Development and Acclimatization of Carbofuran-Degrading Soil Enrichment Cultures at Different Temperatures

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Soil enrichment cultures were prepared by six applications of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) at 10-day intervals to a flooded alluvial soil that had been incubated at 6, 28, and 35 °C. Suspensions from carbofuran-amended soil incubated at 28 and 35 °C effected more rapid degradation of carbofuran than did the respective unamended soil suspension in a mineral salts medium incubated at 28 and 35 °C. Enrichment culture developed at soil temperature of 35 °C was particularly effective in degrading carbofuran to low levels within 5 days of incubation with concomitant evolution of $^{14}CO_2$ from the [*ring*-^{14}C]carbofuran. Evidently, repeat applications of carbofuran to the soil led to enrichment of carbofuran-degrading microorganisms at soil temperatures of 28 and 35 °C, and not at 6 °C, and this enrichment was more pronounced at 35 than at 28 °C.

Information available in the literature on the environmental fate of pesticides has been generated mostly from studies in the temperate environment, because use of pesticides in agriculture and public health has been more extensive in temperate countries than in the tropics and subtropics. But, a steady increase in the use of pesticides, insecticides in particular, in the tropics in recent years has prompted studies on the fate and significance of pesticide residues in the tropical environment. According to available evidence, pesticides that persist in the cooler conditions of temperate countries need not necessarily persist in the hot and humid conditions of the tropics and subtropics (Talekar et al., 1977). One of the major factors responsible for such a difference in pesticide persistence between temperate and tropical conditions is the temperature.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) is used widely for controlling certain major pests of many economically important crops including brown planthopper (*Nilaparvata lugens* Ståhl) in rice and root worm (*Diabrotica longicornis* Say) in corn. More recently, an alarming problem of decreased efficacy of carbofuran has developed in corn fields of the United States and Canada (Fox, 1983). This problem, accentuated by repeat applications of the same pesticide to the same field year after year, is attributed to the buildup of soil microbes that undermine the efficacy of the pesticide (Felsot et al., 1981; Kaufman and Edwards, 1983; Read, 1983, 1986). There are also reports of decreased efficacy of carbofuran against brown planthopper in tropical rice fields of The Philippines (IRRI, 1977). But, no definite link could be established between decreased efficacy of carbofuran against brown planthopper and buildup of carbofuran-degrading microbes in tropical rice fields (Siddaramappa et al., 1978; Venkateswarlu and Sethunathan, 1978). This study is concerned with the effect of temperature on the development of carbofuran-degrading enrichment culture in a flooded soil after repeated applications of carbofuran. Also, the cross adaptation of soil enrichment cultures to different temperatures was studied.

MATERIALS AND METHODS

Chemicals. Nonlabeled (analytical grade, 99.4% purity) and ^{14}C-labeled (labeled uniformly around the benzene ring; sp act. 39.4 mCi/mmol; 98% purity) carbofuran and its hydrolysis product, carbofuran phenol (99.4% purity), were gifts of FMC Corp., Middleport, NY. Purity of [^{14}C]carbofuran was confirmed by thin-layer chromatography before use. All reagents used were scintillation grade.

Soil Enrichment Culture. An alluvial soil (Haplaquept, pH 6.2, organic matter 1.61%, total nitrogen 0.088%) from the experimental farm of Central Rice Research Institute, Cuttack, was used in the study. The soil was air-dried and passed through a 2-mm sieve before use. Soil samples (20 g) were placed in test tubes (200 × 25 mm) and then flooded with 25 mL of sterile distilled water. The soil sample in each test tube was treated with 1 mg of a technical formulation of carbofuran (77.5% purity from FMC Corp., Middleport, NY) and incubated in a BOD incubator at 6 ± 1, 28 ± 2, and 35 ± 1 °C. Every 10 days 1 mg of carbofuran was applied to each test tube for enrichment of carbofuran-degrading microorganisms. Three or four days after the sixth addition, the contents of each test tube, incubated at the three temperatures, were stirred with a glass rod and this suspension served as the soil enrichment culture in all experiments. Simultaneously incubated soil samples at the three temperatures, but without carbofuran, served as the source for suspension from unamended soil.

Mineral Salts Medium. The nitrogen-free mineral salts medium, used in incubation studies with soil enrichment cultures, was the same as used in our earlier studies (Rajagopal et al., 1984b) with the following composition: $MgSO_4 \cdot 7H_2O$, 0.2 g; K_2HPO_4, 0.1 g; $FeSO_4 \cdot 7H_2O$, 0.001 g; $CaSO_4$, 0.04 g; distilled water, 1 L; pH, 6.2.

