Fate of Insecticide Residues. Decomposition of Lindane in Soil

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A mixture of soils was fortified with DDT, dieldrin, lindane, and heptachlor and was analyzed monthly for qualitative changes. Only lindane decomposed during 6 months' exposure in percolated and standing moist soil. The breakdown product was analyzed and identified chromatographically as γ-pentachlorocyclohexene, which was found by insect bioassay to be of the order of 1000 times less toxic than the parent lindane. The dehydrochlorination of lindane was 2 to 3 times greater in moist, acidic-to-neutral soil than in dry soil, and there was some experimental evidence that soil microorganisms were associated with the breakdown. However, specific in vitro microbial tests were inconclusive on this point.

Persistent organochlorine insecticides have been used extensively for more than two decades for crop protection purposes, and their residues are commonly found in the soil. However, knowledge of the fate of the more persistent chlorinated insecticides such as DDT, dieldrin, lindane, and heptachlor in the soil complex is only fragmentary (Edwards, 1966). While this situation may be attributed partly to emphasis on methodology required to implement regulatory legislation on foodstuffs, a contributory factor is undoubtedly a lack of information on the nature of transformation products.

As part of a project aimed at the systematic evaluation and development of more comprehensive methods for the analysis of residues in soils, the authors have established that lindane (γ isomer of hexachlorocyclohexane) will decompose to a less toxic material in soil. The formation of a less toxic material from lindane in soil had been postulated previously as a result of discrepancies between biological and chemical methods of assay (Edwards *et al.*, 1957; Lichtenstein and Schulz, 1959).

The breakdown product has now been identified as γ-pentachlorocyclohexene (PCCH); its toxicity for insects has been found to be of the order of 1/1000 of that of the parent lindane. Furthermore, some evidence has been obtained that a microbial agency may be involved in the decomposition process in soil.

MATERIALS AND METHODS

Soils. All the soil experiments reported here were made with a mixture of five arable soils collected in Ontario (Table I) and stored in a moist condition in a plastic bag refrigerated at 1° C. A mixture was used with the hope of providing a large variety of microorganisms for this study of insecticide behavior. The soil mixture had a pH of 6.8 as determined by a glass electrode method (Atkinson *et al.*, 1958), and chromatographic analyses (Figure 1, Table III) showed it to be free from significant amounts of pesticides.

Solvents. Extra-pure solvents were used in fortifying, extracting, chromatography, and dilution—e.g., Burdick and Jackson Laboratories, Inc., distilled in glass grade.

Fortification of Soils. Soils were fortified with concentrated petroleum ether solutions of Entomological Society of America standard grades of DDT, dieldrin, heptachlor, and lindane, using a rotary evaporator. The lindane standard, labeled as 100% pure, was found by gas chromatographic analysis (GC) to have traces of impurities, including PCCH, and is considered here as 99+% purity.

Preparation of PCCH. The method of alkaline monodehydrochlorination of lindane (Nakajima *et al.*, 1949) was used to prepare PCCH. The product was then purified twice by preparative scale thin-layer chromatography (TLC) using silica gel (Morley and Chiba, 1964) to give a sample of PCCH of 99.9+% purity. This material was used for bioassay tests and as a chromatographic standard.

Extraction Techniques. Microbial cultures and water perfusates (aqueous systems) were extracted with chloroform-methanol according to the method of Bligh and Dyer (1959) for analysis by TLC. For analysis by GC with electron capture detector, soils were extracted with acetone-hexane (1 to 1, v./v.) for qualitative detection of PCCH (Table II). However, acetone residues remaining in the final hexane solution interfered with quantitative estimation of PCCH (Table III), and for this purpose a 1 to 1 (v./v.) solution of acetonitrile-hexane was used for extracting soils.

The general extraction procedure comprised vigorous blending for a total of 10 minutes of from 4 to 10 parts of solvent mixture per 1 part of soil or aqueous medium

Table I. Composition of Soils Mixture Used in Experiments

Soil Type	Source (in Ontario)	Percentage Composition by Weight of Fresh, Moist Soil
Muck	Chatham	40
Clyde loam	Chatham	5
Rubicon sandy loam	Ottawa	40
Lyons loam	Ottawa	5
North Gower clay loam	Ottawa	10

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Table II. Gas Chromatographic Data to Check and Identify γ-PCCH from Different Sources

(Soil extracts, 1 to 1 acetone-hexane)

