more than fish; results that are the reverse of findings by Metcalf and Sanborn (1975). Thus, this investigation has shown that significant differences exist between the nine fractions of toxaphene tested and that future environmental research should probably be directed toward the two most toxic fractions (5 and 6).

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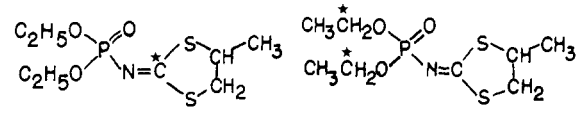
Photodegradation of Cytrolane (Mephosfolan) Systemic Insecticide in the Aquatic Environment Using Carbon-13 as a Mass Tracer

Chia C. Ku, Inder P. Kapoor, Steven J. Stout, and Joseph D. Rosen*

Photodegradation of mephosfolan (Cytrolane, a registered trademark of the American Cyanamid Co.) was studied by exposing approximately equimolar mixtures of either $^{12}C/^{13}C$-imido-labeled or $^{12}C/^{13}C$-ethyl-labeled material to sunlight. Use of the $^{12}C/^{13}C$ mixtures facilitated identification of photoproducts by gas chromatography chemical ionization mass spectroscopy (GC–CIMS) because molecular and fragment ions in the mass spectra appear as doublets. Solvent artifacts and volatile rice paddy water constituents gave mass spectra containing only singlet ions and were ignored. Irradiations were conducted in distilled water, "natural" water obtained from a flooded rice paddy, and a 2% acetone-water solution. The half-lives for mephosfolan were 18, 14, and 7 days, respectively, and indicated that photosensitization by paddy water constituents and acetone was occurring. The major products identified were cyclic *S,S*-propylene dithiocarbonate, 2-imino-5-methyl-1,3-dithiolane, and diethyl phosphate. Minor products were ethyl phosphate, phosphoric acid, and diethyl methyl phosphate.

Cytrolane systemic insecticide [mephosfolan; (diethoxyphosphinyl)dithioimidocarbonic acid, cyclic propylene ester] is used for pest control on cotton, corn, rice, sorghum, and sugar cane, especially in the Middle East and Asia. As part of the study on the environmental chemistry of mephosfolan (Ku et al., 1978), we examined its behavior upon exposure to sunlight. This study was conducted by using approximately equimolar mixtures of either $^{12}C/^{13}C$-imido-labeled (Ia) or $^{12}C/^{13}C$-ethyl-labeled material (Ib), where the asterisks denote ^{13}C. Use of the $^{12}C/^{13}C$ mixtures facilitated identification of photoproducts by GC–CIMS because molecular and fragment ions in the mass spectra appeared as doublets. Solvent artifacts and volatile rice paddy water constituents gave mass spectra containing only singlet ions and were ignored.



Ia

Ib

EXPERIMENTAL SECTION

Synthesis of [^{13}C]Mephosfolan. (*1*) *Imido Carbon Labeled (IL).* One gram of potassium [^{13}C]cyanide (90% enriched, Merck), 738 mg of sublimed sulfur, and 15 mL of 1,2-dimethoxyethane were refluxed for 1 h and then stirred overnight at room temperature. Diethyl chlorophosphate (2.65 g) was added and the mixture was stirred at room temperature for 1 h. Next, 1.66 g of 1,2-propylenedithiol, 2.3 g of potassium bicarbonate, 75 mL of 1,2-dichloroethane, and 15 mL of water were added. The two-phase reaction mixture was refluxed for 3 h and stirred overnight at room temperature. The organic phase was separated and the aqueous phase was extracted twice with 150 mL of 1,2-dichloroethane. The combined organic phases were back-washed with 30 mL of 5% potassium bicarbonate. After separation, the organic phase was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure. The crude product, a yellow oil, was obtained in 59% yield. The oil was dissolved in

Agricultural Division, American Cyanamid Co., Princeton, New Jersey 08540 (C.C.K., I.P.K., S.J.S.) and the Department of Food Science, Cook College, Rutgers University, New Brunswick, New Jersey 08903 (J.D.R.).

