Colorimetric Method for the Determination of Ethion of Residues

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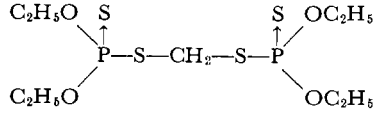
Ethion is extracted from plant tissues with a suitable solvent such as hexane, concentrated by evaporation, and hydrolyzed in ethanolic sodium hydroxide. The diethyl phosphorodithioic acid so formed is determined spectrophotometrically as its complex copper salt, absorbing at 418 mμ. By subjecting crop extracts to preliminary selective hydrolysis and/or chemical cleavage steps, the method has been shown to be specific for determination of ethion residues in the presence of other phosphate insecticides.

THE INSECTICIDE - ACARICIDE *O,O,O',O'*-tetraethyl *S,S'*-methylene bisphosphorodithioate (ethion) has been registered for use on a wide variety of crops. The structural formula for ethion is:



mol. wt. 384.5

Gunther *et al.* (*4*) have reported an infrared spectrophotometric analytical procedure for the determination of ethion residues on and in citrus crops. Ethion residues have also been determined by the enzymatic procedure of Cook and Fallscheer (*1, 3*), which is based upon the cholinesterase-inhibiting properties of the compound. This paper describes a colorimetric chemical method for ethion residue determinations on and in a variety of crops. The procedure is an adaption of the method of Norris *et al.* (*6*) and involves hydrolysis of ethion to diethyl phosphorodithioic acid, followed by spectrophotometric determination of its yellow complex copper salt which absorbs at 418 mμ. By subjecting crop extracts to preliminary selective hydrolysis and/or chemical cleavage steps, ethion may be determined in the presence of other phosphate insecticides.

Analytical Procedure

Apparatus and Reagents. Florisil, 100- to 200-mesh (Floridin Co., Tallahassee, Fla.), not activated.

1% Copper sulfate, ACS Reagent (1.0 gram of $CuSO_4 \cdot 5H_2O$ in 100 ml. of water).

0.2*M* Mercuric chloride solution (2.7 grams diluted to 50 ml. with 2B ethanol).

ACS reagent grade solutions were used. Solvents were distilled through a 1-foot Vigreaux column. Water was purified by double distillation or by demineralization to eliminate traces of metal, particularly copper, which interfere with the general procedure.

Spectrophotometer or colorimeter—any type that responds satisfactorily at 418 mμ.

Calibration. A calibration curve is obtained by adding various amounts of a standard solution of ethion (in hexane) containing 100 μg. per ml. to 40 ml. of hexane and developing the color as outlined in the general procedure, beginning with the step immediately preceding evaporation of solvent from the crop extract. A straight line passing through the origin results. From a calibration curve so obtained, was derived the equation relating amount of ethion to absorbance (*A*):

$$\text{Ethion } (\mu\text{g.}) = 664 \times A_{418} \qquad (1)$$

Extraction of Crops. *n*-Hexane (Skellysolve B) was found to be the preferred solvent for extraction of ethion residues from treated crops. Surface residues were removed by mechanically tumbling the crops with 1 ml. of *n*-hexane per 4 grams of sample for 10 minutes. Interior residues were satisfactorily extracted by grinding the crops in a food blender and tumbling the ground material with 2 ml. of *n*-hexane per gram of sample for 1 hour.

General Procedure

Carbon Disulfide Treatment (5). Into a 500-ml. separatory funnel, place the volume of strippings equivalent to a maximum of 500 grams of crop sample. If an aliquot of less than 100 ml. is taken, dilute to that volume with hexane to provide a convenient bulk for the subsequent extraction step. Add 25 ml. of ethanol plus 0.1 ml. of carbon disulfide and shake for 1 minute. After 5 minutes, add 75 ml. of 2% NaCl solution and shake 1 minute. Separate and discard the bottom aqueous layer.

Hydrolysis. Transfer the hexane phase into a 500-ml., round-bottomed flask, avoiding inclusion of aqueous phase. Place in a 40° C. water bath, stir magnetically, and evaporate under vacuum (water aspirator) into a receiver set in dry ice.

To dissolve the residue, add 5 ml. of cyclohexane and 40 ml. of ethanol. Set in a water bath at 40° C. and allow to come to temperature. Add 2.5 ml. of 6*N* NaOH and stir at 40° C. for 15 minutes. Cool, transfer to a 250-ml. separatory funnel, rinse in with 100 ml. of 2% NaCl, shake for 1 minute, and let stand for 30 minutes.

Add 25 ml. of cyclohexane (Distillation Products, Inc., practical, redistilled) and shake 1 minute. Allow to separate and drain the lower aqueous layer into a clean separatory funnel. Repeat the wash. In each case, discard the cyclohexane phase. Acidify with 3.5 ml. of 6*N* NaCl, shake 1 minute, and allow to stand for 10 minutes for complete phase separation.

Drain the aqueous layer into a clean funnel. Pipet 10.0 ml. of cyclohexane and shake 1 minute. Drain the aqueous layer into a clean funnel. Pipet 5.0 ml. of the cyclohexane phase and filter into a 1-cm. cell for use as a spectrophotometric blank.

