Degradation of Lindane by *Escherichia coli*

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Received for publication 20 November 1974

Lindane was degraded by *Escherichia coli* isolated from rat feces. About 10% of the added lindane was metabolized by the bacterium in Trypticase soy broth containing the pesticide. A single metabolite, 2,3,4,5,6-pentachloro-1-cyclohexene, was detected and identified by gas chromatography and mass spectrometry.

There have been many studies on the degradation of lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) in plants, insects, mammals, and soil and on the subsequent identification of lindane metabolites (4). A *Clostridium* sp. isolated from flooded soils is reported to degrade lindane anaerobically in pure culture within 27 h (2), but no metabolic pathway has been suggested and none of the intermediates have been identified. Other studies also have indicated that lindane does not persist in anaerobic soil environments (3, 5). Yule et al. (6) reported that γ-pentachlorocyclohexene is formed from the metabolism of lindane in soil and suggested that soil microorganisms are associated with the breakdown. However, their specific in vitro microbial tests were inconclusive.

Our attempts to isolate lindane-degrading microorganisms from soil and sewage samples by the enrichment culture technique had been unsuccessful. The recently reported ability of mammalian systems (1) to dehalogenate chlorinated compounds prompted us to screen for microorganisms from rat feces that could dehalogenate and metabolize lindane.

A diluted suspension of a rat feces samples obtained from the animal research laboratory at the Stanford Research Institute was incubated in Trypticase soy broth containing 0.04% lindane. After 4 days of incubation at 28 C on a shaker, 1 ml of the mixed culture sample was transferred to fresh medium containing lindane and incubated for 4 more days. A sample of this culture was streaked on Trypticase soy agar plates, and several pure cultures were isolated. The pure cultures then were tested for their ability to metabolize lindane in Trypticase soy broth. The preliminary results indicated that one isolate was capable of metabolizing lindane, as evidenced by new peaks and decrease in lindane concentration in the gas chromatograms.

One-half milliliter of a 2-day-old lindane-metabolizing culture was transferred to several 250-ml Erlenmeyer flasks containing 50 ml of Trypticase soy broth and 0.04% lindane. Lindane (>99% pure) was dissolved in acetone, and 0.1 ml was added aseptically to media to give the desired concentration. The cultures were incubated on a shaker at 28 C, and the contents of each flask were analyzed periodically for lindane degradation.

The culture sample was acidified with 4 N HCl and extracted with two 50-ml portions of ether. The ether extracts were combined, dried over anhydrous MgSO₄, filtered, and concentrated to approximately 1 ml. 1,1,1-Trichloro-2,2-(p-chlorophenyl)ethane was added as an internal standard, and the resulting solution was analyzed on a Varian 204 gas chromatograph equipped with a flame ionization detector and a glass column (6 feet by 0.25 inch [182.9 by 0.6 cm]) packed with a 50:50 mixture of 4% SE-30 and 6% OV-210 on 80/100 mesh Gas-Chrom Q (Applied Science Laboratory). The following chromatographic conditions were employed: column temperature, 100 to 240 at 8°/min; detector, 300; injector, 150; flow rate, 60 ml/min. Peak areas and retention times were recorded on an Autolab 6300 digital integrator.

The ether extract was derivatized with O,N-bis-trimethyl-silyltrifluoracetamide and analyzed for nonvolatile metabolites. Both derivatized and undervatized samples were examined in an LKB 9000 gas chromatograph-mass spectrometer equipped with a PDP-12

![Fig. 1. Chemical structure of 2,3,4,5,6-penta-chloro-1-cyclohexene.](image-url)
TABLE 1. Degradation of lindane by Escherichia coli isolated from rat feces

| Day | Amt (μmol) | Lindane | γ-PCCH* |
|-----|------------|---------|---------|
| 0   | 52         | 0       | 0       |
| 3   | 52         | 0       | 0       |
| 5   | 50.5       | 0.10    | 0       |
| 7   | 49.8       | 0.15    | 0       |
| 10  | 48.0       | 0.26    | 0       |
| 12  | 46.7       | 0.42    | 0       |

* γ-PCCH, γ-Pentachlorocyclohexene.

computer to determine the presence of chlorinated metabolites.

The bacterium isolated from the rat feces was identified as Escherichia coli on the basis of its morphological and biochemical characteristics. The organism degraded 10% of the added lindane in 12 days. A single metabolite, 2,3,4,5,6-pentachloro-1-cyclohexene (I), was identified by gas chromatography-mass spectrometry (Fig. 1).

Confirmation of structure 1 was achieved by the synthetic preparation of compound 1 by the method of A. van Schoor (Ger. Patent 1,029,373, 1958; Chem. Abstr. 54:16407e, 1960). Gas chromatographic retention times and mass spectra of the synthetic and unknown compounds were identical. Table 1 lists the amount of lindane remaining after each day and the amount of γ-pentachlorocyclohexene formed. Approximately 1% of the consumed lindane appeared as γ-pentachlorocyclohexene, indicating the possibility of multiple steps to the formation of γ-pentachlorocyclohexene or alternate pathways for lindane degradation.

Silylation of the ether extract with O,N-bis(trimethyl-silyl)trifluoroacetamide produced a host of new components in the chromatogram, many of which were identified as hydroxy mono- and dicarboxylic acids. However, none of these new compounds contained chlorine. The inoculated control culture containing no lindane also produced some hydroxy mono- and dicarboxylic acids, but the relative concentrations were much lower than those in culture media containing lindane. This suggests that lindane or its metabolite(s) may interfere in the normal metabolism of the organism, resulting in the accumulation of these compounds.

The present study demonstrates for the first time that lindane is metabolized by a pure culture under aerobic conditions with the formation of the metabolite γ-pentachlorocyclohexene. It has been reported (6) that γ-pentachlorocyclohexene is 1,000 times less toxic than lindane.

We thank Karen Try for technical assistance.
The work was supported by contract DADA 17-73-C-3124 from the U.S. Army Medical Research and Development Command.

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