INSECTICIDE-ACARICIDE RESIDUES

Residues of Ethion on and in Lemons and Oranges as Determined by an Infrared Spectrophotometric Procedure

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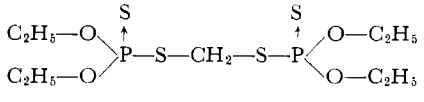
Aging residues on and in lemons and oranges of the compound O,O,O′,O′-tetraethyl S,S′-methylene bisphosphorodithioate, commonly known as ethion, are determined by means of strong infrared absorption bands at 1017 and 959 cm.$^{-1}$. Isolation from interfering substances occurring in grapefruit, lemons, and oranges is accomplished by means of selective absorption on Florisil, and partition extraction from *n*-hexane solutions with acetonitrile. When applied to lemons in a commercial manner, ethion residues had average RL_{50}, half-life, values of 30 and 44 days for wettable powder and emulsive formulations, respectively; when applied to oranges the average RL_{50} values were 42 and 25 days for wettable powder and emulsive formulations, respectively.

THE COMPOUND *O,O,O′,O′*-tetraethyl *S,S′*,-methylene bisphosphorodithioate, commonly known as ethion, possesses nsecticidal and acaricidal properties. In this laboratory, it has been shown to be promising for the control of California red scale, *Aonidiella aurantii* (Mask.), on citrus. The structural formula for ethion is:



Molecular weight 384.5

The infrared characteristics for the chromatographically purified compound (*6*) are shown in Figure 1.

Analytical procedures which have been described for ethion are a semi-specific colorimetric procedure utilizing the yellow copper salt complex with its hydrolysis product, diethyl phosphorodithioic acid, which absorbs at 418 mμ (*5*), and a nonspecific enzymatic procedure based upon the cholinesterase inhibiting properties of ethion (*7*). Because of the advantages of identification and specificity attainable by infrared spectrophotometric techniques (*3*), clean-up for an infrared procedure was devised utilizing both selective absorption of ethion onto Florisil, and selective partition from hexane into acetonitrile. The very intense P—O—C absorption peak at 1017 cm.$^{-1}$ imparts sensitivity to the infrared measurement while the secondary strong P—O—C peak at 959 cm.$^{-1}$ enhances specificity.

This article describes an infrared spectrophotometric analytical procedure for the determination of ethion residues on and in grapefruit, lemons, and oranges, and the magnitudes and behavior of ethion residues on and in lemons and Valencia oranges, field-treated in a commerical manner.

Table I. Partition Ratio of Ethion from *n*-Hexane into Acetonitrile

n-Hexane, Parts	Acetonitrile, Parts	Portion in Acetonitrile, %
1	1	94
2	1	81
4	1	64
10	1	32

Analytical Procedure

Special Reagents. Florisil, 60 to 100 mesh (Floridin Co., Tallahassee, Fla.).

Table II. Recovery of Ethion from Citrus Peel Extractives

Added μG.	Added P.p.m.	Recovered μG.	Recovered P.p.m.	Recovered %
		GRAPEFRUIT		
133	10.0	94	7.1	71
133	10.0	90	6.8	68
63	1.0	46	0.7	73
63	1.0	45	0.7	72
23	0.1	17	0.08	70
		LEMONS		
135	10.0	87	6.5	65
135	10.0	97	7.2	72
69	1.0	46	0.7	67
69	1.0	45	0.7	65
23	0.1	16	0.08	70
		VALENCIA ORANGES		
146	10.0	92	6.3	63
146	10.0	87	6.0	60
100	2.0	72	1.4	72
100	2.0	69	1.4	69
23	0.1	16	0.08	74

Table III. Background Values[a] of Apparent Ethion from Untreated Citrus Peel[b]

Peel, Grams	Absorbance at (A) 1017 cm.$^{-1}$	Absorbance at (B) 959 cm.$^{-1}$	Apparent Ethion μG.[c]	Apparent Ethion P.p.m.
		GRAPEFRUIT		
13	0.047	0.000	3.5	0.3
66	0.030	0.000	0	0
200	0.037	0.000	2.0	0.01
		LEMONS		
13	0.040	0.000	2.5	0.2
66	0.037	0.000	2.0	0.03
200	0.033	0.000	1.5	0.007
		VALENCIA ORANGES		
13	0.036	0.000	2.0	0.2
50	0.052	0.000	5.0	0.1
200	0.030	0.000	0	0

[a] Uncorrected for recovery.
[b] Values based upon weight of peel only.
[c] ½ [A in μg. (standard curve) + B in μg. (standard curve)] = μg. apparent Ethion.

