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INSECTICIDE RESIDUES

Microdetermination of Thiodan Residues

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Thiodan residue is extracted from plant material with *n*-hexane, and the extract is cleaned up, evaporated to dryness, and reacted with aqueous pyridine-alkali. The reaction, carried out in a single test tube, is sensitive to both isomers of technical Thiodan and to Thiodandiol. The method is sensitive to 5 μ g. of Thiodan, requires no special apparatus or reagents, and is adaptable to routine laboratory work when a large number of samples are to be analyzed. It has been used for determining Thiodan residues on sugar beet leaves, alfalfa, and strawberries, and for Thiodan vapor in air.

ANALYTICAL methods suggested for determining Thiodan (6,7,8,9,10,10 - hexachloro - 1,5,5a,6,9,9a - hexahydro - 6,9 - methano - 2,4,3 - benzo-dioxathiepin - 3 - oxide) have been based on chlorine analysis or hydrolysis of Thiodan to Thiodandiol and sulfur dioxide and determination of sulfur dioxide by iodometric or colorimetric procedures (2). The chlorine method is not specific, and the sulfur dioxide method will not determine Thiodan degradation products in pesticide residues. Zweig and Archer (6) have developed a gas chromatographic technique for the analysis of Thiodan residues, and Zweig, Archer, and Rubenstein (7) have combined the gas chromatographic technique with infrared analysis to increase the sensitivity of the method. Thiodan has been found to react with alkali to form a compound that can be evaluated quantitatively with a pyridine-water-sodium hydroxide reagent by a Fujiwara-type reaction (3). The method is sensitive to 5 μ g. of Thiodan and has been used to measure up to 100 μ g. Both isomers of technical Thiodan and Thiodandiol can be measured by this reaction. Thiodan ether will not react. The method requires no unusual apparatus or reagents and is adaptable to the routine laboratory

work associated with a large number of samples. Chlorinated solvents, such as carbon tetrachloride and chloroform, must be absent from the air and the apparatus.

Reagents

Thiodan Standard Solution. Dissolve 0.1063 gram (94% Thiodan, Food Machinery & Chemical Corp., Niagara Chemical Division), in 1 liter of distilled *n*-hexane. One milliliter of this solution contains 100 μ g. of Thiodan. Dilute an aliquot of this solution with *n*-hexane so that 1 ml. contains 10 μ g. of Thiodan.

Cotton, acetone extracted and oven dried.

n-Hexane, redistilled over sodium. Skellysolve B or any other *n*-hexane with a low reagent blank can be used.

Pyridine Solution, 96% in distilled water. Purify the pyridine by refluxing over solid potassium hydroxide for 1 hour (700 ml. of pyridine and 50 grams of KOH), cool, decant into a clean dry flask, and distill. Add 4% of water to the purified pyridine.

Mineral Oil Solution. Dissolve 0.2 ml. of mineral oil (Nujol or equivalent) in 100 ml. of *n*-hexane.

5*N* Sodium Hydroxide Solution. Dissolve 200 grams of reagent-grade sodium hydroxide in distilled water and dilute to 1 liter.

Apparatus

Oil bath (100° \pm 2° C.).
Spectrophotometer.

A modified, Smith-Greenberg, all-glass impinger or equivalent for air samples.

Preparation of Standard Curve

Pipet aliquots of the Thiodan standard solution containing 0 to 100 μ g. into glass-stoppered test tubes. Dilute to a volume of 10 ml. with distilled *n*-hexane. Add 1 ml. of the mineral oil solution and evaporate to dryness with the aid of a gentle stream of air on top of a steam bath, or in a water bath, at about 50° C. Add 5 ml. of the pyridine-water solution and 2 ml. of 5*N* sodium hydroxide, stopper, mix well, and immerse in an oil bath (100° C.) for 3 minutes. Swirl occasionally while the samples are heating without removing them from the bath. At the end of the heating period, remove the test tubes and cool them in an ice-water bath for 1 minute. Loosen the stoppers in the test tubes to prevent freezing. Decant the pyridine solution through a loosely packed cotton plug, in a small funnel, into 1-cm. cells and determine the absorbance at 525 $m\mu$, with distilled water as a reference. A cloudy filtrate indicates that the reaction mixture has not been sufficiently cooled before filtration. If the

