Colorimetric Determination of Guthion Residues in Crops

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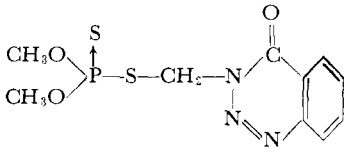
A colorimetric method for the determination of Guthion residues in crops is based on alkaline hydrolysis to anthranilic acid. The anthranilic acid is determined colorimetrically by diazotization and coupling with *N*-(1-naphthyl) ethylenediamine dihydrochloride. Cleanup procedures are described for cottonseed, cole crops, fruit, and milk. The method can be applied to the ethyl homolog of Guthion.

GUTHION, *O,O*-dimethyl *S*-[4-oxo-1,2,3 - benzotriazin - 3(4*H*) - ylmethyl] phosphorodithioate, is a broad-spectrum insecticide which has been introduced recently. It is particularly effective against a wide variety of insects which attack cotton, fruit, and vegetables. In the literature, this material has been referred to as Bayer 17147 and as "Gusathion."

Its structural formula is



The compound is soluble in many organic solvents. Solubility in water is approximately 1 part in 30,000.

In considering suitable means for determining Guthion (Chemagro Corp. trade-mark) residues in crops, several possibilities presented themselves. The compound can be hydrolyzed in alkali to form dimethyl phosphorodithioate (*4*). This compound forms a colored complex with copper similar to that used for the determination of malathion residues (*9*). A colorimetric procedure involving coupling with phenyl-1-naphthylamine in acid solution has been described by Wollenberg and Schrader (*11*). The alkaline hydrolysis of benzazimide to form anthranilic acid has been reported by Finger (*3*). The anthranilic acid formed on hydrolysis can be diazotized and coupled to form a colored complex by a procedure similar to that described by Bratton and Marshall (*2*).

The anthranilic acid method of analysis offered most promise, because the final colorimetric determination has been widely used and is well defined. The reaction involving the formation of a copper complex (*9*) has the disadvantage that the color is not stable. A preliminary study of the phenyl-1-naphthylamine method (*11*) showed that much development work would be required before this procedure would be applicable to routine analyses.

Recently a colorimetric procedure for Guthion residues in cottonseed has been reported by Giang and Schecter (*5*). The Guthion is hydrolyzed with acid and the liberated formaldehyde is distilled and determined colorimetrically with chromotropic acid.

In this paper, a colorimetric procedure based on hydrolysis of Guthion to form anthranilic acid is presented. Extraction and cleanup methods for cottonseed, fruits, cole crops, and milk are also described.

In the development of a satisfactory residue method, due consideration must be given to the possible presence of toxic metabolites and to interference from nontoxic decomposition products. The objective is to include the former compounds in the residue method while omitting the latter.

One would expect the principal toxic metabolite of Guthion to be its oxygen analog. Although metabolic studies (*10*) have shown that this compound is not formed in cotton, procedures have been developed which would include residues of this material if it should happen to be present.

Interference from Guthion hydrolysis products might be expected from 1,2,3-benzotriazin-4(3*H*)-one (I), 3-(hydroxymethyl) - 1,2,3 - benzotriazin - 4(3*H*)-one (II), 3-(mercaptomethyl)-1,2,3-benzotriazin-4(3*H*)-one (III), or *o*-aminobenzoic acid (IV). It has been shown that the first two compounds do not interfere with the method described. Compound III gives 70% of the theoretical amount of color and would interfere, if present. Compound IV would also interfere. The extent of interference due to IV can be determined by carrying a sample through the procedure, omitting the hydrolysis step.

Colorimetric Determination

Hydrolysis Conditions. In the initial studies on Guthion hydrolysis, the Averell and Norris (*1*) method was used without modification to determine the amount of anthranilic acid formed. Finger (*3*) reported that benzazimide is decomposed to nitrogen and anthranilic acid on heating with alkali, but did not describe the conditions which he used for hydrolysis.

At the outset, Guthion was dissolved in isopropyl alcohol and aqueous 1*N* potassium hydroxide was added. Under these conditions yields of anthranilic acid were approximately quantitative in about 10 minutes at room temperature; under the same conditions the Guthion oxygen analog produced only 50 to 70% of the expected color. Accordingly, the effect of the solvent system on this reaction was studied.

