species, but their activity decreased markedly at lower concentrations (100 and 10 ppm). Compounds IVb, C, and g exhibited antifungal activity of the order of Dithane M-45 (a commercial fungicide) at 1000 ppm and inhibited 45–52% growth of both the test fungi even at 10 ppm. However, compound IIIa was far less active than its successor IVa.

Perusal of the screening data (Table III) clearly indicates that there was significant alteration in the antifungal activity with the change in the relative positions of the substituents on phenyl ring. For example, compounds IV bearing the *o*-fluoro group were more active than the corresponding IV with the *p*-fluoro group. Similarly, the 2-chloro group was more effective than the 4-chloro group.

ACKNOWLEDGMENT

We are thankful to the Head, Department of Chemistry, for providing laboratory facilities and to Dr. V. J. Ram for recording the spectra. A.R.M. expresses his sincere thanks to the UGC, New Delhi, for the award of a Teacher Research Fellowship under FIP Program.

Registry No. Ie, 113056-45-4; IIa, 397-60-4; IIb, 449-81-0; IIIa, 113056-46-5; IVa, 113056-47-6; IVb, 113056-48-7; IVc, 113056-49-8; IVd, 113086-35-4; IVe, 113056-50-1; IVf, 113056-51-2; IVg, 113056-52-3; IVh, 113056-53-4; IVi, 113056-54-5; IVj, 113086-36-5.

LITERATURE CITED

Balse, M. N.; Mahajanshetti, C. S. "Condensed Tetrahydrobenzothiazoles: Part II. Synthesis of 2-Aryl-5,6,7,8-tetrahydro-*s*-triazolo[3,2-*b*]benzothiazoles & 3-Aryl-5,6,7,8-tetrahydro-*s*-triazolo[3,4-*b*]benzothiazoles". *Indian J. Chem.* **1980**, *19B*, 260–262.

Bucchel, K. H.; Draber, W. Ger. Offen. 1 940 628, 1971; *Chem. Abstr.* **1971**, *74*, 125698t.

Filler, R.; Kobayashi, Y. *Biomedicinal Aspects of Fluorine Chemistry*; Kodansha & Elsevier Biomedical: Amsterdam, 1983.

Greenfield, S. A.; Seldel, M. C.; Von Meyer, W. C. Ger. Offen. 1 943 915, 1970; *Chem. Abstr.* **1970**, *72*, 100713q.

Hoggarth, E. "Compounds Related to Thiosemicarbazide: Part II. 1-Benzoylthiosemicarbazides". *J. Chem. Soc.* **1949**, 1163–1167.

Horsfall, J. G. "Quantitative Bioassay of Fungicides in the Laboratory". *Bot. Rev.* **1945**, *11*, 357–397.

Okano, S.; Yasujaga, K. Jpn. Pat. 70 17 191, 1970; *Chem. Abstr.* **1970**, *73*, 77251x.

Reisser, F. Ger. Offen. 1 918 795, 1969; *Chem. Abstr.* **1970**, *72*, 43692f.

Vogel, A. I. *A Text-Book of Practical Organic Chemistry Including Qualitative Organic analysis*, 3rd ed.; Longmans: London, 1956; pp 907–910.

Received for review May 22, 1987. Accepted November 30, 1987.

Deltamethrin Residues in an Organic Soil under Laboratory Conditions and Its Degradation by a Bacterial Strain[1]

Shahamat U. Khan,* Ram M. Behki, Richard I. Tapping, and M. Humayoun Akhtar

An organic soil was treated with the insecticide deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylate] labeled with ^{14}C at the methyl position at a level of 10 mg/kg for a laboratory incubation study. At the end of a 40-month incubation period the extractable and nonextractable (bound) ^{14}C residues amounted to 19.5% and 16.3%, respectively, of the initially added ^{14}C. The bound residues were characterized as the parent compound and its metabolite [3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid]. The microbial population including bacteria, actinomycetes, and fungi decreased considerably from the initial numbers. A bacterial species capable of utilizing deltamethrin as a sole source of carbon was isolated by enrichment from the incubated soil.

In recent years, the synthetic pyrethroid deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylate] has become of great interest for use in crop protection because it is very effective in controlling a wide range of insects in agriculture at very low application rates (FAO, 1981). The increased use of this chemical for application to vegetable and field crops requires periodic assessment of its residues behavior and fate in soil. Recent studies have indicated a half-life of deltamethrin in mineral soils in the range of 1–8 weeks (Chapman et al., 1981; Miyamoto and Mikami, 1983; Hill, 1983), and the degradation appears to be mainly mediated by soil microorganisms (Kaufman et al., 1981; Chapman et al., 1981). In organic soils, the degradation of deltamethrin was found to be slower under the anaerobic than aerobic conditions (Zhang et al., 1984). A half-life of deltamethrin of 72 days was observed under aerobic conditions in an organic soil (Zhang et al., 1984).