Special Apparatus. Infrared spectrophotometer and 5-mm. light path sodium chloride cavity cells, approximately 0.4-ml. capacity (Connecticut Instrument Co., Wilton, Conn.).

Procedure. SELECTIVE ABSORPTION. By means of a Kuderna-Danish evaporative concentrator, concentrate an aliquot of *n*-hexane stripping solution, obtained in the usual manner (*2*), to a volume of less than 10 ml. Add this concentrate to a 25 × 100 mm. column of Florisil, which has been prewashed with *n*-hexane, and allow 100 ml. of fresh *n*-hexane to percolate through the column and discard. Then percolate 110 ml. of 10% ethyl ether in *n*-hexane through the column, discard the first 40 ml., and collect the remaining 70 ml. directly in a Kuderna-Danish evaporative concentrator. Concentrate this to a volume of less than 1 ml.

PARTITION DISTRIBUTION. Transfer the residue into a 60-ml. separatory funnel with 25 ml. of *n*-hexane. *Caution. Do not use stopcock grease on the plug of the separatory funnel as it will cause serious interference during the measuring step.* Extract the *n*-hexane solution with 25 ml. of acetonitrile, withdrawing the *n*-hexane with the aid of a suction stick. Wash the acetonitrile solution once with 25 ml. of *n*-hexane, then add the acetonitrile solution to 200 ml. of water in a 500-ml. separatory funnel. *Caution. Do not use stopcock grease.* Extract the resulting aqueous solution with 150 ml. of *n*-hexane, then wash the hexane solution with three 25-ml. portions of water before passing it through anhydrous sodium sulfate into a Kuderna-Danish evaporative concentrator. Rinse the separatory funnel and sodium sulfate with 50 ml. of *n*-hexane and evaporate the extract plus rinsings to dryness, using a jet of air to remove the last traces of solvent.

MEASUREMENT. Dissolve the residue in 0.3 ml. of spectrograde carbon disulfide, transfer this solution to a 5-mm. cavity cell, and record the spectrum from 1100 to 910 cm.$^{-1}$, compensating with carbon disulfide. The absorbances of the peaks at 1017 and 959 cm.$^{-1}$ are determined by the baseline technique. An average of the two individual results, determined from the two absorbance values by means of calibration curves, is used for subsequent calculations.

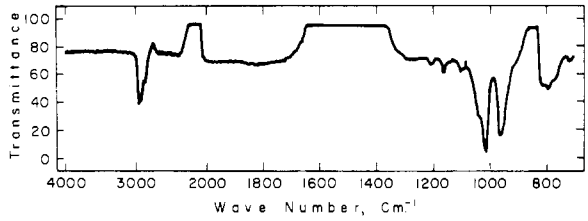


Figure 1. Infrared characteristic of O,O,O',O'-tetraethyl S,S'-methylene bisphosphorodithioate in carbon disulfide solution

Treatment and Processing

Mature Valencia orange trees were sprayed May 11, 1959, and mature lemon trees were sprayed Oct. 5, 1960, with the formulations listed in Table IV. Applications were made as conventional sprays, using a high-pressure reciprocating pump and manually operated spray guns. Sprays were applied at the rate of approximately 2500 gal. per acre to the orange trees (average 90 trees per acre) and 1500 gal. per acre to the lemon trees (average 110 trees per acre).

Mature orange fruit samples for assay of residues were collected 1, 7, 14, 21, 30, 45, 60, 90, and 120 days after treatment. Mature lemon fruit samples for assay of residues were collected 5, 12, 19, 33, and 61 days after treatment. Four shoulder-high fruits (one from each quadrant) were picked from each of eight trees in each plot, and the resulting 32 fruits were processed as a unit sample. The three replicates for each treatment were collected from different plots.

