Determination of Residues of Metasystox-R and Metabolite in Plant and Animal Tissues and Soil

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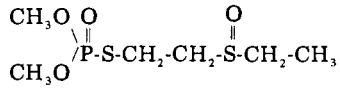
A gas chromatographic procedure is described for the analysis of residues of Metasystox-R and its sulfone metabolite in a wide variety of plant and animal tissues and soil. Varied extraction procedures are described for most efficient removal of residues from specific samples of differing physical characteristics. Following this, residues are partitioned into water and then extracted into chloroform, taking advantage of the dual solubility characteristics of the compounds to effect cleanup. The extract is oxidized to convert all active residues to the sulfone, which is then measured by gas chromatography with alkali-flame detection. Recovery for all sample types is generally 80–100% with limit of sensitivity at least 0.01 ppm.

Metasystox-R, *S*-[2-(ethylsulfinyl)ethyl] *O,O*-dimethyl phosphorothioate, sometimes called oxydemeton methyl or methylisosystox sulfoxide, is a systemic organophosphate insecticide exhibiting marked specificity in its action against aphids, mites, leafhoppers and similar plant sucking insects. It is also unique because of its total water solubility as well as being soluble in most organic solvents. The structural formula is:



Early metabolism studies have well documented the oxidative conversion of Systox and Metasystox-type compounds in biological systems (Muhlmann and Tietz, 1956; Tietz, 1960). The primary concern in developing a suitable residue analysis method for Metasystox-R was to account for the parent compound and its oxidative metabolite with adequate sensitivity and specificity.

The earliest residue analysis procedure for Metasystox compounds was a cholinesterase inhibition method developed by Hensel (1954) for Systox residues. This was unsatisfactory, however, due to the low inhibitory effect of the Metasystox compounds. Later, Laws and Webley (1959) developed a total phosphorus colorimetric procedure for Metasystox residues in plant material.

A number of workers have identified residue amounts of Metasystox-R in the presence of other organophosphorus compounds using thin-layer chromatography, including Eichenberger and Gay (1960), Ragab (1967), Smart and Hill (1967), Guth (1967), and Getz and Wheeler (1968).

Infrared spectroscopy (IR) has been used as a method of quantitation for Metasystox-R. Crosby and Laws (1964) used preparative gas chromatography to separate residues of Metasystox-R from crop extractives prior to measurement by IR.

Gas chromatography has recently become of interest for Metasystox-R residue analysis. Its advantage of sensitivity and specificity over the total phosphorus and other nonspecific methods was obvious. Burke and Holswade (1966) chromatographed Metasystox-R on a mixed phase column of 15% QF-1 and 10% DC 200 on Gas Chrom Q but found it necessary to inject 2000–3000 ng for suitable response with electron-capture detection. Bowman et al. (1969) reported their published method for disulfoton and metabolites in tobacco plants would also detect Metasystox-R and its sulfone, although the procedure was not tested with crop samples. McCully (1970) recognized the entire demeton group of compounds could be analyzed by the disulfoton residue procedure of Thornton and Anderson (1968), although no specific extraction and cleanup procedures or recoveries were described for thiono Systox or Metasystox-type compounds.

A flame photometric method by van der Merwe and Taylor (1971) for demeton-*S*-methyl utilized the precipitation cleanup of Thornton and Anderson (1968) and included some recovery data for Metasystox-R and its sulfone from sorghum foliage.

The procedure described in this paper takes advantage of the dual solubility characteristics of Metasystox-R and its sulfone in extraction and cleanup schemes for a wide variety of crops and tissues. Following initial extraction and partitioning into water and then into chloroform, most extracts are sufficiently clean to be readily oxidized with permanganate with the resulting sulfone measured by alkali-flame gas chromatography. Preliminary work showed both compounds could be separated using gas chromatography. However, a multicomponent analysis has the disadvanatage of the increased possibility of interference from crop extractives or other pesticides.

ANALYTICAL METHOD

Apparatus. A Hewlett-Packard Model 5750 gas chromatograph equipped with a flame ionization detector modified for alkali-flame operation as previously described by Thornton and Anderson (1968) was used. Explosion-proof blender motors were used to minimize the fire hazard from volatile organic solvents.