Degradation Studies. The ability of soil enrichment cultures to degrade carbofuran was studied by inoculating the mineral salts medium containing carbofuran as sole source of carbon and nitrogen with enrichment culture as follows: A 1-mL sample of a solution of 400 μg/mL of nonlabeled analytical-grade carbofuran in acetone or 0.4 mL (2.2×10^5 dpm) of a solution of [^{14}C]carbofuran in acetone was added aseptically to presterilized 100-mL Erlenmeyer flasks. After 24 h to allow the evaporation of acetone at room temperature, 20-mL portions of sterile mineral salts medium were dispensed into the flasks. After equilibration for 24 h, the medium was inoculated with 0.4 mL of soil enrichment culture (suspension from carbofuran-amended soil) developed at soil temperatures of 6, 28, and 35 °C and then incubated at respective temperatures. Another set of the same medium was inoculated with 0.4 mL of the suspension from the unamended soil

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0021-8561/88/1436-0200$01.50/0

that had been incubated at 6, 28, and 35 °C and then incubated at respective temperatures. Uninoculated mineral salts medium served as control. Also, carbofuran enrichment cultures developed at the soil temperature of 6 °C was tested for its ability to degrade carbofuran in the mineral salts medium at 28 and 35 °C. Likewise, cross adaptation of soil enrichment cultures developed at 28 and 35 °C was similarly tested. Nonlabeled carbofuran was used in experiments with soil enrichment cultures developed at soil temperature of 6 and 28 °C while in experiment with enrichment culture developed at 35 °C [^{14}C]-carbofuran was used. At periodic intervals, duplicate samples of each treatment were removed for residue extraction and analysis.

To determine whether very rapid degradation of carbofuran by soil enrichment culture developed at 35 °C is due to microorganisms, this enrichment culture was sterilized by autoclaving at 121 °C for 20 min. The mineral salts medium (20-mL portions) contained in 100-mL Erlenmeyer flasks was supplemented with nonlabeled carbofuran at 20 μg/mL of the medium as described earlier and then inoculated with 0.4 mL of sterilized or nonsterilized soil enrichment culture. Uninoculated medium served as control. After 5-day incubation at 35 °C, the carbofuran remaining in uninoculated and inoculated medium was extracted and then analyzed by colorimetry after separation by thin-layer chromatography.

Extraction. Residues in the medium (20 mL) from each flask were extracted three times with 30-mL portions of chloroform–diethyl ether (1:1) as described earlier (Rajagopal et al., 1984a,b). The extracts pooled from the three extractions were evaporated to dryness at room temperature and the residues redissolved in 1 mL of methanol.

Thin-Layer Chromatography. Silica gel G plates (300 μm) were prepared from 1:2.5 silica gel G–distilled water slurry and then activated at 105 °C for 1 h. The methanol-dissolved residues (200 μL) were spotted on silica gel G plates along standards of carbofuran and carbofuran phenol, and the plates were developed with diethyl ether–hexane (3:4) for a distance of 15 cm and air-dried. The standards were located by spraying successively with 2 N NaOH in absolute methanol and a solution of *p*-nitrobenzene diazonium fluoroborate (5 mg dissolved in 25 mL of methanol and 25 mL of diethyl ether) (Archer, 1976). Silica gel areas coinciding with the standards were scraped into 7.5-mL test tubes for colorimetric analysis of nonlabeled carbofuran or into 5 mL of scintillation cocktail contained in 20-mL low-background scintillation vials to assay the radioactivity in experiments with [^{14}C]carbofuran.

Colorimetry. For colorimetric analysis of nonlabeled carbofuran, the residues in silica gel contained in test tubes were treated with 1.25 mL of 0.3% sodium nitrite solution, 1.25 mL of 0.2% sulfanilic acid in 1 N HCl, and 2.5 mL of 4 N NaOH in a hot water bath (40–50 °C) for 20 min. Silica gel was removed by centrifugation (5000 rpm for 30 min), and the supernatant was made up to 10 mL prior to colorimetric analysis at 490 nm (Venkateswarlu et al., 1977). The same procedure, but without NaOH treatment, was used for estimation of carbofuran phenol. By this method, the detectable limit for carbofuran was 1 μg and recovery of carbofuran from the mineral salts medium fortified with 20 μg/mL was 85–90%.

