Stability of Commercial Formulation of Fenitrothion, Methyl Parathion, and Parathion in Anaerobic Soils

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The relative stability of formulated emulsifiable concentrates of fenitrothion, methyl parathion, and parathion in an anaerobic soil was studied by using two approaches. When the formulated pesticides were applied to 10-day preflooded soil, followed by continued incubation of soil samples under static flooded conditions, degradation of parathion proceeded essentially by nitro group reduction and of methyl parathion and fenitrothion by hydrolysis. In contrast, all three insecticides were degraded by nitro group reduction to their respective amino analogues in the second approach involving direct equilibration of the pesticide with the soil prereduced by flooding for different periods. Interestingly, rapid nitro group reduction of all organophosphates, parathion in particular, occurred within 5 s of their equilibration with a soil prereduced by incubation with rice straw under flooding. Sterilization of the prereduced soil by autoclaving prevented the rapid destruction of the pesticides by the prereduced soil.

The importance of anaerobic degradation as an effective means of detoxification of some pesticides has received increased significance, especially after the demonstration of the extreme susceptibility of hexachlorocyclohexane (HCH) in predominantly anaerobic flooded soil (Raghu and MacRae, 1966). Although chlorinated hydrocarbon insecticides constitute more than 50% of all pesticides used in India and probably other developing countries, use of less persistent, but more toxic, organophosphates has been increasing steadily in recent years. Our knowledge of the behavior of organophosphorus pesticides in tropical environments as exist in most developing countries is, however, restricted to the limited studies on the behavior of diazinon (Sethunathan, 1972) and parathion (Sethunathan et al., 1977) in flooded rice soils.

In most reported studies dealing with the stability of pesticides in flooded soils, air-dried soils were first treated with pesticides and then flooded for different periods. The pesticides were thus exposed to aerobic conditions before the onset of anaerobic conditions generated within few days after flooding. But, in transplanted rice culture, pesticides, applied to the standing crop several days after submergence, immediately come into contact with the soils already in the reduced state. More recently, we found that rapid degradation of parathion occurred upon its equilibration with a soil prereduced by flooding for 30 days or more (Wahid et al., 1980). The present study, which is an extension of the earlier work with parathion, deals with the degradation of agriculturally important organophosphorus insecticides, viz., fenitrothion [*O,O*-dimethyl *O*-(3-methyl-*p*-nitrophenyl) phosphorothioate] and methyl parathion [*O,O*-dimethyl *O*-(*p*-nitrophenyl) phosphorothioate] besides parathion [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate] in a flooded soil.

MATERIALS AND METHODS

Soil. An alluvial soil (pH 6.2; organic matter, 1.61%; total nitrogen, 0.09%), collected from the experimental farm of our institute, was used. The soil was air-dried and ground to pass through a 2-mm sieve before use.

Pesticides and Their Metabolites. Commercial emulsifiable formulations of 46.7% parathion (Folidol), 50% methyl parathion (Metacid), and 50% fenitrothion (Folithion) were gifts from Bayer (India), Bombay. Authentic standards of parathion, methyl parathion, fenitrothion, and their amino analogues, used for identification and quantitation, were obtained from Farbenfabriken Bayer AG, Leverkusen, Germany, *p*-nitrophenol was from Riedel, Hannover, Germany, and 3-methyl-4-nitrophenol was from National Chemical Laboratory, Pune, India.

Relative Stability of Insecticides. The relative stability of fenitrothion, methyl parathion, and parathion in a flooded alluvial soil was studied by using two different approaches. In the first experiment, 20-g portions of the soil were placed in test tubes (200 × 25 mm) and were flooded with 25 mL of sterile distilled water. After 10 days of flooding at room temperature (28 ± 4 °C), 1 mL of an aqueous solution of the commercial formulation of the respective insecticide was added to the flooded soil at a concentration of 500 ppm active ingredient (a.i.). After incubation for different periods, the parent insecticides and their amino analogues in the flooded soil samples were analyzed by gas–liquid chromatography (GLC) and hydrolysis products by thin-layer chromatography (TLC).

