Table IV. Comparison of Two Extraction Procedures for Removal of Parathion Residues[a]

Lb. Active per Acre	IZ Extraction Days 0	IZ Extraction Cut-off 7	IZ Extraction Interval 11	IZT Extraction Days 0	IZT Extraction Cut-off 7	IZT Extraction Interval 11
			Spinach			
0.25	17.69	3.10	1.14	18.93	3.16	1.07
0.50	28.39	6.09	2.33	29.95	4.73	1.91
			Cabbage			
0.50	5.89	0.94	0.35	6.13	0.87	0.30

[a] Expressed in p.p.m. parathion. Average of three replications.

When dealing with an organic phosphate pesticide, such as parathion, some quick and efficient method of storing large quantities of samples must be devised. Placing field-cut samples immediately into a Hobart food cutter for 5 minutes and then quick-freezing answered the storage and possible subsequent breakdown-prior-to-analysis problems. However, when the frozen leafy vegetable sample is thawed immediately prior to extraction, the analyst is faced with considerable quantities of water which must be quantitatively removed without excessive loss of pesticide.

To quantitatively remove the pesticide from the crop and the accompanying water, a rigorous cosolvent extraction blending technique should be employed. This procedure results, not only in efficient residue removal from its crop environment, but also in extracts containing unwanted and interfering extractants. In other words, rigorous cosolvent extraction ensures more or less quantitative residue removal but can accentuate cleanup problems.

The first experiment verified the need for crop blending in the presence of a suitable solvent over merely tumbling the crop end-over-end in the solvent. The advantage of using a cosolvent extraction, such as isopropanol and benzene, to remove pesticides from thawed leafy vegetables was apparent from the results obtained in the second experiment. From the experience gained in this experiment, it was also apparent that column chromatography had definite cleanup advantages when compared with the shaking of the extract in the presence of a mixture of adsorbents for decolorization, etc. Once it was decided to go to the cosolvent extraction technique, additional research was necessary to determine whether or not tumbling in addition to blending extraction was definitely superior. Simultaneously, experimentation with more efficient chromatographic columns for cleanup was undertaken in the third experiment. Results of this study indicated that with the improved chromatographic column for extraction cleanup, there appeared to be very little difference in efficiency of parathion residue removal from frozen spinach, regardless of whether cosolvent blending extraction alone was used or when this treatment was augmented by an additional 30 minutes of end-over-end tumbling. A fourth and last extraction experiment utilizing parathion-treated cabbage tended to verify the results found previously on parathion-treated spinach.

It was concluded from the four experiments evaluated in this study that when extracting a pesticide, such as parathion, from frozen field-treated leafy vegetable samples, the highest and most consistent recoveries were made with cosolvent blending with or without subsequent tumbling. Also, column chromatography employing the proper combinations of adsorbents for the particular pesticide in question was found to be an excellent and reproducible technique for cleanup of highly contaminated extracts.

Literature Cited

(1) Bann, J. M., Division of Agricultural and Food Chemistry, 131st Meeting, ACS, Miami, Fla., April 1957.
(2) Ford, L. A., Ottes, R. T., *J. Assoc. Offic. Agr. Chemists* **43,** 700 (1960).
(3) Gunther, F. A., Blinn, R. C., "Analysis of Insecticides and Acaricides," p. 46, Interscience, New York, 1955.
(4) Jones, L. R., Riddick, J. A., *Anal. Chem.* **24,** 569 (1952).
(5) Klein, A. K., *J. Assoc. Offic. Agr. Chemists* **41,** 551 (1958).
(6) *Ibid.*, **43,** 703 (1960).
(7) Klein, A. K., Laug, E. P., Sheehan, J. D., *Ibid.*, **42,** 539 (1959).
(8) Moddes, R. E. J., Cook, J. W., *Ibid.*, **42,** 208 (1959).

Received for review January 15, 1962. Accepted April 12, 1962. Division of Agricultural and Food Chemistry, 140th Meeting, ACS, Chicago, September 1961. Florida Agricultural Experiment Station Journal Series No. 1418.

INSECTICIDE RESIDUES

Residue Analysis of Phorate by Cholinesterase Inhibition after Oxidation

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The systemic organophosphorus insecticide *O,O*-diethyl *S*- (ethylthiomethyl) phosphorodithioate (phorate) is used in the seed treatment and side dressing of sugar beets, potatoes, and cottonseed for protection from insect attack. Since one may expect to find extremely small residual amounts of the pesticide and its metabolites in the harvested plant tissue, the method of analysis must be highly sensitive.

The recently reported residue method for phorate (*6*) involves the direct enzymatic determination of phorate phosphorothiolate sulfone without prior oxidation, assuming that complete conversion of phorate had already occurred in the plant by a metabolic reaction. This procedure is based on the observation of Bowman and Casida (*2*) and Metcalf *et al.* (*11*) that phorate is metabolized to the corresponding phosphorothiolate sulfone by many plant species. If, however, the oxidation has not gone to completion at the time the analyses are performed, the residue data may be expected to be low, since phorate phosphorothiolate sulfone is the most potent cholinesterase inhibitor of the known phorate metabolites (*1*).