Assay of Radioactivity. The radioactivity was assayed by scraping the silica gel areas corresponding to carbofuran and carbofuran phenol into 5 mL of scintillation cocktail (naphthalene, 60 g; PPO, 4 g; POPOP, 0.2 g; methanol, 100 mL; ethylene glycol, 20 mL; *p*-dioxane to make to L). The radioactivity was assayed in a liquid scintillation counter (Model LSS-20, Electronics Corp. of India Ltd., Hyderabad, India) with an efficiency of 70%. All counts were corrected for background and quenching, if any. Recovery of added radioactivity at 2.2×10^5 dpm of carbofuran was 85–90%, and variations within duplicate estimations never exceeded 5%.

Autoradiography. The thin-layer chromatograms of the solvent extracts of the mineral salts medium containing [^{14}C]carbofuran and its degradation products were exposed to Kodak X-ray No screen film for 20 days in a Siemens metal cassette, and the film was developed with X-ray developer.

Assay of $^{14}CO_2$. Whether the rapid loss of the ring-^{14}C in carbofuran from the mineral salts medium inoculated with soil enrichment culture developed at 35 °C was due to its mineralization to $^{14}CO_2$ was examined in another study. The sterile mineral salts medium (20-mL portions) was supplemented with [^{14}C]carbofuran (2.6×10^5 dpm/flask) in presterilized 100-mL Erlenmeyer flasks and then inoculated with 0.4 mL of soil enrichment culture developed at 35 °C. Uninoculated medium and medium inoculated with the suspension from unamended soil that had been incubated at 35 °C served as controls. Each flask was closed with a rubber bung provided with an inlet and an outlet. Both inlet and outlet were closed with a pinchcock. The air-tight assembly was incubated at 35 ± 1 °C in a BOD incubator. At 5 days after incubation, the inlet was connected to an air generator through a trap containing 2 N KOH (25 mL) to remove $^{14}CO_2$, if any, in the air, and $^{14}CO_2$ evolved, if any, from [^{14}C]carbofuran in each of duplicate flasks was purged into 25 mL of 2 N KOH solution contained in 150-mL flask for 5 min. KOH solution containing $^{14}CO_2$ was shaken with hexane to remove carbofuran or its metabolites, volatilized if any. Analysis of hexane extract showed negligible radioactivity. KOH solution remaining after hexane extraction was acidified with 50 mL of 2 N HCl, and the resulting $^{14}CO_2$ was absorbed in 5 mL of Hyamine Hydroxide contained in a 20-mL scintillation vial for 5 min. Hyamine Hydroxide with dissolved $^{14}CO_2$ was mixed with 5 mL of scintillation cocktail, and radioactivity was assayed by liquid scintillation.

RESULTS AND DISCUSSION

Carbofuran-Degrading Ability of Soil Enrichment Culture Developed at 6 °C. Carbofuran was applied six times to a flooded soil incubated at 6 °C at 10-day intervals. The resulting enrichment culture (soil suspension) was tested for its ability to degrade nonlabeled carbofuran in a mineral salts medium at 6, 28, and 35 °C. During 10-day incubation at 6 °C, no appreciable degradation of carbofuran occurred in the medium inoculated with soil suspension from both carbofuran-amended and unamended soil and in uninoculated medium (Table I). This is expected since microbial activity is generally low at 6 °C. But, carbofuran was not degraded by the enrichment culture even at 28 °C, which permits significant microbial activity. At 35 °C, carbofuran disappeared rapidly and reached 10–15% of the original level in 40 days from the medium inoculated with suspension from carbofuran-amended and unamended soil as well as from uninoculated medium. Substantial loss of carbofuran also from uninoculated medium at 35 °C was presumably due to its volatilization according to a later isotope study. Evidently, repeated applications of carbofuran did not lead to enrichment of carbofuran-degrading microbial population at soil temperature of 6 °C.

