PESTICIDE RESIDUES

Colorimetric Estimation of Malathion Residues

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A colorimetric method has been developed for the estimation of spray residues of S-(1,2-dicarbethoxyethyl) O,O-dimethyl dithiophosphate, commonly known by the generic name malathion, on plant material. Malathion is removed from the plant material by extraction with carbon tetrachloride and decomposed by alkali in carbon tetrachloride–ethyl alcohol solution into sodium dimethyl dithiophosphate and sodium fumarate. The sodium dimethyl dithiophosphate is extracted into water, converted to a copper complex, and extracted into carbon tetrachloride, in which it forms an intense yellow color. The color is measured and the corresponding amount of insecticide obtained from a standard curve prepared by carrying weighed amounts of pure insecticide through the same procedure. Amounts of malathion between 0.25 and 2.5 mg. in 100-ml. aliquots of carbon tetrachloride extracts of plant material may be readily determined. The method is applicable to the analysis of a wide variety of plant materials.

WIDESPREAD USE OF TOXIC ORGANIC PHOSPHORUS COMPOUNDS as insecticides has created a need for sensitive methods for detecting them as spray residues on plant materials. One of the newer compounds of this type which has attained commercial status is *S*-(1,2-dicarbethoxyethyl) *O,O*-dimethyl dithiophosphate, also known as malathon or Insecticide 4049. Recently the generic name malathion has officially been assigned to this compound.

A colorimetric method based upon the rapid decomposition of malathion by alkali to form dimethyl dithiophosphoric acid, and subsequent determination of this decomposition product, was developed.

Reagents

Carbon tetrachloride. Distill commercial products and store in glass. Commercial carbon tetrachloride has been found to contain an unidentified interfering impurity.

Ethyl alcohol, absolute or formula 2B (anhydrous).

Sodium hydroxide, aqueous 6*N*.

Hydrochloric acid, aqueous 7*N*.

Copper sulfate reagent. Dissolve 1 gram of C.P. cupric sulfate pentahydrate in 100 ml. of distilled water.

Malathion, purified material obtainable from the American Cyanamid Co., Stamford, Conn.

Sodium chloride solution. Dissolve 20 grams of C.P. sodium chloride in 1 liter of distilled water.

Special Apparatus

Glass extraction jars, 1-gallon capacity or larger, with plastic screw caps lined with tin or aluminum foil.

Spectrophotometer or photoelectric colorimeter. A Klett-Summerson colorimeter with filter No. 42 (absorption peak 420 mμ) and matched cylindrical test-tube cells 1.2 cm. in diameter was used by the authors for most of the experimental work.

Tumbling machine, motor-driven rollers geared to produce agitation at about 40 r.p.m. For end-over-end tumbling, place glass extraction jars sideways in a large cylindrical container, which can then be placed on rollers. Other apparatus of similar design may be equally applicable.

Procedure

Preparation of Standard Curve Dissolve approximately 0.25 gram (weighed to 0.1 mg.) of pure malathion in ethyl alcohol and dilute volumetrically to 250 ml. with ethyl alcohol. Mix well, then transfer a 25-ml. aliquot to a 250-ml. volumetric flask, and dilute to volume with ethyl alcohol (1 ml. $\cong$ 0.1 mg. of malathion). Using this standard solution, carry aliquots of 0, 2.5, 5, 10, 15, 20, and 25 ml. through the following procedure.

Transfer the aliquot to a 250-ml. separatory funnel containing 100 ml. of distilled carbon tetrachloride, and add ethyl alcohol until the total volume of ethyl alcohol present is 25 ml., then mix well by swirling. Add 1 ml. of 6*N* sodium hydroxide and shake vigorously for exactly 1 minute.

The procedure from this point on should be carried out without interruption. Aqueous alkaline and acid solutions of dimethyl dithiophosphoric acid are stable for short periods only (probably not more than 1 hour). Conditions required to produce maximum decomposition of the insecticide by alkali to dimethyl dithiophosphoric acid are critical. Reaction periods of less than 30 seconds and more than 2 minutes produce low results. Water must be limited to the amount added as 6*N* sodium hydroxide, or results will be low—for example, the use of 95% ethyl alcohol gives results that are low by about 10 to 20%.