Column Conditions			Actual Retention Times, Min.			Relative Retention Times		
Packing and size	Operating temp., ° C.	N_2 flow rate, ml./min.	A, PCCH in soil	B, PCCH synthetic	C, Lindane standard	A/C	B/C	γ-PCCH/ Lindane[a]
5% SE-30 on 60–80 Chromosorb W 5 foot × 1/8 inch, SS	160	45	1.4	1.4	4.2	0.33	0.33	0.34
5% Epon 1001 on 60–80 Gas-Chrom Z 5 foot × 1/8 inch, Al	160	47	1.3	1.3	8.2	0.16	0.16	0.16
10% QF-1 on 60–80 Chromosorb W 5 foot × 1/8 inch, Al	157	45	0.78	0.78	3.0	0.26	0.26	...

Aerograph 204, electron capture detector; detector temp., 197° C.; injector temp., 218° C.; recorder, Westronic LD 11A

[a] From Ishida and Dahm, 1965b.

using a Sorvall Omni-Mix. Extraction was followed by filtration through paper supporting a pad of Supercel, washing twice with at least double the volume of water, separation of immiscible layers with quantitative solvent rinsings at transfer stages, and a final drying with sodium sulfate.

Analytical Techniques. TLC was carried out by the method of Morley and Chiba (1964), using 5 to 10% v./v. acetone in *n*-hexane as developing solvent. GC analysis was made using an Aerograph Model 204 instrument fitted with an electron capture detector. Operating conditions for particular GC analyses are given in Tables II and III.

Soil bioassays were carried out according to the direct method of Edwards *et al.* (1957) using Drosophila. The potencies of lindane and PCCH were compared by a dry-film technique with Drosophila (Yule, 1965) and by topical dosing with acetone solutions to female houseflies (Yule and Smith, 1967).

EXPERIMENTS AND RESULTS

Percolated Soils. Fresh, moist samples of the soil mixtures were fortified with the four insecticides to give an oven-dry concentration of 0.1% by weight, and 30-gram aliquots were loaded into automatic percolating devices (Lees, 1947). The soils were continuously perfused with aerated water for 6 months at room temperature to accelerate chemical and biochemical activity. Half the volume of perfusate was taken monthly, extracted with chloroform-methanol, and analyzed by TLC, while the percolators were made to volume with distilled water and restarted. After 1 month's percolation, the TLC chromatograph of the lindane-fortified soil was found to contain an extra spot with higher R_f than lindane when compared with the chromatographed unfortified soil and lindane standard. This extra TLC spot in the lindane soil persisted and increased in density relative to lindane at the 2- and 3-month sampling and analysis, while none of the other insecticides changed. This result indicated that lindane was being broken down progressively in soil.

Standing Soils. Aliquots of the same fortified soil were held at the original moisture content (20%) in a ventilated jar for 6 months to parallel the percolator experiment. The soils were then extracted with acetone-hexane and analyzed by TLC and GC. The TLC analysis (Figure 1) confirmed that of the four insecticides under study, only lindane had changed qualitatively in the soil mixture (the same results were obtained at 12 months). The R_f of this lindane product corresponded to that of the material obtained in the perfusion experiment, and to PCCH on TLC (Figure 1). GC analysis confirmed the identification of the new material according to its retention time relative to lindane on three different columns (Table II) (Ishida and Dahm, 1965b).

Microbiological Tests. Lindane thus appeared to be readily dehydrochlorinated in acidic-to-neutral soil and in water at pH 8 (Figure 2), although not in burnt-sand perfusions nor in water at the pH range (6 to 7) of the soil nor in solutions kept in daylight and darkness for 3 months to parallel the percolator treatment. For these reasons, a microbial agent was suspected of the decomposition. To check this possibility, pinches of the percolated soil were inoculated into a basal mineral medium (Tonomura *et al.*, 1965) with lindane as the sole C source. By weekly subculturing through this same medium fortified with either glucose or a water extract of soil (pH < 7), and by selective plating and separation of macroscopically different colonies, two species of soil bacteria were isolated which appeared to be capable of dehydrochlorinating lindane in vitro (Figure 2). These were identified as *Bacillus cereus* (also *B. cereus* variety *mycoides*), and a so-far unknown species of Bacillus.

The results of these microbiological tests obtained by TLC analysis of chloroform extracts of broth cultures (Figure 2) were not confirmed by GC analysis of acetone-hexane extractions of further cultures. Furthermore, when the above specific bacterial cultures were fortified at 4 p.p.m. with C^{14}-labeled lindane, no evidence of a radioactive metabolite in the R_f position of PCCH was found by autoradiography of a TLC plate.