Reagents. All solvents were pesticide quality (nanograde). Other reagents were Analytical Reagent grade or equivalent.

Sample Preparation. Grind wet crops, oily crops, and animal tissues in a Hobart food cutter in the presence of dry ice and place the samples in frozen storage overnight to allow the dry ice to sublime. Grind dry samples in a Wiley mill to pass a No. 3 screen.

Sample Extraction. *Extraction of High Moisture Content Crops.* Place 100 g of chopped and mixed sample into a Waring blender jar marked at the 300-mL level. Add 200 mL of acetone and blend for 3 min at high speed. Dilute to the 300-mL mark with water and blend for 1 additional min. Filter through 32-cm Whatman No. 2V fluted filter paper and collect 150 mL of the filtrate in a graduated cylinder. Transfer the filtrate to 1-L separatory

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funnel and add 150 mL of hexane. Shake the funnel for 30 s, allow the phases to separate, and drain the lower (acetone–water) phase into a 500-mL separatory funnel. Extract the acetone–water mixture successively with 200- and 50-mL portions of chloroform. Evaporate the combined chloroform extracts just to dryness on a rotary vacuum evaporator using a 40 °C water bath. Remove last traces of solvent with a gentle stream of air. Proceed to the "Oxidation" section.

Extraction of Oily Crops, i.e., Nut Meats, Cottonseed. Weigh 50 g of chopped and mixed sample into a blender jar and add 300 mL of chloroform. Blend at high speed for 5 min. Filter with vacuum through Whatman No. 42 filter paper covered with a 0.25-in. layer of Hyflo Super-Cel in a Büchner funnel. Wash the filter cake with 100 mL of fresh chloroform. Transfer the extract to a 1-L round-bottomed flask and evaporate the solvent on a rotary vacuum evaporator in a water bath at 40 °C. Proceed to the "Hexane–Water Partition" section.

Extraction of Animal Tissues (Except Fat). Weigh 50 g of chopped and mixed tissue into a blender jar. Add 50 g of powdered, anhydrous sodium sulfate, 10 g of Hyflo Super-Cel, and 200 mL of acetonitrile and blend at high speed for 2 min. Filter with vacuum through Whatman No. 42 filter paper covered with a 0.25-in. layer of Hyflo Super-Cel in a Büchner funnel. Return the filter cake to the blender and reblend with 300 mL of hexane for 2 min. Filter as before, omitting any additional filter aid. Rinse the blender with 100 mL of fresh hexane and use this to wash the filter cake. Transfer the combined filtrate to a 1-L separatory funnel using a few milliliters of fresh acetonitrile to complete the transfer. Shake the separatory funnel for 30 s, allow the phases to separate, and drain the lower phase into a 500-mL round-bottomed flask. Evaporate the combined acetonitrile extract just to dryness on a rotary vacuum evaporator in a 40 °C water bath. Proceed to the "Hexane–Water Partition" section.

Extraction of Fat. Weigh 50 g of chopped and mixed fat tissue into a blender jar. Add 300 mL of hexane and blend for 2 min at high speed. Filter with vaccum through Whatman No. 42 filter paper covered with a 0.25-in. layer of Hyflo Super-Cel in a Büchner funnel. Return the filter cake to the blender and reblend with 200 mL of acetonitrile for 2 min. Filter as before but omit any additional filter aid. Rinse the blender with 100 mL of hexane and use this to wash the filter cake. Transfer the combined filtrate to a 1-L separatory funnel and continue the hexane acetonitrile partition steps as described for animal tissues (above).

Extraction of Milk. Mix milk samples thoroughly to disperse the cream. Place 100 g of milk in a blender jar and extract as described for animal tissues, omitting the addition of granular sodium sulfate.

