

INSECTICIDE RESIDUES

Residue Analysis of a Chlorinated Insecticide (Thiodan) by Combination of Gas Chromatography and Infrared Spectrophotometry

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A combination gas chromatography-infrared spectrophotometry technique is used to analyze quantitatively small traces of a chlorinated insecticide (Thiodan) extracted from plant materials. The advantages of this method are the relative simplicity, the possible application to other compounds, and the simultaneous qualitative and quantitative analyses of the compound under investigation.

QUANTITATIVE analyses of trace amounts of chlorinated insecticides, as may be required for residue analyses in crop materials, have been performed by one of the following techniques: analysis by total chloride (4); bioassay using different test organisms (10); and specific chemical analyses (9). The first two methods suffer from their nonspecificity and the interference of other chlorine-containing or toxic compounds other than the material under investigation. The disadvantages of a specific method of analysis are the cumbersomeness of purification, and in some cases, the unavailability of a truly specific chemical method, especially for newly developed compounds.

Therefore, it was felt desirable to develop a general method of trace analysis for a chlorinated pesticide which might be applicable to a number of such compounds and, at the same time, retain the advantage of great specificity. This objective has now been accomplished by the combination of gas chromatography and subsequent quantitative analysis by infrared spectrophotometry. The use of infrared spectrophotometry also afforded the qualitative identification of the material being analyzed.

As a model compound, the newly developed insecticide Thiodan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) was chosen.

Present methods of trace analysis for Thiodan are by total chloride or by the colorimetric determination of sulfur dioxide after hydrolysis of the parent compound (7). The quantitative analysis of Thiodan in formulations by gas chromatography has been reported re-

cently (12). The method reported here is an extension of this work.

Equipment and Materials

Gas Chromatography. Aerograph Model A-100-C and Burrell Model K-3, equipped with a Honeywell-Brown recorder, 1-mv., 1-second response. Six-foot ($\frac{1}{4}$ inch in outside diameter) stainless steel, spiral columns were packed with Dow Corning No. 11 high-vacuum silicone grease on Chromosorb 35/80 (30% w./w.). All other conditions for gas chromatography have been described (12).

Infrared Spectrophotometry. Model 221 Perkin-Elmer infrared spectrophotometer, equipped with scale expansion. Liquid microcells with sodium chloride windows and a light path of 3.0 mm. were used. A variable thickness liquid absorption cell was used for the reference beam.

Reagents. Mixed hexane and carbon disulfide (analytical reagent grade) were used without further purification. Thiodan standards and a formulation (24.00%) in xylene (Niagara Chemical Division, Food Machinery and Chemical Corp.) were used.

Experimental Methods

Standard Curve. Five microliters of Thiodan standard, dissolved in toluene and containing 4.8 to 120 γ of Thiodan, were injected into the chromatographic column with a 10.0- μ l. Hamilton syringe-type pipet. The column temperature was kept at 225° C. and the gas flow (helium) had an effluent rate of 167 ml. per minute. Under these experimental

conditions, the two isomers of Thiodan (6) had a retention time between 4.0 and 7.2 minutes. During this period the effluent stream was collected by a tapered 1.0 \times 14 cm. glass tube, fitted with a 7/15 standard taper at one end. A small wad of glass wool soaked in carbon disulfide was inserted into the exit end of the tube just prior to the time of collection. At high concentrations of Thiodan, the material was deposited as a white film on the walls of the collector near the collection point.

A slight difference in design between two commercial gas chromatographic apparatus might be mentioned. The Aerograph has a rubber O-ring gasket at the collection port, into which the tapered end of the collection tube is inserted. The Burrell fraction collector consists of a stainless steel male standard tapered joint to which the open end of the collection tube is attached. Just before the removal of the collection tube from the Burrell, the temperature of the collector must be decreased because of different expansion coefficients of the glass and the metal.

