of insecticide residues, the method shows sufficient promise to warrant such an extension. Furthermore, these procedures may possibly be applied to the identification of degradation products of the organophosphates formed in the plant.

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INSECTICIDE RESIDUES

The Detection of Residues of Systox and Its Toxic Metabolites in the Presence of Other Organophosphorus Pesticides

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A method has been developed for detection of residues of Systox and its metabolites in plants. The method is based upon chromatographic separation on paper and subsequent characterization through the use of the color forming agent, 2,6-dibromo-N-chloro-p-quinoneimine. The method has a sensitivity for detection of 0.3 p.p.m. based on a sample of 100 grams and will distinguish residues of Systox and its metabolites in the presence of other organophosphorus pesticides and cholinesterase inhibitors. With the application of a preliminary chromatographic cleanup procedure, the method has been used for the detection of Systox residues in a large number of crops.

THE DETERMINATION of Systox (demeton) residues in plant material has been based in the past on the measurement of cholinesterase inhibition (*8*). In view of the numerous compounds presently registered as pesticides which are either cholinesterase inhibitors themselves or are capable of being converted into cholinesterase inhibitors, this method possesses insufficient specificity to be useful for determining the presence or absence of Systox in plant material of unknown history. The work described in this paper was carried out to develop a detection method for Systox residues in the presence of residues of other cholinesterase-inhibiting pesticides or their metabolites.

The commercial product, Systox (Chemagro Corp.), is a mixture of two insecticidally active isomers: *O,O*-diethyl *O*-2-(ethylthio) ethyl phosphorothioate (I); and *O,O*-diethyl *S*-2-(ethylthio) ethyl phosphorothioate (II). The isomers are present in the commercial material in a ratio of approximately 2 to 1.

Extensive metabolic studies on Systox have been conducted by Metcalf and coworkers (*5*, *7*, *9*, *12*). These studies have shown conclusively that the Systox isomers do not persist as such for more than 1 or 2 weeks after application of the product to plants (*12*). The first step in the metabolism of the thiono isomer in plants is its conversion to the sulfoxide (*5*, *9*). Also, when the thiono isomer is applied to cotton plants, there is an appreciable residue of the thiono isomer sulfone present, even 10 days after treatment (*7*). The thiol isomer is likewise oxidized at the side chain sulfur atom to form both the sulfoxide and the sulfone (*6*). The phosphate derivatives which might be formed from the thiono compounds have not been detected in plants. This is likely due to their rapid rate of hydrolysis. Thus, it is apparent that a generally applicable method for Systox residues should determine not only the parent isomers but their sulfoxides and sulfones as well. The method described here will identify the Systox thiol isomer and the sulfoxide and sulfone of both isomers. The thiono isomer cannot be distinguished from other interfering pesticides by this method.

Compounds listed in Table I are registered as cholinesterase inhibitors. A specific method for Systox must be capable of detecting Systox and its metabolites in the presence of these cholinesterase-inhibiting compounds and/or their toxic metabolites.

The organophosphorus compounds containing the P=S group are converted in the animal body to their oxygen analogs (*3*) containing a P=O group. In this way, phosphorodithioates are metabolized to phosphorothioates, and phosphorothioates to phosphates. Evidence continues to accumulate that the anticholinesterase activity of compounds containing the P=S group is due to their conversion to the oxygen analogs in the mammalian liver (*3*).

In most cases, it has not been demonstrated that the oxygen analog of the compounds listed in Table I are formed in plants. However, Metcalf *et al.* (*13*, *15*) have shown that Di-Syston (trademark of Farbenfabriken Bayer) derivatives containing the P=O group are formed in both cotton and tomato plants. In view of these observations, there is a definite possibility that traces of the oxygen analogs of the compounds in question may be present in plants. Therefore, all of the oxygen analogs of these compounds were considered as possible interferences in the detection of Systox residues.