Immediately add 75 ml. of the sodium chloride solution (cooled to about 15° C.) and shake vigorously for 1 minute. Allow the phases to separate, then draw off the carbon tetrachloride layer and discard it. Add 25 ml. of carbon tetrachloride to the separatory funnel, shake vigorously for 30 seconds, allow phases to separate, and discard the carbon tetrachloride layer. Add 25 ml. of carbon tetrachloride and 1 ml. of 7*N* hydrochloric acid to the separatory funnel, shake vigorously for 30 seconds, allow phases to separate, draw off the carbon tetrachloride layer as completely as possible, and discard it. Using a pipet, add exactly 25 ml. of distilled carbon tetrachloride and 2 ml. of the copper sulfate reagent, shake vigorously for 1 minute, and allow phases to separate. (A smaller amount of carbon tetrachloride may be used to extract the yellow color for measurement if a more sensitive procedure is desired, but the accuracy will be decreased.)

Immediately measure the absorbance of the yellow color in the carbon tetrachloride layer at approximately 418 mμ, using a 1- to 1.5-cm. cell and distilled carbon tetrachloride as the reference solution. (The yellow color of the copper dimethyl dithiophosphate complex in carbon tetrachloride is not stable for more than 5 minutes.) Prepare the standard curve by plotting the absorbance of each of the aliquots against the milligrams of malathion present.

Extraction of Malathion from Plant Material

Total Residue. Cut a representative sample of about 500 to 1000 grams into small pieces and macerate in a Waring Blendor with sufficient water to produce a thick slurry. Transfer the macerate to a 1-gallon jar and add a measured volume of carbon tetrachloride (1 to 3 ml. per gram of sample). Place a tight-fitting tinfoil-lined cap on the jar and extract for 4 hours by end-over-end agitation at the rate of about 40 r.p.m. Remove jar from extraction apparatus, allow mixture to stand until the carbon tetrachloride layer separates, and then siphon it off. If a stable emulsion has formed, which will not break on standing overnight or on gentle agitation with a stirring rod, separate the layers by centrifuging or by the following procedure, which has been found very effective in the case of potatoes.

Macerate and extract the sample as described, but keep the amount of water required to make a smooth slurry at an absolute minimum. After extraction, add anhydrous sodium sulfate to the emulsion up to three times the weight of the sample to make a thick paste or a crumbly mass. Stir the salt in slowly, so that heat of solution does not localize and crack the jar or cause volatilization of solvent. Replace the closure on the jar and tumble the sample 10 minutes more. This treatment should break the emulsion, so that a satisfactory aliquot of the carbon tetrachloride extract can be obtained for analysis. Dry filter the extract to remove water or suspended solids and then take an aliquot for analysis. Analyze the extracts immediately after preparation, in order to avoid possible decomposition of malathion during prolonged storage.

Surface Residue. Place a representative sample of about 500 to 1000 grams in a glass jar, add a measured volume of carbon tetrachloride (1 to 3 ml. per gram), place the cap securely on the jar, and agitate gently for from 5 minutes to 1 hour by mechanical means, as on motor-driven rollers. Materials having relatively small surface areas will require shorter periods of agitation than those having large areas. A short period should be used if a distinction is to be made between strictly surface residues and such "subsurface" residues as may be found in a waxy surface layer.

Figure 1. Standard curve for malathion

Klett-Summerson photoelectric colorimeter
Filter 42, cell diameter 1.2 cm.