It has been suggested that pyrethroids or their metabolites will not persist for lengthy periods in soil (Miyamoto and Mikami, 1983). Buildup of bound (nonextractable) residues has been observed to take place in the short term in soil (Roberts, 1981), but bound residues are considered to be degraded further (Roberts, 1981; Miyamoto and Mikami, 1983). Previously we reported a steady increase in the formation of bound ^{14}C residues over a 6-month incubation period of an organic soil treated with radiolabeled deltamethrin (Zhang et al., 1984). It was also observed that the bacteria and actinomycetes number increased by a factor of approximately 4, whereas the fungal population remained relatively unaffected (Zhang et al., 1984). The present work extends these investigations to determine the fate of deltamethrin in the organic soil in

Land Resource Research Centre (S.U.K., R.M.B., R.I.T.) and Animal Research Centre (M.H.A.), Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

[1] This study was part of a coordinated program of research under the sponsorship of the International Atomic Energy Agency, Vienna, Austria. LRRC Contribution No. 87-24. ARC Contribution No. 1461.

0021-8561/88/1436-0636$01.50/0 Published 1988 by the American Chemical Society

laboratory incubation experiments after more than 3 years. The study also describes the isolation of bacteria from the incubated soil that utilized deltamethrin as a sole source of carbon for growth and degraded the insecticide to 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (Br_2CA).

MATERIALS AND METHODS

Chemicals. Deltamethrin ([^{14}C]methyl labeled) was a gift from Roussel-Uclaf-Procida through its subsidiary Hoechst of Canada Ltd. The radiochemical purity and specific activity of deltamethrin were 98% and 56 mCi/mmol, respectively. A portion of the radiolabeled material was mixed with unlabeled deltamethrin and the resultant mixture dissolved in acetone to give a concentration of 484 μg/mL.

Soil Incubation Experiment. An organic soil (500 g, air dry) described earlier (Zhang et al., 1984) was incubated with labeled (10.3 μCi) and unlabeled deltamethrin at an insecticide concentration of 10 mg/kg. The moisture content of the soil was initially adjusted to 70% of field capacity and maintained at this level by adding distilled water as necessary. The incubation was carried out in a soil flask (Loss et al., 1980) for a period of about 40 months under the experimental conditions described earlier (Zhang et al., 1984).

Determination of Residues. At the end of a 40-month incubation period of microbial population, extractable and nonextractable (bound) residues in soil samples were determined as described in an earlier publication (Zhang et al., 1984).

Determination of Radioactivity. Combustion of dried soil was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. The latter was absorbed in and admixed with appropriate volume of Carbo-Sorb and Permafluor-V. Aliquots of various solutions or extraction were analyzed by liquid scintillation counting.

Chromatography and Analysis. Column chromatography, thin-layer chromatography (TLC), and gas chromatographic (GC) analyses were carried out as described earlier (Zhang et al., 1984).

Enrichment Cultures and Bacterial Isolation. Soil sample (2.0 g) after the incubation period described above was placed in an Erlenmeyer flask to which 0.5 mL of acetone containing 300 μg of deltamethrin was added. The solvent was allowed to evaporate, and the soil was thoroughly mixed. Ten milliliters of sterile basal minimal salts nitrogen medium (BMN) containing 0.07% cycloheximide (Behki and Khan, 1986) was added, and the suspension was incubated with continuous shaking at 29 °C for 10 days. Three successive dilutions (0.5 mL into 10 mL) of the suspension into BMN–cycloheximide–deltamethrin were made every 10 days. Finally 0.1 mL of the aliquot was spread onto BMN–deltamethrin–agar plates. After incubation at 29 °C for 1 week five fast-growing single colonies on the plates were picked up and purified by streaking on BMN–deltamethrin–agar plates. Five purified uniform single colonies were obtained after four passages on agar plates containing deltamethrin. Each of the isolate could grow on deltamethrin as the sole source of carbon and energy as tested in liquid BMN–deltamethrin medium.

The shape and morphology of the bacterial isolates were determined by phase-contrast microscopy. Further characterization, including the determination of the constituents of the cell wall, were carried out as suggested by Keddie and co-workers (1977, 1981).

Biodegradation and Utilization of Deltamethrin by the Cells. Purified cells were suspended in BMN medium containing deltamethrin (30 μg/mL) and glucose (500 μg/mL). The cultures were incubated with continuous shaking for 2 weeks at 28 °C.

Analysis of Deltamethrin and Its Metabolites. An aliquot of the medium (1.0 mL) was extracted with benzene (3.0 mL × 3). The extract was then analyzed as described earlier.

Table I. Extractable and Nonextractable (Bound) ^{14}C Residues in an Aerobically Incubated Organic Soil Treated with [^{14}C]Deltamethrin.