After the sample had been collected, the inside of the glass tube and the outside of the restricted end were washed thoroughly with generous amounts of carbon disulfide (about 25 ml.). The washings were evaporated at room temperature in a stream of warm air, and the dry residue was redissolved in carbon disulfide (0.20 to 5.0 ml.). This solution was then transferred to a liquid microcell for infrared spectrophotometry, and the spectrum was scanned between 8.0 and 8.5 microns using pure carbon disulfide in the reference beam. The setting of the infrared instrument was as follows: slit

program 9.80; scanning time 16; suppression 10; and scale expansion 5 X. The absolute absorbance at 8.37 microns was measured and calculated, using the base-line method (7).

It was important to flush the microcell after each analysis with pure carbon disulfide and to evacuate the empty cell with a vacuum pump for several seconds. Failure to observe this procedure resulted in a spurious absorption band at 8.25 microns. The origin of this band may be due to a volatile reaction product from carbon disulfide and Koroseal, because Koroseal bulbs were employed to empty the microcell. A typical scan for a Thiodan standard solution is shown in Figure 1. Figure 1 also shows the base line used for calculating the absorbance values. Absorbance was plotted *vs.* concentration (γ /ml.) and a straight line was obtained, following Beer's law. For confirmatory work the absorbance at 10.16 microns could also be employed, but because of the lower sensitivity at this wave length, the reading at 8.37 microns was preferred (Figure 1).

Trace Analysis of Thiodan from Crop Material. In order to validate the method of residue analysis a typical agricultural crop was chosen. Winter-Nellis pears with an unknown spray history were surface-extracted with mixed hexane (1000 grams of fruit per 1000 ml. of solvent) using the roller-stripping technique (4). Prior to stripping, 5 p.p.m. of insecticide solution in toluene were added. After 1 hour of stripping, the mixture was dried over anhydrous sodium sulfate and then filtered through Whatman No. 1 paper. One hundred milliliters of this solution, equivalent to 100 grams of crop, were evaporated to complete dryness in a stream of warm air. A small amount of a waxy material remained as a residue. Then 100 μ l. of toluene were added, the solution was warmed slightly to melt the waxes, and the total volume of this solution was measured, usually between 112 and 118 μ l. Fifty microliters of this solution were injected into the gas chromatographic column with a 100- μ l. Hamilton syringe-type pipet, and the procedure for analysis was followed exactly as described above under standard curve.

Other crop materials analyzed for Thiodan by this procedure without further modifications were alfalfa and peaches.

Results and Discussion

Reproducibility of Method. In order to test all possible experimental errors due to pipetting, loss during collection from the chromatographic column, and errors due to infrared instrumentation, 11 samples, containing 120 γ of Thiodan, were analyzed by this procedure.

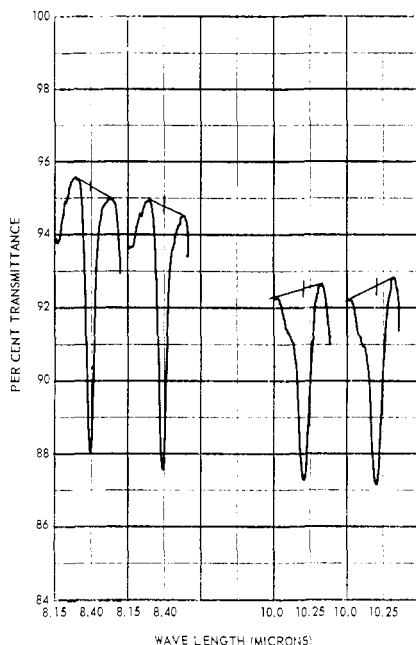


Figure 1. Infrared scan 8.0 to 8.5 and 10.0 to 10.5 microns of Thiodan standard

Note base line is drawn in

Table I lists the absolute absorbance of each sample as well as the duplicate readings of the spectrophotometer. The calculated standard deviation was 0.002 or $\pm 10.2\%$ and included all sources of error enumerated above. When 12 other standard solutions were analyzed directly by infrared spectrophotometry, without prior gas chromatography, an average value of 148.62 γ was obtained, using the standard curve. This indicated a loss of about 23% due to the gas chromatography step. However, because standard curves were obtained by processing the standards by the two-step process (gas chromatography and infrared spectrophotometry), these experimental errors canceled out in the analysis for an unknown amount of Thiodan.

