Persistence and Biodegradation of Diazinon in Submerged Soils

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Only 2 to 6% of the originally applied diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] remained in the soils between 50 and 70 days after application when added to three submerged tropical soils at a rate about seven times that recommended for protecting rice plants from stem borer infestation and certain virus-transmitting leaf hoppers. Losses of the insecticide from sterilized samples of two of the soils were much slower than from nonsterilized samples, indicating microbial participation in diazinon degradation in these two soils. Diazinon disappeared rapidly in sterilized samples of the third soil, an acid clay of pH 4.7, apparently because of its instability under acid conditions. Microbial degradation of the pyrimidine ring of the diazinon molecule was demonstrated by the release of $C^{14}O_2$ from submerged soils treated with diazinon labeled at the 2 position of the pyrimidine ring. *Streptomyces* sp. isolated from diazinon-treated submerged soil could degrade diazinon in shake cultures, but only in the presence of glucose.

Diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] was recently found to control rice stem borers and some leaf hoppers effectively when a granular form of this insecticide, Basudin-10G (F. E. Zuellig, Inc., Makati, Rizal, Philippines), was applied directly to the standing rice field water at a rate of 2 to 3 kg. per hectare of active ingredient (International Rice Research Institute, 1967; Pathak, 1966).

Earlier reports on the persistence of diazinon in nonflooded soils (Getzin and Rosefield, 1966; Gunner *et al.*, 1966b) indicate a fairly lengthy persistence of this compound (more than 160 days) when applied to soil at rates equivalent to 3 to 6 pounds per acre. Information on the fate of diazinon in nonflooded soils indicates that the initial step in the degradation of this insecticide involves a chemical hydrolysis of the heterocyclic-phosphate bond resulting in the formation of 2-isopropyl-4-methyl-6-hydroxypyrimidine which is rapidly degraded by the soil microflora (Getzin, 1967). The participation of the soil microflora appears to be largely in the decomposition of the products of hydrolysis rather than in an attack on the parent insecticide molecule.

Pure culture studies on diazinon degradation, using bacteria of the genus *Arthrobacter* isolated from soil, also indicate that the initial step involves a chemical hydrolysis and that the bacterium is capable of metabolizing the products of diazinon hydrolysis (Gunner *et al.*, 1966a). Gunner *et al.* (1966b) later claimed that the metabolism of diazinon by their soil isolate was conditioned by the solvent used to dissolve the insecticide and by the presence of an additional carbon source.

The practice of inundating soils used in rice cultivation leads to profound changes in the physical, chemical, and biological properties of these soils. One of the most important changes is that within a few days after submergence the soil is nearly devoid of oxygen. This state of anaerobiosis occurs because of the utilization of soil oxygen by the aerobic microflora and is maintained throughout the period of soil submergence because the soil pores are filled with water, which prevents penetration of oxygen into the soil from the atmosphere. Under these conditions anaerobic bacteria constitute the bulk of the active microflora. Therefore, since the microbial decomposition of organic material in flooded soils usually follows anaerobic pathways, the fate of a pesticide such as diazinon should differ greatly in flooded soils and in nonflooded "aerobic" soils.

Since no information was available on the fate of diazinon in flooded soils, the present investigation was aimed at determining the persistence of diazinon in three Philippine soils, and the role played by the microflora of these soils in the degradation of the insecticide.

MATERIALS AND METHODS

Persistence of Diazinon. The persistence of diazinon in three soils of Luzon (Philippines) was determined. The air-dried soils (Maahas clay, pH 6.6, organic matter 2.0%, total N 0.14%; Luisiana clay, pH 4.7, organic matter 3.2%, total N 0.21%; and a clay loam from Pila, pH 7.6, organic matter 1.5%, total N 0.09%) were screened (2-mm. diameter) and placed in 20-gram amounts in large test tubes plugged with cotton. Tubes containing sterilized samples of the three soils were also

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prepared by sterilizing the moistened soils in shallow layers by autoclaving at 121° C. for 1 hour on each of three successive days and then dispensing the soils aseptically into cotton-plugged test tubes in 20-gram amounts.

