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Interim Results of Studies of Microbial Isomerization of Gamma-Hexachlorocyclohexane

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General aspects of Lindane metabolism were reviewed (ENGST et al. 1977 b and 1979).
Isomerization of gamma-HCH to alpha-HCH (BENEZET et al. 1973, NEWLAND 1969, MARTENS 1972, MATSUMURA et al. 1976, STEINWANDTER 1976) and to delta-HCH (NEWLAND 1969) was pointed out.

This paper confirms the microbial isomerisation of gamma-HCH to alpha-HCH and metabolism to gamma-2,3,4,5,6-Pentachlorocyclohexene (PCCH), Tetrachlorobenzene (TeCB) and unknown unpolar metabolites. Facts pointing to an isomerization of gamma-HCH to beta- and delta-HCH were presented.

MATERIALS AND METHODS

A mixed culture of bacteria with Pseudomonas aeruginosa as a main component was used. The organisms were isolated from soil polluted by industrial wastes by enrichment culture using HCH as sole carbon source. In the described experiments the mixed culture was grown under anaerobically conditions in 50-ml-bottles with glass stopper on a mineral salt medium according to TU (1976) with gamma-HCH as carbon source at 28 °C. Gamma-HCH (VEB Fahlberg-List, Mag-

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deburg; 99.8 %; recrystallized three times from absolute ethanol and two times from chloroform) was solved in aceton and added to the medium after sterilisation. The applied concentrations of gamma-HCH were 10, 50, and 100 µg/ml. Growth, determined by measurement of turbidity, started after one day and rised in the case of 100 µg HCH/ml from an extinction at 600 nm of 0.05 at inoculation to 1.2 after 22 days. The cultures were incubated up to 39 days.

The culture medium was brought up to pH 3 (double destilled) hydrochloric acid. The bacterial culture then was extracted with 10 % of the aqueous volume destilled n-hexane using an Ultra-Turrax after saturation with sodium chloride. Second extraction was carried out with the same volume of sodium purified and destilled benzene. All solvents were "gaschromatographic pure". Centrifugation was necessary for separation of organic and aqueous phases after extractions. Benzene and hexane extract were dried by anhydrous sodium sulphate. In these extracts HCH isomers were quantified. For detecting phenolic metabolites aliquots of the extracts were diluted by a saturated solution of diazomethane in n-hexane. For all extracts the identification of metabolites has been carried out[1)]. Gaschromatography was performed using a modell 2100 gaschromatograph (Varian Aerograph) with Sc^3H-ECD. The separations were made in a pyrex glass column (6' x 1/4") packed with 1.5 % OV-17 on Chromosorb G HP 100-120 mesh at a temperature of 170 °C (injector 170 °C; detector 220 °C). The flow rate of nitrogen carrier gas was

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1) for test substances see ENGST et al. (1976).

30 ml/min.

Calibration curve was obtained with in the linearity range by CDS 111(c) using quantities of 100, 250, and 500 pg of each identified HCH isomer. The quantities of each isomer determined in hexane and benzene extract were added up and are presented in Fig. 5 and 6.