The colorimetric method (*9*) for the determination of phorate residues did not prove successful in the authors'

A cholinesterase inhibition method of analysis was adapted for residue determinations and for general screening of phorate, a systemic insecticide, and its oxygen analogs in various plant materials. The chemical oxidation of phorate resulted in a product that was similar to phorate phosphorothiolate sulfone. Results from residue determinations of phorate in cottonseed, sugar beets, and potatoes are reported. Comparison was made for phorate analysis in sugar beet leaves with and without oxidation.

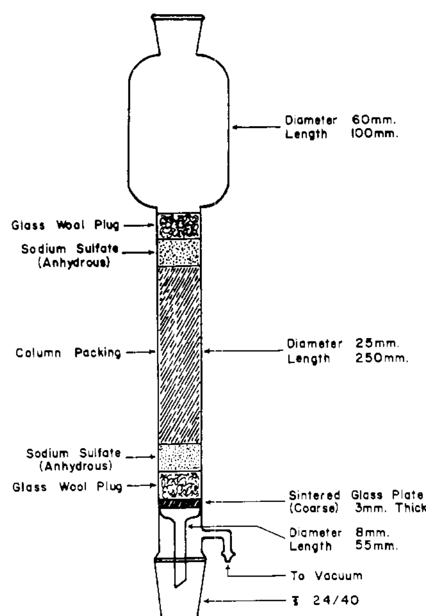


Figure 1. Chromatographic column for cleanup of crops prior to phorate analysis (*15*)

laboratories for the analysis of the pesticide and its metabolites in sugar beet leaves and cottonseeds due to high blanks. Therefore, an enzymatic method based on cholinesterase inhibition was developed. This method utilizes phorate as the reference compound which is oxidized by the peracetic acid method of Patchett and Batchelder (*14*). The chemical oxidation of phorate yields an oxidation product which has high anticholinesterase activity. It is necessary in some cases to purify plant extracts to remove naturally occurring cholinesterase inhibitors.

Experimental

Extraction of Sugar Beet Leaves and Roots. The plant material is chopped to a coarse pulp in a Hobart food chopper. This operation is facilitated by first freezing the green tops in a deep-freeze and then chopping the frozen plant material. Five hundred grams each of the macerated leaves and roots are stripped for 1 hour with 1 liter of chloroform by tumbling in sealed 1-gallon cans at 32 r.p.m. Each can contains a stainless steel baffle to improve the extraction efficiency. The resultant suspension is filtered and dried over anhydrous sodium sulfate. One hundred

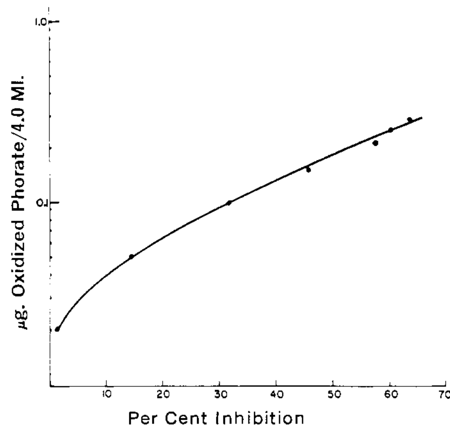


Figure 2. Standard curve for oxidized phorate using horse plasma as cholinesterase source

milliliters of the stripping solution is taken to dryness under reduced pressure at 50° to 60° C., and the remainder is redissolved in 5.0 ml. of benzene. Each milliliter of this solution is thus equivalent to the extractives from 10 grams of plant material.

Extraction of Cottonseed. The cottonseed is chopped with a Wiley cutting mill equipped with a 2-mm., mesh-screened sieve. The plant material is extracted in the same manner as described for the sugar beets except that for each gram of plant material 4 ml. of chloroform is used. One hundred twenty milliliters of the stripping solution is taken to dryness under reduced pressure at 50° to 60° C., and the residue is redissolved in 50 ml. of mixed pentanes (b.p. 30° to 60° C.).

Extraction of Potatoes. One to 2 kg. of whole potatoes are chopped in a Hobart food chopper for about 25 seconds to reduce to appropriate size. Five hundred grams of the chopped potatoes are weighed into a tared, 1-gallon paint can. Three hundred grams of anhydrous sodium sulfate powder is mixed into the pulp, and 1 liter of technical chloroform is placed in the can. The plant material is extracted in the same manner as that described for the sugar beets. Fifty-five milliliters of the stripping solution is taken to dryness under reduced pressure at 50° to 60° C., and the residue is redissolved in 5.5 ml. of benzene. Five milliliters of the benzene solution (equivalent to 25 grams of potatoes) is oxidized and analyzed as described below without purification.