	% of applied ^{14}C	
incubn time, months	extractable	bound
3[a]	72.5	11.0
6[a]	59.0	19.2
40	19.5	16.3

[a] Zhang et al. (1984).

Table II. Extractable ^{14}C Residues in an Aerobically Incubated Organic Soil Treated with [^{14}C]Deltamethrin

	% of applied ^{14}C		
incubn time, months	deltamethrin	Br_2CA	deltamethrin/Br_2CA
3[a]	32.9	29.6	1.1
6[a]	21.4	17.4	1.2
40	8.9	6.0	1.5

[a] Zhang et al. (1984).

RESULTS AND DISCUSSION

The total radioactivity recovered in the soil at the end of the 40-month incubation period amounted to about 35.8% of that initially applied. The disappearance of $^{14}CO_2$ released during the incubation period was small (2.6% ^{14}C of the initially added), thereby indicating very little mineralization. It appears that the loss of most of the radiocarbon occurred due to the evolution of some volatile degradation products during the incubation. By the end of the incubation period it was observed that 54.0% and 44.6% of ^{14}C of the total ^{14}C recovered were present as extractable and bound residues, respectively. The latter corresponded to 16.3% of the ^{14}C added at the start of the incubation experiment (Table I). In an earlier study it was shown that the amount of bound ^{14}C residues formed at the end of about 6 months of incubation was 19.2% of the initially added ^{14}C (Zhang et al., 1984). These observations demonstrate that only a small decrease in the amount of bound ^{14}C residues formed in the methyl-labeled deltamethrin treated soil occurred over a further period of incubation of 34 months. However, during the same period the extractable ^{14}C residues decreased from 59.0% to 19.6% of the initially added ^{14}C.

Analysis of the extracted ^{14}C material by a combination of preparative and analytical TLC and finally by GC revealed the presence of deltamethrin and BR_2CA in measurable amounts (Table II). Thus, by the end of 40 months of incubation, the extractable ^{14}C constituted about 8.9% and 6.0% of the initially added ^{14}C in the form of deltamethrin and Br_2CA, respectively. The band on silica gel TLC plates corresponding to R_f 0.3–0.7 (viewed under UV light) was removed free from the glass support, extracted with methanol, derivatized with freshly prepared diazomethane, and finally analyzed by GC. A small peak at a retention time of 1.8 min corresponding to the methyl ester of 3-phenoxybenzoic acid (PBacid) appeared, but the amount was too small to be quantitated. Some other radiolabeled products that appeared on TLC plates could not be identified because of their low concentrations. Proportions of extractable ^{14}C present in the form of

Table III. Microbial Population in an Aerobically Incubated Organic Soil Treated with [^{14}C]Deltamethrin

incubn time, months	fungi (×10), numbers/g	bacteria and actionomycetes (×10^8), colonies/g
0[a]	6.1	1.8
3[a]	6.4	5.3
6[a]	6.6	7.8
40	0.6	0.2

[a] Zhang et al. (1984).

deltamethrin and Br_2CA at different time intervals are shown in Table II. It is apparent that the parent compound deltamethrin was more persistent than the metabolite Br_2CA under the experimental conditions described. It should be noted that about 10–20% of the extractable radioactivity after different incubation periods could not be identified by the experimental techniques used in this study (Table II).

The identification of the bound ^{14}C residues was carried out by the high-temperature distillation technique (HTD) using acetone, methanol, and Carbosorb as the trapping solutions (Zhang et al., 1984). Amounts of ^{14}C trapped in these solutions were 21.0%, 3.8%, and 6.5% ($^{14}CO_2$) of the total bound ^{14}C whereas about 50.3% ^{14}C was present in the burned solid material left after HTD as described earlier (Zhang et al., 1984). The ^{14}C material in acetone and methanol was subjected to various cleanup procedures and analyzed by TLC and GC to determine the identities of the ^{14}C compounds as described earlier (Zhang et al., 1984). We were able to recover after extensive cleanup about 72.0% of the total ^{14}C material present in acetone and methanol traps for final identification. It was observed that deltamethrin and Br_2CA were present as the main compounds constituting 1.1% ^{14}C and 0.06% ^{14}C, respectively, of the initially added radiolabeled deltamethrin in the soil. A trace amount of PB acid was also suspected to be present, but its identity could not be positively confirmed because of the low amounts. It should be noted that a substantial amount of bound ^{14}C remained unidentified under the experimental conditions used in this study.

