formation and excretion of dichloroethyl glucuronide is known to occur in rabbits administered dichloroethanol (*31*) and in rats administered DDVP (*8*). The observation that labeled carbon dioxide is produced in vivo in rats from DDVP-C^{14} (*8*) indicates that the further metabolic reactions shown in Figure 4 do not account for the complete metabolism of DDVP. The mechanism by which the small amount of dichloroacetic acid observed is produced has not been investigated. An enzyme, which requires DPN, has been described for the metabolism of chloral to trichloroacetic acid (*13*).

The method described for the colorimetric determination of dichloroacetaldehyde might be adapted for residue analysis of certain organophosphorus insecticides. DDVP, Dipterex, and Butonate form blue derivatives which appear to be identical with that formed from dichloroacetaldehyde, whereas most natural aldehydes and ketones yield red derivatives. Under these reaction conditions with 2,4-dinitrophenylhydrazine, Dibrom gave a colored derivative with a different absorption maximum, one very similar to that given by chloral. Presumably acid degradation of these compounds results in carbonyl compounds which react to form dinitrophenylosazones. By suitable chromatography and/or solvent partitioning this method might be made specific for DDVP. The sensitivity might approach the 1-μg. range by modification of the reaction times and volumes, or by increasing the extent of reaction by carrying out the hydrolysis first in dilute alkali and then reacting the product with 2,4-dinitrophenylhydrazine, or by conducting the acid hydrolysis in sealed tubes.

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INSECTICIDE ANALYSIS

Infrared Analysis of Insecticides to Determine Toxaphene Alone or in the Presence of Dichlorodiphenyltrichloroethane (DDT)

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TOXAPHENE is used mainly in emulsifiable concentrates and wettable powders and dusts. To prepare these materials for analysis by infrared absorption, toxaphene and other organic insecticides are separated from inert ingredients by a simple chromatographic procedure for emulsifiable concentrates and by methanol extraction of dusts. These separations gave recoveries of 98% of the active ingredients in several commercial formulations.

Infrared absorption spectroscopy is useful in the analysis of insecticides and pesticides because it offers a simple, accurate, and specific assay of multicomponent mixtures (*1, 2*).

Toxaphene, which is chlorinated camphene containing 67 to 69% chlorine, can be determined quantitatively in this way. A quantitative infrared method for the determination of toxaphene is described herein. A previous article reported the use of infrared spectroscopy for the identification of toxaphene (*5*).

There are three general methods for determination of toxaphene: measurement of total chloride (*6*), colorimetric methods (*4*), and infrared absorption.

The total chloride method has been widely used for both assay and residue work, but it lacks specificity. The major advantage of the colorimetric method is its sensitivity, which makes it useful in residue determinations. The infrared absorption method offers the advantage that it is always specific for toxaphene in the presence of DDT

Infrared absorption spectroscopy is useful in analysis of insecticides and pesticides because it offers a simple, accurate, and specific assay of multicomponent mixtures. A quantitative, infrared method for determining toxaphene and DDT in such formulations is described.

and BHC, whereas the other methods are not. It is designed for the assay of toxaphene in various formulations.

Toxaphene is used mainly in emulsifiable concentrates and wettable powders and dusts. The analysis of each will be discussed separately.

Emulsifiable Concentrates

Emulsifiable concentrates are usually formulated to contain: 40 to 60% of toxaphene in formulations containing 40% of toxaphene, 20% of DDT is sometimes added; 30 to 40% of hydrocarbon solvent (usually mixed xylenes and or kerosine); and 3 to 5% of emulsifier.

Figure 1 shows the infrared spectrum of toxaphene. Toxaphene has a strong band at 7.7 microns that can be used for quantitative analysis. The band at 9.8 microns used to determine DDT does not interfere with determination of toxaphene. In concentrates formulated as described above, solvent and emulsifier interfere with the determination of toxaphene, usually by causing results to be too high. Figures 2*A* and 2*B* show representative spectra of two emulsifiers which are mixtures of nonionic and anionic surfactants that are used in toxaphene concentrates. Both of these spectra exhibit absorption that would interfere with the determination of toxaphene.

To separate these interfering substances (solvent and emulsifier) from the toxaphene, a simple chromatographic separation has been developed; it constitutes the first step in the analytical procedure. Figures 3 *A* and *B* show the infrared spectrum of a typical concentrate before and after cleanup. As indicated in these figures, absorption bands in the concentrate at 8.0, 8.9, and 9.9 microns have been removed from the sample.

Dusts and Wettable Powders

Dusts and wettable powders are usually formulated to contain: 10 to 40% of toxaphene and 5 to 20% of DDT mixed with sulfur and clay.

