The separation of four isomers of benzene hexachloride and their determination in soil by gas–liquid chromatography

The use of gas–liquid chromatography (GLC) for the separation and detection of insecticides in general and benzene hexachloride (BHC) in particular is well known[1–6]. Two of the above procedures[5,6] list the separation of the α, β, γ, and δ isomers; however, these articles were not concerned with the determination of BHC in soil samples and both used thermal conductivity detectors which do not have the sensitivity necessary for trace insecticide analysis.

BHC has eight possible geometrical isomers[7] including one *dl* pair. These are shown in Table I. Of these, all but the ι and θ forms have been observed.

TABLE I

ISOMERS OF BENZENE HEXACHLORIDE

Greek designation	*Melting point*	*Planar designation*	*Equatorial–axial designation*
Alpha	157	↓ ↑ ↑ ↓ ↓ ↑ (*dl*)	a a e e a a
Beta	312	↑ ↓ ↑ ↓ ↑ ↓	e e e e e e
Gamma	111	↓ ↑ ↑ ↓ ↑ ↑	a a a e e e
Delta	132	↑ ↑ ↑ ↓ ↑ ↓	a e e e e e
Epsilon	227	↑ ↑ ↑ ↓ ↓ ↓	a e e a e e
Eta		↑ ↑ ↑ ↑ ↓ ↓	a a e a e e
Theta		↑ ↑ ↑ ↑ ↑ ↓	a e a e e e
Iota		↑ ↑ ↑ ↑ ↑ ↑	a e a e a e

As a result of herbicide studies being made a procedure for the determination of the isomers of BHC in soil samples was needed. Certain questions had to be answered satisfactorily in order for the procedure to work. Among the questions were the following: Could BHC be readily extracted from the soil samples, and would breakdown products and other materials interfere with the determination of BHC?

Apparatus

In order to minimize clean up procedures of the extracted samples a Wilkins model 600 C gas chromatograph equipped with a Wilkins model 600 C electron affinity detector was used for the analysis because of the specificity of the electron affinity detector for halogenated and related compounds. The following modifications were made on this unit. In order to minimize baseline shift due to the sensitivity of the EA detector to temperature changes, the EA detector was removed from the column heating oven and mounted on the back plate of the unit. Connection to the column was made by means of a $^1/_8$ in. O.D. copper tube that was kept as short as possible. The detector and connecting tube were wrapped in an electronthermal No. HT 352 heating tape supplied by a powerstat type 216 variable voltage transformer. The potential of the EA cell was controlled by a voltage controller built in this laboratory. A Sargent model SR one millivolt recorder was used.

Chemicals

Acetone AR (Mallenckrodt Chemical Works, St. Louis, Mo.) was used as a solvent. The BHC was supplied by Eastman Organic Chemicals, Rochester, N.Y. This compound contained the normal distribution of isomers: α, 70 %; β, 5 %; γ, 10–12 %; δ, 13–15 %; ε, 1–2 %. In order to identify the various isomers, pure forms of the individual isomer were obtained from Hooker Chemical Company, Niagara Falls, N.Y.

Operating conditions

Column: $^1/_8$ in. O.D. stainless steel loaded with 0.5 % Apiezon L on Chromosorb P (60–80 mesh)
Column temperature: 145°
Detector temperature: 185°
Detector voltage: 32 V
Injector temperature: 200°
Range: 1
Inlet pressure: 22 p.s.i.g.
Outlet pressure: atmospheric
Attenuation: 16
Carrier gas: N_2
Flow rate: 60 ml/min

Experimental procedure

It was found that BHC was readily soluble in acetone and thus this solvent was used to extract the BHC from the soil samples. After two extractions it was found that the soil still contained a very small amount of BHC. In order to prepare a valid standard sample in the light of this information, the following procedure was used.

To a BHC-free soil sample, an amount of BHC was added to give the desired concentration in the soil. This soil sample was mixed thoroughly and extracted in exactly the same manner as the unknown samples. Each sample was extracted three times using 10 ml of acetone each time. The extracts were combined and diluted to 50 ml. The solution was then filtered through Whatman No. 4 filter paper and analyzed using 1 μl aliquots. The instrument was allowed to stabilize for 12 h at the conditions listed before the analyses were made.

TABLE II

Sample	*Peak height (in.)*			*Concn. found (p.p.m.)*			*Mean and std. dev.*
	1	*2*	*3*	*1*	*2*	*3*	
Std.	0.75	0.73	0.72	2.88	2.80	2.76	2.81 ± 0.04
Soil 1	0.68	0.62	0.68	2.63	2.40	2.62	2.54 ± 0.13
Soil 2	0.56	0.56	0.52	2.16	2.16	1.99	2.09 ± 0.09
Soil 3	0.18	0.18	0.18	1.22	1.22	1.22	1.22 ± 0.00
Soil 4	0.49	0.44	0.44	1.74	1.73	1.73	1.73 ± 0.00

Results and discussion

The precision of analysis of four unknown soil samples based on the α isomer is shown in Table II. Under the conditions used BHC tended to build up in the column on repeated injections and thus a small memory peak was obtained on injection of pure acetone following an injection of BHC. This source of error was minimized by

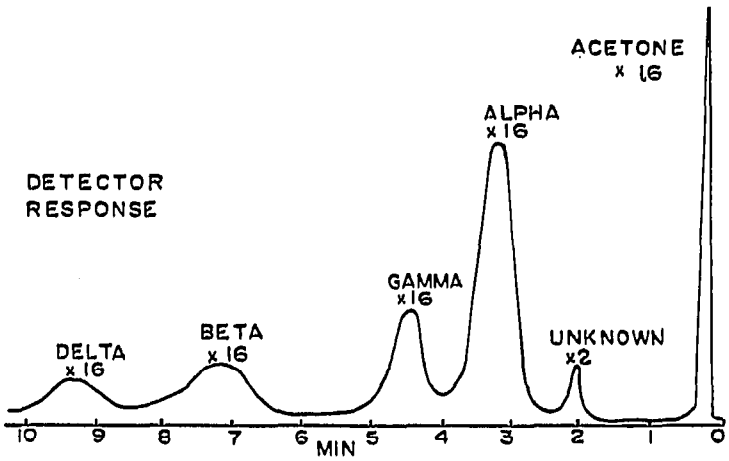

Fig. 1. Typical chromatogram of the α, β, γ, and δ isomers of benzene hexachloride.

purging the column with pure acetone after the injection of both the standard and unknown samples, thus keeping the column uniform for both samples. The limit of detectability, based on the α isomer, is an absolute amount of $2.2 \cdot 10^{-11}$ g per one microliter sample. Fig. 1 shows a chromatogram of the BHC mixture.

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