Cleanup of Sugar Beet Leaves Extract. The extract must be subjected to a chromatographic cleanup to reduce the interferences to near 0.008 p.p.m., which is the sensitivity of the method. The column packing consists of a mixture of 60 grams of anhydrous sodium carbonate, 20 grams of Celite 545, and 10 grams of Darco-G60 charcoal. As seen in Figure 1, a 25 × 250 mm. glass column is used that was originally designed for the analysis of aldrin and dieldrin (*15*). The column is dry-packed with a glass-wool plug, 10 grams of anhydrous sodium sulfate, and 40 grams of the packing mixture. While a moderate vacuum is applied, 50 ml. of benzene is slowly added, and the packing is tamped with a glass rod. As the benzene level approaches the top of the column, another 10 grams of sodium sulfate and a glass-wool plug are added. The column is washed with 50 ml. of benzene, and 2.5 ml. of the concentrated plant extract is added. Four 5-ml. rinses of benzene are followed by 230 ml. so that a total eluate of 250 ml. is collected in a round-bottomed flask. The eluate is colorless and contains only traces of plant pigments. The benzene solution is evaporated in vacuo at 50° to 60° C. to a final volume of 10.0 ml. The time required for this purification is approximately 2 hours.

Cleanup of Cottonseed Extract. Fifty milliliters of mixed pentane (b.p. 30° to 60° C.) solution containing the extractives from 30 grams of cottonseed is transferred quantitatively from a round-bottomed flask to a 500-ml. separatory funnel with three separate 50-ml. rinses of pentanes. This solution is extracted with four 50-ml. aliquots of acetonitrile saturated with pentanes. Fewer than four extractions resulted in lower recoveries. The combined acetonitrile fractions are evaporated to dryness in vacuo at 50° to 60° C. The residue in the round-bottomed flask is dissolved in 5 ml. of pentanes and re-evaporated. This procedure is repeated a second time to ensure complete removal of the acetonitrile. The final residue is dissolved in 5 ml. of pentanes.

A chromatographic column is prepared in the following manner. A piece of glass wool is placed on the fritted plate of the column (Figure 1) followed by 10 grams of anhydrous

sodium sulfate. Two inches of activated florisil (activated for 3 hours at 270° C.) is added to the column and gently packed with a glass rod. A second 2 inches of activated florisil is gently packed into the column followed by 2 grams of anhydrous sodium sulfate. The packed column is moistened with 40 ml. of mixed pentanes, and the residue prepared as described above is immediately placed on the column quantitatively with three 10-ml. rinses of pentanes. The column is eluted with 200 ml. of a solution of 10% ethyl ether in pentanes. The eluant is evaporated to dryness in vacuo at 50° to 60° C., the residue is dissolved in 6 ml. of benzene, and a 5-ml. aliquot of the benzene solution is chemically oxidized.

Oxidation of Phorate and Plant Extractives. Five milliliters of the benzene solution of purified extracts (equivalent to 12.5 grams of sugar beet leaves or 25 grams of cottonseed) is pipetted into a 16 × 150 mm. glass-stoppered test tube. The tube dimensions and other conditions were chosen for best oxidation as discussed by Patchett and Batchelder (*14*). Three milliliters of a mixture of 1 volume of 30% hydrogen peroxide and 5 volumes of glacial acetic acid are added to the benzene layer. A boiling chip is placed in the tube, and the solution is mixed. For each analysis, at least three tubes should be prepared in this manner—a control sample, a treated plant sample, and a standard phorate sample. The tubes are immersed in a water bath at 75° C. and heated for 20 minutes. The tubes are then immersed in an ice bath and cooled for 5 to 10 minutes. The solution in the tube is decanted into a 60-ml. separatory funnel. The benzene layer is washed free of acetic acid with five 1-ml. washes consisting of two 1-ml. washes with a saturated sodium sulfate solution and three 1-ml. washes of distilled water. The final solution is dried over anhydrous sodium sulfate, filtered, and aliquots are analyzed by cholinesterase assay as described below. This oxidation procedure is reproducible and did not result in conditions as described by Miskus and Hassan (*12*).

The stock solution of phorate contains 1 mg. of phorate per ml. of benzene. An aliquot of the stock solution is diluted so that the final concentration is 5 μg. of phorate per ml. of benzene. Five milliliters of this standard phorate solution is oxidized and processed as described above.

Standard Curve and Analysis of Samples. Small aliquots of 1.0 ml. or less of the benzene solutions of the oxidized phorate and plant extractives are pipetted into 10-ml. Griffin beakers, 0.5 ml. of glycerol solution (10% in methanol v./v.) is added, and the solvents are evaporated with a gentle stream of warm air from a hair dryer. The purpose of the glycerol is to serve as a keeper and to prevent the contents of the beakers from going to complete dryness when the solvents are evaporated. A standard series contains the following amounts of phorate per beaker: 0, 0.02, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 μg. Analyses are performed by the potentiometric method measuring cholinesterase inhibition by a change in pH (*8*, *16*).

