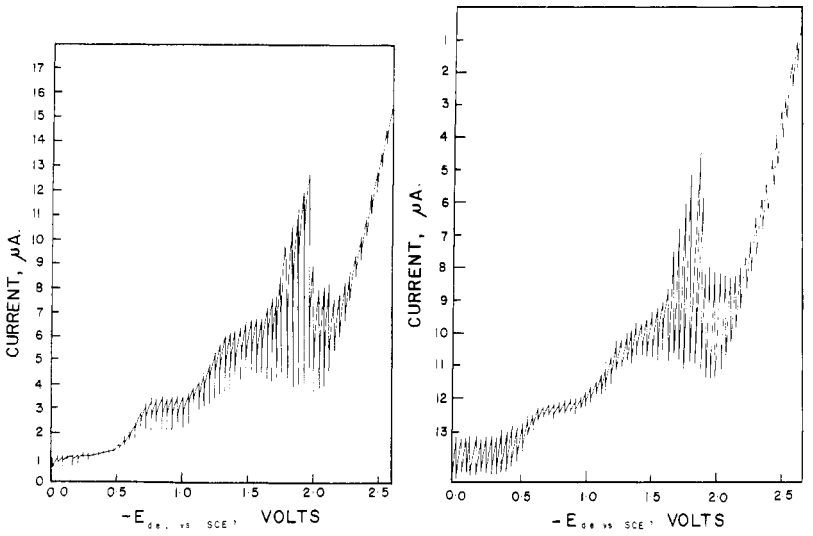

Figure 4. Polarogram

Left. 5.00 × 10^{-4}M 1,1′-ethylene-2,2′-bipyridylium dibromide in 0.1 M KCl
Right. Free radical of 5.00 × 10^{-4}M 1,1′-ethylene-2,2′-bipyridylium dibromide in 0.1 M KCl

and food products. Ion exchange type elimination of interfering materials is readily achieved according to the method of Calderbank *et al.* (*3*) and Calderbank and Yuen (*4*) using KCl or tetra-*n*-butylammonium iodide for eluents. In this manner samples of potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), rutabagas (*Brassica napobrassica*), turnips (*Brassica rapa*), and radishes (*Raphanus sativus*) were analyzed. Recoveries, standards added right after the weighing of the samples, range from 84 to 97% against appropriate blanks. The limit of residue detection with respect to the raw sample is 0.01 to 0.1 p.p.m.

The given procedure is expected to be reliable for the determination of all bipyridylium-type herbicides because the reduction of 2,2′- and 4,4′-bipyridylium ions at the D.M.E. is only slightly influenced by the nature of the *N*-substituents. However, the $E_{1/2}$'s of these herbicides will be modified somewhat by the composition of the supporting electrolytes.

Acknowledgment

The authors thank Alan Calderbank for supplying a sample of Diquat.

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Received for review January 20, 1966. Accepted May 2, 1966.

RESIDUE ESTIMATION

Determination of Demeton Residues by Infrared Spectrophotometry

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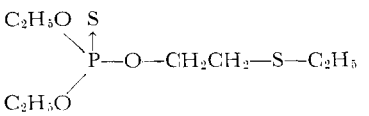
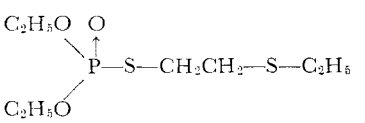
Commercial demeton is a mixture of about 40% of thiol and about 60% of thiono isomers [*O,O*-diethyl *S*-(and *O*)-2-(ethylthio)ethyl phosphorothioate; Systox]. It was first synthesized by Schrader in 1948 (*17*). Ever since the publication of its insecticidal properties in 1952 (*19*), it has attracted wide interest, and it is now recognized as one of the most valuable systemic insecticides (*6, 11, 13, 16, 18*), although it also has contact activity (*13, 16, 18*).

