Determination of Residues of Di-Syston and Metabolites by Thermionic Emission Flame Gas Chromatography

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A specific gas chromatographic procedure for the determination of residues of Di-Syston and its metabolites in various crops and products involves initial extraction, precipitation to remove pigments, and oxidation with potassium permanganate to convert the Di-Syston and metabolites to the corresponding sulfones. Final detection is by thermionic emission flame ionization, which is several hundred times more sensitive to phosphorus-containing compounds than to ordinary organic molecules, allowing little interference from crop extractives. Recovery experiments were run on several crops and products by adding 0.1 p.p.m. of Di-Syston or metabolites at the extraction step. In general, recoveries were in the 75 to 110% range. A number of other phosphorus-containing pesticides were checked for interference and their retention times are reported.

Di-Syston [*O,O*-diethyl *S*-[2-(ethylthio) ethyl] phosphorodithioate], also known as disulfoton and thio-demeton, is a systemic insecticide which is toxic to a wide range of insects, particularly sucking insects. The structural formulas for Di-Syston and all possible oxidative metabolites which are insecticidally active are shown in Figure 1.

The metabolism of Di-Syston in plants was studied by Metcalf *et al.* (1957), who found that Di-Syston was converted initially to the sulfoxide. After longer periods of time increasing amounts of Di-Syston sulfone and the oxygen analog sulfoxide and sulfone were present in extracts of cotton plants. These same metabolites were identified by Bull (1965) in his studies with plants.

Bull (1965) found that in rats the compound was also oxidized to Di-Syston sulfoxide and sulfone and the oxygen analog sulfoxide and sulfone. Hydrolysis or further oxidation resulted only in decomposition products having little toxicity to insects or mammals.

The primary concern in the development of a suitable residue method was to account for the parent compound and its oxidative metabolites with adequate sensitivity. All of the compounds can be separated on a gas chromatograph; however, the sulfoxide peaks tail severely, give poor response, and have such short retention times that they are usually lost in the solvent peak. In addition, a multicomponent analysis increases the possibility of interference from crop extractives or other pesticides.

An ideal method of analysis would be to oxidize Di-Syston and all the metabolites to a single compound, the oxygen analog sulfone. Blinn (1964) achieved this with a similar compound, phorate, using *m*-chloroperbenzoic acid. Giang and Schechter (1966) developed an infrared residue method for Systox which included an oxidation with the same reagent. Under similar conditions, we found that Di-Syston gave only slight conversion with considerable degradation.

This paper describes a residue procedure based on conversion of Di-Syston and its oxygen analog to sulfones, using potassium permanganate as the oxidant. The sulfones are then measured gas chromatographically, employing a potassium chloride thermionic flame detector.

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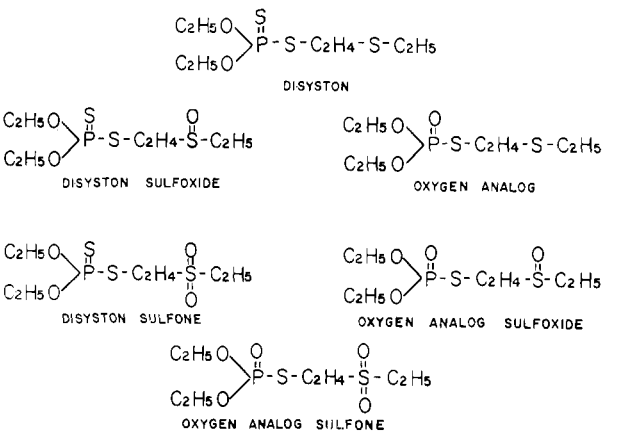

Figure 1. Di-Syston and metabolites

ANALYTICAL METHOD

Apparatus. Gas chromatograph, F & M Model 5750 equipped with flame ionization detector. Modify the detector as follows: Place 0.3 gram of finely powdered analytical reagent potassium chloride in a Parr press with a 1/4-inch diameter chamber and press into a solid pellet approximately 1/4 inch in diameter and 1/4 inch long. Drill a hole half-way through the pellet lengthwise, the same size as the flame jet diameter. Complete the through hole with a 1/32-inch drill bit. Place the pellet firmly on the jet tip. Position the collector electrode approximately 1 mm. above the pellet.

Reagents. Precipitation solution, 1.25 grams of ammonium chloride and 2.5 ml. of orthophosphoric acid (85%) dissolved in water and made up to 1 liter.