A typical calibration curve for oxidized phorate by the potentiometric method, using horse plasma, is shown in Figure 2. The useful range of the standard curve extends from 0.02 to 0.30 μg. oxidized phorate.

Results and Discussion

Activation of Cholinesterase Inhibitors by Oxidation. Formerly the application of cholinesterase inhibition methods to residue determinations was limited to those organophosphates which had a direct in vitro action on the enzyme. Chemical methods were later developed to activate those organophosphate insecticides which themselves had only slight in vitro cholinesterase inhibitor effect.

Giang and Hall (*8*) were among the first to show that parathion could be readily converted with fuming nitric acid to a powerful cholinesterase inhibitor. They assumed that the parathion was converted to its oxygen analog, paraoxon.

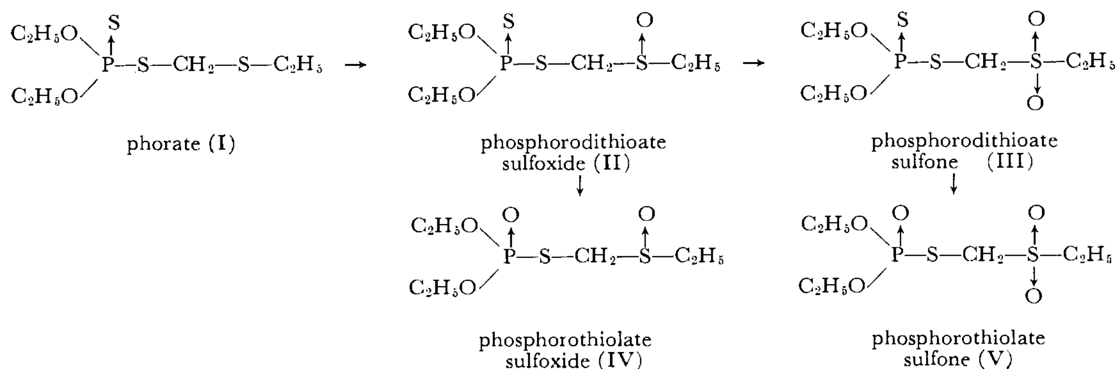
Cook (*4*) reported that malathion and other thionophosphates could be converted on paper chromatograms to powerful cholinesterase inhibitors by spraying with *N*-bromosuccinimide (NBS) solution. Excess NBS interfered with the enzymatic reaction, but this interference could be eliminated by addition of a dilute water solution of phenol before adding the enzyme.

Fallscheer and Cook (*7*) showed that when certain thionophosphates and dithiophosphates were treated with dilute bromine water, they were quickly converted into potent in vitro cholinesterase inhibitors, and that an excess of bromine water did not interfere with the reaction.

Casida *et al.* (*3*) have reported that Schradan could be activated to a potent in vitro inhibitor by neutral permanganate oxidation and by incubation with liver slices and plant tissue. Certain phosphorothionates are not activated by bromine in the cold but may be oxidized with peroxy acids under more severe conditions. Patchett and Batchelder (*14*) have used peracetic acid to convert Trithion (P=S compound) to two P=O oxidation products, *S*-(*p*-chlorophenylsulfinyl) methyl *O,O*-diethyl phosphorothioate and *S*-(*p*-chlorophenylsulfonyl) methyl *O,O*-diethyl phosphorothioate. Giang and Schechter (*9*) oxidized phorate, its oxygen analog, and their sulfoxides to their respective sulfones with perbenzoic acid in order to obtain maximum recoveries for phorate residues when using their colorimetric method. Bowman and Casida (*2*) have proposed a pathway for the conversion of phorate to the phosphorothiolate sulfone which occurs in the cotton plant. A similar scheme may be postulated for the chemical activation process (see bottom of page).

Preliminary experiments using dilute bromine water for the activation of phorate proved unsuccessful. For this reason, oxidation by the peracetic acid method of Patchett and Batchelder (*14*) was attempted. Oxidation products were examined by infrared spectrophotometry, paper chromatography, and cholinesterase inhibition.

Figure 3 presents infrared spectra for phorate (*A*), oxidized phorate (*B*), and phorate phosphorothiolate sulfone (*C*). As can be seen in spectrum *A*, the phorate



