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Persistence of Endrin in Indian Rice Soils under Flooded Conditions

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A radiotracer study was conducted to determine the relative persistence of endrin-^{14}C under flooded conditions in eight Indian rice soils. Endrin decomposed rapidly and reached low levels within 55 days in all soils except in a sandy soil. Interestingly, most rapid degradation occurred in pokkali soil despite its high salt content. The decrease in the total radioactivity partitioned in the chloroform–diethyl ether fraction was less pronounced despite the rapid decline in endrin levels indicating the formation of stable metabolites. Radioautography revealed that endrin was converted to six stable metabolites in all soils except in sandy and kari soils; five compounds were detected in kari soil and three compounds in sandy soil. More rapid degradation of endrin occurred in nonautoclaved samples of three soil types than in autoclaved samples indicating microbial participation in its degradation. The addition of rice straw enhanced the degradation of endrin. Liming the acid soils had no effect on the degradation rate of endrin.

Endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene), a chlorinated hydrocarbon insecticide, is highly effective against common rice insects. Its widespread use in agriculture has caused serious environmental hazards leading to its restricted use in several countries. Such restricted use of endrin could adversely affect the recent intensified efforts to increase food production in developing countries such as India, because substitutes for endrin are costly and often not very effective. Information concerning its fate and persistence in Indian rice soils under flooded conditions is, therefore, both useful and necessary.

Chlorinated hydrocarbon insecticides persist for several years in soils under nonflooded conditions (Edwards, 1972; Pionke and Chesters, 1973). In contrast, some of these chlorinated hydrocarbons such as benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane), DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane), methoxychlor (1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane), and heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,5,5a-tetrahydro-4,7-*endo*-methanoindene) readily break down in a predominantly anaerobic flooded soil ecosystem (Sethunathan, 1973a). Although the degradation of endrin in soil and aquatic systems and by microorganisms isolated from soil, fresh water, lake bottom sediments, and marine environments has been demonstrated (Patil et al., 1970, 1972; Guenzi et al., 1971; Matsumura and Boush, 1971), information on its fate and behavior in flooded soils is rather limited. In one instance, endrin was shown to persist in 3 out of 4 Philippine rice soils under flooded conditions (Castro and Yoshida, 1971). A radiotracer study was therefore conducted to determine the relative persistence of endrin in Indian rice soils including two organic acid sulfate saline soils under flooded conditions.

MATERIALS AND METHODS

Soils. Some of the characteristics of the soils used in this study are listed in Table I. Among the soils used, two saline acid sulfate soils from Kerala, South India, are unique rice soils of extreme acidity and locally known as kari and pokkali soils (Bloomfield and Coulter, 1973).

Labeled Endrin. Uniformly labeled endrin-*1,2,3,4,10*-^{14}C (specific activity, 7.44 mCi/mmol; 98% purity) was obtained from Mallinckrodt, Science Products Division, Radiochemical Department, St. Louis, Mo. The labeled endrin was dissolved in hexane (100 ml) after evaporating off the benzene carrier. An aliquot of the stock solution was evaporated to dryness and the residues were redissolved in ethanol prior to incorporation into the soils.

Soil Incubation Studies. The soils (20 g) were placed in test tubes (200 × 25 mm diameter). Labeled endrin was introduced to the soils in ethanol (0.1 ml) together with 0.5 mg of 95% technical endrin as carrier. The total radioactivity added to the soils was 113×10^4 cpm/20 g of soil. The soils were then flooded with distilled water (25 ml) and incubated at room temperature (28 ± 4 °C). At intervals, two replicate tubes were removed for analysis.

Effect of Rice Straw. Only alluvial soil from the Institute farm was used in this study. The soil (20 g) was thoroughly mixed with rice straw powder ground to pass through 100 mesh screen at 0.5% (w/w) level in test tubes (200 × 25 mm). The labeled endrin was introduced to the soils in ethanol (0.1 ml) with technical endrin (0.5 mg), and after 3 h the soils were flooded with distilled water (25 ml). At intervals, two replicate tubes were removed for analysis.

Effect of Liming. Alluvial soil from the experimental farm of Central Rice Research Institute and kari soil (20 g) were thoroughly mixed with 25 mg and 600 mg of $CaCO_3$, respectively, in test tubes. The labeled endrin was introduced to the soils in ethanol (0.1 ml) with 0.5 mg of technical endrin and, after 3 h, the soils were flooded with distilled water (25 ml). At intervals, two replicate tubes were removed for residue analysis.

