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INSECTICIDE ANALYSIS

Infrared Determination of Dichlorodiphenyltrichloroethane and Benzene Hexachloride in Insecticides

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An infrared spectrophotometric method has been developed for the determination of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p′-DDT) and the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (γ-BHC) in cotton dust insecticides. Direct extraction of the sample with the infrared transparent solvent, carbon disulfide, reduces the time required for analysis as well as errors incurred in sample transfer and solvent evaporation. An average error of ± 0.05% was obtained for single determinations.

During an inspection program by the Alabama State Department of Agriculture, the State Chemical Laboratory was called upon to analyze a large number of cotton dust insecticides for 1,1,1 - trichloro - 2,2 - bis(*p* - chlorophenyl) ethane (DDT, both *p,p′*- and *o,p′*-isomers) and the gamma isomer of 1,2,3,4,5,6 - hexachlorocyclohexane (γ-BHC). The partition chromatographic method of Harris (*8*) was earlier employed in these analyses but was of limited value because in formulations containing sulfur it gave too high results for DDT. Several other methods have been developed for the determination of DDT and BHC in insecticides containing mixtures of the two. A partition chromatographic method of Prat and Colas (*19*) can be successfully used to determine BHC but cannot always be relied upon in the analysis for DDT because the elution zones of hepta- and octachlorocyclohexanes, sometimes present as impurities in BHC, overlap that of DDT. Chemical methods reported by Perkow (*18*) and Weber (*24*) appear to be complicated and are unable to distinguish among the several isomers of BHC found in commercial preparations. A polarographic determination of γ-BHC in the presence of DDT has been described by Wolf (*26*) and a polarographic determination of both γ-BHC and DDT in mixtures of the two was reported by Tamamushi and Tanaka (*22*). The latter investigators were unable to distinguish *p,p′*-DDT from its isomers by this procedure.

A number of articles dealing with the infrared spectrophotometric analysis of technical DDT for the para-, para′-isomer and of technical BHC for the gamma isomer lead to the conclusion that this method might be adapted to the analysis of formulations containing both of these products in a range of concentrations considerably lower than that found in the technical materials.

The infrared spectrum of *p,p′*-DDT as well as that of four related compounds in the range of 1 to 13 microns has been reported by Andrews and coworkers (*1*). Quantitative analyses of samples of technical DDT have been carried out by Downing and associates (*5*) and Henry, Colas, and Prat (*10*).

Spectra for the five isomers of BHC, α-, β-, γ-, δ-, and ε-, in the range 2.5 to 14 microns were published by Kauer, DuVall, and Alquist in a paper describing the isolation of the ε-isomer from crude BHC (*12*). Quantitative procedures for the analysis of technical BHC concentrate have been described by Harris (*2, 9*), Kuratani, Shimanouchi, and Mizushima (*13*), Mecke and Mutter (*16*), Kamada and Tanaka (*11*), Trenner and coworkers (*23*), Larnaudie (*14*), Whiffen and Thompson (*25*), Daasch (*4*), and Marrison (*15*).

This paper deals with a rapid routine infrared spectrophotometric method for the simultaneous determination of *p,p′*-DDT and γ-BHC in insecticidal mixtures containing a high percentage of other constituents such as sulfur, talc, and clay. As the latter two ingredients are insoluble in the solvents used in infrared analyses, an extraction was carried out to separate DDT and γ-BHC from the inert constituents prior to measuring the infrared absorption.

The customary extraction procedure uses the Soxhlet extractor with ethyl ether in an overnight extraction. Although with hexane, the extraction period may be shortened to 2 hours, the higher operating temperature of the extractor brings about additional difficulties. If the sample contains traces of ferric ions or other catalysts, thermal decomposition on the hot walls of the extraction flask above the level of the solution becomes a problem (*7*). Also, contamination with silicone stopcock grease must be avoided since this interferes with DDT determination. Hence, an improved extraction technique was needed to give rapid and reliable results.

Experimental

Preparation of Standards. *p,p′*-DDT (melting point 108.5–109.0° C.) was recovered from a technical grade by repeated recrystallizations from 95% ethyl alcohol. The infrared spectrum of the purified product was in agreement with that reported by Downing and coworkers (*5*).

