Degradation of Lindane-^{14}C in a Mineral Soil and in an Organic Soil

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Lindane, γ-1,2,3,4,5,6-hexachlorocyclohexane (HCH), has been used, per se or as the active ingredient of BHC (benzene hexachloride, in agriculture and public health for nearly three decades as a soil insecticide, foliar spray, seed dressing and for protection of stored grains. Many reports in the literature have alluded to the persistence of lindane in soil, its translocation into crops and contamination of food and animals (ULMANN 1972). Numerous reports indicate that lindane is readily degraded by some insects BROOKS 1972, 1974), plants (SAHA and LEE 1974; KOHLI et al 1976a, 1976b), micro-organisms in synethetic media (MATSUMURA and BENEZET 1973; ELSNER 1972; HAIDER and JAGNOW 1975; ENGST et al 1974; and FRANCIS et al 1975) and animals (CHADWICK et al 1975; FREAL and CHADWICK 1973; KURIHARA 1975; SAHA and BURRAGE 1976) into γ-1,2,3,4,5,6,-hexachlorocyclohexene (HCCH), 2,3,4,5,6-pentachloro-2-cyclohexan-1-ol (PCCOL), γ-2,3,4,5,6,-pentachlorocyclohex-1-ene (PCCH), hexachlorobenzene, pentachlorobenzene, and various isomers of di-, tri-, and tetrachlorobenzenes and chlorophenols, apparently through more than one pathway. Some other reports were reviewed by MATHUR and SAHA (1975). Very little information, however, is available on the degradation of lindane in soil. YULE et al (1967) reported that γ-PCCH was the only identifiable product of a slow decomposition of lindane in soil. TSUKANO and KOBAYASHI (1972) found γ-BTC (γ-3,4,5,6-tetrachlorocyclohexene) in flooded rice field soil treated with lindane. Previous work in this laboratory has shown that lindane is slowly biodegraded to γ-PCCH, γ-BTC, 1,2,4-trichlorobenzene (TCB) 1,2,3,5- and/or 1,2,4,5-tetrachlorobenzene (TtCB) and 1,2,3,4-TtCB in a flooded sandy loam soil (MATHUR and SAHA 1975).

It is well known that soil organic matter content plays an important role in determining the behavior of pesticides in soil (MATHUR et al 1976a, 1976b). Thus pesticides persist longer in organic than in mineral soils (HARRIS and MILES 1975), and their bioavailability is curtailed by adsorption on soil humic compounds. However, it is not known whether there are any qualitative differences in the biodegradation products of pesticides in organic soil as compared to those in mineral soil. This investigation reports on a study on the identification of degradation products of lindane in an organic soil and in a mineral soil, both under aerobic and submerged conditions.

MATERIALS AND METHODS

All solvents were nanogen grade; obtained from Burdick and Jackson Laboratories and used as received. γ-PCCH was prepared in this

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laboratory according to KARAPALLY et al (1973). The reference chlorobenzenes were obtained from commercial sources and γ-BTC from Prof. Norio Kurihara, Radioisotope Research Center, Kyoto University, Kyoto, Japan.

Uniformly labeled lindane-^{14}C (sp. act. 129 μc/mg), obtained from Amersham Searle Co., Toronto, was purified by thin layer chromatography (TLC) on silica gel plates, using 5% acetone in hexane as the developing solvent. The purified material contained more than 99.9% lindane, as indicated by TLC and autoradiography and also by electron capture gas chromatography (ECGC). The purified lindane-^{14}C was diluted with pure non-labeled lindane to give a specific activity of 227 mμc/mg.

Treatment of Soils

The mineral soil used here and its source were described by MATHUR and SAHA (1975), and the organic soil by MATHUR et al (1976a). The plot used as source of the organic soil had not been treated with lindane for the last 10 years and no pesticide during the year the samples for this study were collected (September, 1974).

Four 500 ml wide-mouth Erlenmeyer flasks plugged with cotton were used. Two of these flasks contained 100g portions of the mineral soil while the other two each contained 100g of the organic soil. Both soils had been air-dried and pulverized to pass a 2 mm screen. To each of the four flasks was added an aqueous suspension of 3.27 mg of lindane-^{14}C in 10 ml distilled water containing one drop of Triton X-100, and mixed thoroughly. Soil blanks, not treated with lindane, were carried separately. Enough water was added to two of the treated soil batches (one mineral and the other organic) to maintain them at 2/3 of their respective field capacities. The other two soil samples were maintained under submerged conditions by adding enough water to keep 1 cm thick layers of liquid over them.

All the treated soils were allowed to stand at room temperature in dark for 8 weeks. Water was added periodically to maintain initial weights.