In this investigation all solutions were 0.5*N* with respect to potassium hydroxide. The solvents used were absolute alcohols and 1 to 1 mixtures of these with water. The results of this study are shown in Table I. The absorbances recorded were obtained using 100 γ of the appropriate compound. It is apparent that to obtain color from the oxygen analog equivalent to that formed from Guthion, one must use isopropyl alcohol as the hydrolysis solvent. Alcoholic potassium hydroxide is not a satisfactory solvent for the solution of waxy plant residues. Benzene was very useful in keeping these substances in solution during the hydrolysis. Further studies conducted using a 1 to 1 mixture of benzene and 0.5*N* potassium hydrox-

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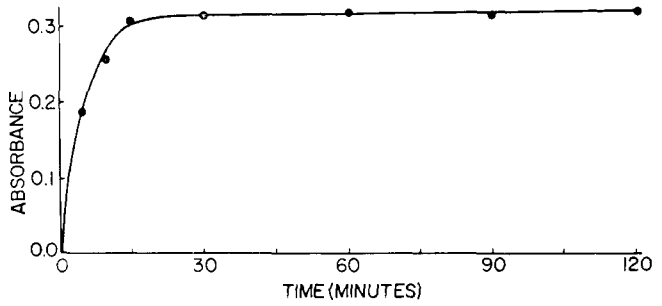

Figure 1. Effect of time of hydrolysis of Guthion on amount of color formed

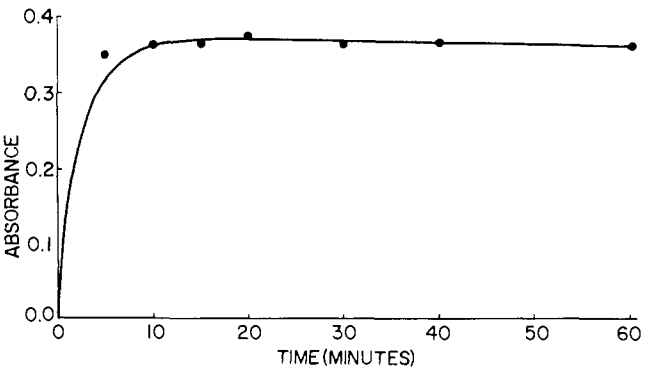

Figure 2. Rate of color formation

ide in absolute isopropyl alcohol showed that dilution with benzene had no effect on the amount of color developed by either Guthion or its oxygen analog.

The time required for completion of hydrolysis was investigated by conducting a series of determinations in which the hydrolysis time was varied from 5 to 60 minutes. In this study, 100-γ samples of Guthion and its oxygen analog were hydrolyzed in a 1 to 1 mixture of benzene and 0.5N potassium hydroxide in absolute isopropyl alcohol. The analytical results for Guthion are shown in Figure 1. Identical results were obtained with the oxygen analog. The hydrolysis is practically complete in 10 minutes at room temperature. To ensure complete hydrolysis, a hydrolysis time of 20 minutes was adopted for routine purposes.

Conditions for Color Development. To find the optimum final acid concentration, determinations were carried out in which 2, 4, 6, 7, 8, 10, and 12 ml. of 3N hydrochloric acid were added to a series of hydrolysis solutions. Maximum color development was obtained with 8 ml. of 3N acid. With less acid, less color was developed. When 10 or 12 ml. of acid were used, the same amount of color was developed as with 8 ml., but the rate of color formation was somewhat decreased.

The rate of color development was investigated using 8 ml. of 3N hydrochloric acid (see Figure 2). Color development is practically complete in 15 minutes. To be certain that small variations in time would have no effect on the amount of color formed, a standing time of 90 minutes was adopted for routine work.

The absorption spectrum of the color formed from Guthion was determined on a Beckman DU spectrophotometer. The absorption maximum is at 555 mμ and this wave length was adopted for routine purposes. When the conditions described above are used, the color obeys Beer's law.