Toxaphene and DDT are extracted from dust with hot methanol. The methanol solution is then evaporated and the active components determined in a CCl_4 solution.

Equipment and Materials

Infrared Spectrophotometry. A Beckman IR-4 infrared spectrophotometer equipped with a NaCl prism was used. The instrument settings were: scanning speed 0.5 micron per minute; gain 3.6%; period 2, resolution dial setting 0.75 mm. at 12.0 microns. A sealed liquid absorption cell with sodium chloride windows and with a light path of 0.1 mm. was used. Carbon tetrachloride (ACS reagent grade) was the solvent used. The reference standards were commercial toxaphene and DDT (reference grade) purified by the procedure outlined by Bunger and Richburg (*2*).

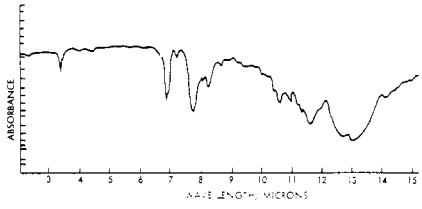

Figure 1. Infrared spectrum of toxaphene

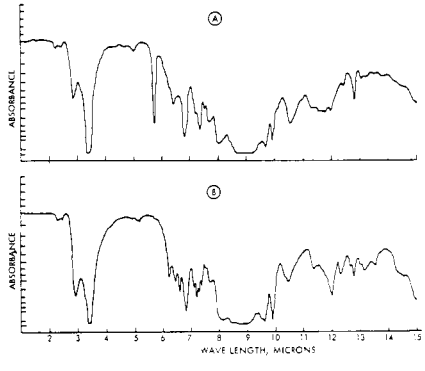

Figure 2. Infrared spectra of two typical emulsifiers used in toxaphene formulations

A. Atlox 4500 (Atlas Powder Co.)
B. Emcol, D-26-24A (Emulsol Chemical Corp.)

Chromatographic Separation. A chromatographic column, size 2 (20 mm.), and a Fisher filtrator were used.

Reagents used were: benzene (C. P.) and Woelm acid alumina (activity grade 1) with 2% of water added.

Extraction Apparatus. A fritted-glass crucible of medium porosity and a Fisher filtrator were used.

Absolute methanol was used as the extraction solvent.

Calibration. Standard solutions of toxaphene in carbon tetrachloride are prepared accurately to about 250, 200, 150, and 100 mg. per ml.

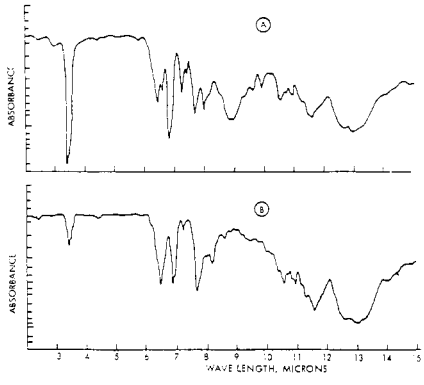

Figure 3. Infrared spectra of an emulsifiable concentrate

A. Before
B. After chromatographic separation

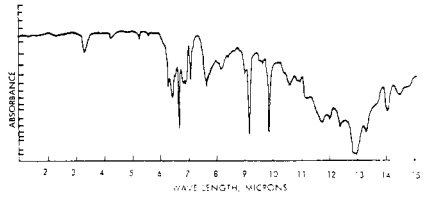

Figure 4. Infrared spectrum of a chromatographed emulsifiable concentrate containing both toxaphene and DDT

With the 0.1-mm. sodium chloride sealed absorption cell, the spectrum from 12 to 5 microns is recorded for each dilution, under the conditions outlined above. A carbon tetrachloride blank should be run. ΔA is calculated for toxaphene as follows:

$$\Delta A \text{ toxaphene} = (A_{7.7 \text{ microns}} - A_{7.0 \text{ microns}})_{\text{soln.}} - \Delta A\ CCl_4 \text{ blank } (7.7 - 7.0 \text{ microns})$$

where $(A_{7.7 \text{ microns}} - A_{7.0 \text{ microns}})$ is the difference between the absorbances of the CCl_4 solution at 7.7 and 7.0 microns and ΔA CCl_4 blank (7.7 − 7.0 microns) is the difference between the absorbances of the CCl_4 blank at 7.7 and 7.0 microns.

A calibration curve of these values of ΔA for toxaphene *vs.* milligrams per milliliter of toxaphene in each of the solutions is plotted.

ΔA for DDT is calculated using the procedure described above for toxaphene. The wave-length maximum is 9.8 microns and the wave-length minimum is 9.0 microns.