Standard Solution. Weigh 0.1 gram of Di-Syston and 0.1 gram of oxygen analog in a 100-ml. volumetric flask. Make to volume with acetone. Transfer 1 ml. of this solution to a 200-ml. volumetric flask, make to volume with acetone, and mix. This flask contains 5 μg. per ml. each of Di-Syston and Di-Syston oxygen analog.

All organic solvents were purified by redistilling in an all-glass apparatus.

Sample Preparation. Grind frozen crop samples in a Hobart food chopper in the presence of an equal weight of dry ice. Place the sample in frozen storage overnight to allow the dry ice to sublime.

EXTRACTION OF CORN AND SOYBEANS. Place 100 grams of the ground sample in a 1-quart blender jar marked at the 600-ml. level, and blend dry for about 3 minutes until the sample is finely granular. Add 15 grams of Hyflo Super-Cel and 200 ml. of acetone and blend for 3 minutes. Filter with vacuum through Whatman No. 42 filter paper. Reblend the filter cake with 200 ml. of chloroform and filter as before. Transfer the combined filtrates to a 1000-ml. separatory funnel. Rinse the filter flask with 100 ml. of fresh chloroform, add this to the separatory funnel, and shake for 30 seconds. Allow the phases to separate and drain the lower phase through a 32-cm. Whatman No. 12 fluted filter paper into a 1000-ml. round-bottomed flask. Rinse the filter paper with 10 ml. of fresh chloroform. Evaporate the filtrate just to dryness on a rotary vacuum evaporator at 40° C.

Transfer the sample residue to a 500-ml. separatory funnel with 200 ml. of Skellysolve B. Rinse the flask with 200 ml. of acetonitrile, add to the separatory funnel, and shake for 30 seconds. Allow the phases to separate, draw off the lower phase into a second 500-ml. separatory funnel containing 100 ml. of Skellysolve B, and shake the second separatory funnel for 30 seconds. Allow the phases to separate and draw off the lower phase into a 1000-ml. round-bottomed flask. Repeat the above extraction twice more with 200-ml. portions of fresh acetonitrile. Evaporate the combined acetonitrile extracts just to dryness on a rotary evaporator at 40° C. Proceed to the precipitation step.

EXTRACTION OF POTATOES, RAW SUGARCANE, SORGHUM FODDER, AND STRAWBERRIES. Place 200 grams of the ground sample in a blender jar, add 375 ml. of acetone, and blend for 3 minutes. Bring to the 600-ml. mark with distilled water and blend for an additional 1 minute. Filter through Whatman No. 12 fluted filter paper and collect 300 ml. of the extract. Transfer the aliquot to a separatory funnel and extract with 300 ml. of chloroform. Repeat the chloroform extraction twice more with 100-ml. portions of chloroform. Evaporate the combined chloroform extracts just to dryness on a rotary vacuum evaporator at 40° C. Proceed to the precipitation step.

EXTRACTION OF PROCESSED SUGAR. Place 100 grams of sample in a 1-liter separatory funnel and dissolve in 200 ml. of water. Continue the analysis, beginning with the chloroform extraction step of the extraction procedure for potatoes, raw sugarcane, etc.

EXTRACTION OF MOLASSES. Place 100 grams of molasses in a blender jar and add 375 ml. of acetone. Dilute to the 600-ml. mark with water and blend for 3 minutes. Centrifuge the molasses samples in 250-ml. centrifuge bottles for 10 minutes at 2000 r.p.m. Take a 300-ml. aliquot of the supernate. Continue the analysis, beginning at the chloroform extraction step of the extraction procedure for potatoes, raw sugarcane, etc.

EXTRACTION OF WHEAT, WHEAT STRAW, AND SORGHUM SEED. Grind the entire sample in a Wiley mill and mix thoroughly. Place 200 grams of sample in a blender jar (use 50 grams for wheat straw). Add 400 ml. of chloroform and blend for 3 minutes. Filter through 32-cm. Whatman No. 12 fluted filter paper and collect 200 ml. of the filtrate in a graduated cylinder. (Place a clean, folded cloth towel over the filter paper to reduce evaporation of chloroform during filtration.) Transfer the filtrate to a 300-ml. round-bottomed flask and evaporate to dryness on a rotary evaporator at 40° C. Proceed to the precipitation step.