Table I. Degradation of Carbofuran in a Mineral Salts Medium Incubated at 6, 28, and 35 °C after Inoculation with Suspension from Unamended and Carbofuran-Amended Soils Previously Incubated at 6 °C[d]

incubn, days	6 °C						28 °C						35 °C					
	control[a]		unamended[b]		amended[c]		control[a]		unamended[b]		amended[c]		control[a]		unamended[b]		amended[c]	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	18.0	nd	18.5	nd	18.0	nd	17.5	nd	17.0	nd	18.5	nd	18.5	nd	18.0	nd	18.5	nd
10	16.0	nd	16.0	nd	16.0	nd	17.0	nd	17.0	nd	17.0	nd	15.0	nd	14.5	nd	14.0	nd
20	15.5	nd	14.0	nd	13.5	nd	17.0	nd	16.5	nd	15.5	nd	9.0	3.0	9.0	3.5	8.0	3.0
40	15.0	nd	14.0	nd	13.5	nd	16.0	nd	15.0	nd	11.0	2.0	3.0	2.5	2.0	2.0	nd	2.5

[a] Mineral salts medium supplemented with 20 μg of nonlabeled carbofuran/mL and not inoculated with soil suspension. [b] Mineral salts medium inoculated with suspension from flooded soil not amended with nonlabeled carbofuran. [c] Mineral salts medium inoculated with suspension from flooded soil treated six times with nonlabeled carbofuran. [d] Key: A, micrograms of carbofuran/milliliter recovered; B, micrograms of carbofuran phenol/milliliter recovered; nd, not detected. Statistical details:temperature (X), incubation (Y), treatment (Z). LSD values at 5% for XY, YZ, XZ, and XYZ are 1.4, 1.4, 1.2, and 2.4 respectively. Interactions between XZ and XYZ are not statistically significant.

Table II. Degradation of Carbofuran in a Mineral Salts Medium Incubated at 28 and 35 °C after Inoculation with Suspension from Unamended and Carbofuran-Amended Soils Previously Incubated at 28 °C[d]

incubn, days	28 °C						35 °C					
	control[a]		unamended[b]		amended[c]		control[a]		unamended[b]		amended[c]	
	A	B	A	B	A	B	A	B	A	B	A	B
0	17.5	nd	17.5	nd	18.0	nd	17.5	nd	18.0	nd	18.5	nd
10	17.0	nd	17.0	nd	16.0	nd	15.0	nd	12.0	3.0	11.0	3.5
20	17.0	nd	14.0	nd	12.5	3.0	9.0	3.0	10.0	2.0	8.5	3.0
40	16.0	nd	13.0	nd	7.0	4.0	3.5	2.5	3.0	2.0	nd	2.5

[a] Mineral salts medium supplemented with 20 μg of nonlabeled carbofuran/mL and not inoculated with soil suspension. [b] Mineral salts medium inoculated with suspension from flooded soil not amended with nonlabeled carbofuran. [c] Mineral salts medium inoculated with suspension from flooded soil treated six times with nonlabeled carbofuran. [d] Key: A, micrograms of carbofuran/milliliter recovered; B, micrograms of carbofuran phenol/milliliter recovered; nd, not detected. Statistical details: temperature (X), incubation (Y), treatment (Z). LSD values at 5% for XY, YZ, XZ, and XYZ are 1.5, 1.9, 1.3, and 2.6, respectively. Interactions between XZ and XYZ are not statistically significant.

Carbofuran-Degrading Ability of Soil Enrichment Culture Developed at 28 °C. Enrichment culture (suspension from carbofuran-amended soil) developed at soil temperature of 28 °C was tested for its ability to degrade nonlabeled carbofuran in the mineral salts medium at 28 and 35 °C. During 40-day incubation at 28 °C, carbofuran was degraded more rapidly in the medium inoculated with suspension from carbofuran-amended soil than in the medium inoculated with suspension from unamended soil (Table II); during the same period, degradation in uninoculated medium was negligible. This showed that repeated applications of carbofuran to the flooded soil incubated at 28 °C led to a significant enrichment of carbofuran-degrading principle. Concomitant with rapid degradation of carbofuran by soil enrichment culture, carbofuran phenol accumulated in the medium. At 35 °C, as in the previous experiment, carbofuran was rapidly lost from the medium inoculated with suspension from carbofuran-amended soil or unamended soil and from uninoculated medium. This rapid loss, possibly due to volatilization of carbofuran at higher temperature, masked the carbofuran-degrading capacity of the enrichment culture when the medium inoculated with suspension from carbofuran-amended soil was incubated at 35 °C.