In the second approach, the soil was first reduced by 30-day flooding and then equilibrated by shaking with the pesticides as follows: 20-g portions of the alluvial soil were flooded with 25 mL of sterile distilled water in 200 × 25 mm test tubes. Also, in another treatment 20-g soil samples were amended with 0.5% rice straw powder before flooding to hasten soil reduction. The flooded soil samples, rice straw amended and unamended, were incubated at 28 ± 4 °C for 30 days to allow soil reduction. The reduced soil samples were transferred to 250-mL Erlenmeyer flasks and the redox potentials measured with a compound platinum–calomel electrode as described earlier (Pal et al., 1979). After measurement of the potentials, 1 mL of an aqueous solution of the commercial formulation of parathion (345 ppm a.i.), methyl parathion (525 ppm a.i.), and fenitrothion (575 ppm a.i.) was added to separate reduced soil samples. The contents in each flask were shaken for 30 min on a Gallenkamp orbital shaker to allow the interaction between the reduced soil and the respective insecticides. Soil samples, flooded at the time of insecticide addition and then shaken for 30 min, served as aerobic controls. For determination of the biological participation in the degradation of pesticides by anaerobic soils, 30-day flooded soil samples were sterilized by autoclaving (121 °C for 1 h for three consecutive days) before equilibration with the respective pesticides. The redox potential of the soil suspension was measured immediately after equilibration, and the insecticides and their amino analogues in the samples were analyzed by GLC.

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Table I. Relative Stability of Parathion, Methyl Parathion, and Fenitrothion in a Flooded Alluvial Soil

	μg of compound recovered/20 g of soil								
	from added parathion			from added methyl parathion			from added fenitrothion		
days after insecticide addition[a]	parathion[b]	amino-parathion[b]	p-nitrophenol[c]	methyl parathion[b]	methyl amino-parathion[b]	p-nitrophenol[c]	fenitrothion[b]	amino-fenitrothion[b]	3-methyl-4-nitrophenol[c]
0	490.4	0	0	485.3	0	0	487.2	0	0
0.5	470.1	0	0	428.1	0	trace	433.4	0	23.2
1	449.9	0	trace	333.7	0	120.0	402.2	0	32.9
2	405.3	0	18.2	219.8	0	98.6	375.6	0	49.2
3	340.8	62.5	0	185.6	0	72.0	269.7	0	44.4
6	213.5	196.2	0	95.5	trace	0	157.3	0	0
12	41.7	218.9	0	58.2	trace	0	68.4	trace	0

[a] Insecticides (500 μg) were applied to the soil after 10 days of flooding. [b] Analyzed by gas-liquid chromatography. [c] Analyzed by colorimetry.

In another study, rice straw amended and unamended soil samples, as prepared in the earlier experiments, were flooded for 10, 20, 30, 60, 90, and 250 days and then shaken with commercial formulation of fenitrothion (575 μg a.i.), methyl parathion (525 μg a.i.), or parathion (345 μg a.i.) for 30 min. After equilibration, the insecticides and their amino analogues in these samples were analyzed by GLC. The redox potentials of the samples were measured before and after equilibration.

Extraction. For analysis of parent insecticides and their amino analogues by GLC, soil plus water in each of duplicate samples was transferred to a 250-mL Erlenmeyer flask. The contents in each flask were shaken first with sequentially added acetone (25 mL) plus methanol (25 mL) plus sodium sulfate (20 g) for 15 min and then with benzene (25 mL) for 45 min. The benzene layer, after appropriate dilution with the same solvent, was analyzed for parent insecticides and their amino analogues by GLC.

For quantification of the hydrolysis products, viz., p-nitrophenol (from methyl parathion and parathion) and 3-methyl-4-nitrophenol (from fenitrothion) by TLC, soil plus water in each of duplicate samples was shaken with 50-, 40-, and 30-mL portions of chloroform-diethyl ether (1:1) for 30 min each in succession as described earlier (Sudhakar-Barik and Sethunathan, 1978). After evaporation of the solvent at room temperature, the residues were eluted in 3 mL of methanol and then analyzed by TLC.

Gas–Liquid Chromatography. The parent insecticides and their amino analogues in the benzene layer were analyzed in a Perkin-Elmer gas chromatograph, Model 3920, fitted with a phosphorus-specific flame photometric detector. The spiral glass column (0.625-cm o.d.; 2-m length) was packed with 2% SE-30 on Gas-Chrom Q (60–80 mesh). The operating conditions were as follows: argon (carrier gas) flow, 40 mL/min; hydrogen flow, 70 mL/min; air flow, 180 mL/min; injector temperature, 210 °C; column temperature, 190 °C; detector temperature, 210 °C. Under these conditions, the retention times of the parent insecticides and their amino analogues were as follows: fenitrothion, 3 min; methyl parathion, 2.5 min; parathion, 3.5 min; aminofenitrothion, 2.75 min; methyl aminoparathion, 2.25 min; aminoparathion, 2.75 min. By the extraction and analytical procedures employed, the recovery of parent insecticides and their amino analogues from aerobic soil samples fortified at 25 ppm ranged between 88 and 97%; the recovery from the reduced soil samples fortified at 25 ppm was 91–94% for parent insecticides and 61–80% for their amino analogues.