EXTRACTION OF COTTONSEED. Grind the entire sample in a Wiley mill and mix thoroughly. Place a 50-gram portion of the sample in a large Soxhlet extraction thimble and cover with a plug of glass wool. Extract the sample for 4 hours in a Soxhlet extractor (1-liter capacity) using 800 ml. of 2-to-1 benzene-ethanol. Evaporate the extract just to dryness on a rotary vacuum evaporator at 40° C. Transfer the sample residue to a 500-ml. separatory funnel with 200 ml. of Skellysolve B. Rinse the flask with 200 ml. of acetonitrile, add to the separatory funnel, and shake for 30 seconds. Allow the phases to separate and draw off the lower phase into a second 500-ml. separatory funnel containing 100 ml. of Skellysolve B. Shake the second separatory funnel for 30 seconds. Allow the phases to separate and draw off the lower phase into a 1000-ml. round-bottomed flask. Repeat this extraction step twice more with 200-ml. portions of fresh acetonitrile. Evaporate the combined acetonitrile extracts just to dryness on a rotary vacuum evaporator at 40° C. Proceed to the precipitation step.

Precipitation of Plant Substances. Dissolve the residue from the extraction step in 15 ml. of acetone. Add 50 ml. of precipitating reagent and let stand for 30 minutes with occasional swirling. Filter with light vacuum through Whatman No. 2 filter paper covered with a $^1/_4$-inch tightly packed layer of Super-Cel in a size 0 Büchner funnel. Rinse the round-bottomed flask with two additional 25-ml. portions of precipitating solution and use these rinses to wash the filter cake. (In the case of cottonseed, soybeans, and wheat grain, use 20 ml. of acetone in combination with each of the 25-ml. rinses to ensure complete transfer of any oily residue to the filter cake.) Transfer the filtrate from the vacuum flask to a 250-ml. separatory funnel. Rinse the vacuum flask with 50 ml. of chloroform, add this to the separatory funnel, and shake for 30 seconds. Allow the layers to separate and drain the lower, organic phase into a 250-ml. round-bottomed flask. Repeat the chloroform extraction with two additional 50-ml. portions of chloroform. Evaporate the chloroform extracts just to dryness on a rotary vacuum evaporator at 40° C.

Oxidation Procedure. Place 2 ml. of the standard (see Reagents) in a 100-ml. round-bottomed flask. This standard is carried through the remainder of the procedure and contains 5 μg. per ml. each of Di-Syston and Di-Syston oxygen analog.

Dissolve the residue from the precipitation procedure in 2 ml. of acetone. Add 5 ml. of 20% (w./v.) aqueous magnesium sulfate solution and swirl to mix. Add 20 ml. of 0.5*N* potassium permanganate solution and swirl to mix. Let stand for 30 minutes at room temperature with occasional swirling, making sure that there is an excess of permanganate the entire time. Transfer the oxidation mixture to a 125-ml. separatory funnel. Rinse the oxidation flask with 20 ml. of chloroform and add this to the separatory funnel containing the oxidation mixture. Shake the funnel 30 seconds to extract. Allow the phases to separate (centrifuge if necessary) and drain the lower layer

through a No. 541 filter paper, containing a teaspoonful of powdered, anhydrous sodium sulfate, into a 250-ml. round-bottomed flask. Repeat the above extraction twice more with 20-ml. portions of chloroform. After the final extraction, rinse the Na_2SO_4 with 20 ml. of chloroform. Evaporate the combined extracts just to dryness on a rotary vacuum evaporator at 40° C. Remove any last traces of solvent with a stream of dry air at room temperature. Dissolve the residue in 2 ml. of acetone.

Gas Chromatographic Procedure. Using a microliter syringe, inject an appropriate aliquot of the sample or standard solution into the gas chromatograph maintained at the following conditions:

Column. 3.5-foot × $^1/_8$-inch borosilicate glass, packed with 10% D.C. 200 and 1.5% QF-1 solution coated on 80- to 100-mesh Gas Chrom Q

Gas flows. Helium carrier gas, 35 ml. per minute; air, 425 ml. per minute; hydrogen, adjust hydrogen flow after other gases are set, so that background current from the flame detector is approximately 1×10^{-10} a.f.s.

Temperatures. Column 210° C.; injection port 225° C.; detector 240° C.

Identify the Di-Syston sulfone and the oxygen analog sulfone peaks by their retention times and measure the area produced on the recorder chart. At the operating conditions employed, the retention time for the Di-Syston sulfone is 5.9 minutes and the oxygen analog sulfone is 4.7 minutes.