Carbofuran-Degrading Ability of Soil Enrichment Culture Developed at 35 °C. Enrichment culture developed at a soil temperature of 35 °C was tested for its ability to degrade [^{14}C]carbofuran in the mineral salts medium at 28 and 35 °C. At 28 °C, the concentration of carbofuran decreased rapidly only in the medium inoculated with suspension from carbofuran-amended soil, but not in the medium inoculated with suspension from unamended soil and in uninoculated medium (Table III). At 28 °C, the amount of carbofuran, recovered from the medium inoculated with soil enrichment culture, reached 26% of the original level in 10 days and undetectable level in 40 days as compared to a recovery of 58.4% from the medium inoculated with suspension from unamended soil and 68% from uninoculated medium at 40 days. Particularly noteworthy was the exceptional capacity of the soil enrichment culture (developed at soil temperature of 35 °C) to degrade carbofuran at 35 °C (Table IV). Even within 5 days of incubation at 35 °C, 90% of the originally recovered carbofuran disappeared from the medium inoculated with suspension from carbofuran-amended soil; during the same period, only 13.4% and 19.1% of carbofuran were lost from uninoculated medium and from the medium inoculated with suspension from unamended soil, respectively. These data demonstrated distinct enrichment of carbofuran-degrading principle in the soil incubated at 35 °C upon repeated applications of carbofuran as noticed earlier with soil incubated at 28 °C. But, enrichment culture developed at 35 °C (Table III and IV) showed a greater potential in degrading carbofuran than the enrichment culture developed at 28 °C (Table II), possibly due to the selective stimulation of carbofuran-degrading microorganisms at higher temperature. In the mineral salts medium incubated at 28 °C, enrichment culture developed at 35 °C (Table III) degraded 74% of carbofuran in 10 days as compared to a loss of 20% of the original level with enrichment culture developed at 28 °C (Table II). Carbofuran is chemically unstable and undergoes rapid hydrolysis under alkaline conditions (Seiber et al., 1978; Rajagopal et al., 1984c). But, the pH of the mineral salts medium during 40-day incubation was near neutral (6.81–7.06). Moreover, enrichment culture developed at 35 °C lost its ability to degrade carbofuran when it was sterilized by autoclaving and then added to the mineral salts medium (Table V). During 5-day incubation at 35 °C, carbofuran declined to less than 20% of the original level in mineral salts medium inoculated with nonsterilized enrichment culture. During the same period the degra-

Table III. Degradation of [^{14}C]Carbofuran in a Mineral Salts Medium Incubated at 28 °C after Inoculation with Suspensions from Unamended and Carbofuran-Amended Soils Previously Incubated at 35 °C

		% of radioactivity recovered[a]/20 mL of medium					
incubn, days	treatment	aq fraction	methanol extr	carbofuran[b]	carbofuran phenol[b]	unidentified metabolite[b]	total rec
0	uninoculated	0.8	89.0	87.0	0.5	0.0	89.8
	unamended	0.9	84.0	81.0	1.5	0.0	84.9
	amended	0.9	85.0	82.0	1.4	0.0	85.9
10	uninoculated	1.4	89.0	84.0	1.9	2.0	90.4
	unamended	1.7	88.0	75.0	5.8	3.0	89.7
	amended	9.1	43.0	26.0	12.5	3.9	52.1
20	uninoculated	2.4	84.0	77.0	3.9	2.3	86.4
	unamended	5.4	81.0	67.3	2.7	3.0	86.4
	amended	10.3	27.3	2.8	18.2	3.0	37.6
40	uninoculated	2.8	83.0	68.0	2.2	5.5	85.8
	unamended	6.4	70.3	58.4	0.4	0.5	76.7
	amended	14.0	14.2	1.7	0.6	0.9	28.2
LSD							
5%		1.8	9.9	7.0	1.6	0.14	
1% (interaction)		2.6	13.8	9.8	2.2	0.19	

[a] [^{14}C]Carbofuran was added at 2.2 × 10^5 dpm/20 mL of medium. [b] After separation of the residues in methanol extract by thin-layer chromatography.