The yield of anthranilic acid under the conditions described was shown to be 85% of the theoretical amount. Anthranilic acid in the hydrolyzate was positively identified by paper chromatography using *tert*-butyl alcohol containing 10% water. Co-chromatography and determination of the absorption spectra of the color complexes from Guthion and anthranilic acid also showed these to be identical.

Application to Crop Residues

Cottonseed. The separation of Guthion from cottonseed presents special problems because of the presence of a large amount of oil as well as gossypol and related pigments. Jones and Riddick (*6*) suggested extraction of a hexane solution with acetonitrile to separate pesticides from glycerides. Kolbezen and Reynolds (*7*) applied this approach to the determination of chlorthion in cottonseed. The efficacy of this system for Guthion was tested using refined cottonseed oil. In this experiment 250 γ of Guthion and 40 grams of refined cottonseed oil were dissolved in 350 ml. of petroleum ether. This approximates the amount of oil which would be obtained from 200 grams of cottonseed. Similar solutions were also prepared, omitting the Guthion and omitting the oil. The petroleum ether solutions were extracted with 50 ml. of acetonitrile for the first extraction and 25 ml. for each subsequent extraction. Each acetonitrile extract was evaporated and analyzed separately.

Four extractions gave a 98.1% recovery of Guthion when oil was omitted but only 90.9% recovery when oil was present. Three additional extractions of the solution containing oil increased the recovery to 94.1%.

At the outset it was observed that the acetonitrile extract contained pigments which interfered with the final color determination. Therefore, the acetonitrile solutions were chromatographed by passage through acid-washed alumina as suggested by Kolbezen and Reynolds (*7*). This was very effective in removal of the interfering materials. Experiment showed that neither Guthion nor its oxygen analog is retained by the column.

In the procedure as finally adopted, the acetonitrile is evaporated on the steam bath. A preliminary experiment with an acetonitrile solution of Guthion showed that no loss is incurred by this treatment.

Fruit. In the extraction of Guthion residues from fruit, two problems were encountered. The extraction of microgram quantities of oil-soluble insecticides from tissues which contain a large amount of water is difficult. In many cases this type of extraction has been carried out by maceration of the plant tissue with a water-immiscible fat solvent. However, fat is more completely extracted from such material by the use of a water-miscible solvent such as acetone or ethyl alcohol. In the procedure adopted, acetone was selected as the solvent for the initial extraction.

The second problem involves the interference of plant pigments which are extracted along with the Guthion. Extracted carotenoid pigments do not show appreciable absorption at 555 mμ and, therefore, do not interfere. However, the small amounts of chlorophyll extracted from green fruit interfere seriously in the final color determination. As separation of chlorophyll from Guthion in an aqueous acetone solution is difficult, it was decided to destroy the chlorophyll by treatment with a small amount of hydrochloric acid. The chlorophyll is rapidly converted to yellow-brown pheophytin-like pigments. In the case of fruit with small amounts of chlorophyll, these pigments cause only slight interference in the final determination.

The basic method, then, consists of the following steps. The tissue is macerated in an excess of acetone, and the insoluble material is removed by filtration. Following dilution with water, sufficient hydrochloric acid is added to make the solution about 0.06N. Guthion residues are extracted from the acetone-water phase with successive portions of chloroform.

The efficiency of chloroform in removing Guthion from aqueous acetone solutions was tested on an apple extract. Known amounts of Guthion were added to the aqueous acetone extract and this

was extracted with one 100-ml. and eight 50-ml. portions of chloroform. The extracts were analyzed separately. Seven extractions gave recoveries ranging from 97 to 114%. The eighth and ninth extractions had little or no effect on the over-all recovery.

Cole Crops. Vegetables and fruits containing high concentrations of plant pigments present a special cleanup problem. The use of solvent systems which have been found to be essential if the pesticide residue is to be recovered, results in extraction of large amounts of pigments from which the residues must be separated. Cole crops present an additional cleanup problem, in that they contain an extractable substance which, upon hydrolysis with alkali, produces a compound that can be diazotized and coupled to form a color similar to that formed by Guthion. The unknown substance apparently produces an aromatic amine on treatment with alkali. The color produced by this interfering material is usually equivalent to 0.25 to 1.5 p.p.m. of Guthion, but values equivalent to 10 or 12 p.p.m. are sometimes encountered. This material must be removed from plant extracts before Guthion residues can be determined.