Calculate the parts per million of residue in a sample by comparing the response obtained for an unknown to the response obtained for a known amount of Di-Syston or oxygen analog standard carried through the procedure from the oxidation step. The standard is a composite of both compounds, but parts per million in the unknown are calculated separately using the corresponding standard peak, because chromatographic response is slightly different for the two compounds. Appropriate corrections are included for sample size, aliquots, and dilutions.

DISCUSSION

The method described measures not only Di-Syston but also its metabolites, Di-Syston sulfoxide, Di-Syston sulfone, Di-Syston oxygen analog, and its sulfoxide and sulfone. Various oxidations were tried in attempts to convert all the compounds to the oxygen analog sulfone; however, the vigorous conditions required caused excessive degradation. Potassium permanganate was finally selected as the oxidant (Tietz and Frehse, 1960). Although it is not vigorous enough to convert the P=S bond of Di-Syston to the P=O, the oxidation quantitatively converts each compound to the respective sulfone in 30 minutes or less at room temperature. The sulfones are more stable, less volatile, analytically more uniform, and more responsive in the thermionic detector than the compounds from which they are derived, lending added sensitivity to the method. Oxidation also converts plant extractives and pigments to a water-soluble form, making them easy to remove. Figure 2 shows the gas chromatographic separation of Di-Syston sulfone and Di-Syston oxygen analog sulfone.

Precipitation (Niessen and Frehse 1963) was included as an additional cleanup step to remove gas chromatographic interferences which could not be eliminated by extraction and oxidation. It also eliminated emulsion problems caused by some crops in the extraction steps following oxidation. The precipitation involves primarily aqueous solvents; therefore the amount of acetone in

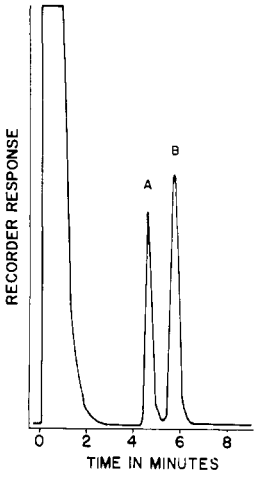

Figure 2. Gas chromatogram of 20 ng. each of Di-Syston oxygen analog sulfone, *A*, and Di-Syston sulfone, *B*

Table I. Recovery of Di-Syston and Its Metabolites from Various Crops and Products

Sample	Compound Added[a]	Recovery, %
Corn kernel	Di-Syston	85
	Di-Syston oxygen analog sulfoxide	122
	Di-Syston oxygen analog sulfone	96
Cottonseed	Di-Syston[b]	102
	Di-Syston oxygen analog sulfoxide[b]	115
	Di-Syston oxygen analog sulfone[b]	108
Grapes	Di-Syston	99
	Di-Syston oxygen analog sulfoxide	99
	Di-Syston oxygen analog sulfone	88
Molasses	Di-Syston	90
	Di-Syston oxygen analog sulfoxide	96
Potatoes	Di-Syston	114
	Di-Syston oxygen analog sulfoxide	76
	Di-Syston oxygen analog sulfone	96
Sorghum grain	Di-Syston	103
	Di-Syston oxygen analog sulfoxide	99
	Di-Syston oxygen analog sulfone	101
Soybeans	Di-Syston	109
	Di-Syston	90
	Di-Syston oxygen analog sulfoxide	81
Strawberries	Di-Syston	83
	Di-Syston oxygen analog sulfoxide	91
	Di-Syston oxygen analog sulfone	98
Sugar, processed	Di-Syston	97
	Di-Syston oxygen analog sulfoxide	105
Sugarcane, raw	Di-Syston	94
	Di-Syston oxygen analog sulfoxide	97
Wheat grain	Di-Syston	92
	Di-Syston oxygen analog sulfoxide	98
	Di-Syston oxygen analog sulfone	90

[a] 0.1 p.p.m. added unless otherwise noted.
[b] 0.05 p.p.m. added.