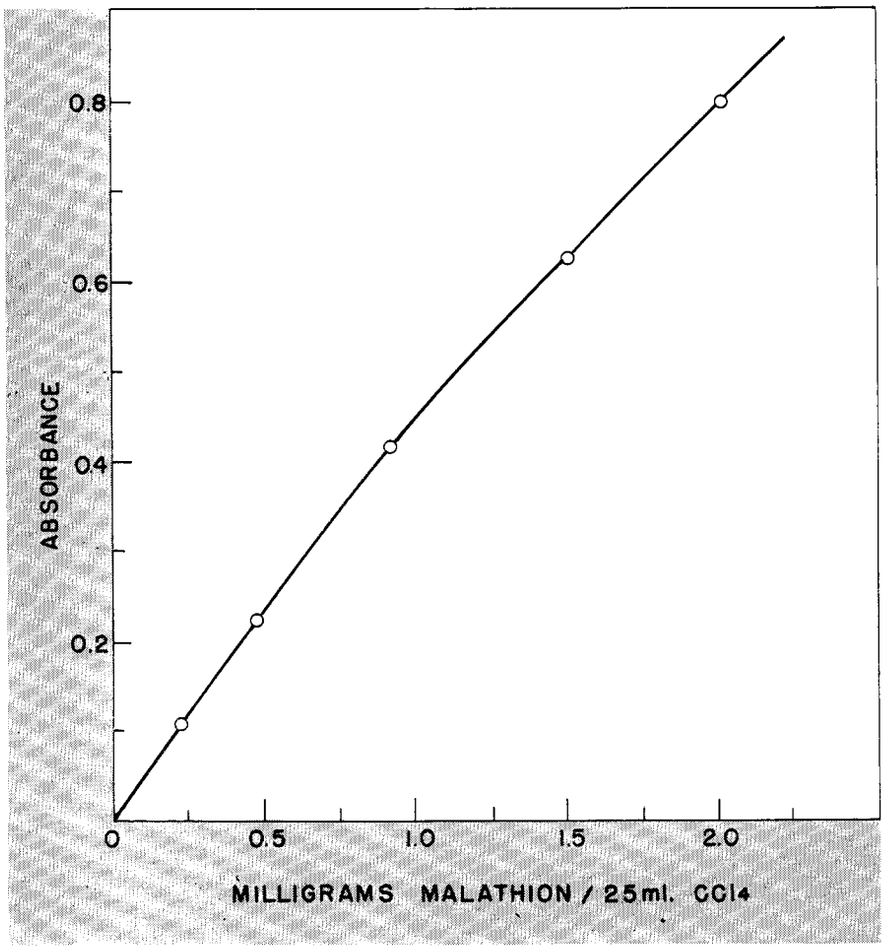

Table I. Recovery of Malathion from Carbon Tetrachloride Solution

CCl_4 Soln. Analyzed, Ml.	Malathion, Mg. Added	Malathion, Mg. Found	Recovery, %
500	0.28	0.25	89
500	0.55	0.55	100
500	0.55	0.55	100
500	0.69	0.69	100
500	0.97	0.93	96
500	1.38	1.42	103
500	1.73	1.73	100
500	2.08	1.98	95

Malathion in Carbon Tetrachloride Extract

Transfer an aliquot of the carbon tetrachloride extract which will contain not more than 2.5 mg. and preferably not less than 0.25 mg. of malathion to a dry 250-ml. separatory funnel and dilute to 100 ml. with carbon tetrachloride. If more than 100 ml. of extract is required for analysis, concentrate in a beaker to about 90 ml. by evaporation on the steam bath with the aid of a jet of air blowing across the surface, transfer to the separatory funnel, and dilute to 100 ml. with carbon tetrachloride. (Experiments should be carried out on each type of material being analyzed, to determine whether insecticide is lost during such evaporations.)

Add 25 ml. of ethyl alcohol to the separatory funnel and mix well by swirling. Add 1 ml. of 6*N* sodium hydroxide and shake vigorously for 1 minute. Immediately add 75 ml. of the sodium chloride solution (cooled to about 15° C.) and shake vigorously for exactly 1 minute. Allow the phases to separate and discard the carbon tetrachloride layer. Add 25 ml. of carbon tetrachloride, shake vigorously for 30 seconds, allow phases to separate, and discard the carbon tetrachloride layer, including the small amount of emulsion layer and suspended solids which may have formed.

Add 25 ml. of carbon tetrachloride and 1 ml. of 7*N* hydrochloric acid to the separatory funnel, shake vigorously for 30 seconds, allow the phases to separate, and discard the carbon tetrachloride layer. Repeat the extraction of the aqueous layer, using 25-ml. portions of carbon tetrachloride each time, until no yellow color is extractable into the carbon tetrachloride phase.

Prove absence of yellow color in the carbon tetrachloride phase by measuring absorbance at 418 mμ.

Extraction of the acidified aqueous layer at this point with carbon tetrachloride removes colored plant pigments and also interfering compounds which may have been formed by alkali decomposition of impurities in the insecticide. For example, diethylmercaptosuccinate, if formed by alkali decomposition and not removed at this point, will interfere.

After the final extraction, draw off

Table II. Recovery of Malathion from Plant Material

Material	Sample Represented by Extract Analyzed, G.	Malathion, P.P.M. Added	Malathion, P.P.M. Found	Recovery, %
	A. Surface Residues			
Apples	500	1.0	1.1	110
	500	2.0	2.1	105
	855	1.0	0.8	80
	690	1.0	0.8	80
Spinach	500	0.5	0.3	60
	500	1.0	0.7	70
	500	2.1	1.5	71
Eggplant	610	1.0	0.9	90
	828	1.0	0.9	90
Tomatoes	1660	0.5	0.4	80
	636	0.5	0.3	60
	925	1.0	0.8	80
Grapes	247	1.0	0.9	90
	504	1.0	0.9	90
String beans	711	1.0	0.8	80
	984	1.0	0.8	80
Tobacco	160	2.0	1.4	70
	231	2.0	1.5	75
Alfalfa	204	1.0	0.9	90
	210	2.0	1.9	95
	B. Total Residues			
Potatoes	250	1.0	0.7	70
	243	1.0	0.6	60
	230	1.0	0.6	60
Canned peaches	329	1.0	0.7	70
Canned lima beans	155	1.0	0.7	70
Canned tomato juice	331	1.0	0.9	90
Canned peas	247	1.2	1.0	83
Strawberries	238	1.0	0.6	60

