To determine the efficiency of the present combustion procedure, a series of biological samples was analyzed and the carbon content of each sample determined from the titration procedure. Similar samples were then analyzed by a modification of the Pregl method (*7*) used for the routine determination of the carbon content of organic compounds. The results shown in Table I indicate good agreement between both methods.

Typical results obtained when a series of duplicate combustions were run on tissues from individual animals are shown in Table II. The individual duplicates checked very closely while there was about a ±6% variation in the overall results obtained with the liver samples from various birds and about a ±3% variation with the muscle samples.

After several years of experience with this combustion method, a general guide has been established as to the optimum sample size of various tissues which can be combusted. The optimum sample size for most animal tissues is about 0.5 to 0.7 gram, but skin or dried blood samples should be about half this size. Plant samples should be 1 to 2 grams.

Typical results obtained when various tissues containing labeled compounds were combusted are shown in Table III. A background sample and standard were counted with each set of samples. The background counts shown in Table III are the average for a 6-month period. In general, the background count with both types of equipment varied less than ±1 c.p.m.

In general, scintillation counting gives the best results because of the large aliquot counted and the increased sensitivity of the method. In general, with agricultural chemicals having a specific activity of 1 mc. of C^{14} per mmole, it is easily possible to detect residues present in the tissues in concentrations of 0.02 p.p m. With barium carbonate counting, the limit of sensitivity is about 0.1 p.p.m. if 1-inch diameter plates are counted. If the level of radioactivity is too low to be detected by this method, the sample of barium carbonate can be placed in a 4.5-inch pan of Dynacon or can be converted to carbon dioxide and counted as a gas in the Dynacon.

The method described has several advantages over methods previously employed in that it permits the rapid combustion of samples containing as much as 100 mg. of carbon in an apparatus which is simple to operate and requires a minimum amount of watching. Since the samples can vary considerably in composition and weight, the operator need not know the history of the sample before combusting it. Because of the simplicity of the method, scientifically trained personnel are not needed to carry out routine combustion. Using two combustion trains, an average operator can do 16 to 20 combustions per day.

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INSECTICIDE RESIDUES

Determination of Residues of *O,O*-Dimethyl-*S*-(*N*-methylcarbamoylmethyl) Phosphorodithioate (Dimethoate) in Fruits and Forage

HENRY F. ENOS, Jr.

Quartermaster Research and Engineering Command, U. S. Army, Natick, Mass.

D. E. H. FREAR

Department of Agricultural and Biological Chemistry, The Pennsylvania State University, University Park, Pa.

An analytical method originally described for the determination of diazinon was adapted for the analysis of dimethoate residues on alfalfa, apples, grapes, and cherries. The method is based on the extraction of the insecticide from a hexane solution with hydrobromic acid, followed by hydrolysis and determination of the evolved hydrogen sulfide as methylene blue.

THE INSECTICIDE dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] is not only effective for the control of aphids and mites on a variety of fruits and vegetables (*1, 5, 9, 11, 12*) but also shows promise as a systemic insecticide for the control of grub on cattle (*3*).

The increasing importance of this insecticide necessitated the development of a rapid, sensitive method for the analysis of residues on agricultural crops. Existing methods were investigated and found to lack the desired sensitivity of 0.1 p.p.m. for the crops under investigation (*1, 8, 10*). Chilwell and Beecham (*2*) have recently reported a method for the residue analysis of dimethoate which appears to have broad application. The authors have described a semiquantitative method for dimethoate in milk (*4*), but there remained a need for a general method for plants which would have the desired sensitivity.

The method of Suter *et al.* (*13*) for the analysis of residues of *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate (diazinon) is relatively simple and is highly sensitive, and it was reasoned that this method could be adapted to the determination of microgram quantities of dimethoate, since both compounds yield H_2S when boiled in an acid solution.