Comparison of PCCH Production in Moist and Dry Soils. Since the results of the pure culture tests were inconclusive, an indirect test of microbial involvement was made. Lichtenstein and Schulz (1960, 1964) have reported that aldrin, heptachlor, lindane, and parathion were less persistent in normal wet soil than in autoclaved wet soil or normal dry soil. They interpreted that these in-

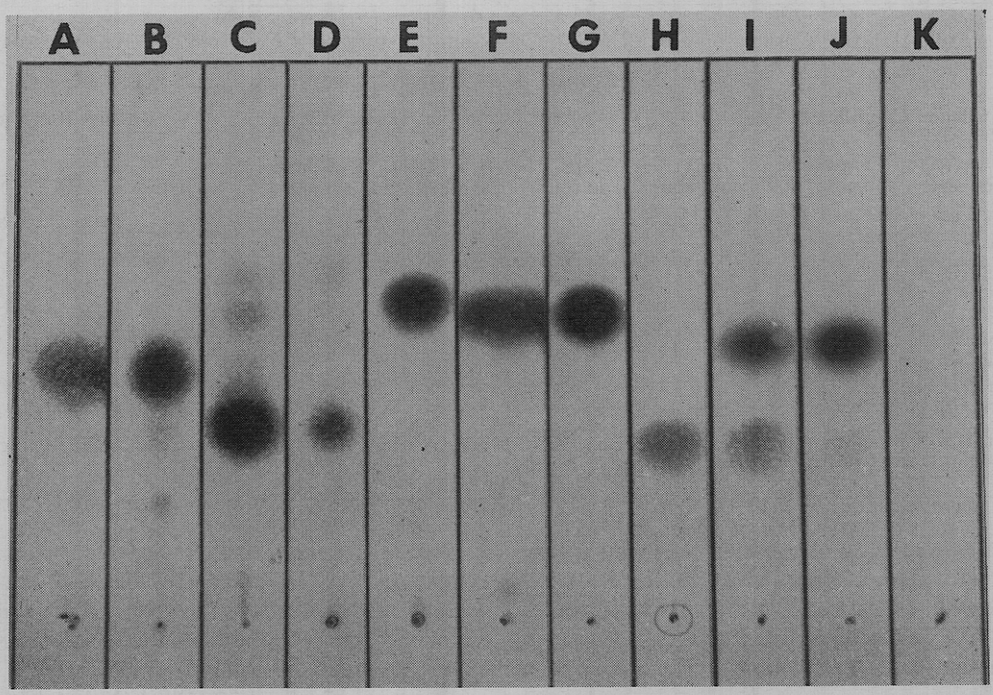

Figure 1. Thin-layer chromatogram of acetone-hexane extracts showing four insecticides after standing 6 months in moist soil with standards

A. DDT soil
B. *p,p'*-DDT standard, 20 μg.
C. Dieldrin soil
D. Dieldrin 85% standard, 40 μg.
E. Aldrin 95% standard, 20 μg.
F. Heptachlor soil
G. Heptachlor 99% standard
H. Lindane 100% standard, 50 μg.
I. Lindane soil
J. PCCH standard
K. Unfortified soil

A and *F*. 0.016 gram air-dry soil/spot. *C* and *I*. 0.08 gram air-dry soil/spot
Extraction by 50% acetone-hexane (v./v.). Developing solvent 5% acetone-hexane (v./v.)

secticides persist longer in soil under conditions where the microbial numbers and/or their metabolic activity are relatively low. Hence, the rate and scale of breakdown of lindane to PCCH in moist and dry soil were compared. The authors varied the moisture content of the soil to avoid physical and chemical changes that could be produced in moist soil (and lindane) by partial sterilization using steam, irradiation, or chemical techniques.

The original moist soil mixture (pH 6.8) was air-dried to give a water content of 5.2%, and 500 grams of this and of an aliquot moistened to 22.4% water by weight were fortified with lindane at 10 p.p.m. and held for 2 months in darkness at room temperature in a stoppered 1-liter flask. Five-gram samples were taken from the bulk at predetermined time intervals, held separately in sealed jars at −20° C., extracted with acetonitrile-hexane, and analyzed by GC. The lindane concentration appeared to have decreased a little, while the amount of PCCH was 2 to 3 times greater at each time interval in the moist soil than in the dry soil (Table III).