Effect of Soil Autoclaving. A laterite and two alluvial soils were used. The soils (20 g) in the test tube (200 × 25 mm) were moistened with 5 ml of distilled water and autoclaved for 1 h at 15 psi on three alternate days. Labeled endrin in ethanol (0.1 ml) with technical endrin (0.5 mg) was introduced. After 3 h, the soils were flooded with distilled water (25 ml). At intervals, two replicate tubes were removed for residue analysis.

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Table I. Characteristics of the Soils Used in the Experiments

Characteristics	Soils							
	Alluvial (1)	Alluvial (2)	Laterite	Laterite clay loam	Red loam	Acid sulfate, saline (Pokkali)[a]	Acid sulfate saline (Kari)[b]	Sandy
Location	Central Rice Research Institute, Cuttack-6, Orissa	Sakhigopal, Orissa	Pattambi, Kerala	Bastar, Madhya-Pradesh	Chipilima, Orissa	Tellichery, Kerala	Vechoor, Kerala	Badagara, Kerala
pH[c]	6.2	4.8	5.0	5.0	5.9	4.2	3.0	6.0
Electrical conductivity[d]	0.6	1.80	0.2	0.81	0.46	8.5	15.0	0.4
Organic matter (%)	1.61	2.38	3.25	1.24	1.89	9.21	27.81	0.02
Total nitrogen (%)	0.09	0.10	0.09	0.09	0.06	0.24	0.36	0.002

[a,b] These unique acid sulfate saline rice soils are known by the local names pokkali and kari (Bloomfield and Coulter, 1973). [c] 1:1.25 soil-water slurry. [d] Electrical conductivity (mmhos/cm) of saturation extract of the soil.

Table II. Persistence of Endrin in Soils under Flooded Conditions

Incubation, days	Endrin[a] recovered, cpm × 10^4/20 g of soil							
	Alluvial (1)	Alluvial (2)	Laterite	Laterite clay loam	Red loam	Pokkali	Kari	Sandy
0	48.8 (68.7)[b]	40.7 (69.0)	48.3 (65.0)	68.8 (102.4)	58.3 (90.1)	35.2 (59.2)	39.0 (58.1)	67.5 (100.3)
25	32.6 (61.4)	16.2 (54.4)	20.4 (73.7)	44.7 (70.6)	58.1 (67.9)	8.4 (29.8)	28.6 (40.8)	58.1 (79.5)
55	5.3 (41.4)	2.3 (41.0)	2.4 (36.5)	23.4 (55.4)	20.2 (48.9)	2.2 (33.6)	12.0 (28.7)	41.5 (68.6)

[a] Endrin-^{14}C recovered after TLC separation of residues in chloroform–diethyl ether fraction. All figures are corrected for background radioactivity. [b] Figures in parentheses represent total radioactivity partitioned in the chloroform–diethyl ether fraction.

Extraction and Residue Analysis. Residues in the soils were extracted with chloroform–diethyl ether (1:1) three times as described earlier for parathion (Sethunathan, 1973b). The solvent extract was evaporated to dryness at room temperature. Residues were redissolved in methanol. An aliquot of this solution was added to 5 ml of liquid scintillator NE 213 (Nuclear Enterprises Limited, Sighthill, Edinburgh, Scotland) which consisted of 2,5-diphenyloxazole (5 g), 1,4-bis[2-(5-phenyloxazolyl)]benzene (0.3 g), and toluene (1000 ml) and the total radioactivity was determined in a liquid scintillation counter (Model LSS 20) (Electronics Corporation of India Ltd., Hyderabad, India). For quantitative determination of the parent compound, the residues dissolved in methanol were separated by TLC together with the authentic compound, employing acetone–hexane (5:95, v/v) as the developing agent. The authentic compounds were located by spraying with an ethanolic solution of 0.085% silver nitrate and 2.5% ammonium hydroxide and subsequent exposure to ultraviolet light. The silica gel areas of the samples in the chromatoplate opposite the authentic compounds were scraped off carefully and transferred to 5 ml of scintillation solution and the radioactivity was determined.