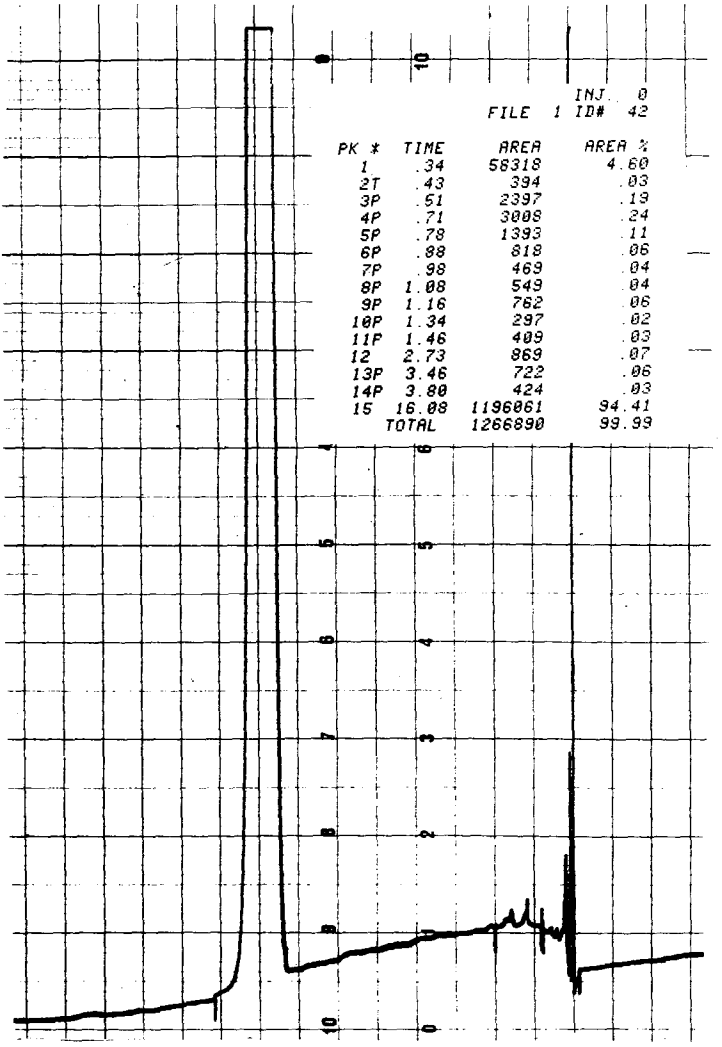

Figure 1. Gaschromatogram obtained from gamma-HCH before incubation

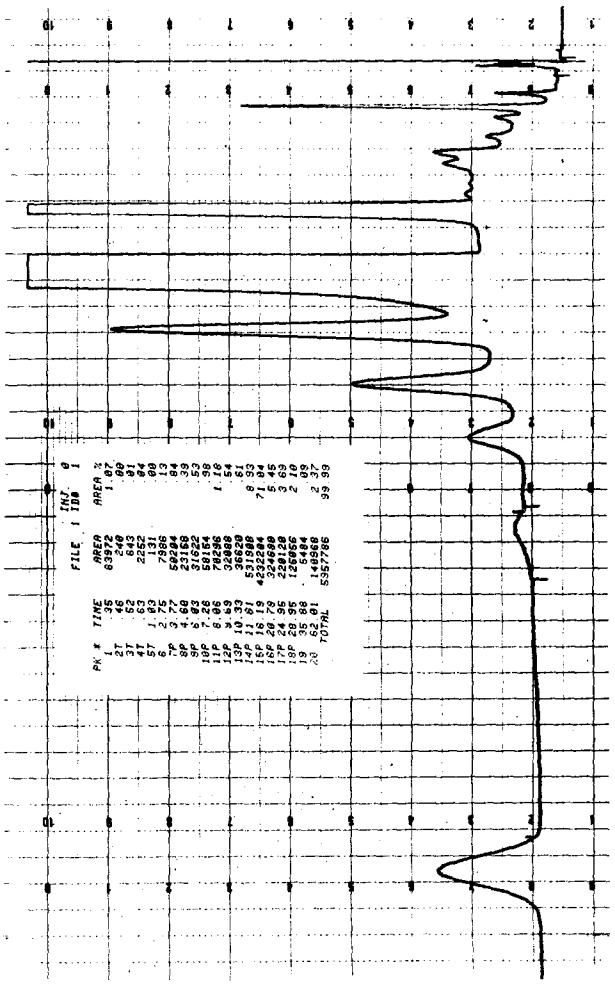

Figure 2. Hexane extract after 3 days of incubation of gamma-HCH

Results and Discussion

Not any isomer of gamma-HCH was detectable in gamma-HCH before incubation (Fig. 1). Fig. 2 presents a gaschromatogram of the hexane extract 3 days after incubation.

Fig. 3, Chromatogram of the corresponding benzene extract confirms quantitative extraction with exception of gamma-HCH.