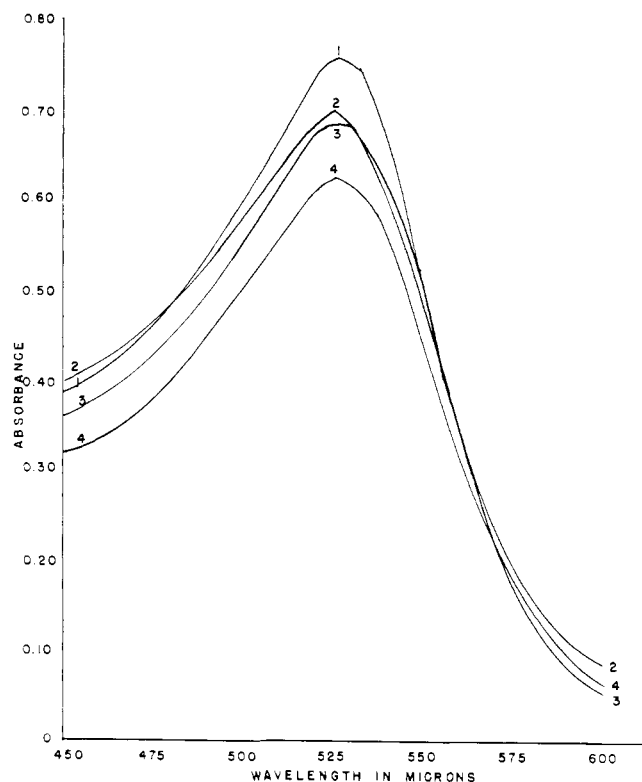


Figure 1. Absorption characteristics of the pyridine-sodium hydroxide reaction products with Thiodan, Thiodan isomers, and Thiodandiol

- (1) Thiodandiol
- (2) High m.p. Thiodan isomer
- (3) Recrystallized technical Thiodan
- (4) Low m.p. Thiodan isomer

cotton is packed too tightly, some color will be removed from the pyridine solution. In this experiment, the average slope for Thiodan (94%) was 0.0092 with a Y -intercept of zero, and the curve followed Beer's Law from 5 to 100 μg . No attempt was made to extend the range. The range in absorbance of standard solutions determined on a Beckman Model B spectrophotometer was from 0.017 for 0 μg . to 0.883 for 100 μg .

The slope of the curve for isomer *A* (m.p. 105° to 107° C.) is slightly greater than that obtained with the recrystallized technical Thiodan, whereas the slope of the curve for isomer *B* (m.p. 203° to 205° C.) is slightly less. The slope of the curve of the Thiodandiol is midway between that of recrystallized technical Thiodan and isomer *A*.

Absorption Characteristics of Thiodan, Thiodan Isomers, and Thiodandiol

Commercial Thiodan is a mixture of two geometric isomers (4). Lindquist and coworkers (5) have suggested that Thiodan residues may contain Thiodandiol. Samples of recrystallized technical Thiodan, Thiodan isomer *A* (m.p. 105° to 107° C.), Thiodan isomer *B* (m.p. 203° to 205° C.), and Thio-

dandiol were obtained (Food Machinery & Chemical Corp., Niagara Chemical Division) for evaluation of the proposed analytical method for Thiodan. The absorption characteristics of the reaction products of the pyridine NaOH reagent with purified Thiodan, Thiodan isomers *A* and *B*, and Thiodandiol are presented in Figure 1. Maximum absorbance of these compounds was between 525 and 527.5 μm .