Radioautograph. The thin-layer chromatograms of endrin residues were exposed to Kodak x-ray no-screen film for 25–30 days in a Siemens metal casette.

Redox Potential (E_h) and pH Measurements. For E_h determination, soil samples (40 g) after appropriate amendments were flooded with 50 ml of distilled water in 100-ml beakers to provide the same soil–water ratio as used in the samples contained in test tubes. E_h of the soils was measured with a portable redox meter Model RM-IF (TOA Electronics Ltd., Tokyo, Japan) fitted with a compound platinum and calomel electrode, type CC-211. After E_h measurements, pH of the soil samples was determined.

RESULTS AND DISCUSSION

Persistence in Different Soils. Analysis after TLC separation of the residues partitioned in the solvent fraction showed that endrin concentration declined rapidly in all soils except sandy soil (Table II). Surprisingly, the decrease was most pronounced in the problem saline acid sulfate soil, pokkali, followed by alluvial (2) and laterite soils. In these soils, the concentration of the insecticide reached low levels of about 5% of the endrin initially recovered after 55 days. Degradation occurred also in red loam, laterite clay loam, and kari (acid sulfate saline) soils, but rather slowly, and about 34% of the added radioactivity was recovered as endrin from these soils even after 55 days. In sandy soil, however, very low degradation occurred during the 55-day incubation period perhaps due to low organic matter content and resulting low microbial activity. In the acid sulfate saline soils used, more rapid degradation of endrin in pokkali soil than in kari soil appeared to be due to a rise in pH from 4.2 to 6.7 within a few days after flooding in the former soil, providing a favorable condition for microbial activity. Despite highly acidic conditions with a pH of about 4.0 in kari soil, even after 55 days of flooding some degradation of endrin occurred. In contrast, γ and β isomers of benzene hexachloride, also a chlorinated hydrocarbon, persisted in this unique soil even under flooded conditions (Siddaramappa and Sethunathan, 1975). But these isomers disappeared rapidly from pokkali soil as noticed with endrin in this study.

It was interesting to note that the total radioactivity in the solvent fraction decreased in most soils during 55-day incubation, but not to the same extent as with endrin loss (Table II). This indicated that endrin was converted to more stable compounds which, like endrin, were partitioned into the chloroform–diethyl ether fraction.

Radioautography of thin-layer chromatograms of endrin residues from the soils revealed that endrin (R_f 0.67) was converted to six compounds (metabolites I to VI) in all soils under flooded conditions except in kari and sandy soils. The R_f values for metabolites I to VI were 0.00, 0.10, 0.22, 0.29, 0.37, and 0.87, respectively. These metabolites were formed in endrin-treated soils as early as 7 days after flooding and persisted even at the end of 55 days. Metabolites I, II, III, and VI were also formed in kari soil; in addition, a compound (Ia) with R_f of 0.07, which was not

Table III. Persistence of Endrin in Autoclaved and Nonautoclaved Soils under Flooded Conditions

Incubation, days	Endrin[a] recovered, cpm × 10^4/20 g of soil					
	Alluvial (1)		Alluvial (2)		Laterite	
	Autoclaved	Nonautoclaved	Autoclaved	Nonautoclaved	Autoclaved	Nonautoclaved
0	59.9 (74.1)[b]	51.6 (85.5)	58.6 (81.7)	32.1 (43.2)	57.5 (87.6)	46.3 (50.9)
7	31.0 (51.6)	32.1 (40.8)	18.9 (56.7)	14.9 (46.7)	21.7 (54.9)	20.7 (50.2)
25	25.8 (53.9)	12.9 (42.3)	23.3 (43.9)	5.2 (48.5)	29.4 (49.7)	12.9 (50.1)
55	24.2 (45.5)	1.3 (38.9)	17.8 (41.4)	2.4 (32.4)	18.5 (44.4)	1.2 (45.7)

[a] Endrin-^{14}C recovered after TLC separation of residues in a chloroform–diethyl ether fraction. [b] Figures in parentheses represent total radioactivity partitioned in the chloroform–diethyl ether fraction.

common in other soils, was also detected. In sandy soil, only metabolites I, Ia, and VI were evident during the 55-day incubation period.