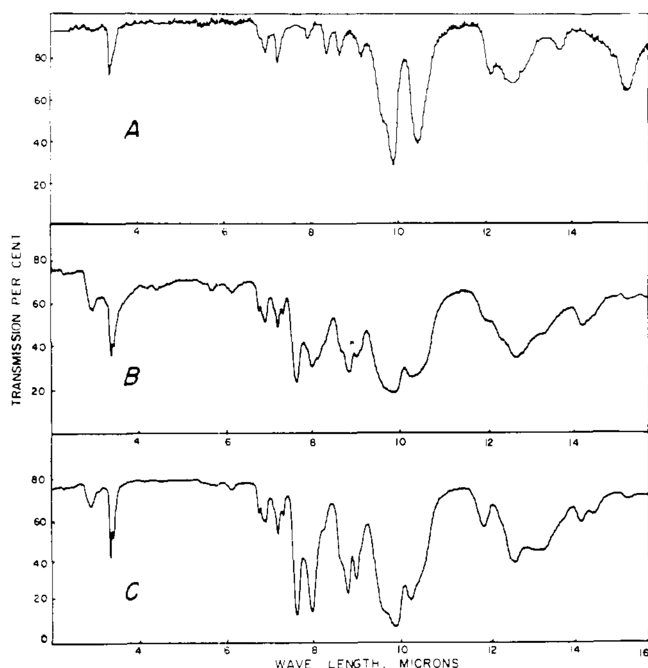


Figure 3. Infrared spectra of (A) phorate, (B) phorate after peracetic acid oxidation, and (C) phorate phosphorothiolate sulfone

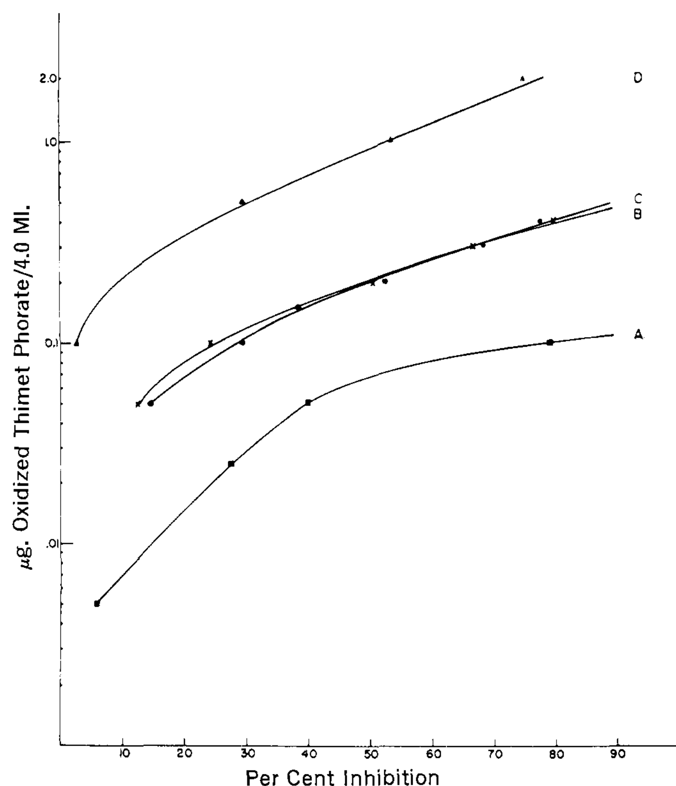


Figure 4. Cholinesterase inhibition curves of four enzyme sources using peracetic acid–activated phorate as inhibitor

(A) housefly head brei; (B) human plasma; (C) horse plasma; (D) honey-bee head brei

has an absorption band at 15.3 microns indicating a P=S group but no absorption bands at 7.7 to 8.0 microns, indicating the absence of a P=O group. The absence of any peaks in the regions of 7.4 to 7.8 and 8.6 to 9.0 microns indicated that no sulfone group was present. Oxidized phorate and untreated phorate phosphorothiolate sulfone have no absorption bands for the P=S group, but do have absorption bands in the regions for the P=O and the sulfone groups as would be expected for these compounds. The latter two spectra appear quite similar to each other indicating that phorate has been converted by peracetic acid oxidation to a product very much like phorate phosphorothiolate sulfone.

A comparison of the paper chromatographic separation of both oxidized and unoxidized phorate phosphorothiolate sulfone and oxidized phorate was made. The reversed-phase paper chromatographic technique was employed by impregnating the paper with a solution of 5% olive oil dissolved in ethyl ether. The mobile solvent system was 1 part toluene and 4 parts hexane saturated with olive oil. The ascending solvent technique was employed. The inhibitor spots were detected by the in vitro cholinesterase inhibition method of Cook (*5*) using horse plasma cholinesterase. The R_f values for all three samples were approximately 0.08 which compares well with those obtained by Metcalf *et al.* (*11*). The fact that a single cholinesterase inhibiting spot was found with the same R_f as that of the authentic phorate phosphorothiolate sulfone was taken as additional evidence that the oxidation products contained the sulfone.

Another parallel chromatogram was then sprayed with the 2,6-dibromo-*N*-chloro-*p*-quinone imine reagent of Menn (*10*). This reagent when tested gave a positive test for phorate, the corresponding phosphorothiolate, and phosphorodithioate sulfone, but was negative for phorate phosphorothiolate sulfoxide and sulfone. Thus, when phorate, its corresponding sulfone, and phorate phosphorothiolate were oxidized with peracetic acid, no spots could be detected with this reagent but were detected by Cook's procedure. These results indicated that the oxidation products were no longer thiono compounds but had been converted to phosphorothiolates.