It has been reported (*14*) that Tri-

Table I. Registered Cholinesterase-Inhibiting Pesticides (17)

Classification	Common Name	Trade Name
Phosphates, Pyrophosphates	1-Methoxycarbonyl-1-propen-2-yl dimethyl phosphate and its beta-isomer	Phosdrin
	Schradan (octamethyl pyrophosphoramide)	
Carbamates	1-Naphthyl *N*-methylcarbamate	Sevin
Phosphonothioates	EPN	
Phosphorothioates	Demeton (a mixture of *O,O*-diethyl *O* (and *S*)-2-(ethylthio)ethyl phosphorothioates	Systox
	O,O-Diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate	Co-Ral
	O,O-Diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	Diazinon
	Methyl Parathion	
	Parathion	
Phosphorodithioates	*O,O*-Dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl] phosphorodithioate	Guthion
	2,3-*p*-Dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate) containing approximately 70% *cis* and *trans* isomers and approximately 50% related compounds	Delnav
	O,O-Diethyl-*S*-2-(ethylthio)ethyl phosphorodithioate	Di-Syston
	Ethion	
	Malathion	
	S-(*p*-Chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate	Trithion
Phosphorotrithioites	*S,S,S* tributyl phosphorotrithioite	Folex

Table II. R_F Values for Systox and Its Metabolites

Compound	R_F
Systox thiono isomer	0
Systox thiono isomer sulfoxide	0.83
Systox thiono isomer sulfone	0.68
Systox thiol isomer	0.42
Systox thiol isomer sulfoxide	0.83
Systox thiol isomer sulfone	0.82

Table III. Recovery of Systox Thiol Isomer and Systox Metabolites from Plant Extracts[a]

Crop	Thiol Isomer	Thiol Isomer Sulfoxide	Thiol Isomer Sulfone	Thiono Isomer Sulfoxide
Celery	61 (0.25)	71 (0.10)	78 (0.05)	71 (0.20)
Peas	94 (0.25)	55 (0.10)	71 (0.05)	80 (0.20)
Peaches	30 (0.25)	54 (0.25)	81 (0.05)	37 (0.25)
Plums	91 (0.25)	68 (0.25)	84 (0.25)	81 (0.25)
Tomatoes	118 (0.25)	97 (0.25)	75 (0.05)	84 (0.25)

[a] Recovery values are given as percentage. Each value is followed by the p.p.m. concentration used in the experiment.

thion is metabolized in plants to its sulfoxide and sulfone. In addition to these compounds, the possible presence of the Trithion oxygen analog and its sulfoxide and sulfone must also be considered.

The basic idea for the method which was finally developed for the detection of Systox residues in crops came from a careful investigation of the hydrolysis of the Systox isomers and their sulfoxides and sulfones in alkali. It was found that alkaline hydrolysis of the thiol isomer of Systox results in formation of *O,O*-diethyl phosphoric acid and ethylthioethyl mercaptan. On the other hand, hydrolysis of the Systox thiol isomer sulfone forms predominantly *O,O*-diethyl phosphorothioic acid and ethyl vinyl sulfone. Muhlman and Schrader (*16*) have reported this reaction for the sulfone for the thiol isomer of Meta-Systox. Hydrolysis of the thiol isomer sulfoxide results in a mixture of the products formed from the other two compounds. The nature of these hydrolyses was shown by reaction of the hydrolysis products on paper chromatograms. In addition, the potassium salt of *O,O*-diethyl phosphorothioic acid could be isolated from the hydrolysate of the thiol isomer sulfone. This material had the reported melting point for *O,O*-diethyl phosphorothioic acid.

The method for detection of the Systox isomers and their oxidation products is based on the fact that all compounds containing a P=S group as well as free phosphorothioic acids form a very intense red color on paper chromatograms when treated with 2,6-dibromo-*N*-chloro-*p*-quinoneimine (*11*).

