Quantitative Determination of Thiodan by Gas Chromatography

GUNTER ZWEIG and T. E. ARCHER

Pesticide Residue Research, University of California, Davis, Calif.

The newly developed insecticide, Thiodan, may be determined indirectly by measuring evolved sulfur dioxide or by infrared spectrography. This paper deals with the quantitative determination of Thiodan isomers by gas chromatography and its adaptation to formulation analysis. No isomerization or decomposition of Thiodan occurs on the column at 250° C. as shown by infrared spectrography, melting point, and elemental analysis of the isolated products. With increased sensitivity the gas chromatographic technique may be adapted to residue analysis of Thiodan.

A NALYTICAL METHODS for the determination of the 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) are based on the hydrolysis of Thiodan to the corresponding alcohol and sulfur dioxide. The released sulfur dioxide is then determined by iodometric or colorimetric procedures (4, 6).

Commercial Thiodan is a mixture of two geometric isomers (3), and the above chemical methods cannot distinguish between these isomers. These methods could also lead to low or erratic results due to the loss of sulfur dioxide, incomplete hydrolysis of Thiodan, and interferences from other oxidizable compounds. For these reasons a new method for the quantitative determination of Thiodan was investigated.

The technique of gas chromatography has recently been adapted to the analysis of various chlorinated and organic-phosphorus insecticides (1). The same technique has now been utilized for the quantitative determination of Thiodan and its isomers in commercial formulations.

Equipment and Materials

Gas Chromatograph Apparatus. Aerograph Model A-100-C, commercial gas chromatograph apparatus; Varian G-10 recorder, 1-mv. range selector switch; hot-wire tungsten filaments.

Column Packing. Dow-11 high-vacuum silicone grease (30% w./w.) on Chromosorb 35/80 using a 6-foot (1/4-inch-diameter) spiral stainless steel column.

Pipets. A 100- and a 10-μl. Hamilton syringe-type pipet were used for quantitative work. For preparative work a 1-ml. syringe was employed.

Infrared Spectrophotometer. A Perkin-Elmer Model 221 infrared spectrophotometer was used for the qualitative and quantitative determinations of Thiodan isomers.

Insecticide Standard Solutions. Analytical Thiodan and partially purified

isomers A and B (obtained from Niagara Chemical Division, Middleport, N. Y.) were dissolved in n-hexane so that 1 ml. contained 10 mg.

Experimental Methods

Gas Chromatography. Appropriate volumes of insecticides, ranging from 2.5 to 50.0 μl. (25 to 500 γ), were injected into the rubber diaphragm of the heated injector block. The column had been previously equilibrated at 250° C. for 24 hours. The filament current was kept at 250 ma., and a helium gas flow of 52.6 ml. per minute was maintained.

Under these conditions a steady base line was obtained with no signal attenuation. The solvent, n-hexane, had a retention time of less than 1 minute, while Thiodan isomers A and B had retention times of 10.5 and 14.0 minutes, respectively. Resolution of the solvent and the two isomers was excellent (Figure 1).

For quantitative evaluation, the areas of the peaks were determined by half the product of the peak height and the width given by the inflection tangents

at the base (2) (Figure 1). The ideal shape of the effluent curves permitted such an approximation. "Corrected concentration" was plotted against "area" and the same straight line re-
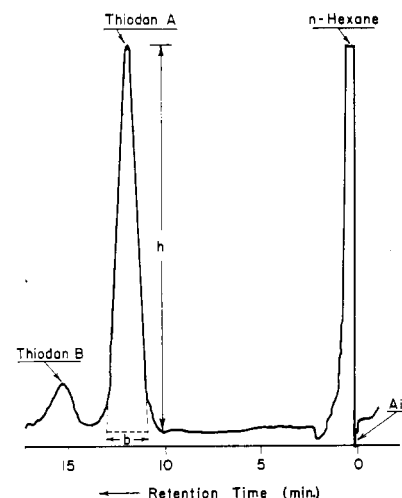