Table IV. Degradation of [^{14}C]Carbofuran in a Mineral Salts Medium Incubated at 35 °C after Inoculation with Suspension from Unamended and Carbofuran-Amended Soils Previously Incubated at 35 °C

		% of radioactivity recovered[a]/20 mL of medium					
incubn, days	treatment	aq fraction	methanol extr	carbofuran[b]	carbofuran phenol[b]	unidentified metabolite[b]	total rec
0	uninoculated	0.9	85.2	79.0	1.3	0.8	86.1
	unamended	1.2	83.0	80.0	1.1	0.6	84.2
	amended	1.0	85.0	80.4	1.8	0.5	86.0
5	uninoculated	1.2	80.7	68.5	1.8	0.8	81.9
	unamdended	1.5	70.5	64.7	2.2	0.4	72.0
	amended	1.5	56.0	7.6	23.9	1.9	57.5
10	uninoculated	1.4	68.9	55.5	1.0	0.5	70.3
	unamended	1.7	64.5	54.2	3.2	0.8	66.2
	amended	1.6	29.2	2.6	9.0	1.3	30.8
20	uninoculated	1.5	56.6	45.6	4.5	1.5	58.1
	unamended	2.0	53.9	42.2	2.6	1.2	55.9
	amended	5.4	20.2	2.0	6.7	1.3	25.6
40	uninoculated	1.5	6.9	4.5	0.2	0.0	7.4
	unamended	5.8	6.1	5.6	0.2	0.0	11.9
	amended	8.9	4.4	0.7	0.3	0.0	13.3
LSD							
5%		0.1	6.0	5.0	0.5	0.06	
1% (interaction)		0.2	8.2	6.9	0.7	0.08	

[a] [^{14}C]Carbofuran was added at 2.2 × 10^5 dpm/20 mL of medium. [b] After separation of the residues in methanol extract by thin-layer chromatography.

dation of carbofuran was negligible in uninoculated medium and in medium inoculated with sterilized enrichment culture. Evidently, rapid degradation in the inoculated medium is mediated by microorganisms.

Rapid loss of carbofuran from the mineral salts medium incubated at 35 °C merits attention. Autoradiograph of the extract of the mineral salts medium at 40 days showed that carbofuran disappeared almost completely from the uninoculated medium incubated at 35 °C; but no degradation product including carbofuran phenol was detected in the autoradiograph. After 40 days, in the uninoculated medium only 13–14% of the added ^{14}C in carbofuran was not accounted for at 6 and 28 °C while more than 92% of the ^{14}C was not accounted for at 35 °C. This would suggest that a rise in temperature from 28 to 35 °C increased the loss of carbofuran by volatilization. But, during the first 5 days of incubation, volatilization loss from the uninoculated medium was not considerable at 35 °C while during the same period carbofuran reached low levels in medium inoculated with enrichment culture at 35 °C (Table IV). This rapid loss of carbofuran from the inoculated medium, but not from the uninoculated medium, during the first 5 days of incubation at 35 °C was, therefore, not due to its volatilization as an intact molecule, but due to its rapid degradation. Autoradiograph at 5 days showed the formation of carbofuran phenol (R_f 0.86) in large amounts and ketocarbofuran (R_f 0.24) as a minor metabolite in the medium inoculated with enrichment culture at 35 °C. In

Table V. Degradation of Carbofuran by Soil Enrichment Culture (Sterilized vs Nonsterilized) Developed at 35 °C in a Mineral Salts Medium[a] at Incubation Temperature of 35 °C

			inoculated			
	uninoculated		sterilized		nonsterilized	
incubn, days	A	B	A	B	A	B
0	18.0	nd	18.5	nd	18.0	nd
5	17.0	nd	16.5	nd	3.5	3.0
LSD						
5%	2.8					
1% (interaction)	4.3					

[a] The mineral salts medium was supplemented with 20 μg of nonlabeled carbofuran/mL. Key: A, micrograms of carbofuran/milliliter recovered; B, micrograms of carbofuran phenol/milliliter recovered; nd, not detected.

Table VI. Degradation of [^{14}C]Carbofuran in a Mineral Salts Medium Incubated at 35 °C after Inoculation with Suspension from Unamended and Carbofuran-Amended Soils Previously Incubated at 35 °C

		% of radioactivity recovered[a]/20 mL of medium						
incubn, days	treatment	aq fraction	CO_2	other volatiles	methanol extr	carbofuran[b]	carbofuran[b] phenol	total rec
0	uninoculated	0.8			86.4	82.1	0.5	87.2
	unamended	1.5			84.8	81.3	0.8	86.3
	amended	1.1			86.0	83.0	0.9	87.1
5	uninoculated	0.9	0.04	0.25	81.6	78.3	1.1	82.8
	unamended	1.4	0.25	0.40	79.7	74.4	1.8	81.8
	amended	1.8	59.80	0.40	9.6	4.8	1.9	71.6
LSD								
5%		0.53			4.7	6.1	0.35	
1% (interaction)		0.80			7.1	9.3	0.52	