Extraction of soils

At the end of the incubation period, contents of the flasks were transferred to centrifuge bottles with jets of water, and centrifuged. The supernatants were poured into separatory funnels and extracted with three 50 ml portions of pentane. The residual soil was extracted 3 times in a mechanical shaker with 150, 100 and 100 ml of pentane/acetone mixture (1:1). Organic soils were extracted six times. The pentane/acetone extract was diluted with 3 times its volume of 1% sodium cloride solution and extracted with 3 x 150 ml pentane. The pentane extracts were combined with that obtained earlier from the extraction of water, dried (anhyd. Na_2SO_4) and concentrated to less than 10 ml by distillation, using a Snyder column. The concentrated solution was made up to 10 ml in a volumetric flask with hexane and two 0.5 ml aliquots were counted for ^{14}C-activity in a scintillation counter. The remaining 9 ml of the solution was chromatographed on a 20 g Florisil (containing 3% water) column and successively eluted with 100 ml pentane, 250 ml 6% ether in pentane and 100 ml of ether. Three fractions, 100, 250, and about 120 ml were collected by letting the column run dry finally. Each fraction was concentrated to less than 100 ml by distillation through a Snyder column, made upto 100 ml in volumetric flasks and their radioactivity contents determined. These fractions were then analyzed by electron capture gas chromatography (ECGC) and gas chromatography/mass spectrometry (GC/MS), after further concentration to suitable volumes.

Gas Chromatography

A Micro Tek gas chromatograph equipped with ^{63}Ni detector was used for ECGC. The column was a 3' x ¼" o.d. glass tube packed with 100-200 mesh gas chrom Q coated with 3% OV-17. The flow rate of the carrier gas (nitrogen) was 45 ml/min and the detector temperature was 224°C. The column oven temperatures were 110°C for the determination of chlorobenzenes; 130°C for γ-PCCH and γ-BTC; and 170°C for lindane.

For gas chromatography/mass spectrometry (GC/MS), a Finnigan gas chromatograph Model 9500 was interfaced to a Finnigan quadrupole mass spectrometer Model 3100 D by a single stage glass jet separator. Data were recorded on a Finnigan Model 600 data acquisition system. A 6ft. x ¼" glass column packed with 100 to 120 mesh Gas Chrom Q coated with 3% OV-17 was used as the gas chromatographic column. The flow rate of the carrier gas, N_2, was 10 ml/min. The column was operated isothermally for 5 min at 80°C and then temperature programmed to 250°C at the rate of 10°/min. A mixture of the suspected degradation products was prepared from standards and the mass spectra recorded under the above conditions.

^{14}C-counting

A Picker Nuclear Ansitron Model II liquid scintillation counter was used. Aqueous samples were counted in 10 ml Aquasol (New England Nuclear, Boston, Mass). Non-aqueous samples were counted in 10 ml toluene containing 0.25g PPO/litre of toluene. Quench corrections were made either by the channels ratio method or by the internal standard method and all counts were corrected for the background.

RESULTS AND DISCUSSION

The recovery of ^{14}C-labeled residues from the two moist soils was nearly 100%, considering the ±2% statistical errors involved in counting radioactivity (Table I). But recoveries of the ^{14}C-activity from the two submerged soils were lower (ca.90%) indicating that some ^{14}C-labeled residues from these soils were not extracted or that some degradation products escaped from the flasks.

The formation of lindane degradation products which were "unextractable" from soil was noted by KOHLI et al (1976b).

TABLE I

Percent distribution of added radioactivity and lindane (in parentheses) in soil extracts and their fractions from column chromatography

	Soil			
	Moist		Submerged	
	Organic	Mineral	Organic	Mineral
Aqueous phase	0.2	0.2	0.4	0.4
Organic phase	103.7 (89.0)	100.3 (84.3)	92.1 (85.7)	88.0 (70.5)
Pentane eluate (of Florisil column)	21.5 (8.2)	18.2 (9.9)	13.4 (8.7)	20.9 (8.4)
6% Ether in pentane eluate	81.2 (80.7)	75.9 (73.2)	77.5 (76.3)	62.3 (60.9)
Ether eluate	0.4 (0.1)	1.6 (1.2)	0.9 (0.7)	2.0 (1.3)

Very little (less than 0.4%) radioactivity was present in the aqueous phase when the acetone/pentane extracts of the soil treatments were partitioned into water and pentane, indicating that hydrophilic degradation products, such as chlorophenols, were not produced in significant amounts at least in the moist soils. In the submerged soils, hydrophilic metabolites, if formed, were rapidly utilized or immobilized by humus (MATHUR and MORLEY 1975, HSU and BARTHA 1974, MATHUR and SAHA 1975). In the column clean-up procedure, about 62 to 81% of the radioactivity in the extract was eluted in the second fraction (6% diethyl ether in pentane). Most of the remaining radioactivity was present in the first fraction (pentane). The last fraction (ether) had very little radioactivity. Analyses of the second fractions by gas chromatography indicated that virtually all the radioactivity present here was due to lindane-^{14}C.