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a minimum of acetone and applied to a 1.5 × 15 in. Florisil F 101 (Fisher Scientific) column, 100/200 mesh, which had been equilibrated with 10% acetone–methylene chloride. The column was eluted first with 300 mL of methylene chloride–acetone (98:2) and then 100 mL of a 95:5 mixture. The column was further eluted with a 90:10 mixture of methylene chloride–acetone. After approximately 500 mL, mephosfolan began eluting. The column effluent was monitored by TLC on silica gel plates using ethyl acetate, as the developing solvent (R_f 0.6). All the fractions containing mephosfolan were pooled and evaporated under reduced pressure to a volume of about 3 mL. The concentrated solution was streaked on 1-mm preparative TLC plates and eluted with ethyl acetate using programmed multiple development (Perry et al., 1975). The pure material gave one spot in two different TLC systems (ethyl acetate and 30% acetone–methylene chloride) and one GLC peak. The proton NMR (60 MHz) and mass spectra of this material were consistent with authentic mephosfolan.

(2) *Ethyl Carbon Labeled (EL).* Equimolar amounts of 2-imino-5-methyl-1,3-dithiolane hydrochloride and phosphorus oxychloride were refluxed approximately 3 h in toluene until the theoretical amount of hydrogen chloride was eliminated and most of the solids disappeared. The mixture was filtered and concentrated to give a brown oil. The structure of this material was shown by infrared and nuclear magnetic resonance spectroscopy to be 2-(dichlorophosphinylimino)-5-methyl-1,3-dithiolane (Addor, 1964). To an ice-cooled mixture of 5 g (0.021 mol) of the brown oil in 20 mL of benzene was added a mixture of 2.5 g (0.054 mol) of ethanol-*1*-^{13}C (Merck) and 4.7 g (0.046 mol) of triethylamine in 5 mL of benzene over a period of 15 min. The solids were filtered off and washed with benzene. The filtrate was evaporated and a yellowish oil, the crude product, was obtained in 71% yield. This material was purified according to the procedure described for imido-labeled compound. The compound was 99+% pure by TLC in solvent systems described earlier.

Syntheses of Suspected Photodecomposition Products. (*a*) *Cyclic S,S-Propylene Dithiocarbonate (II).* 2-Imino-5-methyl-1,3-dithiolane hydrochloride (2 g) was dissolved in 100 mL of water and refluxed overnight. Extraction with methylene chloride followed by drying with anhydrous sodium sulfate and evaporation of solvent afforded a pale-yellow oil (1.2 g) whose structure was confirmed by IR, NMR, and GC–CIMS.

(*b*) *5-Methyl-2-methylimino-1,3-dithiolane.* 2-Imino-5-methyl-1,3-dithiolane hydrochloride (0.1 g) was dissolved in 0.5 mL of methanol. To this solution, 2 mL of ethereal diazomethane was added. The reaction mixture was stirred at room temperature for 30 min before GC–CIMS analysis.

(*c*) *Diethyl Methyl Phosphate.* Sodium bicarbonate (0.1 g) was slurried in 3 mL of methanol in a 5-mL Reacti-vial. Approximately 1 mL of diethyl chlorophosphate was added into the vial slowly. The reaction was kept at room temperature for 30 min before GC analysis.

(*d*) *Dimethyl Ethyl Phosphate.* Sodium bicarbonate (0.2 g) was slurried in 3 mL of methanol in a 5-mL Reacti-vial. Approximately 1 mL of dichloroethyl phosphate was added slowly into the vial with stirring. The reaction was stirred at room temperature for 30 min before GC analysis.

Irradiation and Work-up Procedure. A solution containing 100 ppm mephosfolan (mixture of $^{12}C/^{13}C$ label at the imido carbon with a ratio of approximately 1:1) was prepared in three types of water, i.e., rice paddy water, distilled water, and 2% acetone–water. Each solution was charged into both a 1000-mL Vycor flask (test) and a 500-mL Pyrex flask (control). The control flask was stoppered and wrapped with aluminum foil to keep out the light.

Another solution containing 100 ppm of mephosfolan (1:1 mixture of $^{12}C/^{13}C$ label at the methylene carbon atoms of the ethoxy groups) was prepared the same way as described above to serve as a duplicate sample as well as to assist in the identification of the photodecomposition products.

