Metabolism of Lindane to Tetrachlorobenzene

In addition to the two previously reported isomers of pentachlorocyclohexene, the 1,2,4,5-isomer of tetrachlorobenzene as a metabolite of lindane in susceptible and resistant strains of houseflies was tentatively identified. Products having retention times identical to those of 1,2,4- and 1,2,3-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, and pentachlorobenzene were also formed. Application of γ-pentachlorocyclohexane produced only 1,2,4,5-tetrachlorobenzene and 1,2,4-trichlorobenzene, while application of the second isomer of PCCH yielded the five products. Treatment with 1,2,4-trichlorobenzene, 1,2,3,4- and 1,2,4,5-tetrachlorobenzene, or pentachlorobenzene produced no other organic-soluble materials.

The rapid metabolism of the insecticide lindane (1,2,3,-4,5,6-hexachlorocyclohexane) to less toxic products is considered to be an important aspect of resistance in some insect species (Oppenoorth and Nasrat, 1966). Two isomers of pentachlorocyclohexene (PCCH), the relatively nontoxic monodehydrochlorination product of lindane, have been tentatively identified in housefly extracts. One isomer, γ-PCCH, corresponds to that formed by the mild alkaline dehydrochlorination of lindane (Bradbury and Standen, 1958; Sternburg and Kearns, 1956), whereas the formation of a second isomer appears to be limited to metabolic means (Reed and Forgash, 1968). While the toxicological significance of γ-PCCH is nebulous, there is apparently correlation between the formation of the second PCCH isomer and resistance. In addition, small amounts of trichlorobenzene have been detected in flies following lindane application (Bradbury and Standen, 1958), and both γ-PCCH and 1,2,4-trichlorobenzene are believed to be intermediates in lindane metabolism in rats (Grover and Sims, 1965). While 1,2,3,5-tetrachlorobenzene was reported to be a bacterial metabolite of lindane (Menzie, 1966), an examination of the references did not support this. The objective of this investigation was to re-evaluate the role of metabolic detoxification in the resistance of houseflies to lindane.

EXPERIMENTAL

Three strains of houseflies (susceptible, moderately resistant, and highly resistant) were used to detect correlation between resistance levels and metabolism. Sublethal and lethal dosages of lindane and sublethal dosages of γ-PCCH, the second isomer of PCCH, 1,2,4,5-tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, and 1,2,4-trichlorobenzene were individually applied topically (in acetone) and by injection (in dimethyl sulfoxide) to CO_2-anesthetized female flies. After 4 hours' incubation at 20°C., the treated flies were anesthetized with CO_2, rinsed rapidly with acetone, and homogenized in a Potter-Elvehjem tissue grinder containing acetone and Na_2SO_4. The extracts were prepared by Florisil column purification prior to GLC analysis (Reed and Forgash, 1968). For gas-liquid chromatography a Micro-Tek model MT-220 equipped with electron-capture detection was used. The column was an aluminum, hair-pin type (6.39-mm. × 1.22-meter) packed with 15% F-50 on Gaschrom-Q; the argon-methane flow rate was 80 ml. per minute at 180°C. Data were confirmed using an aluminum, hair-pin type (4.8-mm. × 2.44-meter) column packed with 15% QF-1 on Chromosorb P with a nitrogen flow rate of 70 ml. per minute.

RESULTS AND DISCUSSION

Chromatographs of purified extracts of highly resistant flies treated by topical application with lindane, γ-PCCH, and the second PCCH isomer are presented in Figure 1, *A*, *B*, and *C*, respectively. The peaks depicted were reproducible and symmetrical, with the exception of peak *C*, which occasionally was skewed by the presence of an impurity possessing a slightly longer retention time when the quantity of *C* material was low. The authors detected none of these peaks in extracts of untreated flies. Peak *A*, corresponding to the second isomer of PCCH, was present in quantities up to 5% of the internal organic-soluble materials present following lindane applica-