the carbon tetrachloride as completely as possible. Using a pipet, add 25 ml. of carbon tetrachloride and 2 ml. of copper sulfate reagent. Shake vigorously for 1 minute and allow the phases to separate. Immediately measure the absorbance of the yellow color in the carbon tetrachloride layer at approximately 418 mμ, using a 1- to 1.5-cm. cell and distilled carbon tetrachloride as the reference solution. From the standard curve read the amount of malathion corresponding to the absorbance observed, and calculate to parts per million of malathion in the sample.

Experimental Work

Preliminary experiments indicated that malathion may be decomposed by sodium hydroxide (sodium carbonate, ammonium hydroxide, or triethylamine were found unsatisfactory) into sodium dimethyl dithiophosphate and sodium fumarate in benzene, chloroform, or carbon tetrachloride solutions, provided sufficient ethyl alcohol is added to solubilize the alkali. The three solvents were therefore considered as possible solvents for removal of malathion residues from plant material, and carbon tetrachloride was found to be the most suitable. Commercial methyl chloroform was later investigated because of its lower toxicity, but found to be inferior to carbon tetrachloride because the developed yellow color of the copper complex was not stable. Benzene was eliminated because objectionable losses were observed upon evaporation of malathion solutions and chloroform because of its higher cost and greater solvent power for water.

Transmittance–wave length curves of the yellow colored copper dimethyl dithiophosphate complex in carbon tetrachloride were run on a General Electric recording spectrophotometer, and show a single absorption maximum at about 418 mμ. A typical standard curve prepared as described in the procedure using purified malathion is shown in Figure 1.

In order to test for loss on evaporation, weighed amounts of malathion were dissolved in 500-ml. volumes of carbon tetrachloride and solutions were concentrated to a volume of about 75 ml. by evaporation on the steam bath with the air of a jet of air blowing across the surface. The concentrated solutions were then transferred to separatory funnels and malathion was determined by the procedure described. The recoveries of malathion are shown in Table I.

In order to test the applicability of the method to determining residues of the insecticide, weighed amounts of malathion were added to various types of plant material and the sample was analyzed by the procedure outlined. For surface residues, the malathion was added to distilled carbon tetrachloride, which was then used to extract the samples. For total residues, the malathion in ethyl alcohol was added to the samples during maceration in the Waring Blendor and the slurry was then extracted. The recovery of added amounts of malathion from various types of plant material is shown in Table II.

Discussion

The method described was developed for analysis of spray residues only. For assay of the technical product or commercial formulations, modifications in this procedure have been made, the details of which will be published later. The procedure outlined for extraction of residues from plant material is intended to serve as a general guide only, and should be modified as necessary for application to specific samples.

The recoveries of added amounts of malathion vary in general over a range of 70 to 90% for different kinds of plant material. This fact should be taken into consideration in evaluating numerical results obtained by this method.

Carbon tetrachloride extracts of plant material should be analyzed immediately after preparation in order to eliminate possible losses of malathion due to decomposition on standing. If overnight storage is inevitable, the extracts should be refrigerated.

Before the extracts are subjected to prolonged storage, experiments should be carried out to determine the effect of such storage conditions. In general, malathion solutions are relatively stable between pH 4 and 7.

With the exception of lettuce, which gave a value of 0.3 p.p.m., control analyses on all untreated plant materials investigated in this laboratory have indicated less than 0.1 p.p.m. of "apparent" malathion. Although no significant control or reagent blanks are anticipated, experiments should be carried out on each different type of material being analyzed to establish this point.