All the samples were placed side by side outdoors (Princeton, NJ; July–August, 1977) in such a way as to insure as much exposure to sunlight as possible. At sampling times of 1, 2, 3, 4, and 5 weeks, a 5-mL aliquot was pipetted from each flask into 50 mL of an aqueous solution saturated with sodium chloride. The solution was extracted twice with chloroform. The organic phase was evaporated under reduced pressure and redissolved in 200 mL of acetone. All the acetone solutions were quantitated for mephosfolan on a Tracor 550 gas chromatograph with a flame photometric detector (P-specific). A 3 ft × 2 mm i.d. glass column containing 2% OV-101 on Gas-Chrom Q, 80/100 mesh, was used. Oven, injector, and detector temperatures were 170, 230, and 260 °C, respectively. Air, hydrogen, and helium (carrier) flows were 15, 50, and 60 mL/min, respectively. Samples were analyzed in duplicate with standards in between. The retention time (t_R) for mephosfolan under these conditions was 1.5 min.

For identification of photodecomposition products, the remaining portions of the original test and control samples were extracted three times with chloroform. The solvent of each organic phase was evaporated under reduced pressure and the residue was redissolved in approximately 0.5 mL of acetone (fraction A). The aqueous phase was acidified to pH 2 with 6 N HCl and extracted three times with ether. Both the ether phase (fraction B) and aqueous phase (fraction C) were evaporated to dryness under reduced pressure, and the residues were treated with diazomethane in ether. The methylated reaction mixtures were evaporated to dryness in a stream of nitrogen and each was taken up in 0.5 mL of acetone.

This procedure produced three fractions of the original sample for GC–CIMS analysis.

GC–CIMS Analysis. GC–CIMS analyses were performed on a Finnigan Model 4023 automated gas chromatograph/EI-CI System (Finnigan Corp., Sunnyvale, CA) interfaced with an INCOS 2300 Data System. The 6 ft × 2 mm i.d. glass column was packed with 10% OV-101 on Gas-Chrom Q, 100/120 mesh. Methane gas (17 mL/min) was used both as the GC carrier gas and as the CI reagent gas. The GC oven temperature was held at 50 °C for 4 min initially and then programmed at 10 °C/min to 250 °C, where it was held for another 5 min. The source temperature was 250 °C and the GC–CIMS interface temperature was 200 °C.

RESULTS AND DISCUSSION

Mephosfolan was irradiated by sunlight in three different solutions: distilled water, a 2% acetone–water solution, and "natural paddy water". The third water source was obtained by filtering the water which had been used to flood the soil used in the growth of rice plants (Ku et al., 1978). Photolysis was conducted in this environment in order to determine if it contained any naturally occurring photosensitizers or charge-transfer complexes which would influence the rate or course of the photochemical reaction. The effect of a natural photosensitizer, riboflavin, on the irradiation of chloroanilines was dem-

Table I. Amount (%) Mephosfolan Remaining after Irradiation by Sunlight

exposure time, weeks	distilled water		paddy water		2% acetone		paddy water control	
	IL[a]	EL	IL	EL	IL	EL	IL	EL
1	72.1[b]	66.5	60.0	61.5	38.6	36.0	97.4	97.0
2	66.0	65.4	49.5	50.3	23.5	27.8	94.7	96.6
3	41.4	47.5	39.4	31.6	12.1	13.3	93.8	90.9
4	34.7	32.0	22.6	20.4	5.0	7.2	90.3	83.3
5	26.4	26.0	20.8	17.8	3.8	4.1	89.4	91.8

[a] IL = imido-labeled; EL = ethyl-labeled. [b] Each value is average of duplicate GLC analyses.