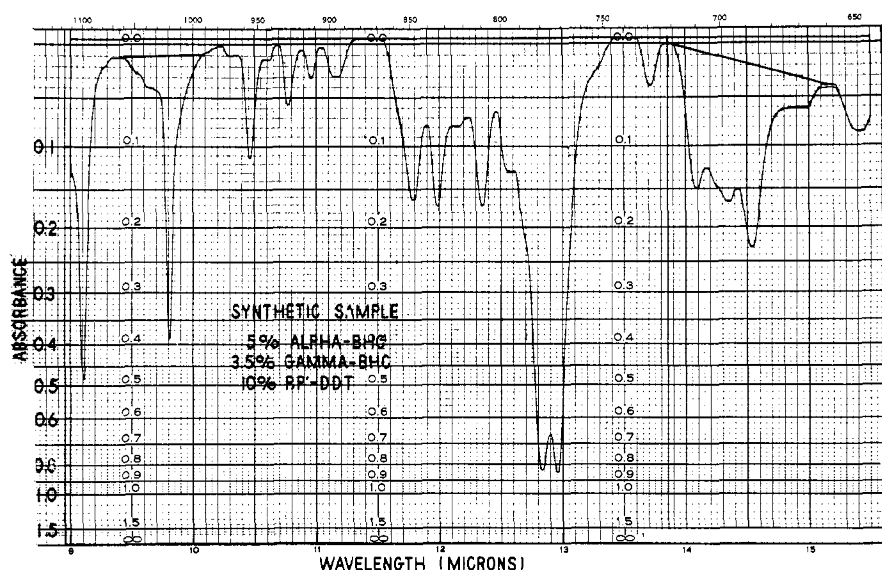

Figure 1. Spectrogram of synthetic sample

Gamma-BHC (melting point 112.0–112.5° C.) was obtained by recrystallization of lindane from chloroform.

Alpha-BHC was obtained from technical BHC by two separate procedures, adsorption on a column of silica gel (*8*), and recrystallization from selective solvents (*20*). The products obtained by the two procedures both melted at 157.5–158.0° C. and gave no depression when mixed. The infrared spectra of γ-BHC and α-BHC were in agreement with those of Daasch (*4*).

Standard solutions in carbon disulfide were prepared for the following ranges of concentration: *p,p'*-DDT, 3 to 11%; γ-BHC, 1 to 4%; and α-BHC, 3 to 16%. The percentages are based on a 5-gram sample and solutions were made up to 100 ml.

Preparation of Sample. The sample was passed through a 40-mesh sieve and mixed for 30 minutes in a twin-shell blender. Duplicate 5-gram portions were weighed into simple fat extraction tubes (*3*) fitted with glass wool plugs and were extracted with carbon disulfide. The tubes emptied directly into 100-ml. volumetric flasks and extraction was continued until the volume of extract reached 100 ml. About 2 hours were required for the extraction.

Infrared Absorption. The region from 8 to 15.4 microns was scanned at a rate of approximately 1.4 microns per minute with a Perkin-Elmer Model 21 double-beam spectrophotometer. The resolution used followed the slit program (schedule 2) recommended for quantitative work by the manufacturer of the instrument (*17*). This corresponds to a spectral slit width which varies from about 0.026 micron at a wave length of 10 microns, to 0.054 micron at a wave length of 14 microns. The cells used were 1 mm. in thickness and this should be checked periodically. Thickness determination by the interference method is accurate to 0.1% or better (*21*). The absorption bands of *p,p'*-DDT at 9.8 microns and that of γ-BHC at 14.53 microns were used, 100% transmittance being balanced at 13.85 microns. The scanning was started at 13.85 microns with the scanning control set on "cycle" so that the 14.53 band was recorded before the 9.8 band. In reversing at 15.4 microns, the pen is thrown off scale and the scanning runs back to 8 microns at maximum speed. By this procedure the balancing, scanning of the 14.53 region and of the 9.8 region are accomplished in the shortest possible time.

The base line method (*27*) was used in determining the absorbances, the line being drawn between 13.85 and 15.15 microns for α- and γ-BHC, and between 9.40 and 10.08 microns in the case of *p,p'*-DDT. These lines are roughly parallel to the solvent curve; a slight absorption here is attributed to small differences in cell thickness. Figure 1 is a spectrogram of a sample containing 5% α-BHC, 3.5% γ-BHC, and 10% *p,p'*-DDT. Base lines have been drawn in.