After examination of many adsorbent-solvent systems, both Guthion and its oxygen analog were separated from all plant pigments except carotenoids by a chromatographic column composed of ascending layers of Super-Cel, a 1 to 2 mixture of Sea Sorb 43 and Super-Cel, alumina (acid-washed), and anhydrous sodium sulfate. Isopropyl alcohol was used as both the initial solvent and the eluting agent. This column was effective in removing the interfering substance from cole crops.

Carotenoid pigments remaining in the solution following hydrolysis of the Guthion to form anthranilic acid can be removed by a benzene extraction of the acidified hydrolyzate.

Milk. The Guthion residues were extracted from milk by blending the milk with acetone and removing the insoluble material by filtration. The acetone-insoluble fraction is re-extracted with benzene. The acetone and benzene extracts are combined and evaporated to dryness. The residue is partitioned between hexane and acetonitrile. The pesticide residue partitions into the acetonitrile, while the fat remains in the petroleum ether. The acetonitrile solution is treated as in the method for cottonseed.

Procedure

Reagents. Alumina, chromatographic grade, acid-washed (Merck).

Sea Sorb 43, chromatographic grade magnesium oxide (Westvaco Division, Food Machinery and Chemical Corp., Newark, Calif.).

Guthion, recrystallized (Chemagro Corp., Kansas City, Mo.).

Super-Cel (Celite Division, Johns Manville, Lompoc, Calif.).

Sample Preparation, Extraction, and Cleanup. COTTONSEED. Place a 200-gram sample of finely ground cottonseed in a large Soxhlet extractor and extract for 16 hours with petroleum ether (boiling range, 30° to 60° C.). Centrifuge or filter the petroleum ether extract to remove finely divided material carried over during the extraction. Wash the residue with fresh petroleum ether and combine washings and extract. Evaporate the extract to about 350-ml. volume on a steam bath and transfer to a 500-ml. separatory funnel. Extract the petroleum ether with 50 ml. of acetonitrile. Re-extract with six successive 25-ml. portions of acetonitrile. Wash the combined acetonitrile extracts with 300 ml. of petroleum ether and then wash this petroleum ether with four successive 10-ml. portions of acetonitrile. Combine the acetonitrile solutions and evaporate on a steam bath in a stream of air to a volume of approximately 10 ml. Pour the concentrated acetonitrile extract onto a column of 2.5 inches of acid-washed alumina. Apply gentle suction and elute with 100 ml. of acetonitrile. Evaporate the extract just to dryness on a steam bath under an air jet. Analyze the residue as instructed under Colorimetric Method.

FRUIT. Grind entire sample in a Hobart food cutter and mix thoroughly. Weigh out a 200-gram sample in a Waring Blendor jar. Blend at high speed with 200 ml. of acetone for 5 minutes. Add 200 ml. of acetone and mix for 5 minutes more at low speed. Transfer to a 1-quart screw-cap jar and dilute to 600-ml. volume with distilled water. Shake vigorously on a mechanical shaker for 30 minutes. Filter the mixture through a pledget of glass wool in a large funnel. If the first portion is turbid, refilter. The resultant filtrate should be crystal clear. Normally 400 ml. can be obtained easily and this is the volume usually taken for analysis. Place the extract in a 1000-ml. separatory funnel and add 3.3 ml. of concentrated hydrochloric acid and 300 ml. of water. Extract with seven successive portions of chloroform, using 100 ml. for the first and 50 ml. for each subsequent extraction. Shake gently during the first extraction, or a stable emulsion may be formed. Wash the combined chloroform extracts with 200 ml. of water. Remove the water and wash it with three successive 25-ml. portions of chloroform. Combine the chloroform extracts and dry by passing through anhydrous sodium sulfate. Evaporate the chloroform extract to dryness on a steam bath in a current of air. Analyze the residue as instructed under Colorimetric Method.