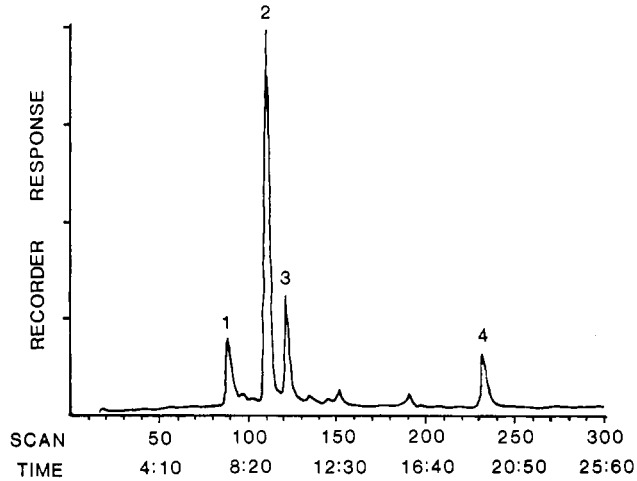

Figure 1. Computer reconstructed gas chromatogram from GC–CIMS analysis of chloroform extract from distilled water ^{13}C-imido-labeled mephosfolan after 5 weeks of solar irradiation (fraction A).

onstrated several years ago by Rosen et al. (1970). More recently, tyrosine and tryptophan have been shown to be at least partially responsible for the photosensitizing activity of agricultural run-off water (Ross, 1974). The Environmental Protection Agency recognizes the effect of photosensitization by requiring studies to be conducted in 2% acetone–water solutions (EPA, 1975).

The amount of mephosfolan remaining in the solutions exposed to sunlight as well as the dark controls was measured weekly over a 5-week period and the results are presented in Table I. Only the dark control of the paddy water sample is presented, but the other control samples were essentially the same. Obviously, thermal and/or microbial decomposition were not as important as solar irradiation under our experimental conditions. Plotting the logs of the mephosfolan concentrations against time gave straight lines, suggesting first-order (or pseudo-first-order) kinetics. Calculating the half-lives from these plots gave values of approximately 7, 14, and 18 days for 2% acetone–water, rice paddy water, and distilled water systems, respectively, indicating that acetone is a very effective sensitizer for mephosfolan. The rice paddy water constituents apparently had some photosensitizing activity but their overall effect was not as spectacular as that of acetone.

Analysis of the photoproducts in the three systems showed qualitative similarities. Analysis of the photoproducts in the paddy water was facilitated by use of 1:1 ^{12}C/^{13}C mephosfolan mixtures. This provided mass spectra with doublet ion peaks for mephosfolan and its photoproducts. Any material which did not show doublet peaks in its mass spectrum was judged to be a volatile paddy water constituent and was not identified.

Identification of Materials in Fraction A. Figure 1 is a computer-reconstructed gas chromatogram of the chloroform extract from the distilled water ^{13}C-imido-labeled mephosfolan (fraction A) after 5 weeks of solar irradiation. An essentially identical chromatogram was obtained from the ^{13}C-ethyl-labeled material. Peaks labeled 1–4 in Figure 1 were identified by mas spectrometry as diethyl methyl phosphate, cyclic *S,S*-propylene dithiocarbonate (II), 5-methyl-2-imino-1,3-dithiolane (III), and mephosfolan, respectively. The first peak gave a mass spectrum (Figure 2A) which exhibited an M + 1 ion at *m/e* 169, indicating that its molecular weight was 168. Fragments at *m/e* 141 and 113 represented successive losses of ethylene. Further support for the assigned structure was obtained from the mass spectrum of peak 1 in the chloroform extract of photolyzed ethyl-^{13}C-labeled mephosfolan (Figure 2B). Because both ethyl groups contained ^{13}C labels, there were two molecular ions, one at *m/e* 169 and the other at *m/e* 171. Loss of ethylene gave fragment ions at *m/e* 141 and 142 because one of the ^{13}C labels was removed. Loss of a second ^{13}C label gave fragment *m/e* 113. The second peak in the gas chromatogram of Figure 1 had doublet M + 1 ions at *m/e* 135 and 136 (due to the carbon-13 label) and a fragment at *m/e* 75 (loss of ^{13}COS) as shown in its mass spectrum in Figure