The molar I_{50} values were determined for phorate and its metabolites after peracetic acid oxidation using horse plasma as the cholinesterase source. As seen in Table I, the molar I_{50} values vary within a range of $\pm 16\%$ which lies within the experimental errors of the method of analysis. The molar I_{50} value for unoxidized phorate using horse plasma cholinesterase is 5.3×10^{-5}.

The effect of reaction time for the oxidation of phorate and the corresponding phosphorothiolate sulfone was studied. For zero time, peracetic acid was added to the benzene solution of phorate phosphorothiolate sulfone and without heating, the mixture was washed with water immediately. Oxidation periods beyond 60 minutes were not studied. As seen in Table II, oxidation times from 0 to 60 minutes for phorate phosphorothiolate sulfone and 20 to 60 minutes for phorate produced no significant changes in cholinesterase inhibition.

Table I. Molar I_{50} Values for Phorate and Its Metabolites

Compound	Oxidation with Peracetic Acid	Molar Concn. for 50% Inhibition
Phorate	No	5.3×10^{-5}
	Yes	1.8×10^{-7}
Phosphorodithioate sulfoxide	Yes	1.4×10^{-7}
Phosphorodithioate sulfone	Yes	1.9×10^{-7}
Phosphorodithiolate sulfoxide	Yes	1.5×10^{-7}
Phosphorothiolate sulfone	Yes	1.5×10^{-7}

The average I_{50} value for phorate phosphorothiolate sulfone and phorate treated with peracetic acid 20 to 60 minutes was found to be $1.7 \times 10^{-7}M$. This is considerably higher than the experimental value for the I_{50} of untreated phorate phosphorothiolate sulfone, $7.2 \times 10^{-8}M$. The apparent loss due to peracetic acid treatment cannot

be ascribed to decomposition since the I_{50} does not change appreciably under the stated conditions during one hour's treatment. These observations are not in agreement with those of Miskus and Hassan (*12*) who observed some losses of phorate due to prolonged heating.

The apparent losses of phorate phosphorothiolate sulfone, therefore, may be caused by the initial separation of the benzene and peracetic acid phases and subsequent water washes. This point could not be checked by conventional potentiometric methods due to the high acid content of these solutions. However, experience with this method has shown that the oxidation of phorate under strictly controlled conditions may not be stoichometric but is entirely reproducible. For 25 separate determinations of the molar I_{50} value of oxidized phorate, an average value of 1.7 ($\pm$0.11) $\times$ $10^{-7}M$ was obtained and confidence limits were calculated on the basis of 95% probability (student's *t*-distribution).

Comparison of Different Cholinesterase Sources. Figure 4 depicts the inhibition curves determined by the ΔpH method of four cholinesterase enzyme sources using oxidized phorate as the inhibitor. The molar I_{50} values of the inhibitor were for horse (*C*) and human (*B*) plasma 1.8 $\times$ $10^{-7}M$, for fly-head brei (*A*) 6.3 $\times$ $10^{-8}M$, and for bee-head brei (*D*) 7.2 $\times$ $10^{-7}M$. The bees used in this experiment were field-raised and may have developed some resistance to pesticides. This may account for the higher I_{50} value of bee-head brei in comparison to the I_{50} value for houseflies.

Recovery Studies. Recoveries were studied by adding appropriate amounts of phorate to the crop at the time of extraction. Results for several crops, e.g., sugar beet tops and roots, cottonseeds, and potatoes are shown in Tables III and IV.

To show that a cleanup procedure was necessary for several crops, the following example is cited. A sugar beet leaf extract (1-gram equivalent) which was not cleaned up gave 47.2% inhibition while a corresponding fortified sample (0.1 p.p.m.) gave only 49.9%. Thus, recovery based on these results amounted to 17.5%. The same sample was then cleaned up by the described procedure giving the following results: check sample, 23.8% inhibition; fortified sample, 44.8%, 87% recovery. These results indicated that the cleanup procedure removed most naturally occurring cholinesterase inhibitors and other masking effects (*13*).

As may be seen in Tables III and IV, recoveries for added phorate to sugar beet leaves and roots, cottonseed, and potatoes resulted in satisfactory yields. Cottonseeds had to be cleaned up by the

Table II. Effect of Time of Treatment by Peracetic Acid on Molar I_{50} Values for Phorate and the Phosphorothiolate Sulfone

Time of Oxidation (Min.)	Molar I_{50} Values: Phorate	Molar I_{50} Values: Phosphorothiolate Sulfone[a]
0	...	1.6 $\times$ 10^{-7}
5	...	1.5 $\times$ 10^{-7}
10	...	1.8 $\times$ 10^{-7}
20	1.8 $\times$ 10^{-7}	1.6 $\times$ 10^{-7}
40	1.5 $\times$ 10^{-7}	1.7 $\times$ 10^{-7}
60	1.7 $\times$ 10^{-7}	1.7 $\times$ 10^{-7}

[a] Amount of horse plasma was chosen so that a ΔpH of 1.5 to 2.0 was attained in 60 minutes at 25° C. with no inhibitor present. I_{50} is the molarity of inhibitor which results in 50% of the activity of the control. I_{50} values calculated upon the basis of the final phosphorothiolate sulfone metabolite.