By a cleanup procedure involving both column and paper chromatography, the Systox thiol isomer and its oxidation products as well as the thiono isomer oxidation products can be separated from all material which might interfere with the color test. The thiono isomer itself is not separated from interfering pesticide residues by this method. By oxidation and hydrolysis on the final paper chromatogram, *O,O*-diethyl phosphorothioic acid can be obtained from both the Systox thiol isomer and its sulfoxide. In this way, the five compounds detected are all converted to *O,O*-diethyl phosphorothioic acid which gives a sensitive color reaction with the reagent (2,6-dibromo-*N*-chloro-*p*-quinoneimine).

In the procedure which was finally adopted, the plant tissue is macerated with distilled water in a Waring Blendor. Systox and its metabolites are separated from the aqueous solution by extraction with chloroform. After removal of the solvent, the extract is purified by passage through a column of acid-washed alumina, as suggested by Fiori (*4*), and followed by paper chromatography on silicone treated paper with ethanol–acetone–water (1:1:2) as solvent. This system has been described by Cook (*2*). After developing the chromatogram, the residues of Systox and its metabolites are detected by successive treatments of the paper with neutral potassium permanganate, sodium bisulfite, ethanolic sodium hydroxide, ethanolic hydrochloric acid, and finally the color reagent, 2,6-dibromo-*N*-chloro-*p*-quinoneimine.

Phosdrin, schradan, Sevin, and the oxygen analogs of Diazinon, parathion, methyl parathion, and EPN cannot interfere because they do not contain a P=S bond. In addition, Diazinon does not pass through the column of acid-washed alumina. EPN, parathion, methyl parathion, Guthion (trademark of Farbenfabriken Bayer), malathion, Trithion, the oxygen analog of Trithion, Trithion sulfoxide, Trithion sulfone, ethion, Folex, and Delnav do not migrate on the paper. As Co-Ral is used only on animals and Systox only on plants, it is not necessary to distinguish these. Only the oxygen analog of Guthion and the sulfoxide and sulfone of the oxygen analog of Trithion remain as potential interfering materials. As has already been pointed out, none of these materials has been shown to be present in plants. However, even if these materials were present, they could be distinguished from the Systox compounds. The oxygen analog of Guthion can be detected on paper after hydrolysis in alkali by diazotization and coupling as described by Meagher *et al.* (*10*). The sulfoxide and sulfone of Trithion give an orange-brown color reaction which can be readily distinguished from the color formed by the Systox compounds. The oxygen analog of malathion will interfere, if present. As Di-Syston forms the same metabolic products as Systox (*13*), residues of these two compounds cannot

be distinguished from each other. Some of the organophosphorus pesticides, such as phorate, which are registered for sale on a "no residue" basis would interfere.

Analytical Procedure

Apparatus and Reagents. Alumina (acid-washed, Merck), chromatographic reagent grade.

2,6 - Dibromo - *N* - chloro - *p* - quinoneimine. 0.5% solution in Skellysolve B saturated with hydroquinone.

Chromatographic Column, 20 × 400 mm.

Chromatographic Paper. Impregnate Whatman No. 1 filter paper (18 × 22 inch sheets) with 5% solution of Dow Corning Silicone 550 in Skellysolve B.

Method. Place 100 grams of plant material and 200 ml. of distilled water in a Waring Blendor. Blend at high speed for 5 minutes. Filter the slurry through a pledget of glass wool and collect 200 ml. of filtrate. Place the extract in a 500-ml. separatory funnel and extract with three successive 50-ml. portions of chloroform. Pour the combined chloroform extracts through 50 grams of anhydrous sodium sulfate in a small funnel fitted with a pledget of glass wool. Rinse the sodium sulfate with an additional 50 ml. of chloroform. On a steam bath under an air jet, evaporate the chloroform solution to a volume of about 10 ml. Complete the evaporation to dryness under an air jet at room temperature.