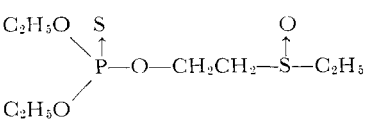
In studies of the metabolism of demeton in plants, Metcalf, Fukuto, March, and their coworkers (*8–10*) found four important oxidation products which are insecticidally active and which have high mammalian toxicity—thiono demeton sulfoxide, thiono demeton sulfone, thiol demeton sulfoxide, and thiol demeton sulfone—the phosphate-sulfoxide and phosphate-sulfone being of lesser significance. Hydrolysis or further oxidation results only in decomposition products having little toxicity to insects or mammals. These authors also found from in vitro enzymatic studies that the sulfones are ten times more potent as inhibitors of cholinesterase than the respective sulfoxides and that pure thiono demeton itself is a weak inhibitor.



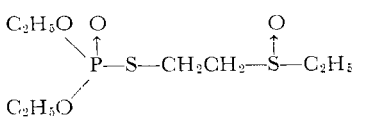
Thiono-demeton



Thiol demeton

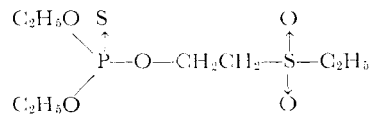


Thiono demeton sulfoxide

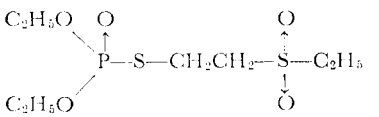


Thiol demeton sulfoxide

An infrared spectrophotometric method for determining residues of demeton and its insecticidally active metabolites in plants consists of extraction of the plant material with a tetrahydrofuran-chloroform mixture, removal of plant pigments with Nuchar C-190-N, oxidation of the demeton residue with *m*-chloroperbenzoic acid in ethyl ether, and further cleanup with a cellulose column. The amount of sulfone resulting from the oxidation is determined by an infrared spectrophotometer equipped with ordinate scale expansion. Recovery studies were made on apples, spinach, string beans, tomatoes, and potatoes.



Thiono demeton sulfone



Thiol demeton sulfone

The most widely used analytical method for determining demeton residues at present is the cholinesterase inhibition method developed by Hensel and coworkers (*11*). Laws and Webley (*15*) in 1958 published a phosphorus colorimetric method for the determination of methyldemeton residues by the measurement of the molybdenum blue complex. Adams *et al.* (*1*, *2*) in 1962 and 1963 modified this method particularly for demeton residues. However, neither cholinesterase inhibition nor total phosphorus methods are specific; one or the other is widely used for the determination of residues of many organic phosphorus insecticides.

The use of infrared spectrophotometric techniques for the determination of pesticide residues has been described by Crosby and Laws (*5*), Blinn (*3*), Frehse *et al.* (*7*), and Gunther, Blinn, and Barkley (*12*). Blinn recently reported the use of *m*-chloroperbenzoic acid as an oxidation reagent for the conversion of phorate to its oxygen analog sulfone (*3*, *4*).

The infrared spectrophotometric method described herein is reasonably specific for the analysis of demeton residues in plants. Although several other insecticides might be converted to sulfones by oxidation and would then interfere with the quantitative evaluation of the demeton sulfone band at 7.55 microns, the presence of additional demeton peaks at 7.94, 8.80, 9.85, and 10.35 microns would prove its presence qualitatively.

The present method involves extraction, cleanup, and oxidation of the residues of demeton (and its sulfoxides) to the respective sulfones and measurement of the sulfone peak in the infrared spectrum at 7.55 microns. The demeton sulfones already present in weathered residues are stable and are not affected significantly by the oxidation employed.

Experimental

Apparatus. Infrared spectrophotometer, a Perkin-Elmer Model 21 equipped with ordinate scale expansion. Instrument settings: resolution, 927; response, 1; gain, 5.6; suppression, 2; and speed, 1 minute per micron.

Sodium chloride cavity cells, 5-mm. light path (Connecticut Instrument Co., Wilton, Conn.) or equivalent.

Rotating evaporator, Rinco or equivalent, attached to a water aspirator.