Table IV. Residue Determinations for Phorate on Cottonseed and Potatoes

Sample Description	Gross P.P.M.	Net[a] P.P.M.	Net Per Cent Recovery
SUGAR BEET ROOTS (5 GRAMS)			
Check	<0.008	...	...
Treatment 1[b]	<0.008	<0.008	...
Treatment 2	<0.008	<0.008	...
Treatment 3	<0.008	<0.008	...
Recovery (0.006 p.p.m.)	0.065	0.065	108
COTTONSEED (2.5 GRAMS)			
Check	0.018	...	...
Treatment 4	0.019	<0.016	...
Recovery	0.096	0.078	98.0[c]
Check	0.031	...	...
Treatment 5	0.022	<0.016	...
Recovery	0.098	0.067	83.8
Check	0.036	...	...
Treatment 6	0.039	<0.016	...
Recovery	0.106	0.070	88.0
POTATOES (2.5 GRAMS)			
Check	0.02	...	...
Treatment 7	0.02	<0.016	...
Recovery	0.10	0.08	100.0

[a] Net p.p.m. corrected for background interference in untreated check samples. Sensitivity of method is 0.016 p.p.m. when analyzing 2.5-gram sample and 0.008 p.p.m. when analyzing 5.0-gram sample.

[b] Treatment 1, 2: 1 lb. active ingredient, phorate (as 5% granules), per acre, two Gandy applications. Harvested 5 months after last application.

Treatment 3: Single application, same as treatment 1.

Treatment 4: 1.9 lb. active ingredient, phorate (as 10% granules), per acre, side-dressing. Harvested 4 months after treatment.

Treatment 5: Side-dressing, 2.3 to 2.1 lb. active ingredient, phorate (as 10% granules), per acre. Harvested 5 to 7 months after treatment.

Treatment 6: Seed treatment, 0.76 lb. active ingredient, phorate, per acre; 8 lb. 44 D per 100 lb. seed. Harvested 5 months after treatment.

Treatment 7: Side-dressing, 2 lb. active ingredient, phorate (as 2% granules), per acre.

[c] 0.08 p.p.m. phorate added for recovery studies with 2.5-gram sample. 0.04 p.p.m. phorate added for recovery studies with 5.0-gram sample.

Table III. Residue Determinations for Phorate in Sugar Beet Leaves by Two Methods[a]

Sample Code	Peracetic Acid Activation	Gross P.P.M.	Net[b] P.P.M.	Net Per Cent Recovery
METHOD 1				
Control	Yes	0.009	...	
Treatment 1[c]	Yes	0.036	0.027	
Treatment 2	Yes	0.023	0.014	
Recovery[d]	Yes	0.044	0.035	87.5
METHOD 2 (*6*)				
Control	No	0.005	...	
Treatment 1[c]	No	0.007	0.002	
Treatment 2	No	0.010	0.005	
Recovery[e]	No	0.013	0.008	95.0

[a] Five grams analyzed.
[b] Method sensitivity: Method 1, 0.008 p.p.m.; Method 2, 0.002 p.p.m.
[c] Treatment 1: 1.85 lb. phorate (5% granules) per acre and 0.95 lb. phorate per acre. Gandy application. Harvested 4 months after application.
Treatment 2: 1.58 lb. phorate (5% granules) single application, same as Treatment 1.
[d] 0.04 p.p.m. phorate added.
[e] 0.008 p.p.m. phorate phosphorothiolate sulfone added.

procedure described under Experimental since otherwise natural inhibitors and masking factors caused extremely low recoveries, similar to the ones discussed above for sugar beet leaves. As may be seen from Table IV, the apparent residue values of these check samples of cottonseeds varied considerably. However, as in many residue analyses, it is important to obtain an untreated sample from the same general area as the sample under investigation, so that residue values may be corrected for background.

Sugar beet roots did not require any cleanup prior to analysis since cholinesterase inhibition of check samples were below 20% and recoveries were 81%.

Whereas a 2.5-gram sample of potatoes could be analyzed satisfactorily without cleanup (15.2% cholinesterase inhibition by check sample), interference from a 5.0-gram sample (36.4% inhibition) persisted even after the above described cleanup procedures. A 2.5-gram aliquot sample was, therefore, chosen for potatoes giving a sensitivity of 0.016 p.p.m. phorate (Table IV).