Thin-Layer Chromatography. p-Nitrophenol (R_f 0.30) and 3-methyl-4-nitrophenol (R_f 0.37) from the thin-layer chromatograms were eluted in 0.1 N NaOH and then assayed at 400 nm after centrifugation (5000g; 10 min) of the suspension to remove the silica gel. By use of this procedure, the recovery of both nitrophenols from soil samples was ~95% at the 10-ppm level.

RESULTS AND DISCUSSION

In the first experiment, fenitrothion, methyl parathion, and parathion were added to 10-day flooded alluvial soil and then incubated under continued flooding for another 12 days. The concentration of all three insecticides decreased fairly rapidly especially after 1 or 2 days of their addition and reached low levels at the end of 12 days (Table I). Methyl parathion appeared to be the most unstable, probably because of its rapid hydrolysis as indicated by the significant formation of p-nitrophenol at 1 day. Hydrolysis was the major pathway in the degradation of fenitrothion as well, but accumulation of its hydrolysis product, 3-methyl-4-nitrophenol, was less pronounced than with methyl parathion. p-Nitrophenol and 3-methyl-4-nitrophenol disappeared subsequently, indicating their further metabolism. But, concomitant with the dominance of hydrolysis, amino analogues of both methyl parathion and fenitrothion were formed only in traces even after 12 days of application (22 days after flooding). In contrast, the degradation of parathion proceeded primarily by nitro group reduction with substantial formation of aminoparathion from 3 days after its application as reported earlier (Sethunathan, 1973).

It is not clear whether the rapid hydrolysis of methyl parathion and fenitrothion (Table I) is chemical, microbial, or both. Chemically, methyl parathion is more rapidly hydrolyzed than parathion and fenitrothion at near neutral pH and alkaline conditions (Nishizawa, 1976). The pH of the soil samples after 10 days of flooding (at the time of insecticide incorporation) was 7.2. But, significant hydrolysis of methyl parathion with substantial formation of p-nitrophenol only after an initial lag of 0.5 day suggests essentially microbial hydrolysis. Likewise, Spillner et al. (1979) provided evidence toward microbial hydrolysis of fenitrothion when significant hydrolysis occurred in nonsterilized, but not in sterilized, forest soils.

In the second experiment, rice straw amended and unamended soil samples were first reduced by 30-day incubation under flooding and then equilibrated on a shaker with fenitrothion, methyl parathion, and parathion. The redox potential after 30 days of flooding decreased to −210 mV in rice straw amended soil and −160 mV in unamended soil (Table II). After 30-min equilibration, the redox potential of amended and unamended soils increased only by 40 mV and the soil was still reduced at the end of equilibration. No appreciable degradation of all insecticides occurred during 30-min equilibration with aerobic

Table II. Change in Redox Potential of a Flooded Alluvial Soil Amended with Rice Straw (0.5%)

days after flooding	redox potential, mV	
	−rice straw	+rice straw
10	+100	−10
20	−40	−130
30	−160	−210
60	−100	−170
90	−110	−190
250	−90	−140

Table III. Relative Stability of Parathion, Methyl Parathion, and Fenitrothion after 30-min Equilibration with Prereduced[a] Alluvial Soil

treatments	insecticide[b] recovered, %		
	parathion	methyl parathion	fenitrothion
aerobic soil	96.5	97.8	96.6
reduced soil			
−rice straw (nonsterile)	49.5	70.2	64.2
−rice straw (autoclaved)	52.9	68.2	60.4
+rice straw (nonsterile)	11.4	3.9	19.3
+rice straw (autoclaved)	44.6	60.4	58.3

[a] Reduced by flooding with water for 30 days. [b] Insecticides applied: parathion, 345 μg; methyl parathion, 525 μg; fenitrothion, 575 μg.