Figure 1. Gas chromatography effluent curve of impure Thiodan A
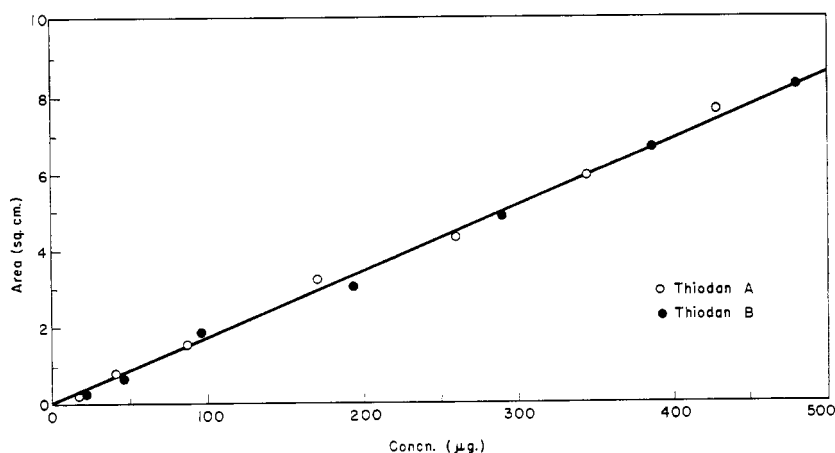
Area $= \frac{1}{2} b \times h$

Figure 2. Calibration curve for Thiodans A and B

sulted over a range of 25 to 500 γ for each isomer, as is illustrated in Figure 2. The necessity for correction is discussed under Results and Discussion.

Miscible formulations of Thiodan were diluted appropriately with n-hexane, and peak areas due to each isomer were directly converted to concentration from the calibration curves. Relative isomer concentration was calculated by the simple equations:

$$\% \text{ isomer A} = \frac{\text{area}_{(A)}}{\text{area}_{(A)} + \text{area}_{(B)}} \times 100 \quad (1)$$

$$\% \text{ isomer B} = 100 - \% \text{ isomer A} \quad (2)$$

Preparation of Pure Thiodan Isomers. For the preparation of several milligrams of pure Thiodan isomers A and B, 1 ml. of the Thiodan solution, containing 10 mg. of mixed isomers, was injected into the column. As each isomer emerged from the column, as indicated by the recorder, it was condensed at room temperature in a U-shaped glass tube, 3 mm. in internal diameter, one end of which was inserted through the collector gasket at the effluent stream. Each isomer was examined by infrared spectroscopy in solution (carbon tetrachloride or carbon disulfide).

Melting points of these pure isomers were determined in an air bath with a glass capillary and were found to be 105–7° C. for A and 203–5° C. for B, in agreement with values reported in the literature (3).

Results and Discussion

Standard Curves. Thiodan isomers A and B (Niagara Chemical Division) were first examined by gas chromatography; Thiodan B contained a small amount of the lower melting isomer when 500-γ quantities were chromatographed. At lower concentrations this impurity was not detectable. Corrections for this impurity were made by the use of Equations 1 and 2. By this method it was determined that Thiodan B contained 3.0% of Thiodan A, and Thiodan A contained 14.1% of Thiodan B (Table I). The contamination of Thiodan A could be detected and measured quantitatively at the 300-γ level.

Gas chromatography was successfully employed to isolate small quantities of pure isomers, as shown by single effluent peaks. The column, however, was not suitable for the isolation of sufficiently large quantities of pure isomers to establish standard curves. Therefore, it was necessary to show that the peak areas, measured by the "half-peak-width" method, were additive. This was demonstrated by the fact that the standard curves, using the corrected values for Thiodan A and B, coincided, as may be seen in Figure 2.

Table I. Standard Curves for Thiodan Isomers

Concn., γ		Peak Areas, Sq. Cm.[a]		% Thiodan A
Apparent	Corrected	A₁	A₂	
		Thiodan A		
500	430	7.6[b]	1.2	86.2
400	344	6.0	0.9	86.4
300	258	4.4	0.7	85.2
200	172	3.3	0	...
100	86	1.5	0	...
50	43	0.7	0	...
25	22	0.2	0	...
				Av. 85.9
		Thiodan B		
500	484	0.2	7.9	2.5
		0.3	8.7	3.7
		0.2	8.4	2.7
				Av. 3.0
400	387	0	6.7	
			6.5	
			6.8	
300	291	0	5.0	
			4.8	
			4.9	
200	194	0	3.1	
			3.1	
100	97	0	2.0	
			1.9	
50	48.4	0	0.8	
			0.7	
25	24.2	0	0.4	
			0.4	

[a] A_1 = Thiodan A. A_2 = Thiodan B.
[b] Average of duplicates.