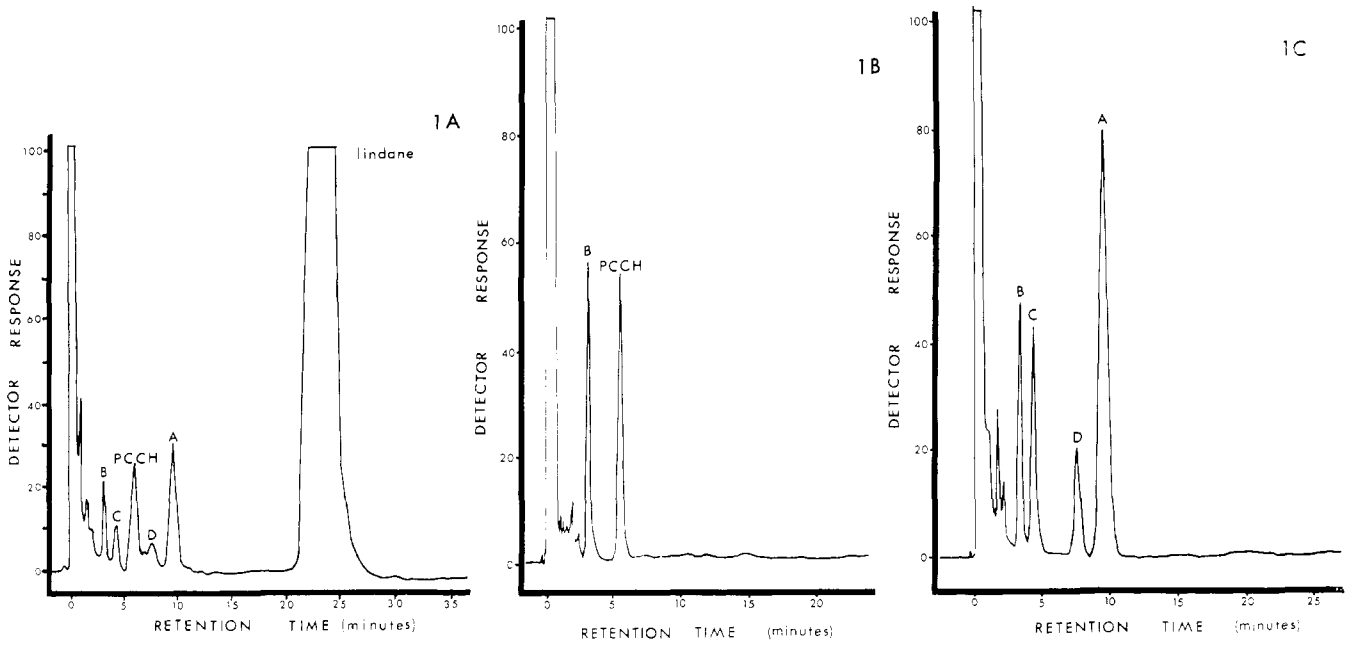


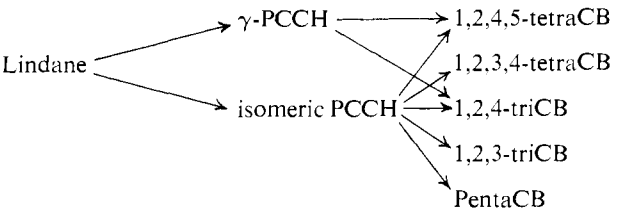
Figure 1. Gas-liquid chromatographs of hexane-soluble products of metabolism

A. Lindane (Reed and Forgash, 1968; copyright 1968 by American Association for the Advancement of Science)
B. γ-PCCH
C. Second isomer of PCCH

tion, while none was detected subsequent to γ-PCCH application. Peak *B* was present following the application of lindane, γ-PCCH, and the second PCCH isomer. Several micrograms of the *B* material were collected from lindane-treated flies by column chromatography and preparative GLC, as was used to collect the second isomer of PCCH (Reed and Forgash, 1968). Mass spectrographic analysis indicated that the material was tetrachlorobenzene and GLC separation showed that its retention time was identical to that of 1,2,4,5-tetrachlorobenzene. This is the basis of the tentative identification. Peak *C*, found only after the application of lindane or the second PCCH isomer, possessed a retention time identical to that of 1,2,3,4-tetrachlorobenzene; peak *D*, found under similar circumstances, had the same retention time as pentachlorobenzene. The two peaks just before the *B* peak in Figure 1, *A* and *C*, possessed retention times identical to those of 1,2,4- and 1,2,3-trichlorobenzene; the single peak just before peak *B* in Figure 1, *B*, has the retention time of 1,2,4-trichlorobenzene. These two compounds were present in more significant amounts following injection tests. No additional products were detected following the application of either 1,2,4,5- or 1,2,3,4-tetrachlorobenzene, 1,2,4-trichlorobenzene, or pentachlorobenzene.

While all of these metabolites were present in relatively small amounts ($\leq 5\%$ of lindane and its derivatives found in the homogenate) following lindane application, they appeared in much greater quantities ($\leq 50\%$) following the application of γ-PCCH and the second isomer of PCCH. The rapid metabolism of the PCCH's indicates their existence as short-lived intermediate metabolites. While preliminary data indicate a correlation between metabolism and resistance, with resistant flies possessing greater metabolic capabilities than susceptibles, a more exhaustive quantitative study is being undertaken. The difference between strains is particularly evident in the rapid metabolism of the intermediates.

The following diagram summarizes the authors' proposal for the in vivo metabolism of lindane to organic-soluble products by houseflies:



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