Tamp a pledget of glass wool followed by 2 grams of Super Cel and 10 grams of acid-washed alumina into a 20 × 400 mm. chromatographic column. Maintain vacuum during the column packing and subsequent steps. Wash the column with 50 ml. of distilled water and adjust the flow rate to about 10 ml. per minute. Dissolve the residue obtained after evaporation of the chloroform in 5 ml. of 95% ethanol. Add 1.0 ml. of distilled water and pour the solution onto the column.

Rinse the walls of the chromatographic column with several small portions of distilled water. Use distilled water as the elution solvent. Collect 150 ml. of eluate from the column. Extract the column effluent with three successive 50-ml. portions of chloroform. Combine the chloroform extracts and evaporate the solvent to about 10 ml. on a steam bath under an air jet. Complete the evaporation of the solvent to a volume of 0.1 ml. under an air jet at room temperature. Rinse the sides of the beaker with 2 to 3 ml. of chloroform and again evaporate at room temperature to a volume of about 0.1 ml.

As an alternative to the above procedure, the chromatographed extracts used for the preparation of samples for quantitative determination of Systox residues by colorimetric determination of phosphorus may be chromatographed on paper by the following method (*1*). Macerate fresh plant material with acetone. After filtering, the solution is extracted with chloroform, the chloroform evaporated, and the residue taken up in acetone and passed through a column containing activated carbon. Because this procedure results in more plant extractives in the final solution, it reduces the sensitivity of the detection procedure.

Transfer the entire sample to silicone-treated filter paper. Develop the chromatogram using ethanol–acetone–water (1:1:2) as solvent. A 3-hour developing time using the descending technique will give good separation. Remove the paper from the chromatographic cabinet and allow the solvent to evaporate at room temperature for 20 minutes.

Spray both sides of the dry chromatogram with 0.1*N* neutral potassium permanganate. Allow the paper to stand for 30 minutes at room temperature. Spray the paper with 0.1*N* aqueous sodium bisulfite or 1% hydrogen peroxide. Spray just to the point of color disappearance. Spray both sides of the paper with 0.1*N* sodium hydroxide in ethanol. Heat the paper for 5 minutes at 100° to 110° C. Spray the undeveloped portion of the paper just ahead of the solvent front with 0.1% ethanolic bromthymol blue. Lightly spray both sides of the chromatogram with 0.1*N* ethanolic hydrochloric acid until the reaction of the indicator is acid. Allow the paper to stand until the ethanol has evaporated. Spray both sides of the chromatogram with the 2,6-dibromo-*N*-chloro-*p*-quinoneimine reagent. Place in an oven at 110° to 120° C. for 3 to 7 minutes. Care must be taken not to overheat the paper as this will darken the paper and obscure the spots.

Discussion

Sensitivity. The method as described will readily detect 10 μg. of any of the following five compounds: Systox thiono isomer sulfoxide, Systox thiono isomer sulfone, Systox thiol iosmer, Systox thiol isomer sulfoxide, or Systox thiol isomer sulfone. This means that if a 100-gram sample of crop is used, the sensitivity would be 0.1 p.p.m. Since the tolerance for Systox in most crops is 0.75 p.p.m., this procedure is sensitive enough for policing purposes.

R_F Values. The R_F values for Systox and its various metabolites with the paper chromatographic system used are shown in Table II.

Recovery. Typical recovery data for four of the compounds from a number of crops are shown in Table III. These recovery data were obtained using compounds labeled with phosphorus-32. The radioactivity was measured on paper chromatograms with a strip counter. In 11 of the 20 cases, recoveries were 75% or better; in seven cases, recoveries were in the 50% to 75% range; and in only two instances were recoveries of less than 50% obtained. Even considering the low recoveries obtained in a few cases, the method is still capable of detecting 0.3 p.p.m. of the Systox thiol isomer or the sulfoxide or sulfone of either isomer. The thiono isomer sulfone was not included in this recovery experiment as radiolabeled material was not available. However, in separate qualitative tests with the unlabeled compound, good recoveries were obtained.

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