Table II. Analysis of Thiodan Formulation by Gas Chromatography

(2.288 mg. of formulated Thiodan in 50 μl. of n-hexane)

No. of Analysis	Peak Areas, Sq. Cm.[a]		
	A₁	A₂	A₃
1	5.6	2.9	0.2
2	6.3	3.1	0.3
3	6.1	3.4	0.3
4	6.4	3.4	0.3
Av.	6.1	3.2	0.3

γ Thiodan (Figure 2) corresponding to
6.1 sq. cm. = 358 γ
3.2 sq. cm. = 185 γ
——————
543 γ (exptl.)

γ Thiodan (theoretical)
2,288 × 0.24 = 549.12 γ (theor.)

[a] A_3 = unidentified impurity.

Qualitative runs were also made with chromatographically obtained isomers; single peaks resulted in each case. These experiments indicated that isomerization of the pure isomers did not occur at column temperatures of 250° C. This was further confirmed by an examination of the infrared spectra and other physical constants.

Analysis of variance of the data in Table I showed no significant difference between replicates at the 5% level and a high degree of reproducibility. One source of error might be the variability
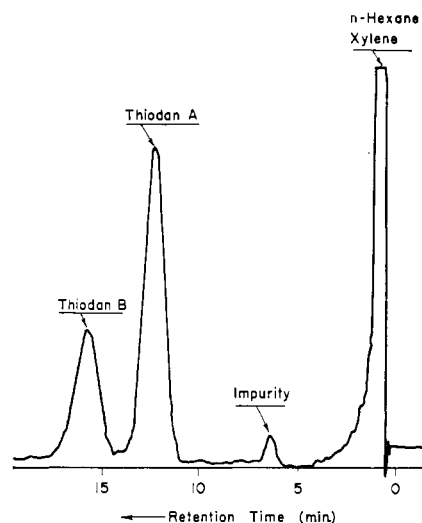

Figure 3. Gas chromatography effluent curve of Thiodan formulation

of pipetting due to the skill of the operator, but this error did not appear on comparing relative concentrations of isomers in a mixture. Excellent reproducibility of this ratio may be seen in Table I. This technique, therefore, may be successfully applied to the determination of isomer ratios, as in production control.

Analysis of Thiodan Formulation. To test the validity of the method described, a formulated preparation of Thiodan was analyzed. This formula-
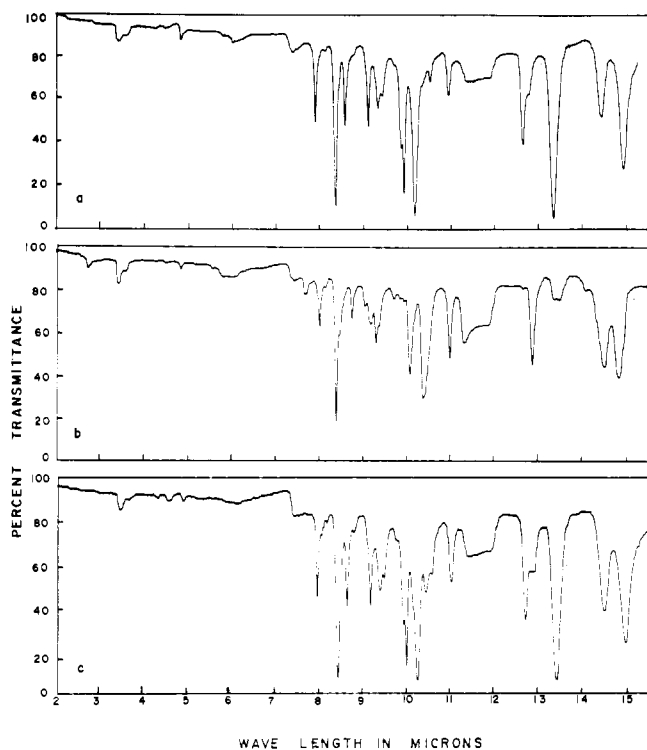
Figure 4. Infrared spectra of carbon disulfide solutions

a. Chromatographically pure Thiodan A (low melter)   b. Chromatographically pure Thiodan B (high melter)   c. Impure Thiodan A   0.03% solutions; 3-mm. liquid microcell

tion bore the following label: Thiodan 24.00% (by weight); xylene 69.00%; inert ingredients 7.00%. A weighed amount of this formulation was dissolved in 10.0 ml. of *n*-hexane, and an analysis was performed on 50-μl. aliquots with four replications. Table II summarizes the results of this analysis— Thiodan 23.69%; Thiodan A/Thiodan B = 1.92. The chromatographic pattern of this formulation shows the presence of a minor impurity which may be due to one of the inert ingredients (Figure 3). Xylene has a retention time similar to that of *n*-hexane (less than 1 minute).