Filter funnel with ⅌ vacuum adapter (Catalog No. FG-1780, Gardner Laboratory, Inc., Bethesda, Md.) or equivalent.

Reagents. Tetrahydrofuran-chloroform mixture (3 to 2 by volume). The tetrahydrofuran, reagent grade, should be distilled (weekly) in the presence of 15 ml. of 10% aqueous ferrous sulfate solution per liter of solvent. (Tetrahydrofuran is toxic by contact and by inhalation, and should be used with care.) The chloroform should be washed twice with water and distilled.

m-Chloroperbenzoic acid (FMC Corp., Carteret, N. J.) solution. Dissolve 100 mg. of *m*-chloroperbenzoic acid per 100 ml. of ethyl ether (use ethyl ether which has been washed with water) containing 1% of benzene. Make fresh just before use.

Coagulating solution. Dissolve 2 grams of ammonium chloride in 1 liter of water containing 2.0 ml. of 85% phosphoric acid.

Sodium metabisulfite ($Na_2S_2O_5$), 0.5% solution. Make fresh just before use.

MN-Cellulose Powder 300 (Brinkmann Instruments, Inc., Westburg, N. J.) or equivalent.

Cotton. Extract cotton in a Soxhlet extractor with acetone; dry in air and then in an oven at 100° C.

Demeton and related compounds were furnished by the Chemagro Co., Kansas City, Mo.

Procedure

Cut the plant sample into small pieces, macerate with a measured volume of tetrahydrofuran-chloroform mixture (about 2 ml. per gram of sample is usually sufficient) in a blender for about 2 minutes, and filter the extract through a large funnel plugged loosely with a pledget of cotton. If the extract contains large amounts of plant pigments, shake with 5 to 10 grams of Nuchar C-190-N and filter again through a fluted filter paper. Measure the volume of organic solvent mixture recovered and evaporate the solvent to approximately 25 ml. with a rotating evaporator by using a water bath at approximately 75° C.

For chromatography, use 10 grams of a Nuchar–Hyflo Super-Cel mixture (3 to 1) for each 100 grams of plant sample. Slurry the mixture with the tetrahydrofuran-chloroform solvent, and pour into a chromatographic tube (about 20 × 400 mm.) containing 1.5 grams of Hyflo Super-Cel in the bottom. Add a layer of 1.5 grams of Hyflo Super-Cel to the top, and pack the column by applying a light vacuum. Wash the column with 100 ml. of the solvent mixture.

When the solvent level reaches the top of the Hyflo Super-Cel, quantitatively transfer the concentrated plant extract to the column with small portions of solvent, start collecting the eluate, and apply enough vacuum so that the speed of percolation will be about 80 drops per minute. When the level of the solvent mixture reaches the top of the column, carefully add more solvent and collect about 200 ml. of eluate. Evaporate the eluate to dryness in a 75° C. hot water bath with a rotating evaporator, removing the last traces of solvent by applying a gentle vacuum at room temperature.

For oxidation, add 25 ml. of the freshly prepared *m*-chloroperbenzoic acid solution to the flask containing the dried residue. Swirl the contents well, stopper the flask, and let the oxidation proceed in an ice water bath for 45 minutes. Extract the oxidation solution with 50 ml. of the coagulating solution by vigorously shaking for at least 1 minute in a clean 125-ml. separatory funnel. When the layers separate, transfer the aqueous layer into a second separatory funnel. Add 10 ml. of freshly prepared sodium metabisulfite solution to the solution in the second funnel and extract by shaking with 20 ml. of redistilled pentane. Drain the aqueous solution into a third separatory funnel, and extract it with another 20 ml. of pentane (sodium metabisulfite solution need not be added to the third funnel). By applying a slight vacuum, filter the aqueous layer slowly through 5 grams of the MN-Cellulose Powder 300 slurry packed in a 20 × 60 mm. filter funnel equipped with a vacuum adapter directly into a 250-ml. separatory funnel. The filter funnel should not be allowed to dry, or the packing will crack and cause channeling.