An aqueous solution of diazinon was prepared using a liquid technical solution of the insecticide (89% active ingredient, supplied by Geigy Agricultural Chemicals, Basel, Switzerland) which had a final concentration of 40 μg. per ml. of diazinon. This solution was sterilized by passing it through a 0.45-micron pore size Millipore filter (Millipore Corp., Bedford, Mass.). The sterilized diazinon solution was then added to each of the soil tubes in 25-ml. amounts which waterlogged the soils and provided a column of standing water 5 cm. deep.

The test tubes containing the treated soil samples were placed in pots containing flooded soil in such a way that the soil and water surface in the test tubes and pots coincided. The pots were kept in the greenhouse (30° ± 3° C.) for the duration of the experiment.

After 1, 15, 25, 50, and, in the case of the Pila soil, 70 days' incubation in the greenhouse, three replicate tubes of each treatment were withdrawn, the diazinon was extracted from the soils, and the amount of insecticide remaining in the soils was determined quantitatively by gas chromatography.

This method of diazinon extraction from the soils was the same as that for the extraction of benzene hexachloride isomers from soil (MacRae *et al.*, 1967), except that the final solvent was nanograde hexane instead of petroleum ether. The extraction procedure comprised vigorous shaking of the contents of each tube, for 10 minutes, with 200 ml. of acetone and then with 20 ml. of hexane in a 1-liter volumetric flask. The volume was finally made up to 1 liter with 2% Na_2SO_4 solution. The hexane layer was suitably diluted prior to injection into the gas chromatograph. Recoveries of 93 to 96% from standard soil, water, and diazinon mixtures were obtained.

The identification of diazinon was based upon the R_f value, and quantities were calculated by relating peak heights to those of standards (in the range 0 to 10 nanograms) analyzed by the same method. The gas chromatograph was an Aerograph Model 200 (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) fitted with dual columns and electron-capture detectors. The spiral glass columns were 5 feet × ⅛ inch and packed with 5% DC 200 silicone on Chromosorb G (30- to 60-mesh). The operating conditions were: column temperature 175° C., detector temperature 200° C., injection port temperature 225° C., column pressure 60 p.s.i., and detector cell voltage 90 volts d.c. The carrier gas was prepurified nitrogen at a flow rate of 25 to 28 ml. per minute. Under these conditions diazinon had a retention time of 3.8 minutes.

Degradation of C^{14}-Labeled Diazinon. To measure microbial degradation of diazinon in submerged soils, the evolution of $C^{14}O_2$ from two Philippine soils (Maahas clay and clay loam from Pila) treated with C^{14}-labeled diazinon (labeled at position 2 on the pyrimidine ring, specific activity 4.0 μc. per mg.) was followed. The air-dried soils were screened (2 mm.) and placed in large test tubes in 20-gram amounts. A solution of C^{14}-labeled diazinon, sterilized by filtration, was prepared, and 3 ml. of this solution (730,000 c.p.m. per ml.) were added to soil samples. In addition, 20-ml. amounts of an aqueous solution of unlabeled diazinon (containing 780 μg. of diazinon) were added to the soil samples. The total volume of 23 ml. of diazinon solution was enough to wet the soils completely and provide a column of water 5 cm. in height over the soil surface. The soil tubes were placed in a 30° C. water bath connected to an aeration train that permitted carbon dioxide–free air to be drawn over the surface of the water columns. Any evolved carbon dioxide was trapped in 10 ml. of 1.0*N* NaOH. Samples of untreated, nonsterilized soils and C^{14}-diazinon–treated sterilized soils were included in the aeration trains as controls. Three replicates of each treatment were prepared.

At 15, 30, and 50 days after the addition of C^{14}-diazinon, the radioactivity of the evolved carbon dioxide was determined. The methods employed to release the carbon dioxide from the alkali trap and to count the activity using a liquid scintillation counter have been described (MacRae *et al.*, 1967).