[a] [^{14}C]Carbofuran was added at 2.6×10^5 dpm/20 mL of medium. [b] After separation of the residues in methanol extract by thin-layer chromatography.

a follow-up study, 59.8% of the ring-^{14}C in added carbofuran was released as $^{14}CO_2$ in 5 days when the mineral salts medium inoculated with soil enrichment culture developed at 35 °C was incubated at 35 °C (Table VI); during the same period, evolution of $^{14}CO_2$ was negligible in uninoculated medium and in medium inoculated with suspension from unamended soil. These data indicate that substantial loss of ^{14}C from the uninoculated medium during 40-day incubation at 35 °C is due to volatilization of intact carbofuran molecule, and the rapid loss of ^{14}C from the medium inoculated with soil enrichment culture developed at 35 °C is due to its accelerated mineralization to $^{14}CO_2$.

According to an earlier report, degradation of carbofuran and its mineralization to the end product CO_2 proceeded more rapidly at 35 °C than at 15 and 27 °C (Ou et al., 1982). Our studies showed that soil incubation at 35 °C during repeat additions of carbofuran triggered the development of a very active enrichment culture capable of mineralizing carbofuran with greater ease than at 28 °C. Microorganisms have been implicated in the degradation of carbofuran in flooded soil (Rajagopal et al., 1984a–c), and possibly, 35 °C is more favorable than 28 and 6 °C for the proliferation and activity of carbofuran-degrading microorganisms.

CONCLUSIONS

According to earlier studies in our laboratory (Rajagopal et al., 1984a,b), repeat additions of carbofuran to a flooded soil at room temperature (25–30 °C) have led to the enrichment of a microbial community capable of degrading this insecticide as a sole source of carbon and nitrogen in a mineral salts medium. However, these enrichment cultures degraded carbofuran at a slow rate, only 43–53% of the added carbofuran being degraded in 20 days. None of these enrichment cultures mineralized carbofuran past carbofuran phenol to CO_2. Likewise, the rate of degradation of carbofuran in enrichment cultures developed at soil temperature of 28 °C in this study was slow. Interestingly, soil incubation at 35 °C during repeat applications of carbofuran yielded an enrichment culture with an exceptional capacity to degrade 85–90% of the added carbofuran in 5 days. Moreover, this enrichment culture developed at 35 °C readily mineralized carbofuran to CO_2. By far, this is the most active carbofuran-degrading enrichment culture developed in our laboratory. Results obtained in this study indicate that intensive use of the same pesticide may lead to more rapid buildup of very active pesticide-degrading enrichment population under hot–humid conditions of the tropical environment than under temperate environment.

ACKNOWLEDGMENT

We thank H. K. Pande (Director) for permission to publish this work; Lacy Daniels, Department of Microbiology, University of Iowa, for the gift of Hyamine Hydroxide; FMC Corp., Middleport, NY, for the gift of carbofuran (nonlabeled and ^{14}C-labeled) and carbofuran phenol; and the Council of Scientific and Industrial Research, New Delhi, for the award of research fellowship to S.P.

Registry No. Carbofuran, 1563-66-2.

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Received for review October 27, 1986. Revised manuscript received August 14, 1987. Accepted September 21, 1987. This research was financed, in part, by the Department of Environment and Forests, Government of India, New Delhi (Contract No. 33(2)/84-EN.I).

Photochemistry of Pesticides. 8.[1] Photodegradation of 2,4,5-Trihaloimidazoles in the Presence of Singlet Oxygen

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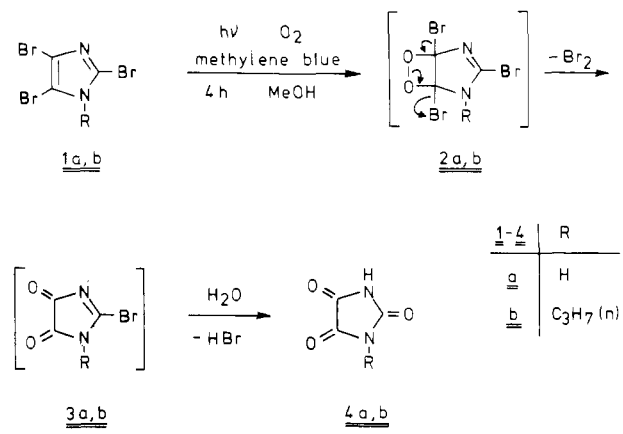
Sensitized photooxidation of 2,4,5-tribromoimidazoles **1a,b** leads in a rapid degradation reaction to parabanic acid derivatives **4a,b**. Similarly, 2,4,5-tribromo-1-(4-chlorobenzoyl)imidazole (**1c**) affords parabanic acid (**4a**). However, photolysis of 2,4,5-trichloroimidazoles **7a–d** results in a ring cleavage to give dimethyl oxalate (**8**), ammonium chloride, and alkylureas **11a,b**.