Bioassay Tests. The purified synthetic PCCH was approximately 1/1000 as potent as the parent lindane against Drosophila and Musca (Metcalf, 1955).

Replicates of 5-gram samples of the moist and dry soils (Table III) were bioassayed directly with Drosophila. However, only very low mortalities were produced after 48 hours exposure, although chemical analysis showed that at least 7 p.p.m. of lindane was present in the soils

Table III. Lindane Breakdown in Dry and Moist Soils
(GC analysis of 1 to 1 acetonitrile-hexane extracts)

Incubation Period, Days	γ-PCCH, P.P.M.	Lindane, P.P.M.
	Dry Soil	
0	Trace (<0.01)	7.6
8	0.033	7.4
16	0.070	7.6
32	0.16	7.2
64	0.20	7.2
	Moist Soil	
0	Trace	8.6
8	0.10	7.2
16	0.18	7.2
32	0.36	7.6
64	0.61	7.0

Column. QF-1, 5% on Chromosorb W, 60 to 80 mesh
Standard.[a] PCCH, 0.028 μg./ml., 5.0-μl. injection
Lindane, 0.020 μg./ml., 2.0-μl. injection
Sample.[a] PCCH, 5 grams of soil/200 ml. of soln., 5.0-μl. injection
Lindane, 1 gram of soil/200 ml. of soln., 2.0-μl. injection
Conditions. C, 172° C.; D, 207° C.; I, 210° C.
Attenuator 4 × at E.C.1
N_2 flow rate:
column, 30 ml./min.
detector, 60 ml./min.

[a] Lindane and PCCH were determined separately because the concentrations and sensitivities of these two compounds were very different.

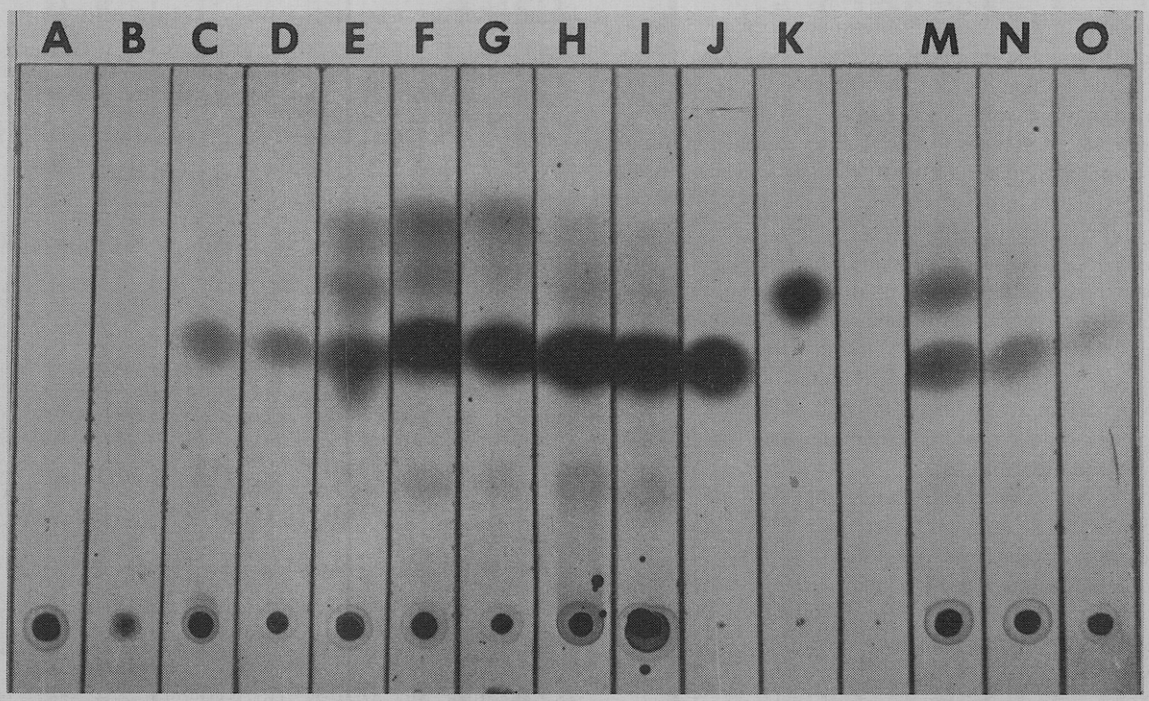

Figure 2. Thin-layer chromatogram of chloroform-methanol extracts of cultures of bacteria isolated from soil showing bacterial and chemical breakdown of lindane to PCCH after 3 weeks' incubation at 25° C.