The method as modified consists of extracting the plant material with either chloroform or aqueous acetone and then, after a suitable cleanup procedure, re-extracting the dimethoate from *n*-hexane with hydrobromic acid. Upon boiling the acid solution, the dimethoate sulfur is converted to hydrogen sulfide

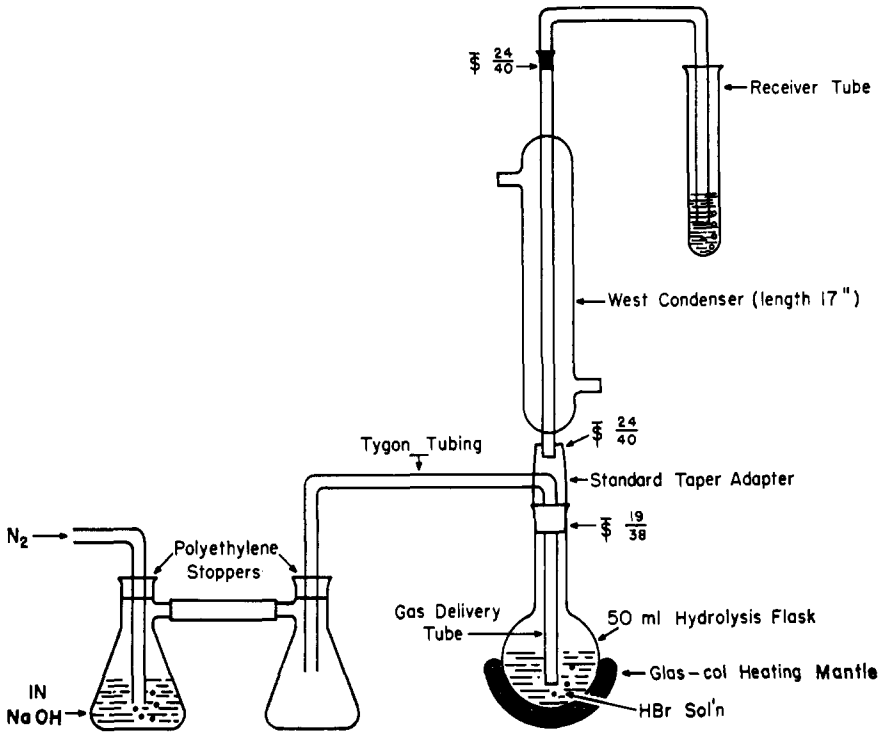

Figure 1. Hydrolysis and distillation apparatus

which is collected in zinc acetate solution and determined spectrophotometrically as methylene blue. Several modifications have been made to the method of Suter *et al.* (*13*) to improve its sensitivity and reproducibility.

Method

Apparatus. The hydrolysis and distillation apparatus is shown in Figure 1. Spectrophotometer, Beckman Model DU or equivalent.

Reagents. Chloform-acetonitrile mixture (9 + 1 v./v.) made from redistilled chemicals.

Hydrobromic acid, 48% reagent grade diluted with triple-distilled water to 47.0 to 47.5% HBr and stored over red phosphorus.

Zinc acetate reagent, made by adding 5 ml. of 12% NaOH to 130 ml. of 1% aqueous zinc acetate solution. Use triple-distilled water.

Ferric chloride reagent, 0.62 gram of $FeCl_3 . 6H_2O$ plus 9.6 ml. of concentrated HCl diluted to 100 ml. with triple-distilled water.

N,N - Dimethyl - *p* - phenylenediamine hydrochloride reagent, 0.1 gram (Eastman No. 492) diluted to 100 ml. with (1 + 1 v./v.) hydrochloric acid.

n-Hexane, center cuts of redistilled Skellysolve B.

All aqueous reagents should be made with triple-distilled water to avoid the presence of traces of copper (*6*).

Preparation of Standard Curve

Add 100 ml. of *n*-hexane to each of five 250-ml. separatory funnels, and introduce known amounts of dimethoate solution in chloroform (reagent grade, redistilled). To cover the range of the method, 0, 10, 25, 50, and 100 μg. are suggested.