COLE CROPS. Prepare and extract samples according to the method described for fruit. Prepare a chromatographic column (20 × 400 mm.) by introducing successively 2 grams of Super-Cel, 5 grams of a 1 to 2 mixture of Sea Sorb 43 and Super-Cel, 10 grams of alumina (acid-washed), and 20 grams of anhydrous sodium sulfate. Maintain suction during packing of column and tap gently to assist packing after each addition. Dissolve the residue remaining after evaporation of the chloroform in 25 ml. of isopropyl alcohol. Boil gently, if necessary, to effect solution. Cool the solution and pour it onto the chromatographic column just as the last of the equilibrating isopropyl alcohol passes into the sodium sulfate layer. Wash with isopropyl alcohol until the total volume of effluent is 160 ml. Evaporate the column effluent to dryness on a steam bath under a current of air. Analyze the residue as instructed under Colorimetric Method.

MILK. Weigh 100 grams of milk into a Waring Blendor jar. Add 200 ml. of acetone and 5 grams of Super-Cel. Blend at high speed for 5 minutes. Filter with suction through a fine filter paper. Return the residue to the jar and add 200 ml. of benzene. Blend for 5 minutes at high speed. Filter as before. Discard the filter cake. Transfer the acetone and benzene extracts to a 1000-ml. separatory funnel. Shake vigorously. After separation, discard the lower aqueous layer. Clarify the acetone-benzene solution by adding 2.0 grams of Super-Cel and refilter. A crystal-clear solution should be obtained. Evaporate to dryness on a steam bath under a current of air. Dissolve the residue in 300 ml. of hexane and transfer to a 1000-ml. separatory funnel. Extract with acetonitrile as described for cottonseed. Evaporate the acetonitrile to dryness on a steam bath in a current of air. Analyze the residue as instructed under Colorimetric Method.

Colorimetric Method

Dissolve the residue obtained by one of the methods described above in 10 ml. of benzene. Add 10 ml. of 0.5*N* potassium hydroxide in absolute isopropyl alcohol and allow to stand for 20 minutes at room temperature. Add 8 ml. of 3*N* hydrochloric acid to acidify the solution. Add 15 ml. of water and 50 ml. of benzene. Transfer to a 250-ml. separatory funnel and shake vigorously. After separation of the phases, draw off the aqueous layer into a 50-ml. glass-stoppered graduated cylinder. Wash the benzene with 15 ml. of distilled water and add the washings to the cylinder. Discard the benzene phase. Dilute the aqueous solution to 50 ml. with distilled water and mix thoroughly. If the solution is turbid at this point, add Super-Cel and filter. Transfer 20-ml. aliquots of the solution to two 25-ml. glass-stoppered graduated cylinders. Add 1 ml. of 0.25% aqueous sodium nitrite solution to each cylinder. Mix and let stand for 10 minutes. Add 1 ml. of 2.5% aqueous sodium sulfamate solution to each cylinder. Rinse the stopper free of nitrite by inverting the cylinder and then, when the cylinder is returned to an upright position, remove the stopper. Repeat several times. Let the solution stand for 10 minutes. Add 2 ml. of a 1% aqueous solution of *N*-(1-naphthyl) ethylenediamine dihydrochloride to only one of the cylinders. Dilute solution in both cylinders to 25

Table I. Effect of Solvent System on Hydrolysis of Guthion and Its Oxygen Analog

Alcohol	Analog	Absorbance[a] 0.5N alcoholic KOH	Absorbance[a] Alcohol + 1N KOH (aqueous)
Methanol	P--O	0.017	0.210
	P--S	0.066	0.359
Ethyl alcohol	P--O	0.162	0.325
	P--S	0.444	0.428
Isopropyl alcohol	P--O	0.432	0.341
	P--S	0.427	0.427
n-Amyl alcohol	P--O	0.140	0.334
	P--S	0.182	0.484

[a] Absorbance values obtained using 100 γ of appropriate compound.