Standard curves were prepared by plotting the absorbance of the compound against the per cent of this compound

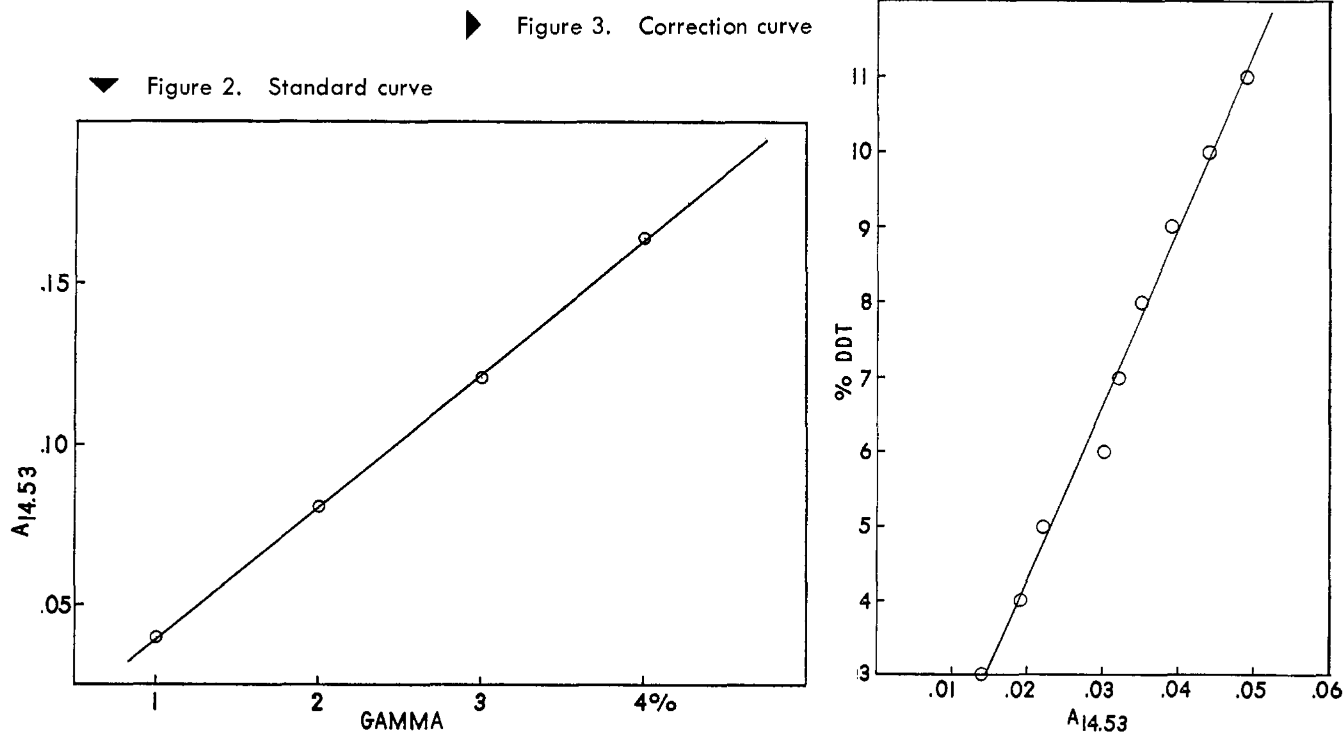

Figure 3. Correction curve

Figure 2. Standard curve

Table I. Analysis of Commercial Samples

Sample	% DDT	% γ-BHC
1	4.53 ± 0.02	3.33 ± 0.02
2	3.75 ± 0.01	3.17 ± 0.02
3	5.13 ± 0.04	2.99 ± 0.02

based on a 5-gram sample. Figure 2 shows the standard curve for γ-BHC at its principal band, 14.53 microns. In each case where interference occurs, a correction curve was plotted. For example, *p,p'*-DDT gives a weak absorption band at 14.55 microns which interferes in the determination of γ-BHC. Figure 3 is a plot of the absorbance of various concentrations of *p,p'*-DDT at the wave length of the γ-BHC absorption band, 14.53 microns.

It was unnecessary to correct the absorbance of DDT for α-, β-, γ-, δ-, or ε-BHC because these compounds show good transparency in the 9.8 micron region.