The differential rates of endrin degradation in soils could not be explained entirely by pH changes since the pH of all soils except kari soil shifted to near neutral values within 2 to 3 weeks after flooding. The redox potentials (E_h) of most soils reached negative values of −120 to −160 mV within 2 to 3 weeks after flooding; only sandy and kari soils showed positive potentials of +70 and +255 mV, respectively, after 25 days of flooding. A low potential is known to favor anaerobic biodegradation of DDT (Guenzi et al., 1971; Glass, 1972; Parr and Smith, 1974) and γ and β isomers of benzene hexachloride (Siddaramappa and Sethunathan, 1975). In this study also, the degradation of endrin was rapid in soils capable of attaining negative potentials within a few days after flooding. However, some degradation occurred in sandy and kari soils despite positive potentials; it is not clear whether the degradation in these soils is chemical or biological.

Persistence in Autoclaved and Nonautoclaved Soils. To determine the role of microbial degradation of endrin in flooded soils, its relative persistence in autoclaved and nonautoclaved samples of three soil types (one laterite and two alluvial soils) was compared. More rapid degradation of endrin occurred in nonautoclaved samples than in autoclaved samples irrespective of the soils tested, indicating microbial participation (Table III). As in the earlier experiment, the insecticide reached negligible levels in 55 days in nonautoclaved soil samples. The insecticide decreased also in autoclaved soils, but relatively slowly and about 30.4 to 40.4% remained unaltered in all autoclaved samples after 55 days. This indicates that endrin degradation in flooded soils proceeds by both chemical and biological phenomena.

With regard to the total radioactivity recovered from the solvent extract, the difference between autoclaved and nonautoclaved samples was not as large as compared with differences in endrin concentration. Apparently, the chemical and biological breakdown products of endrin were more stable than the parent compound.

Radioautography revealed marked differences in the number of decomposition products of endrin in autoclaved and nonautoclaved soils. The nonautoclaved soils produced more breakdown products of endrin than autoclaved soils. Thus, metabolites I to VI were detected in all three nonautoclaved soils (Figure 1). In all autoclaved soils, compounds I and VI were also formed and one additional compound, Ia (R_f 0.07), was detected only in autoclaved soils. This indicated that metabolites II, III, IV, and V were products of microbial decomposition of endrin in the soils, whereas Ia detected only in autoclaved soils was formed by chemical reaction. It cannot be speculated from the present work whether compounds I and VI were produced by either chemical or biological means or by a combination of both in nonautoclaved soils. Degradation products of endrin formed in both autoclaved and nonautoclaved soils showed biological and chemical stability leading to considerable radioactivity in the solvent fraction even after 55 days despite a rapid loss of endrin. The degradation products could not be identified because of the nonavailability of authentic compounds.

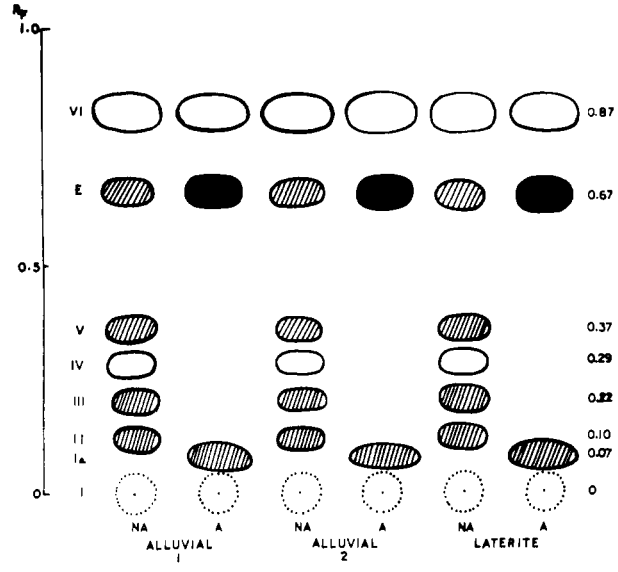

Figure 1. Thin-layer chromatogram of endrin-^{14}C and its metabolites formed after 25 days of incubation with nonautoclaved (NA) and autoclaved (A) soil samples under flooded conditions. Spot E represents undecomposed endrin: black spots show strongest radioactivity; shaded spots, medium radioactivity; open circles with solid line, weak radioactivity; circle with dotted line, very weak radioactivity.