Table II. Recovery of Guthion and Its Oxygen Analog from Crops

Crop	Compound Added	Added, P.P.M.	Found, P.P.M.[a]	Mean Recovery, %
Cottonseed		0.05	0.05 ± 0.01 (5)	100
		0.10	0.9 ± 0.01 (2)	90
		0.20	0.18	90
		0.40	0.32	80
Apples		0.25	0.23	92
		0.50	0.51 ± 0.01 (2)	102
		0.75	0.75 ± 0.04 (2)	100
		1.00	0.91	91
Peaches		0.50	0.50 ± 0.00 (2)	100
Pears		0.50	0.44 ± 0.02 (2)	88
Apricots		0.50	0.44	88
Cabbage[b]		0.20	0.21 ± 0.03 (2)	105
		0.30	0.31 ± 0.01 (2)	103
		0.40	0.43 ± 0.02 (2)	108
		0.50	0.48	96
	Oxygen analog	0.20	0.15	75
		0.30	0.21	70
		0.40	0.36	90
		0.50	0.48	96
Cauliflower[b]		0.20	0.20	100
		0.30	0.28	94
		0.40	0.40	100
	Oxygen analog	0.20	0.22	110
		0.30	0.40	133
		0.40	0.43	107
Broccoli[b]		0.20	0.21	105
		0.30	0.27	90
		0.40	0.34	85
		0.50	0.49 ± 0.03 (2)	98
	Oxygen analog	0.50	0.37 ± 0.02 (2)	74

[a] Values followed by average deviation from mean and in parentheses by number of determinations.
[b] Zinc reduction step included in analytical method used for these samples.

ml. with distilled water and mix thoroughly. Allow to stand for 90 minutes at room temperature and compare the absorbances at 555 mμ in a suitable spectrophotometer or colorimeter. Read both solutions against a solvent blank. The solution from which the coupling reagent is omitted will permit correction for residual color in the extract.

Determine the concentration of Guthion in the samples by comparison with a calibration curve prepared using highly purified Guthion.

In the case of cole crops, the recovery of Guthion and its oxygen analog can be improved by introduction of a zinc reduction step following the hydrolysis and extraction with benzene. When this modification is desired, add 0.2 gram of zinc dust to the solution after dilution to 50 ml. Shake the sample vigorously two or three times during a 15-minute reaction period at room temperature. Filter through a Whatman No. 12 fluted filter paper. Place 20-ml. aliquots of the filtrate in 25-ml. graduated cylinders and proceed according to the instructions above.

Discussion

Recovery Experiments. Recovery experiments were conducted on each of the methods described above (Table II), in which known amounts of Guthion were added to the raw agricultural commodity. While such experiments do not indicate the efficiency of the initial extraction system, they show whether or not the material is lost in the steps subsequent to extraction. Approximately 75% of the recovery values shown fall between 90 and 110% and are considered excellent for this type of procedure. In only three cases were such values less than 80%. It is apparent that the recovery values for the Guthion oxygen analog are not quite as good as those obtained for Guthion itself.

Sensitivity and Precision. Using the method as described above, 8 γ of Guthion in the final 25 ml. of solution will give an absorbance of 0.063 with the standard tubes (approximately 2 cm.) in an Evelyn photoelectric colorimeter. This is considered the limit of sensitivity of this method. This corresponds to a concentration of 0.15 p.p.m. when a 200-gram sample and the dilutions and aliquots described are used. Sensitivity can be increased to 0.05 p.p.m. by using a cell of 5-cm. path length in a Beckman DU spectrophotometer.

A study of the precision of the method showed that in the concentration range up to 1 p.p.m. an average deviation of about 5% of the mean can easily be obtained. With larger residues, the precision is greater.

That compounds I and II are not hydrolyzed under the conditions used for Guthion is surprising. The stability of compound I is attributed to enol-keto tautomerism involving a shift of hydrogen from the nitrogen in position three to the carbonyl oxygen group in position four. This does not explain the stability of compound II. It may be that, in this case, very rapid release of formaldehyde results in the formation of I.

A study showed that approximately 80% of compound III is removed by the cleanup procedures. On this basis, compound III would produce interfering color equivalent to 15 to 20% of the amount present.

The variations in the rates of hydrolysis of Guthion and its oxygen analog in various solvents merit some consideration. The oxygen analog of Guthion is more rapidly hydrolyzed than Guthion (*8*). Because Guthion is hydrolyzed in alkali to form the *O,O*-dimethyl phosphorodithioate, it must be assumed that compound II is also formed. If the rate of formation of compound II is greater for the oxygen analog than for Guthion, it would explain the lower color yield from the oxygen analog in a partially aqueous system. This would require the assumption that the hydrolysis rate of the oxygen analog is greatly decreased in absolute isopropyl alcohol. No explanation is proposed for the fact that the absolute isopropyl alcohol is more effective than absolute methyl or ethyl alcohol.