Extract the ether solution in the first separatory funnel again with two successive 50-ml. portions of the coagulating solution. Extract each aqueous solution in the second separatory funnel by adding an additional 10 ml. of the sodium metabisulfite solution and extracting with the same 20 ml. of pentane left from the first extraction. In the third separatory funnel extract again with the pentane left from the first extraction. Filter each portion of the aqueous solution through the filter funnel containing the cellulose powder.

Extract the combined coagulating solution extract with three 25-ml. portions of chloroform. Wash each chloroform extract in three other 60-ml. separatory funnels with 15 ml. of water in each. After complete separation of the layers in the last separatory funnel, filter the chloroform extract through a thick pledget of cotton into a 125-ml. Erlenmeyer flask, and wash the cotton with a little additional chloroform. Evaporate the solvent almost to dryness in a 75° C. hot water bath with a rotating evaporator. Remove the last traces of chloroform by applying a slight vacuum at room temperature. The chloroform must be completely removed to avoid any peaks from this solvent in the infrared absorption spectrum, but the vacuum should be applied no longer than necessary to avoid volatilization of the residue.

Chill the flask in an ice bath and dissolve the dried demeton residue in 0.75 ml. of ice-cold spectral grade carbon disulfide. Transfer the solution with a long-needle syringe to a 5-mm. cavity cell, stopper tightly, and scan the spectrum from 7.00 to 11.00 microns, compensating with carbon disulfide in the reference beam. Determine the height of the peak at 7.55 microns by the base line method, using a line drawn from about 7.50 to about 7.60 microns (depending on the instrument calibration and the nature of the curve). The edge of the carbon disulfide absorption region occurs just before 7.4 microns.

Prepare a standard curve from aliquots of a standard demeton solution in chloroform with the same procedure.

Results and Discussion

To check the method, known volumes of standard chloroform solutions of demeton and its related compounds were pipetted into 125-ml. Erlenmeyer flasks, evaporated, oxidized, and analyzed. The infrared absorption spectra are shown in Figures 1 to 4.

One hundred grams of the plant sample were chopped and placed in a blender. An aliquot of a standard chloroform solution containing equal amounts of thiono demeton, thiol demeton, and the respective sulfoxides and sulfones was added to the plant sample. The sample was then extracted, cleaned up, oxidized, and analyzed. Results obtained with apples, spinach, string beans, tomatoes, and potatoes are shown in Table I.

