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Note

Analysis of HCB and BHC isomer residues in food

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The application of γ-1,2,3,4,5,6-hexachlorocyclohexane (γ-BHC) as an agricultural and household insecticide has been continued although the use of DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) had been curtailed or even prohibited in most European countries. As a result, the environment has been contaminated with other compounds that have cumulative properties. This applies not only to γ-BHC, but also to other BHC isomers. Moreover, hexachlorobenzene (HCB), used for the treatment of cereals, contributes greatly to this problem. Pentachloronitrobenzene containing HCB can serve as a source of environmental contamination by HCB.

There has therefore been increasing interest in the determination of BHC, its isomers and HCB, mainly in the food chain. Up to 1965, the studies were concerned only with the γ-BHC isomer, but more recently attention has been paid to other BHC isomers[1–4]. However, a problem arose as a consequence of the coincidence in gas–liquid chromatography (GLC) of HCB and α-BHC[5–7].

In this work, we attempted to separate and determine individual BHC isomers in the presence of HCB residues using GLC and thin-layer chromatography (TLC). The procedures for extraction and purification from food products and biological materials have been described elsewhere[8].

EXPERIMENTAL AND RESULTS

For the determination of chlorinated insecticide residues by GLC using an electron capture detector, the following liquid phases were tested: 2% methyl silicone polymer SE-30, 2.5% silicone oil XE-60 and 5% silicone grease DOW-11; however, they were found to be unsuitable for the determination of the four BHC isomers.

In the separation of the BHC isomers in the presence of DDT and its metabolites, the best results were obtained with a mixed column of 1.5% methyl phenyl silicone OV-17 and 2% silicone oil QF-1. Fig. 1 illustrates a chromatographic record obtained with different amounts of individual substances according to their electron affinities, so that approximately equal chromatographic peaks were obtained.

The analysis was performed under following conditions: temperature of the column, 200°; temperature of the detector, 220°; temperature of the injection volume, 220°; nitrogen flow-rate, 100 ml/min; detector voltage, 70 V; and chart paper speed, 0.5 cm/min. A 1-μl aliquot from a sample condensed to a volume of 1–5 ml (according to the expected insecticide content) was applied to the device.

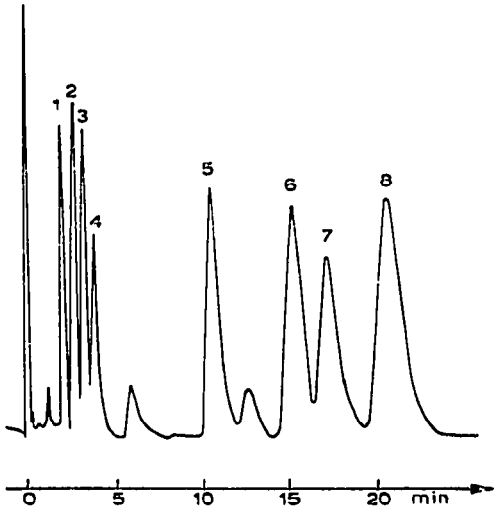

Fig. 1. Separation of BHC isomers in the presence of DDT and its metabolites by GLC. Column: 1.5% OV-17 + 2% QF-1 on Chromosorb W, 80–100 mesh. For other GLC conditions, see text. Peaks: 1 = α-BHC; 2 = γ-BHC; 3 = β-BHC; 4 = δ-BHC; 5 = *p,p'*-DDE; 6 = *o,p'*-DDT; 7 = *p,p'*-DDD; 8 = *p,p'*-DDT.

The residue contents were calculated by comparing the heights of the peaks with the calibration curve obtained from at least three standard mixtures of different concentrations in a range so that linear relationships occurred between the peak heights of individual isomers (for α-, γ- and δ-BHC, 0.12–0.03 μg/ml; for β-BHC, 0.6–0.15 μg/ml)[3].