The changes in the microbial population of soil treated with deltamethrin are shown in Table III. It was observed earlier that numbers of bacteria and actinomycetes increased steadily over the first 6-month period in the treated soil whereas no appreciable change occurred in the untreated soil (Zhang et al., 1984). However, the number was considerably reduced in the treated soil at the end of a 40-month incubation period (Table III) and increased in the control soil (9×10^8 colonies/g). Although the fungal population remained relatively unaffected during the first 6-month incubation period, it decreased almost 10 times in the treated and 7 times (0.9×10^4 numbers/g) in the control soil at the end of 40 months (Table III).

All of the five bacterial isolates growing on BMN–deltamethrin–agar plates showed similar characteristics and appeared to belong to the same genus. They were gram-positive nonmotile, pleomorphic long rods. On a stationary phase the cells were coccoid and reverted to rods when subcultured in fresh nutrient broth. The cell wall sugars were predominantly arabinose and galactose. The cell wall preparations also contained diaminopomelic acid as a major constituent. The isolates were tentatively assigned to the *Rhodococcus* group.

The isolates utilized deltamethrin as a sole source of carbon and energy. Disappearance of deltamethrin after 1 and 2 weeks of incubation is shown in Table IV. In the absence of bacterial isolates, nearly quantitative recoveries

Table IV. Deltamethrin Degradation by Bacterial Cultures in a BMN Medium

	% deltamethrin metabolized			% deltamethrin metabolized	
culture	1 week	2 weeks	culture	1 week	2 weeks
1	35.7	59.7	4	41.3	64.8
2	43.2	64.2	5	44.4	72.5
3	41.0	64.1			

(90–97%) of deltamethrin were obtained. The main product formed after incubation was Br_2CA.

Our studies demonstrate a long-term persistence of deltamethrin and its metabolites in an organic soil. The results show that about 16% of the initially applied radioactivity was present in the soil in the bound (nonextractable) form even after more than 3 years of incubation, and this bound radioactivity still contains the parent pesticide as well as its metabolites. Obviously, analysis of the extractable residues alone cannot be considered as a valid criterion to determine the long-term persistence of deltamethrin in soils. While it is generally assumed that soil microorganisms play a major role in the degradation of pyrethroids, we have isolated a bacterial species from a soil capable of utilizing deltamethrin as a sole source of carbon. Further research is under way to determine the intracellular localizations of genes responsible for this degradation.

ACKNOWLEDGMENT

The capable assistance of Iris Young and W. R. McDowell is greatly appreciated.

Registry No. Deltamethrin, 52918-63-5; 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 53179-78-5.

LITERATURE CITED

Behki, R. M.; Khan, S. U. "Degradation of Atrazine by *Pseudomonas*: N-Dealkylation and Dehalogenation of Atrazine and Its Metabolites". *J. Agric. Food Chem.* **1986**, *34*, 746.

Chapman, R. A.; Tu, C. M.; Harris, C. R.; Cole, C. "Persistence of Five Pyrethroid Insecticides in Sterile and Natural, Mineral and Organic Soil". *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 513.

FAO (Food and Agricultural Organization) *FAO Plant Prod. Prot. Suppl.* **1981**, *26*, 122.

Hill, B. D. "Persistence of Deltamethrin in a Lethbridge Sandy Clay Loam". *J. Environ. Sci. Health, Part B* **1983**, *B18*, 691.

Kaufman, D. D.; Russell, B. A.; Helling, C. S.; Kayser, A. J. "Movement of Cypermethrin, Decamethrin, Permethrin, and Their Degradation Products in Soil". *J. Agric. Food Chem.* **1981**, *29*, 239.

Keddie, R. M.; Cure, G. L. "The Cell Wall Composition and Distribution of Free Mycolic Acids in Named Strains of Coryneform Bacteria and in Isolates from Various Natural Sources". *J. Appl. Bacteriol.* **1977**, *42*, 229.

Keddie, R. M.; Jones, D. In *The Prokaryotes, a Handbook on Habitats, Isolation and Identification of Bacteria*; Starr, M. P., Stolp, H., Truper, H. G., Balous, A., Schelegal, H. G., Eds.; Springer-Verlag: Berlin, 1981.

Loss, M. A.; Komstson, A.; Kearney, P. C. "Inexpensive Soil Flasks for ^{14}C-Pesticide Degradation Studies". *Soil Biol. Biochem.* **1980**, *12*, 583.

Miyamoto, J. M.; Mikami, N. "Degradation of Pyrethroid Insecticides in the Field". *Int. Congr. Pestic. Chem., 5th* **1983**, *2*, 193.

Roberts, T. R. In *Progress in Pesticide Biochemistry*; Wiley: Chicester, U.K., 1981; p 115.

Zhang, L. Z.; Khan, S. U.; Akhtar, M. H.; Ivarson, K. C. "Persistence, Degradation, and Distribution of Deltamethrin in an Organic Soil under Laboratory Conditions". *J. Agric. Food Chem.* **1984**, *32*, 1207.

Received for review March 4, 1987. Accepted October 15, 1987.