Preparation of Samples

Sugar Beet Leaves. Samples of frozen sugar beet leaves (consisting of 179 and 716 square centimeters of leaf surface) were shaken for 3 minutes with 50 ml. of distilled *n*-hexane on a mechanical shaker. The agitation during this time reduced the frozen leaf tissue to a fine enough state to be considered a total rather than surface extraction. After the sample was shaken, a small amount of sodium sulfate was added, and the extract was filtered through a filter paper containing additional sodium sulfate. The flask and filter paper were rinsed three times with *n*-hexane.

Strawberries. One hundred grams of the berries were weighed into a 1-pint jar, 100 ml. of *n*-hexane added, and the jar sealed. The jar was shaken vigor-

ously at 5-minute intervals for 30 minutes and the extract decanted into bottles. This procedure constituted a surface extraction.

Air Samples. Samples were collected in a two-stage, modified, Smith-Greenberg all-glass impinger with distilled *n*-hexane as the solvent. The vacuum source was a Venturi-type pump activated by a large-sized carbon dioxide cylinder. The air was drawn into the first impinger, washed with distilled *n*-hexane, then drawn into the second impinger, and washed in a similar manner. The volume of air drawn through the impinger was measured on a precalibrated gas meter. The hexane washes were concentrated in a flask fitted with a Snyder column on a steam bath and then transferred to test tubes for analysis.

Cleanup of Extracts

No cleanup was necessary for the extracts from sugar beet leaves, strawberries, or the *n*-hexane solution from the air samples.

Alfalfa. Samples were chopped with a vegetable cutting mill and thoroughly mixed. A 200-gram aliquot was taken, returned to the cutting mill, and mixed with sodium sulfate to obtain a crumbly mixture. This sample was transferred to a 1-gallon bottle with 600 ml. of *n*-hexane and the jar sealed and tumbled for 2 hours. The extract was decanted into bottles. This was considered to be a surface extraction.

Cleanup of Alfalfa Extract. An aliquot containing less than 100 μg . was evaporated to dryness by warming to about 50° C. and sweeping with a gentle stream of air. The residue was redissolved in 20 ml. of ethyl ether, transferred quantitatively to a 50-ml., glass-stoppered, centrifuge tube, and the volume adjusted to about 35 ml. One gram of activated carbon (Nuchar) was added and the sample carefully shaken for 1 minute. The tube was then centrifuged at 2000 r.p.m. for 4 minutes and the clear, colorless supernatant liquid decanted into a 250-ml. flask. The amount of carbon may be increased, if necessary, to remove all of the color. After decanting the supernatant liquid, 30 ml. of ethyl ether was added to the centrifuge tube, the tube shaken for 1 minute, centrifuged again, and the liquid decanted into 250-ml. flask. The ether wash was repeated three times.

Analysis of Extracts

After any necessary cleanup, the procedure given for preparation of the standard curve was followed. One milliliter of the mineral oil solution was added to an aliquot of the extract and the sample evaporated to dryness on a steam bath or in a water bath at about

Table I. Recovery of Thiodan Added to Various Crops

Sample	Extraction	Cleanup	Sample Size	µg./Sample		% Recovery
				Added	Found	
Sugar beet leaves	Total	None	179 ^a	5	4.7	94.0
			179	10	11.7	117.0
			716	10	9.6	96.0
Alfalfa	Total	Ether solution decolorized with Nuchar	100 ^b	200	180.0	90.6
			100	500	508.0	101.6
			200		458.5	91.7
			200		478.5	95.7
Strawberry	Surface	None	100 ^b	75	82.0	109.3
			100		70.5	94.0

^a Square centimeters of leaf surface.

^b Grams of sample.

