil samples after ten days of each of the three applications of carbofuran phenol were treated with U-phenyl-^{14}C-carbofuran (30x10^4 dpm/20 g soil). ^{14}C-Carbofuran was added also to the soil samples not previously treated with carbofuran phenol. At periodical intervals after ^{14}C-carbaryl or ^{14}C-carbofuran addition, duplicate samples were removed for residue analysis after extraction and separation by thin-layer chromatography.

Residues of ^{14}C-carbaryl or ^{14}C-carbofuran and their metabolites from soil were extracted thrice with chloroform-diethyl ether (1:1) and the residues were redissolved in 2 ml of methanol after evaporating the chloroform-diethyl ether solvent fraction at room temperature (Rajagopal and Sethunathan 1984). For quantitative determination of parent compounds and their metabolites, the residues dissolved in methanol were separated on chromatoplates coated with silica gel-G (300 µm thick) using diethyl ether-hexane (3:4) as the developing solvent (Rajagopal and Sethunathan 1984). The silica gel areas of the samples corresponding to authentic compounds were scraped into 5 ml of scintillation solution (naphthalene, 60 g; PPO, 4 g; POPOP, 0.2 g; methanol, 100 ml; ethylene glycol, 20 ml; and p-dioxane to make up to 1 litre) for assay of radioactivity. An aliquot of the aqueous phase (from soil) after the third extraction was added to 5 ml of liquid scintillator. The radioactivity was determined in a Liquid Scintillation Counter Model LSS-20 (Electronics Corporation of India Ltd., Hyderabad, India) and corrections were made for background activity. The initial recovery of ^{14}C-carbaryl and ^{14}C-carbofuran, by this procedure, from soil ranged from 82-87%. Variations within duplicate samples never exceeded 5%.

$^{14}CO_2$ evolved from ^{14}C-carbaryl or ^{14}C-carbofuran applied to the soil was trapped in 2 ml of 1 N KOH contained in small vials hung from rubber stoppers used for closing the test tubes. The alkali trap was assayed for $^{14}CO_2$ and other volatiles (parent compound or its metabolites) (Rajagopal and Sethunathan 1984). Unextractable (soil-bound) residues in the soil were estimated by combustion to $^{14}CO_2$ as described earlier (Rajagopal and Sethunathan 1984).

RESULTS AND DISCUSSION

Carbaryl disappeared more rapidly from the soil pretreated with 1-naphthol than from the control soil never exposed to 1-naphthol (Table 1). The accumulation of 1-naphthol and bound residues formed from added ^{14}C-carbaryl was greater in soils pretreated with 1-naphthol than in untreated soil concomitant with faster degradation of parent molecule in pretreated soil.

Similarly, carbofuran degradation was more rapid and significant in soils pretreated with carbofuran phenol than in untreated soil (Table 2). Concomitantly, soils pretreated with carbofuran phenol accumulated greater amounts of carbofuran phenol and bound residues from ^{14}C-carbofuran than untreated soil.

Although carbaryl and carbofuran declined fairly rapidly, the decrease in the total radioactivity recovered from the soil was small due to the insignificant formation of the end product, $^{14}CO_2$ from the ^{14}C in carbaryl and carbofuran, even after prolonged incubation. Most of the ^{14}C in carbaryl and carbofuran applied was accounted for as 1-naphthol + soil-bound residues and carbofuran phenol + soil-bound residues. No metabolite other than 1-naphthol or carbofuran phenol was detected in the radioautographs.

The data presented in this study demonstrate that hydrolysis of carbaryl and carbofuran in an ecosystem can be accelerated following the application of their respective hydrolysis products to the ecosystem. This is analogous to the accelerated degradation of parathion in a flooded soil upon repeated application of its hydrolysis product, p-nitrophenol (Sundhakar-Barik et al. 1979). But 1-naphthol and carbofuran phenol, accumulated in the soil in large amounts even after prolonged incubation (Tables 1 and 2). Evidently, these hydrolysis products, unlike p-nitrophenol, were not fully utilized as energy sources by microorganisms in the soil. Yet, the application of these hydrolysis products accelerated the degradation of carbaryl and carbofuran in the soil. The mechanism involved in this accelerated degradation of carbaryl and carbofuran in the soil after repeated application of their hydrolysis products is not clear and this could be an exciting area for further follow-up.

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Table 1. Degradation of ^{14}C-carbaryl in flooded laterite soil as influenced by repeated applications of its hydrolysis product, 1-naphthol

Days after ^{14}C-carbaryl application	No. of 1-Naphthol application[2]	% of applied radioactivity recovered/20 g soil[1]							
		CO_2	Other volatiles	Aqueous phase	Methanol extract	Carbaryl	1-Naphthol	Soil-bound	Total recovery
0	0	0	0	0.4^a	84^a	80^a	0.7^a	2.5^a	87
	1	0	0	0.5^a	85^a	81^a	0.8^a	1.8^a	86
	2	0	0	0.5^a	84^a	80^a	1.0^a	2.2^a	87
	3	0	0	0.3^a	85^a	82^a	0.9^a	1.6^a	87
20	0	0.11^a	0.03^a	2.5^a	59^a	41^a	15^a	12^a	74
	1	0.12^a	0.03^a	2.7^a	62^a	38^a	21^a	14^a	79
	2	0.15^a	0.04^a	3.5^a	58^a	29^b	25^b	17^a	79
	3	0.18^a	0.04^a	4.3^a	55^a	20^c	32^c	20^a	80

[1] (Naphthyl-1-^{14}C) carbaryl was added at 25 x 10^4 dpm/20 g of soil. For each sampling date means followed by common notations are not significantly different at 5% level.

[2] 1-Naphthol (100 μg) was applied at 15 day intervals. ^{14}C-Carbaryl was applied at 10 days after the last application of 1-naphthol (at 50 days after flooding for all treatments).

Table 2. Degradation of ^{14}C-carbofuran in flooded laterite soil as influenced by repeated applications of its hydrolysis product, carbofuran phenol

Days after ^{14}C-carbofuran application	No. of carbofuran phenol applications[2]	% of applied radioactivity recovered/20 g soil[1]							
		CO_2	Other volatiles	Aqueous phase	Methanol extract	Carbofuran	Carbofuran phenol	Soil-bound	Total recovery
0	0	0	0	0.4^a	82^a	79^a	0.8^a	1.2^a	84
	1	0	0	0.5^a	81^a	78^a	1.0^a	0.9^a	82
	2	0	0	0.5^a	83^a	80^a	0.6^a	0.7^a	84
	3	0	0	0.4^a	82^a	80^a	0.7^a	0.9^a	83
20	0	0.08^a	0.03^a	4.0^a	55^a	40^a	12^a	15^a	74
	1	0.09^a	0.05^a	3.8^a	53^a	36^a	15^a	18^a	77
	2	0.08^a	0.04^a	4.5^a	50^a	25^b	21^b	20^a	75
	3	0.10^a	0.04^a	5.1^a	49^a	19^c	26^c	22^a	76

[1] (U-phenyl-^{14}C) carbofuran was added at 30 x 10^4 dpm/20 g soil. For each sampling date means followed by common notations are not significantly different at 5% level.

[2] Carbofuran phenol (100 µg) was applied at 15 day intervals. ^{14}C-Carbofuran was applied at 10 days after the last application of carbofuran phenol (at 50 days after flooding for all treatments).

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