soil provided by flooding at the time of insecticide addition as reflected in a recovery of ~97% of the original insecticide levels (Table III). In contrast, the three organophosphorus insecticides degraded fairly rapidly on their equilibration for 30 min with soil prereduced by 30-day flooding; interestingly, the degradation was more pronounced with soils amended with rice straw. The insecticides levels reached 4–19% of the original levels with rice straw, amended soil as compared to 34–70% with unamended soil at the end of 30-min equilibration. Addition of organic matter such as rice straw increases the microbial activity and hastens the drop in potentials in flooded soils (Ponnamperuma, 1972). More intense microbial activity and relatively low redox potentials (Table II) in rice straw amended soils probably favored the rapid destruction of the three insecticides. Evidence for microbial participation was provided when sterilization of the prereduced soil samples by autoclaving before equilibration with insecticides increased the stability of the insecticides (Table III).

In another experiment, rice straw amended and unamended alluvial soil samples were flooded for different periods (10–250 days) before treatment with fenitrothion, methyl parathion, and parathion for 5 s or 30 min. As in the previous experiment, rice straw amended soil effected more rapid transformation of all the three insecticides than the unamended soil. Thus, the interaction between reduced soil and organophosphorus insecticides led to a significant conversion of all parent molecules to their respective amino analogues even within 5 s of their equilibration with rice straw amended soil and after 30-min equilibration with unamended soil (Table IV). Also, rice straw amended and unamended soils differed considerably with regard to the period of flooding required to produce significant soil–insecticide interaction. Rice straw amended soil could effectively perform the nitro group conversion of the insecticides even after 10-day flooding as compared to the over 30-day flooding period required for unamended soil. Probably, nitro group reduction of these insecticides is favored by more reduced conditions in rice straw amended soil (Table II). But, redox potentials may not fully explain the rapid interaction between insecticides and rice straw amended soil, because 30-day flooded unamended soil with a potential of −160 mV was certainly less active in degrading the insecticides than 10-day flooded rice straw amended soil with a potential of −10 mV. It is likely that products of anaerobic decomposition of rice straw participated in the accelerated degradation of the insecticides in rice straw amended soil. The decrease in the concentration of insecticides upon their equilibration with preflooded soils was not always accompanied by the substantial formation of amino analogues. For example, insecticide levels declined after 5 s of contact with unamended soil flooded for different periods or after 30-min equilibration with unamended soil flooded for 10

Table IV. Degradation of Parathion, Methyl Parathion, and Fenitrothion on Their Equilibration with an Alluvial Soil Preflooded for Different Periods

treatments	μg of compound recovered/20 g of soil					
	from added parathion[a]		from added methyl parathion[a]		from added fenitrothion[a]	
	5 s	30 min	5 s	30 min	5 s	30 min
10-Day Preflooded Soil						
−rice straw	210.4 (0)[b]	165.4 (tr)	490.2 (0)	488.3 (tr)	478.0 (0)	463.7 (tr)
+rice straw	173.0 (54.9)	78.5 (119.0)	454.9 (50.0)	145.4 (182.5)	363.3 (0)	286.8 (57.2)
20-Day Preflooded Soil						
−rice straw	225.5 (0)	203.7 (0)	355.3 (0)	337.3 (0)	359.4 (0)	328.7 (0)
+rice straw	163.7 (68.8)	101.8 (127.8)	261.5 (42.5)	92.7 (182.5)	288.0 (tr)	189.5 (126.7)
30-Day Preflooded Soil						
−rice straw	200.0 (0)	171.0 (tr)	331.7 (tr)	350.8 (tr)	346.5 (0)	320.9 (tr)
+rice straw	140.7 (45.5)	39.2 (227.2)	306.2 (60.0)	19.2 (325.0)	308.0 (tr)	96.3 (203.1)
60-Day Preflooded Soil						
−rice straw	169.8 (tr)	143.0 (9.0)	305.6 (0)	298.7 (67.7)	330.0 (0)	310.9 (39.2)
+rice straw	136.9 (68.7)	19.0 (287.0)	276.9 (175.0)	10.7 (324.7)	294.0 (99.0)	67.4 (282.3)
90-Day Preflooded Soil						
−rice straw	201.6 (0)	194.3 (66.4)	391.8 (0)	220.8 (101.6)	348.0 (tr)	271.6 (98.0)
+rice straw	186.7 (28.4)	79.0 (156.8)	201.8 (162.5)	74.2 (262.6)	191.0 (145.0)	101.0 (247.9)
250-Day Preflooded Soil						
−rice straw	262.0 (0)	211.0 (39.4)	444.7 (0)	198.0 (96.7)	482.7 (0)	465.4 (tr)
+rice straw	269.2 (tr)	174.6 (49.2)	368.0 (tr)	136.7 (112.5)	399.4 (tr)	339.5 (30.6)