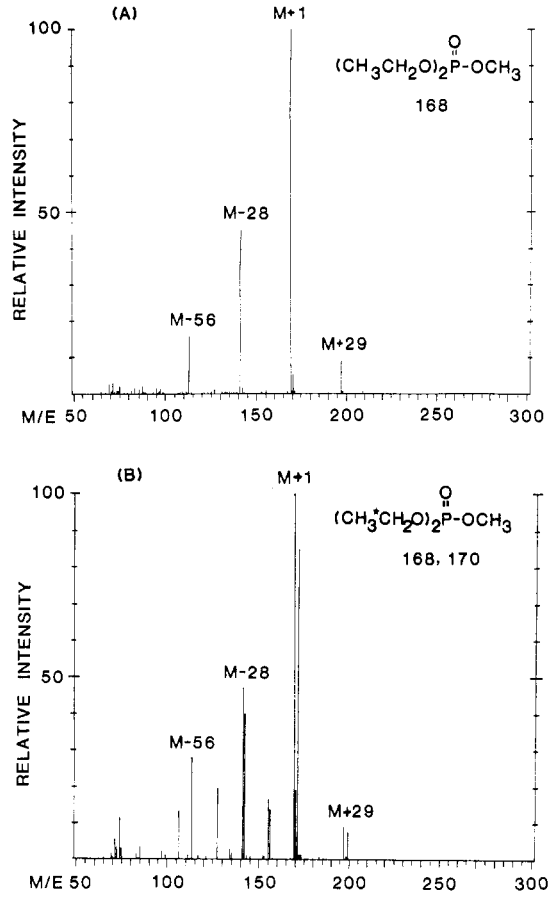

Figure 2. CI(CH_4) mass spectrum of diethyl methyl phosphate from (A) ^{13}C-imido-labeled and (B) ^{13}C-ethyl-labeled mephosfolan photolysates.

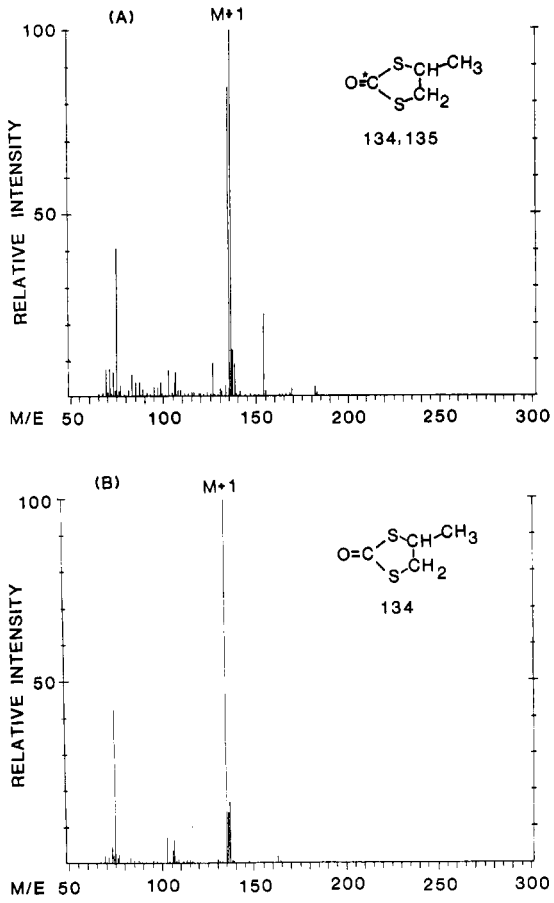

Figure 3. CI(CH_4) mass spectrum of cyclic S,S-propylene dithiocarbonate from (A) ^{13}C-imido-labeled and (B) ^{13}C-ethyl-labeled mephosfolan photolysates.

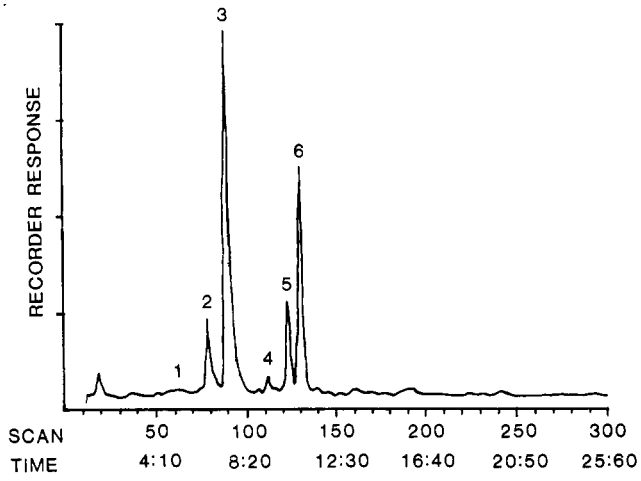

Figure 4. Computer-reconstructed gas chromatogram from GC–CIMS analysis of the diazomethane-treated aqueous phase, after extraction with chloroform and then ether, from the distilled water ^{13}C-imido-labeled mephosfolan after 5 weeks of solar irradiation (fraction C).