Extraction of Eggs. Break one egg into a blender jar and record the weight. Add 15 g of Hyflo Super-Cel and 200 mL of acetone and blend for 2 min at high speed. Filter with vacuum through Whatman No. 42 filter paper covered with a 0.25-in. layer of Hyflo Super-Cel in a Büchner funnel. Rinse the blender jar with 300 mL of chloroform and use this to wash the filter cake. Transfer the filtrate to a 1-L separatory funnel using a few milliliters of fresh chloroform to complete the transfer. Shake the funnel for 30 s, allow the phases to separate, and drain the lower, organic phase through a 32-cm Whatman No. 2V fluted filter paper into a 1-L round-bottomed flask. Rinse the filter paper with 5–10 mL of fresh chloroform. Evaporate the organic extract just to dryness on a rotary vacuum evaporator in a water bath at 40 °C. Dissolve the residue from the previous steps in 300 mL of hexane and transfer to a 500-mL separatory funnel. Rinse the flask with 50 mL of acetonitrile and add to the separatory funnel. Shake the separatory funnel for 30 s, allow the phases to separate, and drain the lower phase into a 300-mL round-bottomed flask. Repeat the extraction twice more with fresh 50-mL portions of acetonitrile. Evaporate the combined acetonitrile extracts just to dryness on a rotary vacuum evaporator in a water bath at 40 °C. Proceed to the "Hexane-Water Partition" section.

Extraction of Soil. Weigh 50 g of air-dried and mixed soil into a Soxhlet extraction thimble. Weigh a companion sample into a beaker for moisture determination if residues are to be reported on a dry weight basis. Place the thimble into a Soxhlet extraction apparatus and extract for 4 h using 300 mL of a 1:1 chloroform–methanol (v/v) mixture at a rate of five–six exchanges per hour. Cool and evaporate the extract just to dryness on a rotary vacuum evaporator in a water bath at 40 °C. Proceed to the "Oxidation" section.

Hexane–Water Partition. Dissolve the residue from the previous steps in 400 mL of hexane and transfer to a 500-mL separatory funnel. Rinse the flask with 100 mL of water and add to the separatory funnel. Shake the funnel for 30 s. Allow the phases to separate and drain the lower, water phase into a clean 250-mL separatory funnel. Extract the water with 100- and 50-mL portions of chloroform and drain into a 250-mL round-bottomed flask. Evaporate the chloroform extract just to dryness on a rotary vacuum evaporator at 40 °C.

Oxidation. Place 5 μg of Metasystox-R standard in a 100-mL round-bottomed flask in 2 mL of acetone solution and carry through the oxidation procedure. Dissolve the sample residue from the previous steps in 2 mL of acetone. Add 5 mL of 20% (w/v) magnesium sulfate solution and 25 mL of a 0.1 M $KMnO_4$ solution, washing down the sides of the flasks during the addition. Mix and let stand with occasional swirling for 30 min, making sure there is an excess of permanganate the entire time. Transfer the oxidation mixture to a 125-mL centrifuge type separatory funnel. Rinse the oxidation flask with 25 mL of chloroform and add this to the separatory funnel containing the oxidation mixture. Shake the separatory funnel for 30 s to extract, allow the phases to separate (centrifuge if necessary), and drain the lower phase through 15 to 20 g of powdered, anhydrous sodium sulfate retained in a powder funnel with a loose plug of glass wool. Collect the filtrate in a 250-mL round-bottomed flask. Repeat the extraction twice more with fresh 25-mL portions of chloroform. After the final extraction, rinse the sodium sulfate with 5–10 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.

Gas Chromatographic Analysis. Dissolve the standard and sample residues from the previous steps in 2 mL of acetone and inject an appropriate aliquot into the alkali-flame modified gas chromatograph maintained at the following conditions: column, 3.5 ft × $^1/_8$ in o.d. (approximately $^1/_{16}$ in. i.d.) borosilicate glass column, packed with 10% DC 200 and 1.5% QF-1 solution coated on 80–100 mesh Chromosorb W (HP); gas flows, helium carrier gas, 60 mL/min; hydrogen, adjust hydrogen flow after other gasses are set so that at least a one-half scale peak results from a 10-ng standard injected; temperatures, column, 210 °C; injection port, 225 °C; detector, 240 °C.