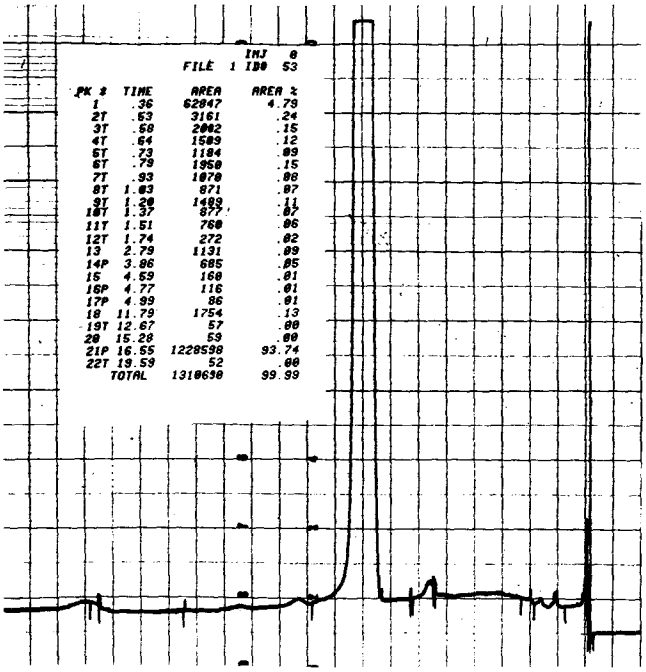

Figure 3. Benzene extract obtained after hexene extraction

Following peaks (Fig. 2) can be assigned to reference substances:

1,2,3,4-TeCB	RT	2.75 min
gamma-2,3,4,5,6-PCCH	RT	3.77 min
alpha-HCH	RT	11.61 min
gamma-HCH	RT	16.19 min
beta-HCH	RT	20.79 min
delta-HCH	RT	24.95 min

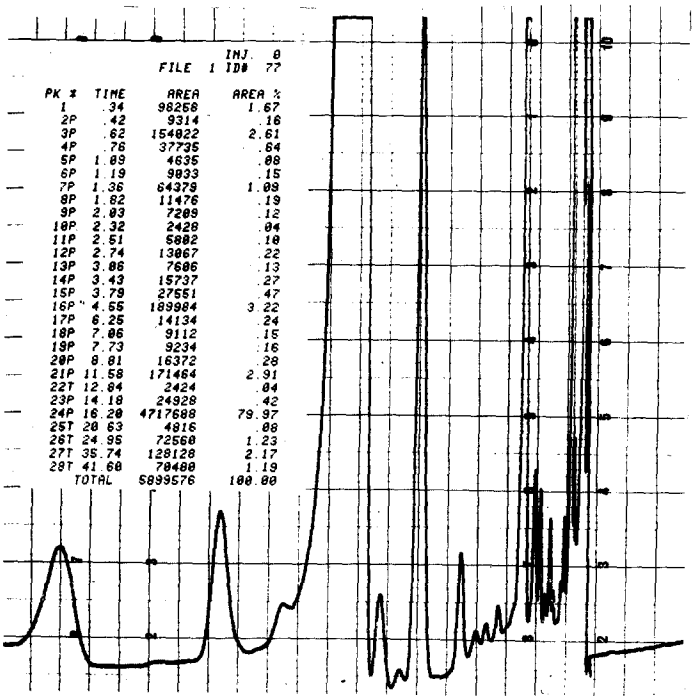

Figure 4. Hexane extract 22 days after incubation of gamma-HCH. After methylation of extract

In not any extract Pentachlorobenzene, delta-PCCH or Hexachlorobenzene were detectable.

Fig. 4 presents an extract after 22 days of incubation and subsequent methylation of the extract for detecting of phenolic metabolites. Polychlorophenols were not detectable. Not any differences were notable in comparison with the unmethylated extract.

Some unknown metabolites are detectable in the following cases: Fig. 2 RT 35.88 min, and RT 62.01 min; Fig. 3 RT 41.60 min.

Concentration of the alpha- and delta-isomer increased till 6 days and then decreased (Fig. 5). Formations of alpha- and beta-HCH were the greatest in culture medium of a low initial gamma-HCH concentration (Fig. 6).