A. culture medium (Tonomura *et al.*, 1965) + 0.1% glucose
B. *A* + bacterium of *I*
C. *A* + lindane, sterile
D. *A* + lindane, sterile
E. *A* + lindane + *B. cereus*
F. *A* + lindane + *Bacillus* sp.?
G. *A* + lindane + *B. cereus* var. *mycoides*
H. *A* + lindane + museum specimen of *E*
I. *A* + lindane + museum specimen of *G*
J. Lindane standard, 100 μg.
K. PCCH standard
M. Buffered water + lindane, pH 8.0
N. Buffered water + lindane, pH 7.0
O. Buffered water + lindane, pH 6.0

Chloroform extract of 25 ml. medium applied per spot. Developing solvent 10% acetone-hexane

(Table III). Apparently, the soil mixture deactivated (Edwards *et al.*, 1957; Harris, 1966) the added lindane to a greater extent than could be explained by the quantity converted to PCCH.

DISCUSSION

Lindane is, in general, only moderately persistent in soils compared with other chlorinated insecticides, and this property may be associated with its relative volatility and solubility in water (Edwards, 1966). However, Edwards *et al.* (1957) and Lichtenstein and Schulz (1959) found a large discrepancy between bioassay and chemical analysis of lindane residues in soils and proposed that some breakdown of lindane to a less toxic material was taking place.

The present authors have found that lindane breaks down to γ-PCCH in a soil mixture, which is approximately 1/1000 as toxic as the parent lindane. Since γ-PCCH would also react positively (Bradbury and Standen, 1957) in the colorimetric tests used in the earlier soil analyses (Edwards *et al.*, 1957; Lichtenstein and Schulz, 1959), the same breakdown process probably was involved. The scale of detoxification of lindane in soil found by these authors appears to be much larger than in the present case (Table III). However, the soils and experimental conditions were different in the three studies, and there is little indication of the field significance of the 9% conversion of lindane that occurred under laboratory conditions in the present study. Furthermore, the lindane-PCCH conversion might be supplemented by chemical breakdown in alkaline soils (Figure 2).

Lindane is detoxified mainly by an enzymatic process through PCCH to more polar metabolites and conjugates in several Arthropod species (Bradbury, 1957; Clark *et al.*, 1966; Ishida and Dahm, 1965a; Sternburg and Kearns, 1956) and the rat (Grover and Sims, 1965). The present study has shown that lindane can be dehydrochlorinated to PCCH in moist, acidic-to-neutral soils.

However, no evidence of further breakdown products such as tri- and dichlorobenzenes in the organic phase of soil extractions has been found by TLC (Figure 2) or under gas chromatographic conditions set for detecting PCCH and lindane.

The conflicting analytical results obtained with microbial cultures may be due to technique differences in extraction and detection with the aqueous systems. Or, the occurrence of PCCH in vitro may be dependent on the microbial population and metabolic activity, which were largely uncontrolled in these tests. However, lindane appeared (TLC) to be metabolized in at least one in vitro test (Figure 2), and there also is indirect evidence that soil microorganisms are involved in the decomposition of lindane in soil. The named species of bacteria were isolated from soil where the lindane breakdown was shown chemically (TLC) to be in progress, and these nonautotrophic or-

ganisms could survive and multiply in an inorganic culture medium with lindane supplied as the sole C source. Furthermore, the lindane-PCCH reaction was increased and accelerated in moist soil compared to dry soil held in darkness (Table III). Stevenson (1956) showed that microbial numbers and activity are generally much lower in drier soils, and since lindane was found previously not to be dehydrochlorinated in water at pH 6 to 7 over 3 weeks (Figure 2) or 3 months (burnt-sand percolators), the evidence provided by this latter soil comparison supports the hypothesis that a microbial agency is involved in the decomposition of lindane to PCCH in soil. Recently, Raghu and MacRae (1966) found by GLC analysis that γ-BHC is more persistent in sterilized flooded soils from Philippine rice fields than in unsterilized flooded soils and propose that biodegradation may occur.

In conclusion, this work shows that lindane can be dehydrochlorinated and detoxified to γ-PCCH in moist, acidic-to-neutral soils, and the authors propose that certain soil bacteria may be associated with the breakdown. Direct evidence about microbial involvement could be provided only by controlled biochemical tests (Tonomura *et al.*, 1965).

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