The methods described can be applied directly to the determination of residues of the ethyl homolog of Guthion.

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INSECTICIDE MEASUREMENT

Determination of Toxaphene by a Spectrophotometric Diphenylamine Procedure

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A confirmatory method for the positive identification of toxaphene in small residues has not previously been reported. A sensitive method for the qualitative and quantitative determination of toxaphene in residues and in formulations is based on fusion with diphenylamine in the presence of zinc chloride. The greenish blue reaction product, dissolved in acetone or glacial acetic acid, has an absorbance maximum at 640 mμ. Toxaphene in the range of 20 to 700 γ can be readily determined by spectrophotometric measurement. When combined with cleanup procedures, the method has been applied to extracts of crops and foods and to various formulations, including mixtures with sulfur or DDT.

THE need for an accurate method for the specific determination of micro quantities of toxaphene in food and forage crops and in milk and beef has become increasingly apparent with the more widespread use of this insecticide. Toxaphene is chlorinated camphene containing 67 to 69% chlorine. Microdetermination by a sensitive method for organic chlorine (*5*) has been widely used for determining toxaphene residues, but this method lacks specificity. Macrodetermination by an infrared method (*4*) is specific, but has not been employed as a residue method because of low sensitivity. A qualitative colorimetric test with pyridine and methanolic potassium hydroxide has been reported (*3*). A spectrophotometric method based upon the reaction of toxaphene with thiourea in the presence of alkali, developed by Hornstein (*2*), is quantitative in the 0.5- to 5.0-mg. range. However, there is need for a more sensitive method.

The reaction of DDT with diphenylamine in the presence of zinc chloride has been reported to give a reddish orange color with an absorbance maximum at 490 mμ (*1*). Toxaphene reacts with these reagents at high temperature and in the absence of solvents to give a greenish blue complex which is soluble in acetone or glacial acetic acid and exhibits an absorbance maximum at 640 mμ; the absorbance–wave length curve is shown in Figure 1 and the spectrophotometric constants are given in Table I. This reaction has been made the basis of a sensitive quantitative method for both residue and assay analyses. Toxaphene in the range of 100 to 700 γ can be readily determined with an accuracy and precision to ±2%; in the 20- to 100-γ range, ±7% is usual. Naturally occurring organic chlorine and most chlorinated insecticides do not interfere; however,

Table I. Sensitivity Constants for Toxaphene Spectrophotometric Procedure

Absorptivity, a, liter/gram cm.	17.0
Molar absorptivity, ϵ, liter/mole cm.	7.0×10^3
Absorbance concentration, γ/ml. (γ/ml. required to give absorbance of 1.00 in 1-cm. cell)	58.8

Measurements made at 640 mμ

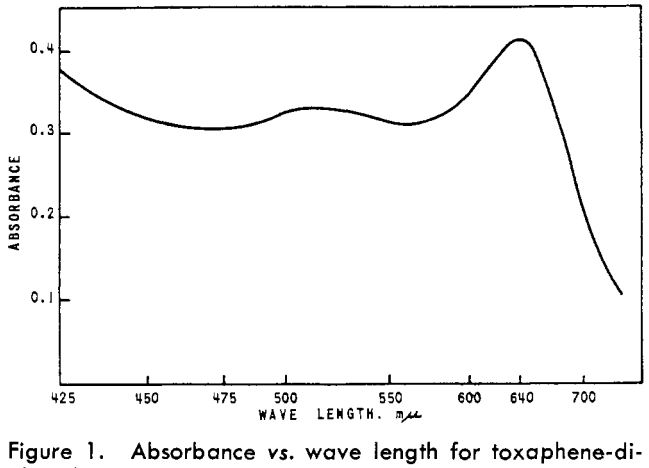

Figure 1. Absorbance *vs.* wave length for toxaphene-diphenylamine reaction product