Microbial Degradation of Diazinon. A species of the genus *Streptomyces* was isolated from diazinon-treated flooded soils for its ability to degrade the insecticide. The enhanced growth of *Streptomyces* sp. in flooded soil was noted earlier to be clearly visible as a zone of brown pigment at the surface of diazinon-treated, flooded soils (Sethunathan and MacRae, 1969). To determine whether the isolate could degrade diazinon, the organism was grown in a mineral medium containing 1% glucose (Pridham and Gottlieb, 1948) to which a filter-sterilized aqueous solution of diazinon was added. Samples for diazinon analysis were removed after a 10-day period of incubation at 30° C. and immediately following inoculation. The culture was shaken continuously during the growth period. The pH of the medium was 6.5. The uninoculated medium plus diazinon served as the control. Two replicates of each mixture were prepared. For extraction of diazinon, 1 ml. of the mixture was shaken first with 1 ml. of acetone and then with 1 ml. of hexane. The hexane layer was suitably diluted prior to injection into the gas chromatograph.

Because the results of Gunner *et al.* (1966b) indicated the need for an additional carbon source in the metabolism of diazinon by their isolate, short-term experiments in which a dense suspension of the *Streptomyces* sp. was provided with diazinon as the sole source of carbon in phosphate buffer were performed. The *Streptomyces* sp. was grown in the mineral medium containing 1% glucose and diazinon for one week at 30° C., after which the growth was harvested by centrifugation, washed, and finally resuspended in a 0.01*M* phosphate buffer at pH 7.1. Diazinon was added to the cell suspension and to the buffer alone, the latter serving as the control. Both mixtures were sampled at the start of the experiment and again after 24-hour

incubation in a shaking water bath maintained at 30° C. The samples were extracted and the amount of diazinon present was determined by gas chromatography.

RESULTS AND DISCUSSION

Persistence of Diazinon in Submerged Soils. The disappearance of diazinon residues from both sterilized and nonsterilized submerged soils followed first-order kinetics. Half-life ($T½$) values of 8.8 and 33.8 days were obtained for nonsterilized and sterilized Maahas clay, respectively, while the corresponding values for the clay loam from Pila were 17.4 and 43.8. Thus, diazinon disappeared more rapidly from the nonsterilized than from the sterilized samples (Figures 1 and 2), indicating that the soil microflora may play an important role in influencing the persistence of the insecticide.

The results obtained from the acid Luisiana clay (Figure 3), on the other hand, show that the loss of the insecticide was more rapid in the sterilized soil samples. Half-life values of 9.9 and 0 days were recorded for nonsterilized and sterilized soils, respectively. This result may possibly be explained by the instability of diazinon in acid solutions and by the fact that the aerobic pH of Luisiana clay is acid. The absence of biological activity in the sterilized samples of Luisiana clay would mean that the soil would not undergo reduction and therefore, in spite of flooding, the pH would remain at 4.7. In fact, no change in pH was observed in sterilized Luisiana clay, 2 weeks after submergence. The nonsterilized samples would undergo reduction following flooding. The pH would then rise and would retard the chemical hydrolysis of the heterocyclic-phosphate bond of diazinon. A significant rise in pH (approximately 2 pH units) of nonsterilized soil of this type has been shown to take place within 3 weeks after flooding (Ponnamperuma, 1965).

Losses of diazinon from sterilized samples of Maahas clay and clay loam (Figures 1 and 2) may have been due to chemical hydrolysis and, to a certain extent, to volatilization. Increased losses of diazinon in the nonsterilized samples of these soils indicate biological action in the hydrolysis and indicate that some segment of the microflora was capable of performing the initial attack on the insecticide molecule. However, the process of soil reduction following soil submergence may lead to the establishment of some physical or chemical condition of the soil which favors the chemical hydrolysis of the heterocyclic-phosphate bond. In this case, the microflora may play an indirect role in the initial degradation of the insecticide molecule.

Whereas the microflora seem to play a significant role in the degradation of diazinon in Maahas clay and clay loam, there is little evidence of a microbial role in diazinon degradation in Luisiana clay. The extremely rapid loss of the insecticide from Luisiana clay