The sensitivity of the method is 20 γ per ml. and a total volume of 0.2 ml. is required to fill the microcell, so that the absolute sensitivity is 4 γ . One difficulty in obtaining even greater sensitivity was the slow bleeding of the stationary phase (Silicone Dow 11) when using a comparatively new column. A column which had been in continuous use over a 6-month period resulted in readable peaks at a concentration of 4.8 γ per ml. Furthermore, a scale expansion of "20 X" by the infrared spectrophotometer would increase the sensitivity by a factor of 4. However, in the present work only the scale expansion factor of "5 X" was employed. If other scale expansions are used, new standard curves must be constructed for each because the scale expansion factors are not exactly linear (8).

Table I. Calculation of Standard Deviation for Analysis of Thiodan

(All samples contained 60 γ of Thiodan per ml. of carbon disulfide solution)

Absorbance at 8.37 Microns		
0.018	0.022	0.020
0.017	0.024	0.021
0.027	0.024	0.020
0.022	0.022	0.022
0.022	0.021	0.020
0.021	0.021	0.021
0.023	0.019	0.021
0.024		

$$m = 0.21$$

$$\Sigma x = 0.473$$

$$n = 22$$

$$\sigma = \pm 0.002 \pm 10.2\%$$

Table II. Recovery Study of Thiodan Added to Crop Material

Sample	P.P.M.		Recovery, % ^a
	Added	Found	
Pear AR 1 ^b	5.00	4.32	86.4
AR 2	5.00	4.93	98.6
ARR 1	5.00	4.30	86.0
ARR 2	5.00	4.33	86.6
ARR 165 ^c	5.00	5.75	115.0
ARR 165	5.00	5.34	106.8
Peach ARR 168 ^d	5.00	4.90	98.0
ARR 168	0.96	0.88	91.7

AR = addition of Thiodan before processing of crop.

ARR = sample to which known amounts of Thiodan were added after extraction and concentration just prior to gas chromatography.

^a 43.47 grams of plant material; value corrected for an average wax concentration of 15% in final concentrate.

^b Winter-Nellis pear.

^c Bartlett pears.

^d Lovell peaches.

Residue Analysis from Crop Materials. Gas chromatography by itself may be used for trace analyses of insecticides by measuring the area of the retention-time curves. However, peak areas corresponding to less than 25 γ of Thiodan have been shown to be unreliable, using the hot-wire Katharometer detector (72). Direct analysis by gas chromatography alone may not be possible if there are interfering substances present in the plant material that have similar retention times as Thiodan. This was observed in the case of several untreated pear samples which exhibited at least two components within the retention time period of 4.0 to 7.2 minutes. These components were collected and did not show the characteristic infrared absorption peak of Thiodan at 8.37 microns. However, when the effluent stream from a treated sample was collected during the predetermined retention-time period, an uncontaminated infrared absorption peak due to Thiodan could be measured at 8.37 microns.