50° C. with a gentle stream of air. For ether extracts, the volume was reduced to about 5 ml. with the aid of a three-ball Snyder column, transferred to a test tube, and carefully evaporated to dryness. Five milliliters of the pyridine solution and 2 ml. of 5*N* sodium hydroxide were added, the tube was stoppered, and the mixture heated in a 100° C. oil bath for 3 minutes with occasional swirling, cooled for 1 minute in an ice-water bath, filtered through a loosely packed cotton plug, and the absorbance determined with a spectrophotometer at 525 mµ. Samples of the crop being analyzed that had not been treated with Thiodan were also analyzed as a check or control.

Recovery of Thiodan from Extracts

Known amounts of purified Thiodan were added to check or control samples prior to extraction with *n*-hexane and the percentage recovery of the added Thiodan was determined. The results are presented in Table I. In an effort to approximate the hexane extracts of air, two samples (500 ml. of *n*-hexane) were fortified with 5 and 25 µg. of Thiodan,

respectively. These samples were concentrated, with the aid of a three-ball Snyder column, on a steam bath, transferred to test tubes, and analyzed in the manner described. Recoveries were 5.4 µg. (108%) and 24.8 µg. (99.2%), respectively.

Interferences

Other compounds may possibly interfere with this analytical method (1). One hundred micrograms of a number of insecticides were analyzed by this method to check for possible interferences. No interference was found for ovex, DDT, aldrin, Tedion (2,4,4',5-tetrachlorodiphenyl sulfone), dieldrin, endrin, methoxychlor, and Kepone [decachlorooctahydro - 1,3,4 - metheno-2*H* - cyclobuta(cd)pentalen - 2 - one]. Chlorobenzilate (ethyl 4,4' - dichlorobenzilate), heptachlor epoxide, and toxaphene gave interferences equivalent to 0.4, 0.7, and 4.0 µg. of Thiodan. Chlordane and heptachlor gave interferences equivalent to 11.0 and 25.0 µg. of Thiodan, and Kelthane [4,4' - dichloro - *alpha* - (trichloromethyl)-benzhydrol] gave an interference equivalent to 73.5 µg.

PESTICIDE RESIDUES

Total Organic Chloride Content in Butterfat by a Rapid Method of Neutron Activation Analysis

CHLORINATED insecticides are chiefly aryl organic molecules that have attached chlorine atoms. For example, the insecticide DDT is 50% chlorine by weight and only about 18% chlorine in atom per cent; the insecticide toxaphene is 68% chlorine by weight and about 30% chlorine in atom per cent. If these chlorinated organic compounds are present, even at low concentrations, in fodder and are ingested by dairy animals, some

of the chlorinated organics may be released in milk by these animals (7). Therefore, determinations of total organic chloride in butterfat yield the total chlorinated insecticide content of milk. Neutron activation analysis would be suitable for a quick screening test for possible insecticides in milk and dairy products such as cheese and ice cream.

At present, analytical procedures such as chromatographic (4) and colorimetric

Discussion

Ammonium hydroxide, aluminum hydroxide, and barium hydroxide, when substituted for the sodium hydroxide, gave no color even when the heating time was increased. Sodium hydroxide and potassium hydroxide from 0.5 to 3.0*N* produced the desired color, but a precipitate formed very quickly and this interfered with the reading of the absorbance. Sodium hydroxide gave a higher absorbance than potassium hydroxide. Sodium hydroxide above 5*N* gave colors in the orange range and a lower absorbance. Three minutes of heating at 100° C. with 5*N* sodium hydroxide gave the optimum absorbance.

The method requires no special apparatus or reagents, is rapid, and is adaptable to routine microdeterminations of Thiodan residues in or on food and forage crops.

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(5, 6) techniques are used to ascertain the insecticide content of milk and dairy products. The information derived from these products is specific for quite a number of insecticides (3). Specific sensitivities, as low as 0.03 p.p.m. of DDT, are claimed for chromatographic techniques and about 1 p.p.m. for the total chlorine coulometric technique.

Neutron activation analysis of chlorine induces radioactivity in chlorine nuclei.