The unwashed fruits were peeled; peel and pulp (edible portion) were processed separately with *n*-hexane in the manner previously described (*2*) to afford final stripping solutions. Aliquots of the

Table IV. Residue Values (P.P.M.) for Ethion on and in Triplicate Samples of Field-Sprayed Lemons and Oranges

Days after Treatment	Dosage[a] 1 Pound 25% W.P./100 Gal.		Dosage[a] 4 Pounds 25% W.P./100 Gal.			Dosage[b] 1/4 Pint E.C. + 1 3/4 Gal. Oil Emulsive/100 Gal.		Dosage[c] 1 3/4 Gal. P.F./100 Gal.	
	Peel[d]	Pulp[e]	Unwashed peel[d]	Pulp[e]	Washed peel[f]	Peel[d]	Pulp[e]	Peel[d]	Pulp[e]
				LEMONS					
5	4.3, 5.2, 3.6	n.d.	10.4, 8.8, 10.4	n.d.	...	4.4, 5.4, 3.8	n.d.	...	...
12	3.5, 2.9, 5.2	n.d.	9.0, 9.9, ...	n.d.	...	5.5, 5.8, 6.5	n.d.	...	...
19	3.6, 3.3, 2.3	n.d.	8.0, 9.1, 7.9	n.d.	...	4.6, 4.7, 2.9	n.d.	...	...
33	1.2, 0.9, 1.0	n.d.	5.0, 4.2, 3.6	n.d.	...	1.3, 1.6, 2.1	n.d.	...	...
61	1.6, 1.7, 1.9	n.d.	2.8, 4.6, 3.7	n.d.	...	2.4, 2.6, 2.4	n.d.	...	...
				VALENCIA ORANGES					
1	2.7, 3.1, 3.5	...	14.5, 22.0, ...	...	11.8, 16.4, 25.0	3.6, 5.2, 5.4	...	5.5, 6.1, 3.8	...
7	3.8, 4.7, 4.1	n.d.	30.7, 24.0, 13.0	n.d.	8.3, 5.8, 9.2	7.4, 6.4, 5.5	n.d.	4.6, 4.9, 5.6	n.d.
14	2.4, 2.4, 3.5	...	32.2, 25.1, 31.7	...	18.1, 26.3, ...	5.5, 7.8, 4.0	...	2.8, 3.6, 6.8	...
21	5.3, 4.9, 3.1	n.d.	26.2, 18.0, 16.6	n.d.	15.5, 17.7, ...	5.6, 4.0, 5.2	n.d.	5.0, 8.2, 8.6	n.d.
30	3.1, 5.8, 3.8	...	17.7, 17.2, 15.6	...	21.5, 13.9, ...	5.8, 3.5, 5.1	...	2.4, 5.6, 3.4	...
45	1.9, 2.7, 3.3	...	8.5, 9.6, 10.3	...	10.5, 9.2, ...	2.8, 3.9, 3.0	...	2.5, 3.3, 2.5	...
60	1.5, 1.6, 1.4	n.d.	8.1, 7.8, 8.1	n.d.	8.0, 11.0, ...	0.9, 0.6, ...	n.d.	0.4, 1.4, 0.4	n.d.
90	1.5, 1.2, 1.0	...	6.1, 6.5, 3.4	...	3.9, 5.2, ...	0.4, 0.4, 0.7	...	4.8, 0.4, 0.8	...
120	0.6, 0.5, 0.5	n.d.	3.8, 3.8, 4.2	n.d.	4.2, 1.3, ...	0.7, n.d., n.d.	n.d.	0.3, n.d., n.d	n.d.

[a] W.P.—wettable powder analyzed by Niagara Chemical Division, Food Machinery & Chemical Corp. (*1*), to contain 25.33% of ethion by bromate titration of chromatographically purified ethion

[b] E.C.—emulsifiable concentrate analyzed by Niagara Chemical Division, Food Machinery & Chemical Corp. (*1*), to contain 87.29% of ethion by bromate titration of chromatographically purified ethion, mixed with light-medium oil emulsive.

[c] P.F.—proprietary formulation analyzed by Niagara Chemical Division, Food Machinery & Chemical Corp. (*1*), to contain 2.6% of ethion in light-medium oil by bromate titration of chromatographically purified ethion.

[d] Values based on weight of peel only. (Mature lemons have 30.0 ± 8.5 weight % peel from 632 measurements. Mature Valencia oranges have 18.7 ± 6.3 weight % peel from 297 measurements.) All values corrected for recovery (lemons, 74%; oranges, 65%) and triplicated "n.d." background values at each picking date. Values of n.d. means less than 15 μg. or less than 0.3 p.p.m. with the size samples utilized.

[e] Values based on weight of pulp (edible portion) only. All values corrected for recovery (lemons, 60%; oranges, 74%) and triplicated "n.d." background values at each picking date. Values of "n.d." means less than 15 μg. or less than 0.2 p.p.m. with the size samples utilized.