Table II lists the recovery studies of

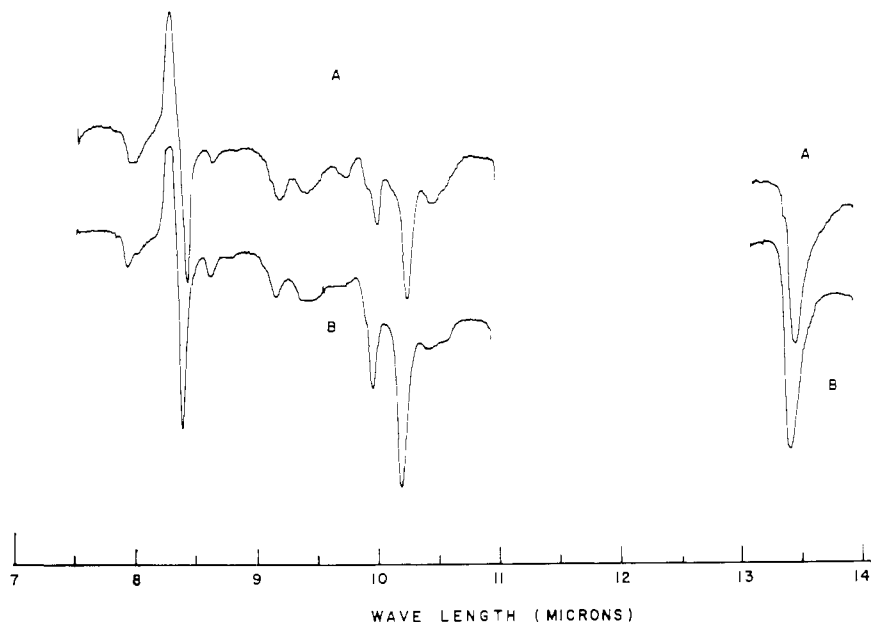


Figure 2. Infrared absorption curves of Thiodan

A. Isolated from Bartlett pear
B. Analytical standard in carbon disulfide solutions

several fortified pear and peach samples. The recoveries ranged consistently from 86 to 115%.

A further advantage of the combination of gas chromatography and infrared spectrophotometry is illustrated in Figure 2. The infrared spectrum depicted in this figure shows the identification of Thiodan from a pear sample to which Thiodan had been added. This method thus provided simultaneously a quantitative and qualitative analysis of the material under investigation. The chromatographic column should be properly aged, as bleeding of the stationary phase would interfere in qualitative identification. Silicone Dow 11 exhibits strong absorption bands in the regions of 7.9, 8.6 to 9.9, and 11 to 12 microns. These bands are especially cumbersome when scale expansion is applied.

Choice of Solvents for Infrared Spectrophotometry. A number of solvents were investigated for suitability in the 8.0- to 8.5-micron region. Some of the solvents which show some transmittance in this region are acetonitrile, carbon tetrachloride, chloroform, cyclohexane dichloromethane, methylcyclohexane, and carbon disulfide. It was possible to obtain a satisfactory 100% transmittance line using scale expansion only with carbon disulfide. Furthermore, it was possible to use carbon disulfide (analytical reagent grade) without any further purification. Gunther and Blinn have also used carbon disulfide for the determination of Tedion by infrared spectrophotometry (5).

Comparison with Other Methods.

The combination gas chromatography-infrared method was compared with two other methods available for trace analysis of Thiodan (7). For this purpose several alfalfa samples were analyzed in the authors' laboratories by the method described above. Table III summarizes these results and those obtained by the sulfur dioxide and total chloride methods in another laboratory (2). In all cases, except one (sample 221), the results were in good agreement with the sulfur dioxide method. Total chloride seemed to give consistently higher results than either of the other two methods.

Future Research. Preliminary research at this and other laboratories (3, 11) has indicated the feasibility of the separation of a number of insecticides by gas chromatography. It might be possible, therefore, to combine gas chromatography and infrared spectrophotometry for the analysis of each, and possibly a number of insecticides simultaneously. The prerequisites are that these compounds be separated by gas chromatography and that they possess specific infrared absorption bands for quantitative analysis. If two compounds were not resolved chromatographically, it might even be possible to analyze them by infrared as a multicomponent mixture (7).

Acknowledgment

The authors thank the Niagara Chemical Division, Food Machinery and

Table III. Comparison of Thiodan Analyses by Three Methods

(Alfalfa samples)

Sample No.	Parts Per Million		
	Total chloride ^a	Sulfur dioxide	Gas chromatography-infrared
221	0	0	0.11
213	1.1	0.03	0.06
214	2.5	0.07	0 ^b
217	3.3	0.38	0.44
219	5.4	2.06	2.86
221	11.0	6.21	10.11

^a Analyses (7) made by Niagara Chemical Division, Food Machinery and Chemical Corp., Richmond, Calif.

^b Less than 0.05 p.p.m.

Chemical Corp., Middleport, N. Y., for a grant-in-aid to the University of California which made this research possible. Thanks are rendered to Jerry Boucher, Francis Allie, and Richard Martin for their technical assistance.

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Received for review February 23, 1960. Accepted June 8, 1960. Division of Agricultural and Food Chemistry, 138th Meeting, ACS, New York, N. Y., September, 1960.