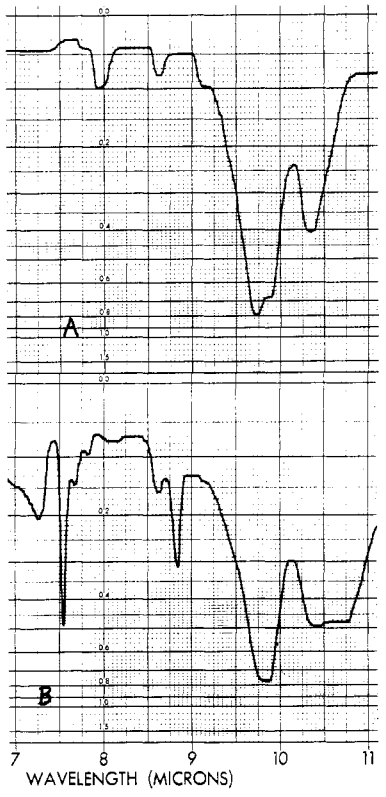


Figure 1. Infrared spectra of O,O-diethyl O-2-(ethylthio)ethyl phosphorothioate

A, before oxidation; B, after oxidation

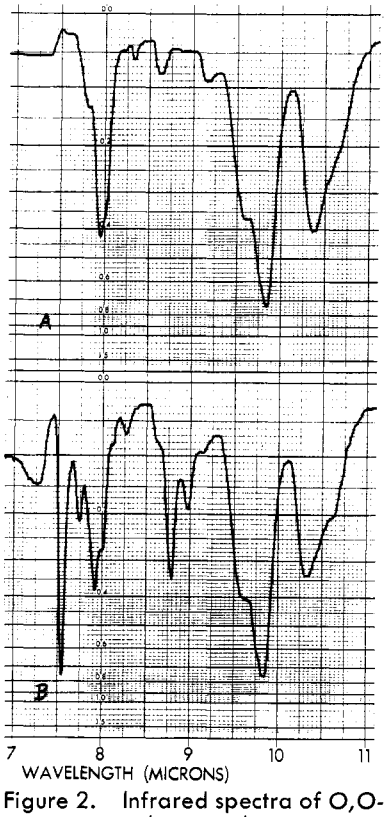


Figure 2. Infrared spectra of O,O-diethyl S-2-(ethylthio)ethyl phosphorothioate

A, before oxidation; B, after oxidation

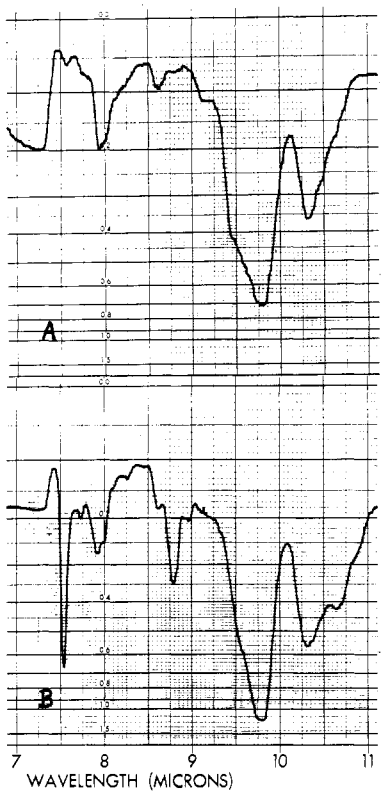


Figure 3. Infrared spectra of O,O-diethyl S(and O)-2-(ethylsulfinyl)-ethyl phosphorothioate mixture (1 to 1 ratio)

A, before oxidation; B, after oxidation

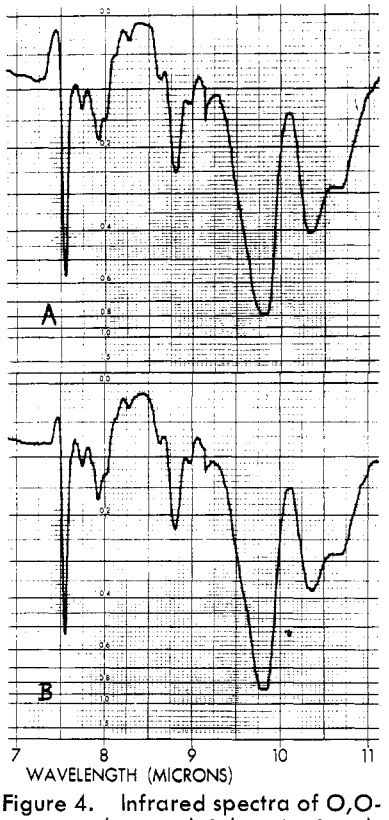


Figure 4. Infrared spectra of O,O-diethyl S(and O)-2-(ethylsulfonyl)-ethyl phosphorothioate mixture (1 to 1 ratio)

A, before oxidation; B, after oxidation

Table I. Recovery of Demeton Added to 100 Grams of Plant Material[a]

Plant	Added, μg.	Recovered[b] μg.	Recovered[b] %
Apple	20	16.6	82
	60	58.5	98
Potatoes	20	15.2	76
	60	56.0	93
Spinach	20	16.0	80
	60	56.5	94
String beans	20	15.6	78
	60	61.0	102
Tomatoes	20	16.2	81
	60	56.6	94

[a] Control samples gave no peaks at 7.55 microns. [b] Average of two or three analyses.