Correction factors to compensate for minor mutual overlapping of the two materials should be applied. These corrections can be plotted in curves

similar to the calibration curves (ΔA *vs.* milligrams per milliliter). The magnitudes of the interferences are small.

Procedure for Emulsifiable Concentrates

Preparation of Chromatographic Column. The chromatographic tube is assembled on the Fisher filtrator. Aluminum oxide is added under full suction from a water aspirator sufficient to give a packed column height of 5 cm. The adsorbent is settled by tapping the side of the column with a wood rod and the alumina is leveled.

Procedure for Chromatographic Separation. Into a small beaker, 0.3 to 0.5 gram of sample is accurately weighed and 5 ml. of benzene is added with stirring.

A tared 100-ml. beaker is placed in the filtrator. With suction off, the benzene solution is transferred to the chromatographic column. The beaker is rinsed with 10 ml. of benzene which is added to the column.

With suction on, the column is eluted with 50 ml. of benzene and allowed to go to dryness.

The beaker containing the benzene solution is removed from the filtrator and the benzene is evaporated in a gentle stream of nitrogen gas on a steam bath. The beaker is cooled and weighed, and the residue is saved for infrared examination.

Procedure for Wettable Powder and Dusts

Procedure for Separations. The dust or wettable powder is weighed into a fritted-glass crucible and extracted with boiling methanol as described by Graupner and Dunn (*4*). The sample size should be such that 200 mg. of the chlorinated organic compounds are available for infrared examination. Each sample is extracted with a total of 60 ml. of boiling methanol in several increments; an aspirator is used to speed filtration. The methanol extract is condensed by evaporation in a gentle stream of air on a steam bath to approximately 25 ml., cooled, and filtered to remove the precipitated sulfur. The filtrate is collected in a weighed beaker. The flask and funnel are rinsed with methanol and the washings added to the filtrate. The filtrate is carefully evaporated to dryness in a gentle stream of dry air. *Caution:* The beaker should be removed from the steam bath as soon as the methanol is gone, since both toxaphene and DDT may be lost on prolonged heating. The beaker is cooled and the residue is weighed. If a wettable powder containing more than 3% of a wetting or dispersing agent is extracted, the residue will probably need to be chromatographed as described above for emulsifiable concentrates.

Infrared Procedure

An amount of the chromatographed concentrate or dust extract sufficient to give a solution of 200 mg. per ml. is weighed accurately and dissolved in carbon tetrachloride. The infrared absorption from 12 to 5 microns is recorded with the same absorption cell and under the same instrument conditions as outlined above.

ΔA is calculated for both materials in the same manner as described under Calibration and the concentration (milligrams per milliliter) of each material is obtained by reference to the proper calibration curve.

Calculation

$$\text{Fraction of sample recovered from chromatographic column or extract dust} \times \frac{\text{mg./ml. of each material from calibration curve}}{\text{mg./ml. of sample in solution}} \times 100 = \% \text{ of each component}$$

Discussion

The infrared spectrum of a chromatographed emulsifiable concentrate containing toxaphene and DDT is shown in Figure 4.

As indicated before, the absorption band at 7.7 microns is the most prominent and can be used for quantitative measurement. The absorption band at 9.8 microns was selected for DDT because it is strong and has little or no interference from toxaphene. Also Bunger and Richburg (*2*) pointed out that this absorption is observed in both the para - para' - isomer and ortho - para'-isomer. The absorption band at 13.3 microns indicates the presence of *o,p'*-DDT. If this isomer is present, the 9.8-micron band can still be used for determination of total DDT (*3*) because both isomers absorb to nearly the same extent.

Table I. Determination of Toxaphene in Commercial Formulations

Sample[a]	Composition, % Toxaphene	Composition, % DDT	Found, % Toxaphene	Found, % DDT
1	50	..	49.5	...
			49.5	
2	60	..	59.0	...
			58.9	
3	40	20	39.0	19.5
			39.2	19.5
4	40	20	39.1	19.5
5	60	..	59.1	...
6	40	..	39.6	...
7	40	20	39.3	19.4
8	10	5	10.2	5.7
9	10	5	10.2	5.5

[a] Samples 1 through 5 are emulsifiable concentrates, 6 through 9 are dusts.

Precision and Accuracy

The accuracy of the method has been tested by checking the result of multiple determinations on several commercial formulations. Results of these analyses are shown in Table I.

The reproducibility was evaluated by performing replicate determinations on 4 different days. Day-to-day variations were slight. If daily variations were assumed negligible, the precision at the 95% confidence limits for duplicate determinations was 0.5% absolute for a mixture.

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