Table IV. Effect of Rice Straw on the Persistence of Endrin in Flooded Alluvial Soil

Incubation, days	Radioactivity recovered, cpm × 10^4/20 g of soil			
	Rice straw amended soil		Unamended soil	
	Total[a]	Endrin[b]	Total[a]	Endrin[b]
0	82.5	47.5	68.7	48.8
25	44.8	15.8	61.4	32.6
55	34.5	2.7	41.4	5.3

[a] Total radioactivity partitioned in the chloroform–diethyl ether fraction. [b] Endrin-^{14}C recovered after TLC separation of residues in the chloroform–diethyl ether fraction.

Effect of Rice Straw. The addition of rice straw enhanced the degradation of endrin in a flooded alluvial soil. The decrease in endrin level in rice straw amended soil was more than twofold as compared to that in unamended soil at the end of 25 days (Table IV). The same trend continued until 55 days. Although the solvent fractions of both amended and unamended soils even after

25 and 55 days showed considerable radioactivity, a decrease in total radioactivity was more rapid with rice straw amendment. Evidently, rice straw accelerated the degradation of not only endrin, but also its breakdown products.

Effect of Liming. It was noticed earlier (Table II) that of the two acid sulfate soils used, the degradation of endrin was slow in kari soil in contrast to its rapid breakdown in pokkali soil, perhaps due to the extremely low pH of the former soil even under flooded conditions. Whether an increase in soil pH by liming would accelerate the degradation of endrin in kari and alluvial soils was studied. The radioactivity counts recorded for endrin in limed and unlimed kari soils were 23.2 and 21.7 cpm $\times 10^4$/20 g of soil after 25 days whereas the corresponding values for alluvial soils were 14.7 and 12.9. Similarly, with regard to total radioactivity recovered in the solvent fraction, no appreciable difference was noticed between limed and unlimed soils. Liming was thus not very effective in increasing the degradation rates of endrin and/or its breakdown products in both soils in spite of a favorable pH in limed soils resulting in an increase in bacterial numbers (Rao et al., 1975). Recent studies in this laboratory showed that both γ and β isomers of benzene hexachloride persisted in this unique acid sulfate soil under flooded conditions even after liming (Siddaramappa, 1975). In contrast, Parr and Smith (1974) reported that liming a muck soil from pH 5.3 to 6.8 caused a rapid conversion of DDT to DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane) in a moist anaerobic environment.

CONCLUSION

The data presented in this report demonstrate the instability of endrin in most Indian soils tested under flooded conditions in contrast to the reported stability of endrin in three out of four Philippine rice soils. Degradation of endrin in flooded soils is mediated by both chemical and biological means; biological degradation is more extensive. Interestingly, metabolites formed showed resistance to further degradation chemically or biologically. Unless these metabolites are characterized with regard to their chemical nature and toxicity and unless means of inactivating these metabolites in a flooded soil environment are developed, the use of endrin in rice poses problems of environmental pollution despite its rapid breakdown to several metabolites under flooded conditions. The rapid degradation of endrin in two unique acid sulfate saline rice soils, particularly in pokkali soil of Kerala, is of applied significance since substantial portions of rice soils in coastal areas of India are highly saline and data regarding pesticide behavior in such environments are rather limited.

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Electrochemical Reduction and Anaerobic Degradation of Lindane

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The anaerobic reduction of lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) in sewage sludge and soil is compared with its electrochemical reduction in dimethyl sulfoxide (Me_2SO). Benzene was found to be an anaerobic reduction product along with the previously recognized γ-BTC (γ-3,4,5,6-tetrachlorocyclohexene). The electrochemical reduction of lindane at a mercury coated platinum electrode has a single wave at −1.520 V vs. SCE. γ-BTC was found to be an intermediate in the reduction to benzene at this potential. No other intermediate chlorinated compounds were detected in either type of reduction. This is another example of the modeling of anaerobic reduction of halogenated compounds by electrochemical reduction.

Organochlorine insecticides, which have been used extensively for the last 25 years, are generally considered to be persistent compounds. However, Hill and McCarty (1967) reported that under anaerobic conditions (such as in sewage sludge, in the sediment of river or lake bottoms, or in the soil of flooded fields) some of the pesticides have very short lifetimes. Lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane), for instance, was shown to have a half-life of approximately 1 day.

In our laboratory we have been studying the electrochemical reduction of organochlorine compounds, especially those of environmental concern (Farwell et al., 1973, 1975a,b). During the course of these investigations we

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