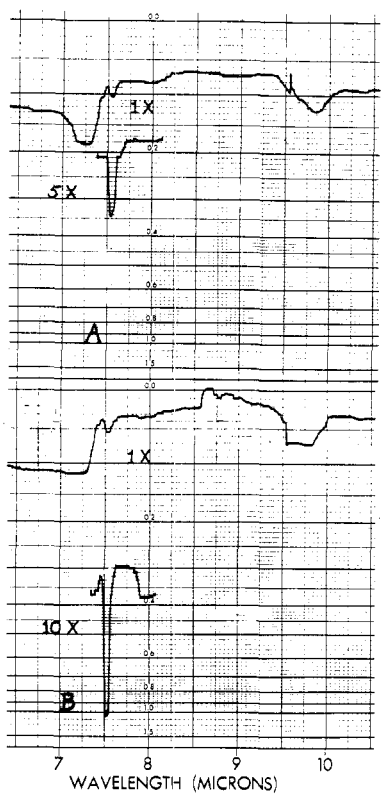


Figure 5. Infrared spectra of 20 μg. of demeton mixture (demeton mixed with its sulfoxides and sulfones in equal amounts) added to 100-gram apple sample

1X (without scale expansion), 5X scale expansion, and 10X scale expansion

We found that tetrahydrofuran is one of the best solvents for extracting the thiono and thiol demeton isomers as well as the more polar and more water-soluble insecticidally active metabolic products from plant samples. Furthermore, its relatively low boiling point allows rapid concentration of the extract. The purpose of adding chloroform to the tetrahydrofuran is to increase its efficiency in extraction of demeton residues and to decrease the solubility of aqueous plant juice in the solvent extract. When anhydrous sodium sulfate or sodium chloride is used for drying the extracts, it impairs the sulfone absorption peak at 7.55 microns for unknown reasons; therefore, these drying agents were not used.

Alcohol present in the chloroform or ethyl ether should be removed by washing the solvents with water. If alcohol is present in the oxidation solution, it tends to increase the absorption height in the region of the P-O-C peak at 9.80 microns.

In the cleanup procedure, chromatography with Nuchar–Hyflo Super-Cel as the adsorbent removes most of the plant pigments and some of the interferences. The plant waxes and other extraneous materials, which do not seem to cause trouble in the oxidation procedure, are removed later by filtering the coagulating solution through the cellulose adsorbent. Silicon grease on stopcocks or on the joint of the rotating evaporator can seriously interfere with the infrared measurement and must be avoided. In general, the use of Teflon stopcocks in the separatory funnels is recommended.

The amount of *m*-chloroperbenzoic acid needed in the demeton oxidation procedure is not critical. However, it was found that 25 ml. of the ethyl ether–benzene solution is sufficient, and oxidation in an ice bath for 45 minutes is about optimum. The addition of sodium metabisulfite solution to the second separatory funnel is necessary for complete reduction of excess *m*-chloroperbenzoic acid; *m*-chloroperbenzoic acid itself has an absorption band at 7.76 microns, which is very close to the absorption peak of the demeton sulfones.

A calibration curve for the oxidation products of demeton (sulfones) in 0.75-ml. aliquots of carbon disulfide solutions conforms to Beer's law from 25 to 750 μg. at 7.55 microns with a slope of 0.1 absorbance unit per 75 μg. in our 5-mm. cavity cells. Greater sensitivity can be achieved by concentration of the carbon disulfide solution with microsize cells or by using 5× or 10× ordinate scale expansion, as shown in Figure 5. With 5× scale expansion the slope of the calibration curve in 5-mm. cavity cells was 0.5-cm. peak height per microgram.

Potassium bromide disks do not give as good peaks at 7.55 microns for demeton sulfones as do carbon disulfide solutions, and they are very difficult to use quantitatively.

Phorate did not interfere in our method, and Di-Syston gave only slight interference (about 10%), although it is possible that its sulfone metabolites formed in crops would give higher interference.

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Received for review March 17, 1966. Accepted May 16, 1966. Division of Agricultural and Food Chemistry, 150th Meeting, ACS, Atlantic City, N. J., September 1965. The use of trade or proprietary names does not necessarily imply the endorsement of these products by the U. S. Department of Agriculture.