[a] Insecticides added: parathion, 345 μg; methyl parathion, 525 μg; fenitrothion, 575 μg. [b] Values in parentheses indicate the amino analogue of the respective insecticide; tr, trace.

or 20 days; however, neither amino analogues and other products sensitive to the P-specific detector nor hydrolysis products were detected. It is not clear, in the absence of isotope studies, whether this decrease in the levels of parent molecules was due to their binding by the soil or their conversion to other metabolites.

The degradation of fenitrothion and methyl parathion by hydrolysis in the first experiment (Table I) and by nitro group reduction in a subsequent experiment (Table IV) merits further discussion. The soil used in this study registered a redox potential of +100 mV after 10 days of flooding (at the time of insecticide addition) and −40 mV after 20 days of flooding (Table II). Evidently, significant nitro group reduction of parathion can occur at a potential of around −40 mV as reflected in the considerable accumulation of aminoparathion even at 6 days after application (16 days after flooding) in the first experiment (Table I). Under the same conditions, nitro group reduction of fenitrothion and methyl parathion was negligible. But, the same soil, with further reduction after prolonged flooding for 60–90 days, could effect substantial accumulation of amino analogues of fenitrothion and methyl parathion as it did with parathion (Table IV). Probably, nitro group reduction of dimethyl phosphorothioates occurs at a potential lower than that required for similar conversion in parathion. According to earlier reports, the degradation of fenitrothion in soils proceeds essentially by hydrolysis under nonflooded conditions (Takimoto et al., 1976; Spillner et al., 1979) and by nitro group reduction in predominantly anaerobic flooded soil (Takimoto et al., 1976). This is probably analogous to the hydrolysis of fenitrothion in flooded soil with relatively high potential in the first experiment (Table I) and its nitro group reduction under more reducing conditions (Table IV).

Of the two pathways implicated in the degradation of the organophosphorus insecticides used in this study, hydrolysis can be both chemical and microbiological while nitro group reduction is essentially microbiological. Chemical hydrolysis proceeds fairly rapidly under alkaline conditions in the order methyl parathion > fenitrothion > parathion (Munnecke, 1976). But, in the enzymatic hydrolysis, parathion was the least persistent when a cell-free suspension from a mixed bacterial culture readily hydrolyzed parathion but not methyl parathion and fenitrothion (Munnecke, 1976). Likewise, the data presented in this study showed that parathion is the least persistent of all three insecticides when the major pathway in their degradation is nitro group reduction. Thus, the relative stability of the selected organophosphorus insecticides is related to the pathway operating and the agents (chemical and/or microbial) involved in their degradation.

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Volatilization and Exudation Losses of Three *N*-Methylcarbamate Insecticides Applied Systemically to Rice

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Rice seedlings were treated by a root-soak systemic method or by foliage spray with carbofuran, carbaryl, or aldicarb. Distribution of residues was followed for 10 days in a small glass chamber provided with air flow and illumination for 12 h each day. The plant culture medium, plant parts, outflow vapor trap, and chamber walls were analyzed for parent carbamate. For carbofuran, physical loss of systemically absorbed residue occurred by root exudation (1775 μg or 35.6% of the initial residue in plant tissue) and volatilization (290 μg or 5.8% of the initial residue in plant tissue). Comparable data for carbaryl and aldicarb were 1928 μg (22%) and 2280 μg (14%), respectively, for root exudation and 367 μg (4.2%) and 920 μg (5.6%), respectively, for volatilization. A rapid translocation of all three insecticides to leaves occurred after treatment; residues moved through the leaf tip to the outside leaf surface by guttation and were available for volatilization. The relative importance of steps contributing to volatilization was related to insecticide stability, water solubility, and vapor pressure.

Systemic *N*-methylcarbamate insecticides such as carbofuran have been shown to be effective against a number of important rice pests when applied to paddy soil or water (Pathak and Dyck, 1973; International Rice Research Institute, 1975). Treatments which place carbofuran within or near the root zone of the rice plant favor absorption and provide more effective and longer lasting control of foliage feeding insects than that obtained with broadcast applications to the paddy water (Aquino and Pathak, 1976). An

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