Beta- and ε-BHC do not interfere with the determination of γ-BHC and the concentration of δ-BHC in the final solution is usually so small as to be negligible. However, one should always check the spectrum for the presence of δ-BHC (principal band at 13.22 microns), for its interference with the determination of γ-BHC is of the same order of magnitude as that caused by α-BHC. Corrections applied to the absorbance of γ-BHC for the presence of *p,p'*-DDT and α-BHC, and of α-BHC for the presence of γ-BHC were small and three successive approximations were always sufficient. For this reason, five of the usual steps in the series of approximations may be eliminated, since they contribute nothing to the final value of the concentration of γ-BHC. Let $A_{9.8}$, $A_{14.36}$, and $A_{14.53}$ represent the observed absorbances of the test solutions at 9.8, 14.36, and 14.53 microns, respectively. The respective concentrations of *p,p'*-DDT, α-BHC, and γ-BHC corresponding to these uncorrected absorbances are obtained from the standard curves. Corrections are applied to these absorbances by using the correction curves. Let $A_{DDT(14.53)}$ and $A_{\alpha\text{-BHC}(14.53)}$ represent the absorbances of DDT and α-BHC at 14.53 microns when they are present in the concentrations indicated by the uncorrected absorbances. The first approximation is applied to γ-BHC only.

$$A'_{\gamma\text{-BHC}(14.53)} = A_{14.53} - A_{DDT(14.53)} - A_{\alpha\text{-BHC}(14.53)}$$

= first corrected absorbance for γ-BHC at 14.53 microns

The concentration corresponding to this absorbance is obtained from the standard curve for γ-BHC. Then the correction curve, concentration γ-BHC *vs.* absorbance at 14.36 microns, was used to correct the absorbance of α-BHC for that due to the presence of γ-BHC. This is a "second approximation" rather than a "first approximation" on α-BHC because the concentration of γ-BHC used was that obtained from the first corrected absorbance of γ-BHC rather than from the uncorrected absorbance.

$$A''_{\alpha\text{-BHC}(14.36)} = A_{14.36} - A'_{\gamma\text{-BHC}(14.36)}$$

= second corrected absorbance of α-BHC

$A'_{\gamma\text{-BHC}(14.36)}$ = absorbance at 14.36 microns caused by γ-BHC in concentration indicated by first correction

The corresponding concentration of α-BHC is obtained from the α-BHC standard curve. The absorbance produced at 14.53 microns by this concentration of α-BHC, $A''_{\alpha\text{-BHC}(14.53)}$, is obtained from a correction curve and is used with the following equation to arrive at the final corrected absorbance of γ-BHC, $A'''_{\gamma\text{-BHC}(14.53)}$:

$$A'''_{\gamma\text{-BHC}(14.53)} = A_{14.53} - A_{DDT(14.53)} - A''_{\alpha\text{-BHC}(14.53)}$$

The corrected concentration of γ-BHC, $C'''_{\gamma\text{-BHC}}$, is read from the standard curve for γ-BHC. The uncorrected absorbance, $A_{9.8}$, is used for determining the concentration of DDT, C_{DDT}, from the DDT standard curve.

$$\%\ \gamma\text{-BHC} = \frac{5}{\text{wt. sample}} \times C'''_{\gamma\text{-BHC}}$$

$$\%\ \text{DDT} = \frac{5}{\text{wt. sample}} \times C_{DDT}$$

Discussion

Extraction of the samples directly with an infrared transparent solvent eliminates the time consuming step of evaporation and redissolving. Continued extraction with an additional 100 ml. of carbon disulfide failed to remove detectable amounts of DDT and γ-BHC, indicating the effectiveness of the extraction procedure.

Marrison analyzed hexachlorocyclohexane for the alpha, beta, gamma, and delta isomers using nitromethane as a solvent in the analysis for the gamma isomer and methyl acetate for the remaining ones (*15*). He rejected the more transparent carbon disulfide as a solvent because of its high volatility (boiling point 46° C.) and because it "has poor solubility for all the isomers." However, Daasch in earlier work found that satisfactory concentrations of the α-, γ-, δ-, and ε-isomers could be obtained in carbon disulfide (*4*). The solubility of the gamma isomer was given as 0.1995 gram per ml. at approximately 25° C. and the authors have confirmed this result. This concentration is approximately 15% by weight of γ-isomer in carbon disulfide and compares with the value of 16% which Marrison set for the upper limit for his solutions of γ-isomer in nitromethane. As the authors' solutions are more dilute than those used by Marrison, carbon disulfide is satisfactory from the point of view of solubility. As for the possibility of concentration of sample during a run brought about by the evaporation of solvent from the cell, no observable change has occurred when polyethylene stoppers were used in stainless steel cell frames. Figure 1 is a double scan of the region of the spectrum of γ-BHC and *p,p'*-DDT employed, and the superposition of the curves shows no evidence of sample concentration.

