Lindane: Metabolism to a New Isomer of Pentachlorocyclohexene

Abstract. *Two of the hexane-soluble products of metabolism of lindane in susceptible and resistant houseflies were tentatively identified as isomers of pentachlorocyclohexene by gas-liquid chromatography and mass spectroscopy. One isomer was identical with the pentachlorocyclohexene obtained by mild alkaline dehydrochlorination of lindane and is apparently the form found in previous studies of metabolism; the second is now reported for the first time.*

The mechanism (or mechanisms) of insect resistance to lindane (1,2,3,4,5,6-hexachlorocyclohexane) has not been established; however, it has been theorized that rapid metabolism to less-toxic products is an important feature in some resistant species (*1*). Pentachlorocyclohexene (PCCH), a relatively nontoxic compound, has been identified as the basic product of detoxification of lindane in susceptible and resistant strains of houseflies. With regard to the metabolic capabilities of houseflies, reports in the literature are conflicting. Rather substantial quantities of PCCH were indicated by colorimetric techniques in lindane-treated flies (*2*), while later studies, using isotopic dilution with γ-PCCH and colorimetric determination of nitrated derivatives, showed that much smaller amounts were formed (*3*). From these results it has been assumed that γ-PCCH is the only isomer produced metabolically (*4*). As a first step in reevaluation of the role of metabolic detoxification in the resistance of house flies to lindane we have studied the metabolism of lindane to hexane-soluble products. We have tentatively identified one such product as a previously unreported isomer of PCCH.

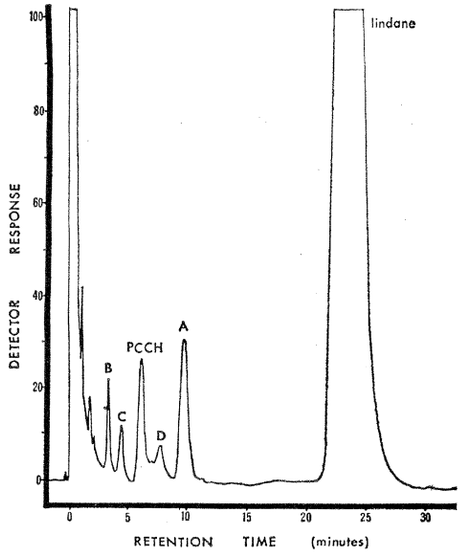

Fig. 1. Gas-liquid chromatograph of the hexane-soluble products of metabolism of lindane by houseflies.

Highly resistant, moderately resistant, and susceptible strains of houseflies were used to facilitate detection of correlation between resistance and detoxification. Sublethal doses of lindane (in acetone) were applied to the dorsa of female flies anesthetized with CO_2. After 4-hour incubation at 22°C, the treated flies were anesthetized with CO_2, rapidly rinsed with hexane, and homogenized in a Potter-Elvehjem tissue grinder containing acetone and Na_2SO_4. Particulate matter was removed from the homogenate by centrifugation. The acetone was removed from the supernatant by evaporation under partial vacuum, and the residue was redissolved in hexane and passed through an activated Florisil column for removal of polar impurities. Portions of the purified eluate were analyzed by gas-liquid chromatography (*5*).

A chromatograph of a purified extract of lindane-treated flies appears in Fig. 1. None of the peaks depicted were detected in extracts of untreated flies; nor were peaks labeled γ-PCCH or *A* found in extracts of fly homogenates to which lindane had been added. Extracts of homogenates containing added γ-PCCH yielded only the γ-PCCH peak. Furthermore live flies could not produce the *A* material from γ-PCCH; thus it was concluded that the *A* material is a primary product of lindane metabolism in houseflies. After treatment with equal amounts of lindane, resistant-fly homogenates contained up to 5 percent of the organic-soluble compounds in the form of the *A* material while the susceptibles contained less than 1 percent. Preliminary data indicate correlation with resistance. The additional peaks (Fig. 1, *B–D*) also are believed to be metabolites of lindane.

Because we could not synthesize the *A* material by alkaline dehydrochlorination of α, β, γ, δ, and η isomers of hexachlorocyclohexane, we had to use houseflies as a source of adequate amounts of the *A* material for identification. It was obtained in the following manner: Highly resistant flies (about 1000) were confined to lindane-coated beakers for 6 hours and then homogenized in acetone. The particulate matter was filtered out, the acetone was removed by evaporation, and the residue was extracted with hexane. The hexane solution was passed through a Florisil column, and the fraction containing the *A* material was purified with a second column. Final purification was by preparative gas-liquid chromatography, the material in the *A* peak being collected in a glass capillary. A portion of this sample was removed from the capillary and rechromatographed; if one peak resulted, the material in the capillary (a white crystalline product at room temperature) was analyzed by mass spectrography (*6*).

While the *A* material produced a mass spectrograph identical with that of γ-PCCH with respect to peak locations, there were significant differences in some peak height ratios. This information, in conjunction with the fact that the retention time of *A* on gas-liquid chromatography was distinct from that of γ-PCCH, indicated that the *A* material is isomeric with γ-PCCH. We tried to obtain additional information about the molecule, using nuclear magnetic resonance and infrared spectrometry, but the data were inconclusive, most likely because of the small amount of sample and the presence of interfering hydrocarbon that did not affect the mass spectrograph.

WALTER T. REED
ANDREW J. FORGASH
Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey 08903

References and Notes

1. F. J. Oppenoorth and G. E. Nasrat, *Entomol. Exp. Appl.* **9**, 223 (1966).
2. J. Sternburg and C. W. Kearns, *J. Econ. Entomol.* **149**, 548 (1956).
3. F. R. Bradbury and H. Standen, *J. Sci. Food Agr.* **9**, 203 (1958).
4. J. R. Smith, *Ann. Rev. Entomol.* **7**, 465 (1962).
5. For gas-liquid chromatography we used a Micro-Tek model-MT-220 equipped with electron-capture detection. The column was an aluminum, hairpin type (6 mm by 1.22 m) packed with 15 percent F-50 on Gaschrom-Q; the argon-methane flow rate was 80 ml/min at 180°C.
6. The mass spectra were obtained by Fred Gollob, Gollob Analytical Service, Inc., Berkeley Heights, N.J., on a CEC 21-104 mass spectrometer.
7. This work, supported by the T. J. Headlee Fellowship and PHS research grant CC-00102, was partly reported before the 1967 meeting of the eastern branch of the Entomological Society of America. This is a paper of the journal series, New Jersey Agricultural Experiment Station, Rutgers–The State University, New Brunswick, N.J. We thank R. K. Cruver for assistance.

24 April 1968