Analysis of this formulation by infrared spectroscopy, measuring the absorption peak at 8.40 microns, gave a concentration of 23.43% of Thiodan (5). Thus, the two methods of analysis were shown to be in good agreement.

INFRARED SPECTROSCOPY. Chromatographically pure Thiodan B when dissolved in carbon tetrachloride yielded an infrared spectrum identical to that published in the literature (3).

The infrared spectrum of a solution of Thiodan B in carbon disulfide yielded two additional bands at 12.8 microns (strong) and 13.35 (weak) (see Figure 4, *b*). These bands may be associated with the carbon-chloride bond, as carbon tetrachloride absorbs strongly in the 12- to 14- micron region.

Figure 4 also illustrates the difference in infrared spectra of the chromatographically pure and contaminated samples of Thiodan A. The contami-

nated A shows an additional absorption band at 10.45 microns which is absent from the spectrum of pure A. As gas chromatographic analysis showed that the impurity was due to the B isomer, this band may correspond to a strong absorption peak of pure B in this region. This 10.45-micron band may also be present in the published spectrum of Thiodan A, indicating a possible contamination with B (3).

Figure 4, *a* also shows three additional absorption bands for Thiodan A when examined in carbon disulfide. These bands are at 12.63, 12.74 (weak), and 13.28 microns and may again be associated with the carbon-chloride bond.

POSSIBLE ISOMERIZATION OR DECOMPOSITION BY GAS CHROMATOGRAPHY. The possibility existed that either Thiodan isomer could be interconverted at the temperature of the chromatographic column (250° C.). However, chromatographically isolated Thiodans A and B yielded single effluent peaks when rechromatographed at concentrations at which 2 to 3% conversion could have been detected.

Elemental analysis of chromatographically pure Thiodan B gave the following results: Found, % C 25.29; % H 1.55; % S 7.37. Calculated, % C 26.55; % H 1.47; % S 7.87. This was taken as evidence that Thiodan B was not decomposed on the chromatographic column. Furthermore, determination of the retention times of possible decomposition products gave: Thiodan A 10.5 min-

utes, Thiodan B 14.0 minutes, Thiodan alcohol (Hooker Chemical Corp., Niagara Falls, N. Y.) 9.5 minutes, and Thiodan ether (Hooker) 5.3 minutes. Mixtures of these four compounds were well resolved under the stated experimental conditions.

When pure Thiodan isomers were chromatographed, perfectly symmetrical retention curves were obtained, and no additional peaks due to Thiodan alcohol or ether were observed. These observations then precluded the possible isomerization or decomposition of Thiodan isomers during gas chromatography at elevated temperatures.

Future Research

Preliminary experiments with an ionization detector (Sr^{90}) and amplifiers indicate that a much higher sensitivity, on the order of 0.1 to 1.0 γ of Thiodan, may be achieved by gas chromatography.

Experiments are in progress in this laboratory combining gas chromatography and infrared spectroscopy for determination of Thiodan isomer residues in raw agricultural crops.

Literature Cited

(1) Coulson, D. M., Cavanagh, L. A., Stuart, J., J. AGR. FOOD CHEM. **7,** 250–1 (1959).
(2) Keulemans, A. I. M., "Gas Chromatography," Chap. I, p. 16, Chap. II, p. 32, Reinhold, New York, 1957.
(3) Lindquist, D. A., Dahm, P. A., *J. Econ. Entomol.* **50,** 483–6 (1957).
(4) Niagara Chemical Division, Food Machinery and Chem. Corp., Richmond, Calif., bulletin, "Colorimetric Microdetermination of Thiodan Residues."
(5) Rubenstein, D., Zweig, G., Univ. of Calif., Davis, Calif., unpublished data.
(6) Stange, H., Organic Chemicals Dept., Food Machinery and Chemical Corp., Princeton, N. J., private communications.