3A. The spectrum of the same peak from ethyl-^{13}C-labeled mephosfolan (Figure 3B) showed the absence of ^{13}C doublets. Confirmation of the assigned structure (II) for this material was obtained by cochromatography with synthesized material. The third peak was identified as 2-imino-5-methyl-1,3-dithiolane (III) based on the similarity of its mass spectrum and retention time to peak 5 in fraction C (Figure 4). Peak 4 was identified as mephosfolan by comparison of MS with the authentic standard.

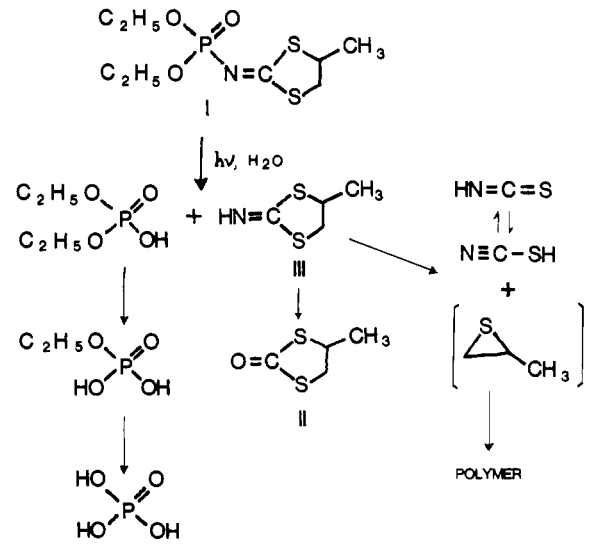

Figure 5. Proposed pathway of photolysis of mephosfolan.

Identification of Materials in Fraction B. GC–CIMS analysis of the diazomethane-treated ether extract of the aqueous phase revealed that the following compounds were present: trimethyl phosphate, dimethyl ethyl phosphate, diethyl methyl phosphate, and traces of II. Since these compounds resulted from treatment with diazomethane, the photoproducts originally present (in addition to II) were phosphoric acid, ethyl phosphate, and diethyl phosphate. The latter was the major constituent of this fraction. Confirmation of structure was obtained by evidence of cochromatography with authentic materials.

Identification of Materials in Fraction C. The materials that remained in the aqueous layer after extraction with chloroform and then ether were (after water evaporation) treated with diazomethane. Figure 4 is the computer-reconstructed chromatogram of the ^{13}C-imido-labeled photolysate of this fraction. The first three peaks were identical with those in fraction B and were due to the presence (before diazomethane treatment) of phosphoric acid, ethyl phosphate, and diethyl phosphate. Again diethyl phosphate was the predominant phosphate ester observed. A small peak, no. 4, identified as II, eluted next. This was followed, no. 5, by a material whose mass spectrum exhibited two M + 1 ions at m/e 134 and 135 in the imido-labeled photolysate and a single M + 1 ion at m/e 134 in the ethyl-labeled photolysate. The base peak in both spectra was 75 and the material was tentatively identified as 2-imino-5-methyl-1,3-dithiolane (III). Confirmation of the assigned structure was obtained by cochromatography with authentic 2-imino-5-methyl-1,3-dithiolane hydrochloride. The latter is known to lose hydrochloric acid in the GC injector port. The next elutant, no. 6, had a doublet M + 1 ion at m/e 148 and 149 (imido-labeled material) and a single M + 1 ion at m/e 148 (ethyl-labeled material). Again, m/e 75 was the base peak. The material, identified as 5-methyl-2-methylimino-1,3-dithiolane (confirmed by cochromatography with synthetic material), had to arise from reaction of III with diazomethane. The last eluting peak gave the same mass spectrum when ethyl-labeled or imido-labeled photolysate was injected (M + 1 at m/e 249, base peak at m/e 71, additional peaks at m/e 207, 193, 179, and 151). Since no doublet peaks were observed, this material could not have been a mephosfolan photoproduct.