Color Development. Recombine remaining cyclohexane with the aqueous phase, add 2.0 ml. of 1% copper sulfate solution and shake for 2 minutes. Separate phases and filter the cyclohexane layer into a matched 1-cm. cell. Measure absorbance at 418 mμ against the aforementioned blank.

The magnitude of the residue in micrograms is obtained by substituting the absorbance reading into equation 1.

Chromatographic Column Cleanup (4)

(Use only when satisfactory blanks and/or recoveries are not obtainable from the General Procedure.)

Evaporate the hexane extract to less

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Table I. Interferences from Other Phosphate Insecticides

Other Insecticides	Other Insecticides, μg.	Ethion, μg.	Absorbance	Apparent Ethion μg.	Apparent Ethion %
...	...	200	0.306	203	101[a]
...	...	302	0.475	314	104[b]
Trithion	218	...	0.003	Nil	0[a]
	318	...	0.005	Nil	0[b]
	334	112	0.156	102	92[b]
Guthion	300	...	0.078	50	16[a]
	300	...	0.012	4	4[b]
	300	106	0.155	102	96[b]
Malathion	315	...	0.100	66	22[a]
	315	...	0.000	0	0[b]
	211	213	0.311	204	97[b]
Phorate	280	...	0.015	Nil	0[a]
	280	...	0.004	Nil	0[b]
	280	336	0.498	325	97[b]
Delnav	250	...	0.208	136	55[a]
	376	...	0.185	122	33[b]
	340	336	0.691	450	135[b]
Methyl parathion	300	...	0.001	0	0[b]
Parathion	300	...	0.003	0	0[b]
Diazinon	300	...	0.003	0	0[b]
Demeton (systox)	300	...	0.001	0	0[b]

[a] Using general ethion analytical procedure.
[b] Using general ethion analytical procedure plus dilute NaOH solution wash.

Table II. Interferences of Delnav and Recoveries of Ethion Alone and in Combination with Delnav

Sample Size, μg.	Absorbance	Apparent Ethion μg.	Apparent Ethion %
	DELNAV		
340	0.014	9	3
227	0.007	4	2
113	0.000	0	0
	ETHION		
348	0.490	320	92
232	0.358	233	101
116	0.165	109	94
	DELNAV + ETHION		
340 + 348	0.555	361	103
227 + 232	0.372	243	105
113 + 116	0.180	118	102

Table III. Interferences of Other Dithiophosphates and Recoveries of Ethion from Various Crop Extracts

Crop Extract	Sample Size, Grams	Ethion Added μg.	Ethion Added P.P.M.	Other Insecticide Added	Ethion Recovered μg.	Ethion Recovered P.P.M.	% Recovery
Strawberry	200	412	2.06	Thimet, 396 μg. Trithion, 445 μg. Guthion, 400 μg. Malathion, 405 μg. Delnav, 454 μg.	340	1.70	83
Pepper (surface)	400	412	1.03	Same as above	343	1.72	84
Strawberry (whole)	50	309	6.02	Thimet, 297 μg. Trithion, 334 μg. Guthion, 300 μg. Malathion, 405 μg. Delnav, 340 μg.	326	6.52	105
Pepper (pulp)	50	309	6.02	Same as above	262	5.25	85
Strawberry (surface)	200	206	1.03	Thimet, 198 μg. Trithion, 222 μg. Guthion, 200 μg. Malathion, 270 μg. Delnav, 227 μg.	188	0.94	91
Strawberry (surface)	200	206	1.03	None	190	0.95	92

than 10 ml. and add the concentrate to a 25 × 100 mm. column of Florisil which has been prewashed with 200 ml. of redistilled hexane. Percolate 100 ml. of hexane through the column followed by 200 ml. of 10% ether-in-hexane solution. Discard the first 40 ml. after adding the 10% ether-hexane solution and collect the next 70 ml. Continue the analyses as described under Carbon Disulfide Treatment.

Pretreatment for Removal of Interfering Phosphate Insecticides

(Use only if treatment of samples with another phosphate insecticide is known or suspected.)

Place an appropriate aliquot of crop extract into a 500-ml., round-bottomed flask, and evaporate to dryness under aspirator vacuum at less than 40° C. Add 5 ml. of cyclohexane to dissolve the residue followed by 25 ml. of ethanol. Place the flask in a water bath at 40° C. and allow contents to come to temperature. Add 10 ml. of 0.2*M* mercuric chloride (in ethanol) and stir the solution for 30 minutes with a magnetic stirrer. Transfer the contents to a 250-ml. separatory funnel and rinse in with 100 ml. of *n*-hexane followed by 75 ml. of 2% sodium chloride solution. Shake for 1 minute, separate and discard the water phase, and wash the remaining hexane phase once with 50 ml. of 2% sodium chloride solution.