Extract each separatory funnel twice by shaking for 1 minute with 6-ml. portions of hydrobromic acid. Transfer the acid layers to a 50-ml. hydrolysis flask, which is then connected to the hydrolysis-distillation apparatus (Figure 1). In the receiver tube, place 7 ml. of zinc acetate reagent, and bubble nitrogen washed with 1*N* sodium hydroxide through the apparatus for 10 minutes to displace all air in the system. Heat the flask by means of the heating mantle, and gently boil the solution for 2 hours, with nitrogen bubbling through the system continuously.

To develop the color, add 1.4 ml. of *N,N*-dimethyl-*p*-phenylenediamine reagent to the zinc acetate reagent in the receiver tube, followed by 0.3 ml. of the ferric chloride reagent. Stir the mixture well with the delivery tube to permit complete reaction of all H_2S, allow to stand for 15 minutes, make to volume of 14 ml. with distilled water. Then mix thoroughly and read in a spectrophotometer at 670 mμ against a reagent blank. A typical standard curve is shown in Figure 2.

Extraction of Fruits

Macerate a 200-gram sample of grapes or cherries in a 1-gallon Waring Blendor with 200 grams of anhydrous sodium sulfate and 350 ml. of chloroform-acetonitrile mixture. Pour off the liquid and re-extract twice more with 200-ml. portions of chloroform-acetonitrile mixture. The extracts are combined and treated as described under Cleanup.

Apples are extracted by the method of Meagher *et al.* (*7*). Blend a 200-gram sample in a water-cooled, 1-gallon Waring Blendor with 200 ml. of acetone. After 5 minutes, add an additional 200 ml. of acetone and continue blending for 5 more minutes. Dilute the extract to 600 ml. with water and shake mechanically for 1 hour. Filter through a pledget of glass wool and collect about

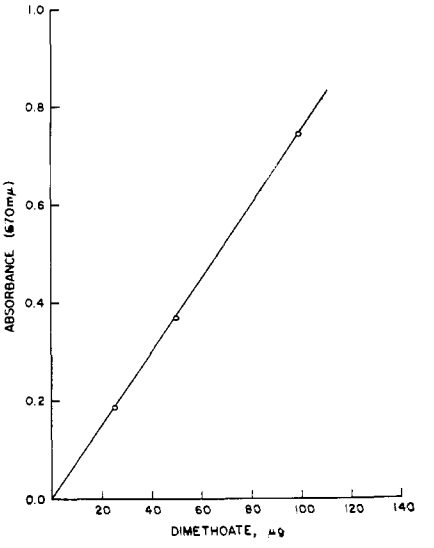

Figure 2. Standard curve for dimethoate

Table I. Recovery of Dimethoate

Dimethoate Added, μg.	Dimethoate Recovered, μg.	Treated P.P.M. Level	Recovery, %
	ALFALFA		
0	3.0	0.03	...
6000	3700	60.0	61.7
3000	2150	30.0	71.7
1500	1150	15.0	76.7
100	50	0.1	50.0
	APPLES		
0	2.0	0.01	...
1000	545	5.0	54.5
500	253	2.5	50.4
250	178	1.3	71.2
125	95	0.6	76.0
62.5	35	0.3	56.0
25.0	19	0.1	76.0
	CHERRIES		
0	2.0	0.01	...
50	31.0	0.2	62.0
25	23.0	0.1	84.0
10	7.0	0.05	70.0
	GRAPES		
0	2	0.01	...
1000	806	5.0	80.6
700	580	3.5	82.9
500	350	2.5	70.0
25	20	0.1	80.0
10	9	0.05	90.0

Table II. Residues (P.P.M.) of Dimethoate in Treated Crops

Rate of Application	Days after Last Spray													
	0	1	3	7	8	10	15	19	22	29	37	42	50	55
						ALFALFA								
1 lb./acre	53.9					1.6		0.2						
1.5 lb./acre	60.9		26.8			2.0		0.2						
						APPLES								
1 pt./100 gal.	2.0		1.1		0.9		0.8		0.3			0.4		0.3
2 pt./100 gal.	3.8		3.8		1.9		0.9		0.6			0.6		0.4
3 pt./100 gal.	5.8		3.7		2.6		1.4					1.0		0.5
						GRAPES								
2 lb. 25% W.P.		6.8			5.0		3.1		1.2	0.7	0.3		0.2	
						CHERRIES								
2 lb. 25% W.P.		3.6		1.5		0.8								