Residue Determinations. Actual residue determinations on treated crop samples are shown in Tables III and IV. In only one crop analyzed were detectable amounts of residues found.

Other crops which have also been analyzed for phorate residues by this procedure with minor cleanup modifications include apples, hops, lima beans and pods, and red kidney beans. Recoveries and sensitivity were in the same range as for cottonseeds (Table IV).

Comparison of Two Residue Methods. Table III contains data for phorate residues in sugar beet leaves obtained by two cholinesterase inhibition methods. The plant material was cleaned up as previously described. Samples were analyzed by two separate cholinesterase inhibition procedures, the first procedure presently described while the second procedure was that of Curry (*6*). In the latter procedure, phorate phosphorothiolate sulfone was used as a standard, and the crop extract was analyzed directly without chemical activation. Check material was fortified prior to cleanup with either phorate (Method 1) or phorate phosphorothiolate sulfone (Method 2), and recoveries were 87.5 and 95.0%, respectively (Table III). Since phorate (least polar) and the corresponding phosphorothiolate sulfone (most polar) resulted in high recoveries through the cleanup procedure, it was assumed, therefore, that all other intermediate metabolites would behave in a similar manner. As can be seen from the data in Table III, the residue results obtained by Method 2 were lower, indicating that not all the phorate present in the sugar beet leaves has been oxidized by the plant material.

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Literature Cited

(1) American Cyanamid Co., New York, N. Y., "Thimet Technical Manual," 1957.
(2) Bowman, J. S., Casida, J. E., J. Agr. Food Chem. **5**, 192 (1957).
(3) Casida, J. E., Allen, T. C., Stahmann, M. A., *J. Am. Chem. Soc.* **74**, 5548 (1952).
(4) Cook, J. W., *J. Assoc. Offic. Agr. Chemists* **38**, 826 (1955).
(5) *Ibid.*, p. 150.
(6) Curry, A. N., Kress, L. M., Paylor, R. A. L., J. Agr. Food Chem. **9**, 469 (1961).
(7) Fallscheer, H. O., Cook, J. W., *J. Assoc. Offic. Agr. Chemists* **39**, 691 (1956).
(8) Giang, P. A., Hall, S. A., *Anal. Chem.* **23**, 1830 (1951).
(9) Giang, P. A., Schechter, M. S., J. Agr. Food Chem. **8**, 51 (1960).
(10) Menn, J. J., Erwin, W. R., Gordon, H. T., *Ibid.*, **5**, 601 (1957).
(11) Metcalf, R. L., Fukuto, T. R., March, R. B., *J. Econ. Entomol.* **50**, 338 (1957).
(12) Miskus, R., Hassan, S., *Ibid.*, **52**, 353 (1959).
(13) Orgell, W. H., Vaidya, K. A., Dahm, P. A., *Science* **128**, 1136 (1958).
(14) Patchett, G. G., Batchelder, G. H., J. Agr. Food Chem. **8**, 54 (1960).
(15) Shell Chemical Co., Agricultural Chemicals Division, New York, N. Y., "Manual of Methods for the Determination of Pesticide Chemical Residues in Agricultural Products, Animal Products, and Soil," 1961.
(16) Zweig, G., Archer, T. E., J. Agr. Food Chem. **6**, 910 (1958).

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INSECTICIDE RESIDUES

Colorimetric Method for the Estimation of Dimethoate Residues

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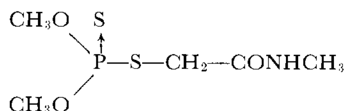
The recent interest in dimethoate [*O,O* - dimethyl *S* - (*N* - methyl carbamoylmethyl) phosphorodithioate], also known as Rogor in European countries, as a systemic insecticide has created the need for a simple but sensitive analytical method of detecting and estimating residues of this compound in milk and treated plants. Dimethoate is highly effective against some aphids, mites, and cattle grubs, and has low toxicity to warm-blooded animals and humans (*1, 3–5, 9*).

Dimethoate is a white, crystalline, solid material, m.p. 51–52° C. (*1, 3, 9*), with the following structural formula:



This compound is very soluble in most organic solvents except saturated hydrocarbon solvents, such as petroleum ether or *n*-hexane, in which it is only slightly soluble. Its solubility in water is about 1 to 2% at room temperature. Dimethoate is relatively stable in dilute acid and in water at room temperature, but it hydrolyzes rapidly in aqueous alkaline solutions to methylamine and thioglycolic acid in addition to the thiophosphate moiety.

Several methods for analysis of residues have been described. Santi and Bazzi (*12*) published a colorimetric method based on analysis for phosphorus for the determination of dimethoate residues present in olive oil. Chilwell and Beecham (*3*) adapted a similar phosphorus method for estimating residues in sprayed crops. In 1956, Bazzi, Pietri-Tonelli, and Santi (*2*) worked out a method for residues of this compound by oxidizing methylamine, which is formed by hydrolysis of dimethoate, to formaldehyde, and colorimetrically