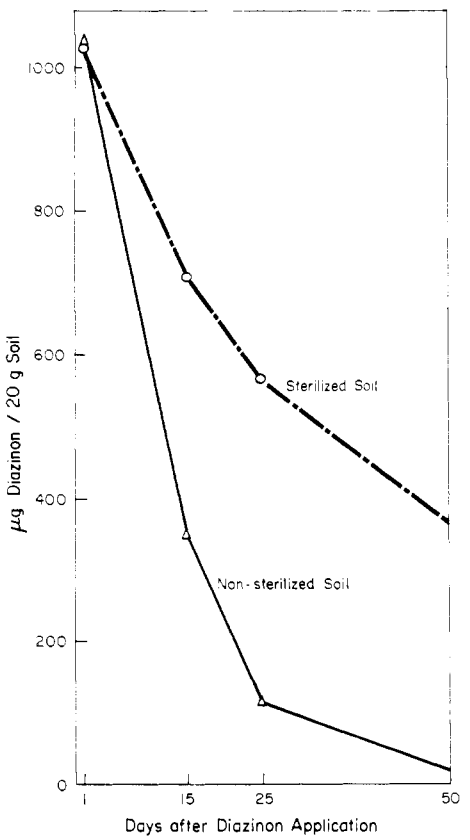

Figure 1. Persistence of diazinon in submerged Maahas clay

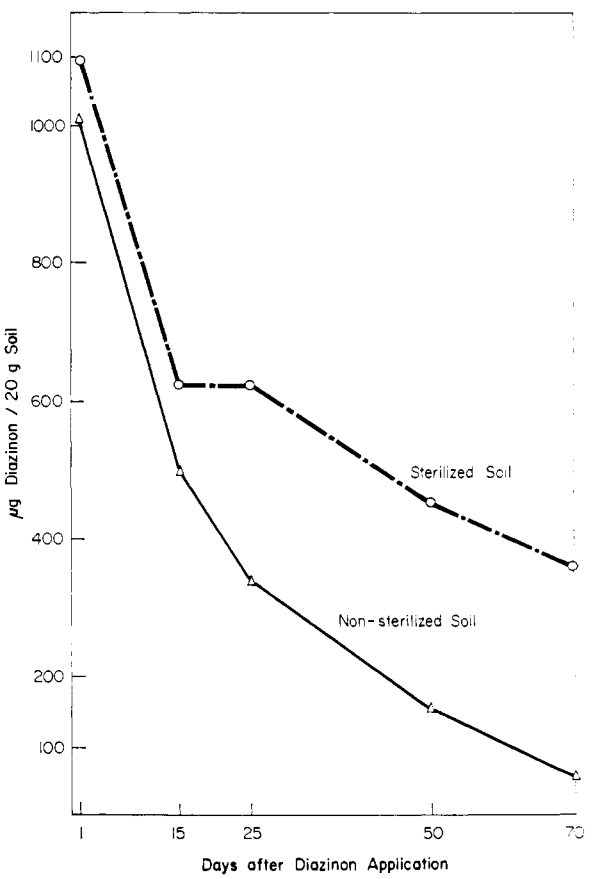

Figure 2. Persistence of diazinon in submerged clay loam (Pila)

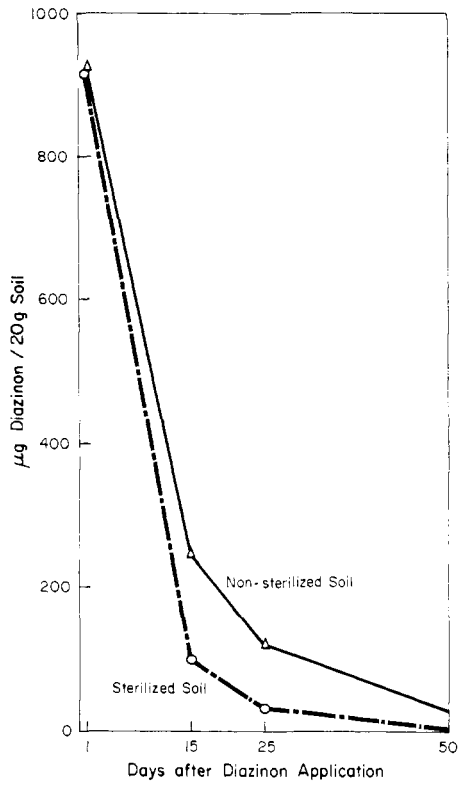

Figure 3. Persistence of diazinon in submerged Luisiana clay

indicates that diazinon may not be very effective for protection of crops grown on this or similar soils. However, in the case of rice grown in submerged soils, it may be possible to delay diazinon application for 3 weeks until such soils have undergone reduction and a pH has been reached at which diazinon is more stable. Alternatively, a more stable insecticide, such as lindane, might offer sufficient protection to the rice crop until the pH of the soil has risen and diazinon may be applied.