Similar GC–CIMS analyses of the photolysate from rice paddy water and acetone gave the same results, qualitatively. The major photoproducts in distilled and paddy

water were diethyl phosphate, cyclic S,S-propylene dithiocarbonate and 2-imino-5-methyl-1,3-dithiolane, suggesting that the major photolytic process involved hydrolysis of the phosphorus–nitrogen bond. In acetone, the major products were diethyl phosphate and cyclic S,S-propylene dithiocarbonate. This suggests that acetone, in addition to accelerating the photohydrolysis of mephosfolan, also effectively sensitized the photohydrolysis of II to III. A suggested scheme for the photolysis of mephosfolan appears in Figure 5.

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Sorption–Desorption of α, β, and γ Isomers of Hexachlorocyclohexane in Soils

P. A. Wahid and N. Sethunathan*

Sorption and desorption of α, β, and γ isomers of hexachlorocyclohexane (HCH) by 12 soils of varying characteristics were studied using uniformly ^{14}C-labeled isomers. Soils showed striking differences in their ability to sorb HCH with sorption ranging from 40 to 96% of the total HCH added. At low equilibrium concentrations no appreciable differences in sorption occurred between the three isomers with respect to individual soil samples; but at higher concentrations sorption of the three isomers within a soil was not always comparable. Organic matter was the most important factor governing the sorption and desorption of HCH isomers. Sorption increased and desorption decreased with increasing soil organic matter content. The sorption of HCH isomers with unit increase of soil organic matter content from 0 to 3.4% was 41 times more than at organic matter contents over 3.4%. In the absence of organic matter, clay content and free iron oxide were implicated in γ-HCH sorption and free iron oxide alone in β-HCH sorption, while no significant correlation existed between α-HCH sorption and clay content or free iron oxide. Desorption of all three isomers showed a hysteresis effect.

Hexachlorocyclohexane (HCH) is used widely for controlling major insect pests of rice. Commercial and technical formulations of HCH are comprised of α, β, δ, ϵ, and γ isomers of which the γ isomer alone is insecticidal. The β isomer is a minor constituent, but, following extensive use of HCH in Japan, it accumulated through the food chain because of its persistence and, as a result, occurred more frequently and in larger amounts than other isomers in agricultural commodities, milk samples in particular (Tomizawa, 1977). Consequently, the use of HCH was banned or restricted in Japan in recent years. In tropical developing countries, such a ban on HCH use would adversely affect the intensive crop production and health programs because HCH is relatively cheap, highly effective, and indigenously available. Price considerations and nonavailability of substitutes would thus delay the replacement of HCH in most of the developing countries. For example, India has the capability of producing HCH in surplus of the projected demand and HCH alone accounts for more than 50% of all pesticides used in the country. In view of the overall importance of HCH to Indian agriculture, an isotope study was undertaken to compare the relative sorption/desorption of α, β, and γ isomers of HCH in Indian rice soils, widely differing in their physical and chemical characteristics.

Laboratory of Soil Microbiology, Central Rice Research Institute, Cuttack 753006, India.

MATERIALS AND METHODS

Soils. Twelve soils from rice-growing tracts in India were the same as used in an earlier study (Wahid and Sethunathan, 1978) and differed widely in their physicochemical characteristics. The soils have been identified by number and their physicochemical characteristics have been described earlier (Table I, Wahid and Sethunathan, 1978). Included were a Pokkali soil (13) and two Kari (14 and 15) soils from the major rice-growing tract of coastal Kerala in India which have extractable S contents of 0.2, 0.8, and 0.5% and are characterized as acid sulfate soils (Bloomfield and Coulter, 1973). Soils were air-dried, ground to pass a 100-mesh sieve, and stored in air-tight bottles.

Labeled HCH Isomers. Uniformly labeled α, β, and γ isomers of HCH were obtained from Amersham Radiochemical Centre, Bucks, United Kingdom, and had specific activities of 48, 33, and 45 mCi/mmol. The purity of all isomers was confirmed by thin-layer chromatography. The benzene carrier was evaporated and the [^{14}C]HCH isomers were equilibrated with distilled water for 24 h prior to their addition to the soils.

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