[f] Fruit hand-washed in dilute Triton X-100 solution and air dried before processing.

Table V. Persisting RL_{50} Values for Residues of Ethion on and in Peel of Field-Treated Lemons and Valencia Oranges

Fruit	Dosage[a], Pound/100 Gal. Water	RL_{50}, Days
Lemons	1[b]	25
	4[b]	36
	1/4-pint E.C. + 1 3/4-gal. oil emulsion	44
Valencia oranges	1[b]	42
	4[b]	43
	1/4-pint E.C. + 1 3/4-gal. oil emulsion	25
	1 3/4-gal. proprietary formulation	25

[a] For exact data, see Table IV. [b] 25% W.P.

stripping solutions were analyzed for ethion by the infrared technique described above.

Separate aliquots of fruit from the 4-pound dosage (Table IV) plots were hand-washed in a dilute Triton X-100 solution before processing to assess in the usual manner (*4*) the degree of adherence *vs.* rate of penetration of the fruit by ethion.

Discussion

Analytical Procedure. Efficiencies of the unit cleanup procedures were determined separately in the absence and in the presence of citrus extractives. Selective adsorption of ethion on Florisil affords 84% recovery of ethion in the eluate; the fate of the remaining 16% was not investigated. From the partition ratios of ethion from *n*-hexane into acetonitrile (Table I), recovery of ethion from the partitioning steps is 89%. The over-all recovery of ethion from the combined steps is therefore 74%. Recoveries of ethion added to citrus peel extractives in *n*-hexane, reported in Table II, ranged from 60 to 74%.

Interference due to citrus peel extractives in *n*-hexane was not proportional to the amount of peel represented (Table III). As mentioned, stopcock grease seriously interferes with the infrared measuring step. Teflon plugs or water-lubricated plugs are recommended for separatory funnels.

A calibration curve for ethion in 0.3-ml. aliquots of carbon disulfide solutions conforms to Beer's law from 15 to 300 μg. of ethion both at 1017 $cm.^{-1}$ (slope, 17 μg. of ethion per 0.1 absorbance unit) and at 959 $cm.^{-1}$ (slope, 34 μg. per 0.1 absorbance unit) in a 5-mm. cavity cell. Upward extension of this range is achieved by diluting the residue with larger volumes of carbon disulfide.

Residue Values. Table IV lists residue values for ethion on and in the peel of field-treated lemons and Valencia oranges from replicated plots. Persisting RL_{50} values (formerly referred to as half-life values) were calculated from the data in Table IV and are listed in Table V.

Residue values on those fruits which were hand-washed in dilute detergent solution prior to processing to simulate commercial practice demonstrate the rapid penetration of ethion into the fruit waxes and oils, and thus the impracticability of residue removal by washing at harvest time.

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INSECTICIDE RESIDUES

Procedure for the Purification of Fat Samples Prior to Analyses for DDT, DDE, and Rhothane

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CONTROL LABORATORIES require clean-up techniques which are applicable to a wide range of foods. These must be efficient enough in the removal of pigments, waxes, fats, and other extraneous materials to allow the analyst to employ paper chromatography and other screening techniques and to study a number of pesticides simultaneously. They should be applicable to metabolites and degradation products, as well as to the parent compounds. Chlorinated pesticides find their way into milk and animal depot fat, and to detect trace quantities of these materials, a procedure which will handle large samples is imperative. A procedure developed in this laboratory (*1*) for the removal of waxes and pigments from plant extracts has been modified and applied to fat extracts containing as much as 100 grams of fat. The present article describes the technique and presents data on the determination of DDT, DDE, and Rhothane residues in a number of animal fats.

Method

Apparatus. Cold bath. A tank which will hold acetone to a height of 7 to 8 inches and two or more 1000-ml. Erlenmeyer flasks is satisfactory. A stainless steel tank, insulated on the outside with 1/4 inch of powdered cork and 1/4-inch plywood has been used. Fisher utility clamps were screwed into the side of the box to hold the Erlenmeyer flasks. The bath was filled to a height of 7 to 8 inches with acetone or methanol and cooled to between −70° and −78° C. by adding dry ice directly to the cooling solvent. An excess of solid chunks of dry ice is left in the tank during the operation.

Büchner funnel, porcelain funnels with outside diameter 142 mm. and plate diameter 126 mm. Sintered glass (me-