Fifty days after diazinon application, approximately 2 and 5% of the amount of insecticide originally applied to Luisiana clay and Maahas clay, respectively, were recovered (Figures 1 and 3). The persistence of diazinon in the clay loam was longer, but only 6% remained in the soils after 70 days. Since the amount of diazinon used in these experiments was approximately seven times that recommended for the protection of a rice crop, it seems unlikely that diazinon would accumulate in rice cultivation soils. The results also indicate that the persistence of diazinon may be shorter in flooded soils than reported for nonflooded soils (Getzin and Rosefield, 1966; Gunner *et al.*, 1966b).

Degradation of C^{14}-Labeled Diazinon. The results of this study (Table I) show that the microflora of flooded soils play a role in the degradation of the pyrimidine ring of the diazinon molecule, thus resulting in the oxidation of some of the pyrimidine ring of the diazinon molecule carbon to $C^{14}O_2$. The oxidation was slow—only 0.4 to 0.7% of the total C^{14} added to the soils was released as $C^{14}O_2$ during the 50-day incubation

Table I. Release of $C^{14}O_2$ from C^{14}-Labeled Diazinon Applied to Submerged Soils

Soil	Radioactivity in Evolved CO_2, C.P.M.[a] 15 days	30 days	50 days
Maahas clay	2700	6500	15,000
Clay loam (Pila)	1700	4400	8,100

[a] All figures adjusted for background and for activity obtained for sterile controls and given as cumulative counts. Range for sterile controls 45 to 130 c.p.m.

period. This was surprising in view of the results of Getzin (1967), who found that 35% of the C^{14} of labeled diazinon applied to nonflooded soil was released as $C^{14}O_2$ over a period of 20 weeks. However, because of the anaerobic nature of the bulk of the flooded soil profile, some of the C^{14} may have been released as methane.

Release of $C^{14}O_2$ was more rapid from Maahas clay than from clay loam (Table I), in agreement with the results obtained from the gas chromatographic determination of the persistence of the parent insecticide molecule in these two soils (Figures 1 and 2).

Microbial Degradation of Diazinon. When *Streptomyces* isolate was grown in the presence of 1% glucose and diazinon, the amount of diazinon in the growth medium declined from 11.75 to 3.23 μg. per ml. during the 10-day period of incubation. Diazinon in the uninoculated control fell from 9.57 to 6.7 μg. per ml. over the same period of incubation. The pH of the inoculated and uninoculated media were 6.3 and 6.5, respectively, at the end of 10-day incubation period. The greater loss of diazinon in the inoculated medium was due to the activity of the isolate.

However, in shorter term experiments where diazinon was the sole source of carbon, the loss of diazinon from the cell suspension was equal to the loss from the control and the organism was not able to initiate the attack on the diazinon molecule in the absence of the additional carbon source, glucose. While these findings agree with those of Gunner *et al.* (1966b) with respect to the need for an additional carbon source, it seems likely that the participation of the *Streptomyces* isolate in diazinon degradation is at first an indirect one, possibly involving the generation of some condition in the growth medium, during the metabolism of glucose, which is conducive to the chemical hydrolysis of the heterocyclic-phosphate bond. This activity may be followed by a more direct attack on the cleavage products by the microorganisms. Recently, diazinon has been shown to be rapidly degraded by synergistic action of an *Arthrobacter* sp. and a *Streptomyces* sp. (Gunner and Zuckerman, 1968).

Although there is still a lack of unequivocal evidence for the enzymatic attack on the diazinon molecule by microorganisms, it is clear that the activities of certain microbial species can accelerate the initial phase in the detoxication of the insecticides, and in soils species of microorganisms exist that can decompose the prod-

ucts of the initial step in the degradation of the diazinon molecule. Both factors are of considerable practical importance in the consideration of the persistence of diazinon in soils.

ACKNOWLEDGMENT

The authors thank Susan R. Hizon for her help, and Geigy Agricultural Chemicals, Basel, Switzerland, for a gift of labeled diazinon.

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Received for review October 28, 1968. Accepted January 3, 1969. Study supported in part by a grant from the National Science Development Board of the Philippines.