Add 25 ml. of ethanol and 0.1 ml. of carbon disulfide to the washed hexane solution. Shake for 1 minute and allow to stand for 5 minutes. Add 1 ml. of 6*N* NaOH to the solution and shake exactly 1 minute. Without delay, dilute the solution with 75 ml. of 2% sodium chloride solution and mix thoroughly by shaking. Separate the hexane phase and continue the analysis as outlined under Hydrolysis. The pretreatment described has been shown to render the method specific for ethion in the presence of the following thiophosphate insecticides: demeton, Delnav, Diazinon, Guthion, malathion, methyl parathion, parathion, phorate, Trithion.

Discussion

The ultimate limit of sensitivity is considered to be 25 μg. of ethion, but a more practical limit would be 50 μg. The procedure described has been arranged for the analysis of 500-gram crop samples, thus giving a sensitivity of 0.05 to 0.1 p.p.m. Where residues are known to be substantially greater than 0.05 to 0.1 p.p.m., correspondingly smaller aliquots of stripping solution can be taken. Except at the very lowest residue levels, blank values from untreated crops are negligible. In apples, for instance, the highest blank encountered was equivalent to only 0.05 p.p.m. of ethion.

The general method has been applied to apples, pears, peaches, tomatoes, onions, beans, green peppers, cucumbers, strawberries, melons, cherries, grapes, cotton foliage, cottonseed, grapefruits, oranges, plums, prunes, eggplants, cranberries, and blueberries.

Cleanup steps as described have been utilized only when necessary to render the basic method satisfactory for all crops tried and specific for ethion in the presence of other phosphate insecticides.

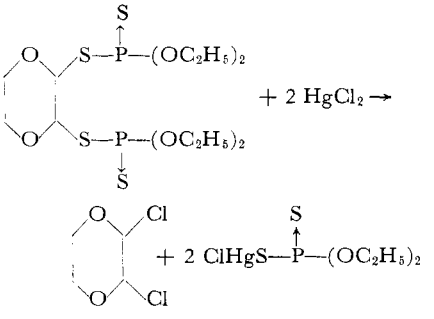
The Florisil chromatographic column treatment has been used for crops which produce extracts containing materials that interfere with hydrolysis and color development and result in poor recovery values. Crops for which this step has been particularly useful include oranges, grapefruits, peaches, and grapes. The column cleanup step may be used routinely for all analyses but has been found unnecessary for a great many crops. The scheme developed for removing interferences from other phosphate insecticides has been used only when samples were known to have received treatments with another phosphate insecticide.

The major difference between the general ethion procedure and the malathion method described by Norris *et al.* (*6*) is the condition of hydrolysis. Ethion was fairly resistant to alkaline hydrolysis. Following the directions for malathion of shaking a carbon tetrachloride solution for 1 minute with 1 ml. of 6*N* sodium hydroxide did not effect ethion hydrolysis. To hydrolyze the more stable ethion, a one-phase system (ethanol), maintained for 15 minutes at 40° C. with 2.5 ml. of 6*N* sodium hydroxide, was necessary. Varying the hydrolysis time from 10 to 30 minutes did not affect the outcome of the analysis.

Malathion was almost completely destroyed by the vigorous hydrolysis conditions and the expected phosphorodithioic acid entity was almost nondetectable by the copper complex technique. By using the more vigorous hydrolysis conditions, runs were made to determine interferences which might be found from the presence of other phosphate insecticides. Runs were also made utilizing a preliminary mild hydrolysis treatment similar to the hydrolysis step described in the malathion method (*6*). As shown in Table I, interferences from all phosphates tried were essentially nil with the exception of Delnav. Experiments run with combinations of ethion and various other phosphate insecticides showed no new interferences or detrimental effects.

Delnav continued to give positive value interferences. Each microgram of Delnav appeared as 0.3 to 0.4 μg. of ethion. More strenuous alkaline hydrolytic conditions failed to eliminate or further reduce this value.

To remove interferences resulting from Delnav, a chemical cleavage step was utilized. As shown by Dunn (*2*), carbon to sulfur bonds can be cleaved readily by treatment with mercuric chloride:



This reaction is fairly specific for thioacetals—Delnav is a thioacetal, while ethion is not. The cleavage step in the analytical procedure involved addition of 10 ml. of 0.2*M* mercuric chloride (in ethanol) to an ethanol solution (25 ml.) of the insecticide and stirring at 40° C. for 30 minutes. The ethanol solution was then diluted with 75 ml. of 2% NaCl solution and extracted once with 100 ml. of hexane. The ethion hydrolysis and color development was continued with the Delnav-free hexane solution. Loss of ethion appeared to be negligible. False ethion values attributed to Delnav were shown to be less than 5%, which were within the limits of the accuracy of the residue procedure. Data in Table II were obtained with Delnav and ethion alone and in combination with one another.

By utilization of both a mercuric chloride treatment and a dilute sodium hydroxide wash, the ethion colorimetric procedure was shown to be specific for ethion in the presence of other dithiophosphate insecticides. The mercuric chloride cleavage to remove Delnav interferences was carried out first, followed by the alkaline wash as described above.

Recoveries of ethion from several crop extracts were made utilizing the alkali and mercuric chloride treatments. Ethion was added alone and in combination with other dithiophosphate insecticides, and no detrimental effects were noted either from the procedure changes or from the presence of other phosphates (Table III).

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