400 ml. of filtrate. Record the exact volume. Place the filtrate 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. Wash the combined 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 washes and dry by passing through anhydrous sodium sulfate. Proceed as described under Cleanup.

Extraction of Forage Crops. Grind a representative 100-gram sample of fresh alfalfa or clover in a food chopper or Wiley Mill with 100 grams of anhydrous sodium sulfate. Place the sample in a plastic bag, seal, and store in a freezer until friable. Transfer the crumbled sample to a Soxhlet extractor and extract with 400 ml. of chloroform for 3 hours. Proceed as described under Cleanup.

Cleanup

Concentrate the plant or fruit extracts in a flask attached to a rotary vacuum evaporator to ca. 25 ml. Add 100 ml. of *n*-hexane, mix, and allow to stand for a few minutes, and filter the turbid solution through Whatman No. 3 filter paper into a 250-ml. separatory funnel. Rinse the flask with 25 ml. of acetonitrile and add this to the separatory funnel. Shake vigorously for 1 minute and allow the layers to separate. Add 30 ml. of distilled water and gently mix by inverting the separatory funnel two or three times. (Emulsions form easily at this point so vigorous mixing should be avoided.) Occasionally, three layers form on standing; transfer the lower layer to a 250-ml. flask and wash out the remaining acetonitrile with an additional 15-ml. portion of water. Repeat the extraction and washing steps with two additional 25- and 30-ml. portions of acetonitrile and water, respectively.

Remove the acetonitrile by evaporation in a rotating vacuum evaporator, and transfer the turbid solution to a 250-ml. separatory funnel; rinse the flask with 15 ml. of chloroform and add this to the separatory funnel. Gently mix the contents of the funnel and allow the layers to separate, then draw off the lower layer into a 50-ml. Erlenmeyer flask, filtering through 8 grams of anhydrous sodium sulfate on a folded Whatman No. 42 filter paper. Repeat the extraction of the aqueous phase with two additional 15-ml. portions of chloroform. Concentrate the chloroform extracts to ca. 10 ml. in a rotary vacuum evaporator, and transfer to a 25-ml. volumetric flask and make to volume with chloroform.

Transfer a suitable aliquot to a 250-ml. separatory funnel containing 100 ml. of hexane. From this point, follow the procedure described for preparing the standard curve.

Results and Discussion

This method has been used successfully on alfalfa, apples, cherries, and grapes. Points on the standard curve (Figure 2) are reproducible within ±2 and ±5 μg. at the lower and higher levels, respectively.

The addition of more than 25 ml. of dimethoate solution in chloroform prior to the HBr extraction causes low recoveries; if 25 ml. or less is added, the average recovery at this point is 95%. The modifications introduced improve the reproducibility of Suter's original procedure.

Samples of untreated crops analyzed by the present procedure showed apparent dimethoate values equivalent to 2 to 3 μg. of the compound. On the basis of a 200-gram sample, this would be an apparent blank value of 0.01 p.p.m.

Over-all recoveries of known amounts of dimethoate added to samples of alfalfa, apples, cherries, and grapes are given in Table I. The recoveries, although variable, were no more so than one can expect to encounter in the diazinon method.

Table II gives the results of analyses of crops sprayed in the field with dimethoate and sampled at intervals thereafter. These are corrected for blank values but not for recoveries. On the four crops studied, dimethoate residues usually drop below 1 p.p.m. in less than 30 days following application.

Compounds other than dimethoate which yield hydrogen sulfide on hydrolysis will interfere in this determination. This includes diazinon and thiocarbamate pesticides.

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