More lindane was recovered from the organic soil than from the corresponding mineral soil. Thus after incubation for 8 weeks about 89% and 86% of the added lindane-^{14}C were recovered from the moist and submerged organic soils, respectively, as compared to 84% and 70% from the corresponding mineral soil treatments (Table I), suggesting that lindane was biodegraded at a slower rate in the organic soil than in the mineral soil. This may be at least partly due to lower bioavailability of lindane in organic soil due to stronger adsorption by the humus in this soil. This was perhaps also responsible for the need to extract the organic soil six times as compared to three times in the case of the mineral soil.

The gas chromatograms of the first fractions (pentane eluate of Florisil column) on the OV-17 column at 110° and 130°C indicated the presence of chlorobenzenes, γ-PCCH and γ-BTC (Table II) in the four soil extracts. The identities of these metabolites were proved by gas chromatography/mass spectrometry as stated in a previous study with the mineral soil (MATHUR and SAHA 1975). In addition to the products previously reported (ibid) it appeared that m and/or p-dichlorobenzene (DCB), 1,3,5-TCB and pentachlorobenzene were also produced during lindane degradation in soils. All of these compounds have been reported previously as metabolites of lindane in insects, plants, micro-organisms or animals (SIEPER 1972, BROOKS 1974, KOHLI et al 1976a, 1976b, CHADWICK et al 1975 and ENGST et al 1974).

There were differences in the metabolites detected in the mineral and organic soils incubated under moist and submerged conditions. In the previous study, 1,2,4-TCB, 1,2,3,5 and/or 1,2,4,5-TtCB, 1,2,3,4-TtCB, γ-BTC and γ-PCCH were detected in a submerged mineral soil incubated for 6 weeks (MATHUR and SAHA 1975). In the present study with the same soil for 8 weeks, 1,2,3,5-and/or 1,2,4,5-TtCB were not detected. Instead, small amounts of m and/or p-DCB and 1,3,5-TCB were present. Dichlorobenzene(s) may be formed from further degradation of product(s) formed earlier.

Although, in keeping with several previous studies (SIEPER 1972), more lindane was degraded in submerged mineral and organic soils (30 and 15%) than in moist soils (16 and 11%) such a difference was prominent only in the mineral soil. Due to the greater potential for biological activity in organic soils, these are more likely than mineral soils to contain microenvironments which are depleted in oxygen, thus allowing some anaerobic bacteria to be active in such sites. Anaerobes have been attributed to be mainly responsible for the degradation of lindane in soil (SIEPER 1972, SETHUNATHAN and YOSHIDA 1973).

As expected, the microflora of organic and mineral soils seem to follow similar pathways for degrading lindane. However, the amount and variety of metabolites present at any stage may be different in the two types of soils e.g. pentachlorobenzene appeared to be present only in the organic soil.

Evidence presented here supports the suggestion (MATHUR and SAHA 1975) that lindane metabolism in soil is as complex as in animals, insects and plants. It also confirms the report made by YULE et al (1967) that γ-PCCH is the main metabolite of lindane present in an aerobic (moist) mineral soil. In a moist organic soil, however, other metabolites are also present in considerable proportions.

TABLE II

Percent distribution of total metabolites detected in 100g portions of soils treated with 3.27 mg lindane-^{14}C

Metabolites*	rt.**	Soil			
		Moist		Submerged	
	min.	Organic	Mineral	Organic	Mineral
m &/or p-DCB	0.85	32.0	3.1	33.3	1.2
1,3,5-TCB	1.60	8.0	ND	5.6	10.2
1,2,4-TCB	2.45	1.3	ND	2.5	13.4
1,2,3,5-&/or 1,2,4,5-TtCB	5.60	2.3	ND	1.8	ND
1,2,3,4-TtCB	8.00	ND	ND	ND	2.7
PtCB	16.80	4.2	ND	1.7	ND
γ-PCCH	4.25	36.9	96.0	45.8	63.8
γ-BTC	4.50	21.7	0.9	9.2	8.8

* DCB = dichlorobenzene; TCB = trichlorobenzene; TtCB = tetrachlorobenzene; PtCB = pentachlorobenzene; PCCH = 1,2,3,4,5-pentachlorocyclohex-1-ene; BTC = 3,4,5,6-tetrachlorocyclohexene.

** retention times at column temperature 110°C for chlorobenzenes and 130°C for γ-PCCH and γ-BTC on 3% OV-17 on chrom Q.

ND - not detectable

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