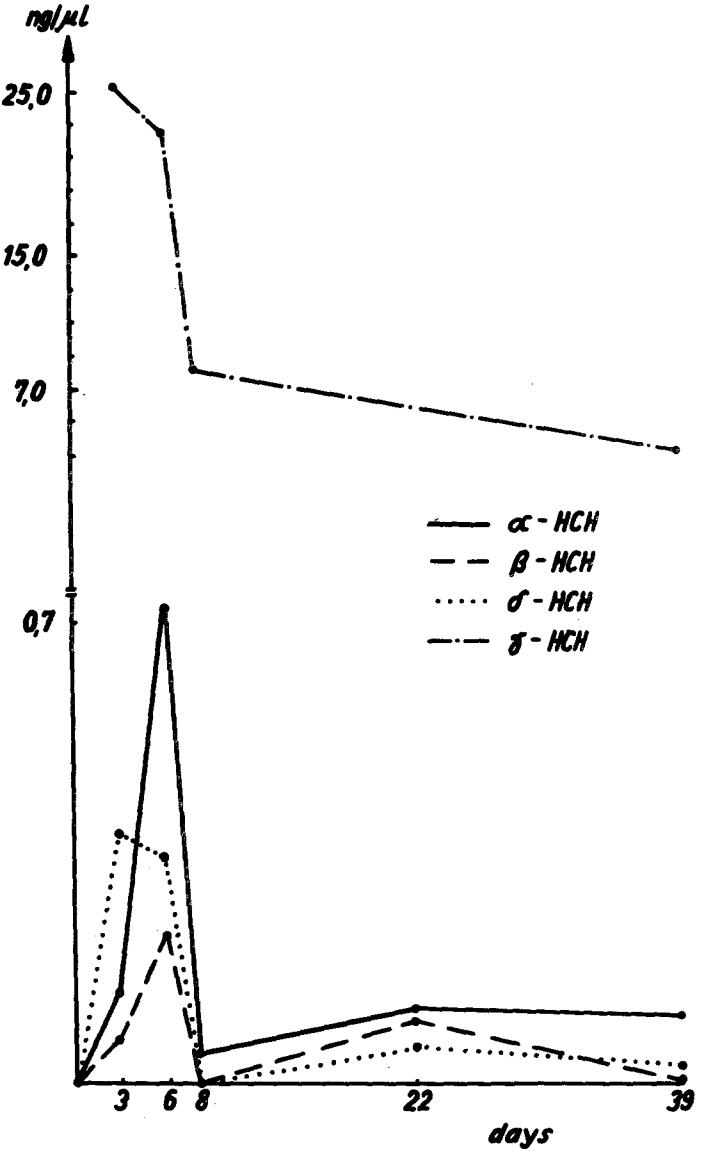

Figure 5. Changes in concentrations of HCH isomers between the beginning of gamma-HCH incubation and the 39th day

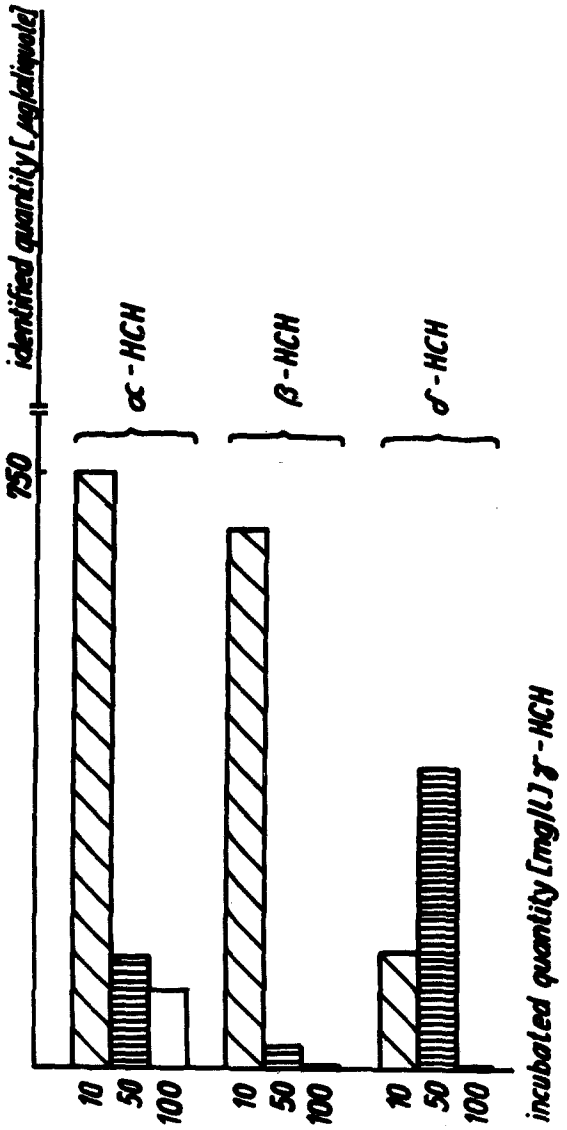

Figure 6. Formation of isomers after 3 days of incubation of gamma-HCH. Dependence of concentration from incubated gamma-HCH quantities

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