In recent years, there has been an increased interest in the environmental photochemistry and photodegradation of heterocyclic herbicides, pesticides, and insecticides (Gunther and Gunther, 1971; Kearney and Kaufman, 1975, 1976; Meallier and Coste, 1981; Ruzo, 1983; Chen, 1985; Moorman et al., 1985; Mukerjee, 1985). Following our current studies on the photochemistry of pesticides (Mahran et al., 1983; Abdou et al., 1985, 1986; Wamhoff et al., 1985), we have become interested in the photochemical behavior and decomposition of 2,4,5-tribromoimidazole (**1a**), 2,4,5-trichloroimidazole (**7a**) (Lutz and De Lorenzo, 1967, 1969; Wade and Landram, 1968; Wasco, 1969; Draber et al., 1970; Martin and Pissiotas, 1971; Pissiotas, 1971, 1972; Steimig and Fischer, 1971; Steimig and Adolphi, 1973; Stensio et al., 1973; Büchel and Erdmann, 1976; Schulze and Klein, 1977, and their N-substituted derivatives **1b,c** and **7b–d** (Boots Pure Drug Co., Ltd., 1967; Rutz and Gubler, 1968; Martin and Pissiotas, 1970; Drabek and Pissiotas, 1972; Büchel, 1976; Takahashi and Ando, 1978) under various conditions.

Compounds **1a** and **7a** as well as the related N-substituted derivatives **1b,c** and **7b–d** have been recognized as potent biocides with special application as herbicides, pesticides, bactericides, miticides, and insecticides (cf. aforementioned literature). However, up to now no investigations have been carried out on the photolysis of **1a–c** and **7a–d**. It has been shown that the parent compound imidazole is capable of slow photooxidation in methanol and in the presence of singlet oxygen to give dimethoxyhydantoin (Wasserman et al., 1968; Wasserman and Lipshutz, 1979), whereas 4,5-diphenylimidazole gives a mixture of 4,5-dimethoxy-4,5-diphenylhydantoin and 5-methoxy-4,5-diphenylhydantoin (Wasserman et al., 1968). In addition, it has been reported that sensitized or direct photooxidation of lophin (2,4,5-triphenylimidazole) gives dibenzoylbenzamidine (Dufraisse et al., 1957, 1964). Other substituted imidazoles, such as 2-methyl- or 1,2-dimethylimidazoles (Matsuura and Ikari, 1969), histidine (Ochiai et al., 1968), 4-phenyl- and tetraphenylimidazole (Wasserman et al., 1968; Wasserman and Lipshutz, 1979) have been studied as well.

Scheme I



RESULTS AND DISCUSSION

In our investigation, we have found that singlet oxygen photolysis of a 1% solution of 2,4,5-tribromoimidazole (**1a**) in methanol and in a Pyrex vessel using methylene blue as sensitizer affords parabanic acid (**4a**) as the only reaction product in 80–85% yield after a considerably short irradiation time (4 h). The identity of **4a** was established by comparison with an authentic specimen (Biltz and Schiemann, 1926). Br_2 and HBr were also identified as decomposition products during the irradiation. Similarly, upon photosensitized oxidation of 1-propyltribromoimidazole (**1b**) propyl parabanate (**4b**) was obtained in ca. 80% yield and its constitution confirmed (Baerlicher and Ebert, 1972). **4a,b** were also isolated and identified when the irradation was carried out in dry toluene or chloroform; furthermore, photolysis of **1a** with UV light as well as in the presence of visible light, without sensitizer, and with continuous circulation of dry air, leads after 35 h to parabanic acid (**4a**). No reaction was, however, observed in a comparative experiment without exposure to UV irradiation. This indicates that both oxygen and light are

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[1] Part 7: Abdou, W. M.; Born, L.; Hulpke, H.; Mahran, M. R.; Sidky, M. M.; Wamhoff, H. *Phosphorus Sulfur* **1987**, *29*, 179.

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0021-8561/88/1436-0205$01.50/0