In samples of animal food products (butter) and mainly in those of biological materials (human fat tissue), a peak appeared that interferred with the α-BHC peak, so that in certain instances the quantitative analysis of α-BHC was not possible (Fig. 2: the highest peak in front of α-BHC). The peak was identified as HCB. The identification was verified by TLC. As HCB occurred in almost all samples of the food chain and biological material, an attempt was made to determine it quantitatively. For this purpose, a column filled with 2.5% XE-60 on Chromosorb W, 80–100 mesh, was used. This column was found to be suitable for the separation of HCB from BHC isomers at 180° (Fig. 3). All the other conditions of analysis remained unchanged.

In the literature, we found objective criteria for the quantitative determination of only γ-BHC residues in foodstuffs. We therefore investigated the sensitivity, the degree of accuracy and the recovery of the method for all four isomers. For the sensitivity at a wave height of 3 cm (12% of the recorder deviation), it was possible to determine as little as 0.0001 mg/kg, *i.e.*, $3 \cdot 10^{-11}$ g in 0.3 g of the material tested. The recovery ranged between 85.6 and 98.5% and was greatly affected by the way the extracts were condensed. The vapour pressures of these isomers are higher than those of other chlorinated insecticides.

In model experiments, the great importance of condensing the extracts just to dryness by means of a vacuum evaporator was demonstrated. Large losses can occur owing to prolongation of the evaporation time (even by 3 min, especially if a flow of air is used to dry the extracts).

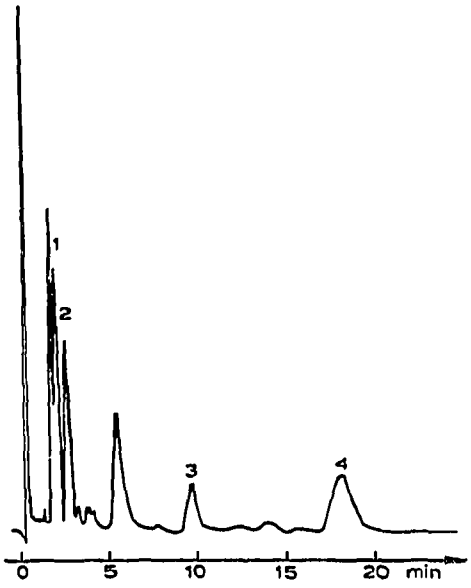

Fig. 2. Interference of HCB with the α-BHC peak in a sample of butter. HCB is the highest peak in front of α-BHC. GLC conditions as in Fig. 1. Peaks: 1 = α-BHC; 2 = γ-BHC; 3 = p,p'-DDE; 4 = p,p'-DDT.

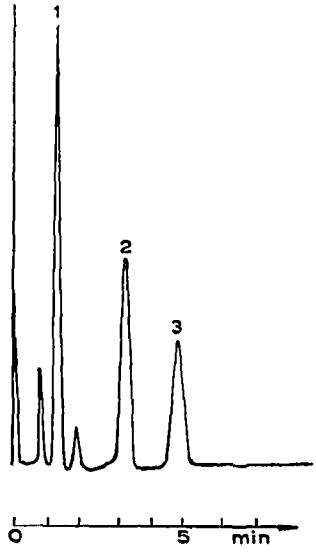

Fig. 3. Separation of HCB from α-BHC by GLC. Column: 2.5% XE-60 on Chromosorb W, 80–100 mesh. For other GLC conditions, see text. Peaks: 1 = HCB; 2 = α-BHC; 3 = γ-BHC.

Fig. 4. Separation of HCB from BHC isomers by TLC. Left-hand spots (order of the spots from bottom to top): δ-BHC, β-BHC, γ-BHC and α-BHC. Right-hand spot: HCB. For TLC conditions, see text.

The accuracy, expressed as the coefficient of variation, ranged between 3.00 and 10.42%.

The identity of the peaks of the individual BHC isomers was also verified by TLC on ready-made Silufol silica gel plates developed in *n*-heptane and detected with silver nitrate added by 2-phenoxyethanol[9]. HCB was also identified on Silufol plates impregnated with 5% of liquid paraffin with double development in 96% ethanol (Fig. 4). Under such conditions, all other chlorinated insecticides move at the front of the chromatogram.

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