Large slit widths are not generally recommended because the Lambert-Beer law plot is linear only for monochromatic radiation. However, the following conditions were favorable toward minimizing the deviations from other sources and the reduced noise level obtainable with the wider slits and lower gain setting was remarkable. The bands are fairly broad and are sufficiently well isolated to make high resolution unnecessary. The largest deviations from the Lambert-Beer law occur at higher concentrations and all solutions were dilute—for example, a 5-gram sample of insecticide containing 3% γ-BHC yields a test solution of 0.15 gram γ-BHC in 100 ml. of carbon disulfide or approximately 0.12%. Intermolecular association is negligible, owing to the low concentration of solute and to the fact that the dipole moment of the solvent is zero. This is an advantage of carbon disulfide over nitromethane as a solvent, since the dipole moment of the latter is 3.42 Debye units (*6*).

The reproducibility of the method has been tested by checking the results of multiple determinations made on commercial samples. Results of eight analyses on each of three such samples are shown in Table I. The average error of about ±0.02% was obtained for both γ-BHC and DDT. Sample 1 contained

Table II. Analysis of Synthetic Samples

Sample	Composition				Found	
	% S	% α-BHC	% γ-BHC	% DDT	% γ-BHC	% DDT
4	0	5.1	2.87	4.90	2.88	4.90
5	40	4.9	3.02	4.92	3.08	4.98
6	0	12.4	3.46	5.04	3.49	4.95
7	0	5.0	3.17	9.42	3.18	9.40
8	0	12.1	2.57	9.45	2.67	9.40
9	40	11.9	3.62	4.90	3.68	4.85

40% sulfur and samples 2 and 3 were sulfur-free.

Synthetic samples were made by mixing weighed amounts of p,p'-DDT, α-BHC, γ-BHC, sulfur, and talc. These were then extracted and analyzed. The results of single determinations are shown in Table II. The average errors for γ-BHC and for p,p'-DDT were again nearly equal and were slightly less than $\pm 0.05\%$.

The band of DDT at 9.8 microns was selected because it is a strong absorption band, it is well isolated, and it is produced by both the para-para' isomer and the ortho-para' isomer. The presence of o,p'-DDT may be detected by the absorption band at 12.3 microns. If this isomer is present, one can still use the 9.8-micron band for the determination of total DDT (*5*), as both isomers absorb to nearly the same extent. Although these isomers are not equal in their insecticidal properties, no distinction is made between them in the guaranteed analysis of present samples in Alabama. Several compounds related to DDT also absorb at 9.8 microns and the spectrum should be checked qualitatively to assure the absence of significant quantities of these interfering substances. Spectra for the several commonly found related compounds are given by Downing (*5*). In only a few of the samples analyzed to date have significant amounts of these compounds been detected.

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OLIGOSACCHARIDE PRODUCTION

Concentration Effects in the Enzymatic Conversion of Lactose to Oligosaccharides

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A study was made of hydrolyzing conditions conducive to high oligosaccharide yields by the action of *Saccharomyces fragilis* lactase on lactose. With pH, temperature, and enzyme concentration held constant, lactose substrates from 5 to 50% were hydrolyzed. The percentage of lactose converted to oligosaccharides increased as the starting lactose concentration increased. This relationship held up to a limiting lactose concentration of 35% (w./v.), at which a maximum conversion of 44.6% was obtained. At starting lactose concentrations of 22 to 50%, the quantity of oligosaccharides present (at the time at which the oligosaccharide concentration reached a maximum value) was linearly related to the sum of the galactose and glucose concentrations as well as to the starting substrate concentration.

THE HYDROLYSIS OF LACTOSE by acid was first reported in 1812, and its hydrolysis by a lactase preparation in 1883 (*9*). That lactase is a hydrolyzing enzyme capable of splitting the glycosidic bond of lactose to form galactose and glucose is common knowledge. That lactase is also capable of synthesizing di- and oligosaccharides was not known until recently.

The advent of paper chromatography permitted broad advances in elucidating the action of lactase on lactose. The report in 1951 by Wallenfels (*7*), the first on the presence of oligosaccharides, was followed by the contributions of Aronson (*1*), Pazur (*4*), and Roberts and McFarren (*5*).

The formation of 11 galactosyl oligosaccharides (Nos. 1 to 11, Figure 1) is the result of a transgalactosidation reaction in which galactose in the form of a galactosido-enzyme complex reacts