Compounds other than malathion which are converted to the *O,O*-dimethyl dithiophosphoric acid by the alkali treatment will interfere with the method described. Known compounds of this type are *O,O,O,O*-tetramethyl trithiopyrophosphate, and the disulfide of dimethyl dithiophosphoric acid—namely, *O,O,O,O*-tetramethyl 2,3-dithiatetraphosphane-1,1,4,4-dithiotetroate. Either one or both of the half esters of malathion—namely, *S*-[(1-carboxy-2-carbethoxy)ethyl]*O,O*-dimethyl dithiophosphate and *S*-[(2-carboxy-1-carbethoxy)-ethyl]*O,O*-dimethyl dithiophosphate—also are converted to *O,O*-dimethyldithiophosphoric acid. The compound

S-(1,2-dicarboxyethyl)*O,O*-dimethyl dithiophosphate is not converted to *O,O*-dimethyl dithiophosphoric acid by the alkali treatment and, therefore, does not interfere.

Cuprous ion interferes by forming with the dithiophosphate a colorless complex that is more stable than the cupric complex. Readily oxidizable materials—e.g., mercaptans(thiols)—if not removed prior to addition of the copper reagent, will reduce the cupric ion and thus give low results. In the procedure described, mercaptans and other acidic impurities are removed by carbon tetrachloride extractions made on acidified aqueous solution just before addition of copper reagent.

Small amounts of the following metallic ions do not interfere: iron(II), iron (III), zinc, nickel(II), cadmium, aluminum, tin (II), and lead. Wettable sulfur, fermate, parzate, cuprocide, aramite, toxaphene, parathion, ovotran, DDT, and methoxychlor do not interfere.

Plant materials which have been analyzed by the method described include alfalfa, apples, barley, beets, broccoli, cauliflower, cottonseed, cranberries, cucumbers, eggplant, grapes, green beans (foliage and pods), kale, lettuce, lima beans (fresh and canned), mustard (fresh and canned greens), onions, peaches (canned), peas (canned), peppers, potatoes, spinach, strawberries, string beans (fresh and canned), Swiss chard, tobacco, tomatoes, and tomato juice (canned).

Ginsburg, Filmer, and Reed (*1*) have applied the method to the analysis of corn, lima beans, lettuce, and onions; Kolbezen (*2*) to analysis of dates, walnut meats, pears, oranges, lemons, milk, avocados, and cantaloupe; and Westlake and Butler (*3*) to analysis of apples, pears, peaches, spinach, snap beans, cucumbers, broccoli, potatoes, strawberries, and peas.

Acknowledgment

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PESTICIDE RESIDUES

Chemical Determination of Aldrin in Crop Materials

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Analytical methods for the determination of aldrin residues on the order of 0.1 p.p.m. in agricultural crop materials were needed to obtain data for government requirements. Two methods have been developed. Both involve the extraction of aldrin from the plant matrix by means of a hydrocarbon solvent, followed by separation of the aldrin from dissolved glycerides, if necessary, by saponification and from most of the other dissolved biological materials by adsorption chromatography. The aldrin is then determined in the concentrate either by an improved modification of the phenyl azide–photometric procedure of Danish and Lidov or by determination of chlorine by combustion procedure of Agazzi, Peters, and Brooks. Tests have been made on a wide variety of insecticide-free plant materials and apparent aldrin values of less than 0.08 and 0.05 p.p.m. have been obtained by the chlorine and photometric methods. Recovery of aldrin added in known amounts to extracts of the plant materials generally has been found to be accurate to a few hundredths part per million, calculated on the basis of the crop material. The photometric method has a high degree of specificity for aldrin, whereas the chlorine method is influenced by certain other common chlorine-containing insecticides which are not separated from aldrin.

RELIABLE METHODS for the determination of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydroendo-exodimethanonaphthalene (aldrin) at concentrations on the order of 0.1 p.p.m. in plant materials were needed to satisfy requirements for government registration. Two specific methods, an infrared absorption method (*5*) and a phenyl azide–photometric method (*4*), had been proposed for the determination of microgram quantities of aldrin and were potentially of use. The infrared method appeared less promising for use with plant materials than the photometric method because of the much lesser absorbance of aldrin in the infrared region than of aldrin as the colored product in the visual region in the photometric method (molar absorptivities of approximately 3.6×10^2 and 5.4 and 10^4 liters per mole cm., respectively). In addition, the interference due to absorption of radiation by many biological materials is greater in the infrared region than in the visible region.

Two less specific methods have also been proposed for the determination of microgram quantities of aldrin—the microbioassay method of Sun and Sun (*10*) employing insects, and the quartz tube combustion–amperometric chloride ion titration method of Agazzi, Peters, and Brooks (*1*). The bioassay technique for the determination of aldrin residues in biological materials requires facilities not

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