Table II. Retention Times of Various Organophosphorus Compounds

Compound	Retention Time, Min.[a] Before oxidation	After oxidation
Azodrin	1.5	No peak
Bidrin	1.5	No peak
Ciodrin	5.0	No peak
Cygon (dimethoate)	1.8	1.8
Cygon oxygen analog	1.1	1.1
Def	6.3	6.3
Delnav	1.8	1.8
Diazinon	1.9	1.9
Dibrom	1.4	No peak
Dicapthon	3.8	3.8
Di-Syston	1.9	5.9
Di-Syston sulfoxide	No peak	5.9
Di-Syston oxygen analog	1.4	4.7
Di-Syston oxygen analog sulfone	4.7	4.7
Di-Syston oxygen analog sulfoxide	No peak	4.7
Dylox	No peak	No peak
EPN	14.9	14.9
Ethion	8.4	8.4
Folex	6.3	6.3
Guthion	17.3	17.3
Guthion oxygen analog	14.0	14.0
Guthion (ethyl)	22.4	22.4
Guthion (ethyl) oxygen analog	18.2	18.2
Imidan	14.0	14.0
Imidoxan	7.6	14.3
Imidoxon	11.3	11.3
Malathion	3.3	3.3, 2.8
Malathion oxygen analog	2.8	2.8
Meta-Systox-R	3.5	3.5
Methyl parathion	2.8	2.8
OMPA	2.1	2.1, 4.0
Parathion	4.0	4.0
Phosdrin	No peak	No peak
Phosphamidon	2.4, 3.0	2.4, 3.0
Supana	4.7	No peak
TEPP	1.1	5.0
Thimet	1.2	3.5
Thiono Systox	0.9	2.8
Trithion	9.6	18.1
Zytron	3.4	3.4

[a] Where no peak was obtained, possibly response was early enough to be lost in solvent peak.

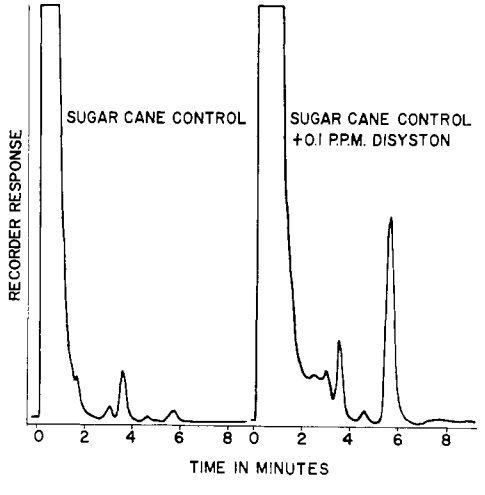

Figure 3. Gas chromatogram of Di-Syston in sugarcane extract

the rinse solution must be increased for oily crops such as corn and wheat (as noted in the stepwise procedure) to ensure complete recovery of Di-Syston. The pad of Super-Cel should be tightly packed in the Büchner funnel to eliminate channeling of the extract, thus lessening the effective cleanup.

The solution coating technique (Parcher and Urone, 1964) used to prepare the gas chromatographic column packing was necessary to produce a column on which the oxygen analog sulfone could be chromatographed without tailing or adsorption. In addition, the column was "no flow" conditioned overnight, followed by conditioning at operating conditions at least 4 hours before use. Several columns have been prepared with identical results, indicating the procedure to be reproducible.

A standard curve was run to determine linearity of response in the gas chromatograph for both Di-Syston sulfone and the oxygen analog sulfone. Response was linear for both over at least a hundredfold range up to 1 p.p.m. Any samples containing residues in excess of this figure should be diluted and reinjected, to ensure that response falls along the linear portion of the curve.

Recovery experiments were run on a large number of different crops and products by adding known amounts of Di-Syston or its metabolites to the samples at the blending or extraction step. In general the recoveries were run at the 0.1-p.p.m. level. Where samples were fortified with metabolites other than Di-Syston, a correction factor for molecular weight was applied to the area obtained before calculating per cent recovery. Results are listed in Table I for several recoveries, including Di-Syston, Di-Syston oxygen analog sulfoxide, and Di-Syston oxygen analog sulfone. Recovery of these compounds, representing the extremes in chemical and physical characteristics, indicates that the other metabolites can be recovered as well. A typical chromatogram for the recovery of Di-Syston from sugarcane is shown in Figure 3.

To determine the specificity of the method for Di-Syston, an interference study was conducted (Table II). Because of the specificity of the thermionic detector employed, only organophosphorus compounds were tested as possible interferences. The list includes all organophosphorus compounds registered on a tolerance and "no residue" basis plus a number of others that were available. The individual compounds were added just prior to the oxidation step and then carried through the procedure The retention times were measured and compared to those obtained by direct injection of the unoxidized compounds. After oxidation, none of these compounds appeared to interfere with the procedure.

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