Identify the Metasystox-R sulfone peak by its retention time and measure the area or peak height produced on the

Table I. Recovery of Metasystox-R and Its Sulfone from Representative Samples

Crop	Ppm added[a]	Recovery, %[b]	
		Metasystox-R	Sulfone
Apples	0.05-0.10	108.4 ± 10.3(8)	94.0 ± 6.6(6)
Grapes	0.05-0.10	103.0 ± 5.2(3)	84.7 ± 2.9(3)
Lettuce	0.05-0.50	92.7 ± 7.8(10)	98.7 ± 10.8(12)
Nut meat	0.05-0.10	99.5 ± 13.1(6)	101.6 ± 9.1(5)
Animal tissue	0.05-0.10	93.8 ± 7.8(17)	92.2 ± 8.4(13)
Animal fat	0.05-0.10	93.0 ± 4.2(5)	94.4 ± 3.7(5)
Bovine milk	0.005-0.01	93.3 ± 6.5(6)	96.5 ± 13.0(6)

[a] Control values were negligible compared with the fortified levels in all cases. [b] Average percent recoveries are followed by the standard deviation and the number of individual determinations. All recoveries were fortified before extraction.

recorder strip chart. At the gas chromatographic conditions employed, Metasystox-R sulfone has a retention time of 3.5 min.

Calculate parts per million of residue in a sample by comparing the response obtained for the unknown with the response obtained for a known amount of Metasystox-R standard started at the oxidation steps, including appropriate factors for sample size, aliquots, and dilutions. An appropriate dilution of Metasystox-R sulfone, if available, may be directly injected into the gas chromatograph for use as a standard. Oxidation of Metasystox-R itself is recommended because the pure sulfone is not readily available to all workers.

DISCUSSION

Sulfoxide compounds such as Metasystox-R are, in general, difficult to chromatograph because of adsorption and tailing. Many workers including Burke and Holswade (1966) have tried to overcome this problem with massive conditioning injections to build up sensitivity. The method described in this paper utilizes permanganate oxidation to convert Metasystox-R to the sulfone which exhibits much less tailing and is easier to chromatograph. In addition, all active residue is concentrated into one peak adding further simplicity and sensitivity.

Room temperature oxidation is quantitative using 0.1 M potassium permanganate (Tietz and Frehse, 1960) for 30 min. Oxidation also converts most tissue extractives and pigments to a water-soluble form, making them easy to remove. The unique water soluble character of the Metasystox compounds allows cleanup procedures to be relatively simple. Initial crop or tissue extracts may be partitioned back and forth between water and various polar and nonpolar organic solvents to yield extremely well-purified extracts prior to oxidation. Oxidized extracts are relatively free from severe crop or tissue interferences on the gas chromatograph.

Recovery experiments were run on a large number of different crops and various animal tissues by fortifying the samples with 0.005 to 5 ppm of Metasystox-R or its sulfone prior to blending. Representative recovery values for a general cross section of the crops and tissues are presented in Table I. Control values in each case were negligible compared with the level at which the recovery check was run. In addition to the samples shown in Table I, the method has been used successfully since 1967 for the following crops and tissues: alfalfa, barley, beans, broccoli, brussels sprouts, carrots, cattle tissues (brain, fat, heart, kidney, liver, muscle, and milk), cauliflower, cherries, clover, grass, mint, onions, peas, plums, poultry tissues (fat, giblets, muscle, eggs), soils, sorghum, strawberries, sugar beets, tomatoes, and wheat. Representative control and recovery chromatograms are shown in Figure 1 for recovery of 0.1 ppm Metasystox-R from walnut meat. Chromatograms for other types of samples were similar.

Table II. Chemicals Tested for Possible Interference with the Metasystox-R Residue Analysis Procedure

Chemical name	Ppm level tested[a]
Azodrin	5.0
Bensulide	5.0
Bidrin	5.0
Chlorfenvinphos	5.0
Ciodrin	5.0
Co-Ral	1.0
Dasanit	5.0
DDVP	5.0
Def	5.0[b]
Delnav	5.0
Diazinon	40.0[b]
Dibrom	5.0
Dicapthon	5.0
Dimethoate	5.0[b]
Di-Syston	12.0
Diuron	2.0
Dyfonate	5.0[b]
Dylox	240.0
EPN	5.0
Ethephon	36.0
Ethion	5.0
Ethrel	36.0
Fenthion	18.0
Famphur	0.1
Folex	5.0
Gardona	110.0
Guthion	5.0
Imidan	40.0
Kelthane	25.0
Malathion	135.0[b]
Methyl parathion	5.0[b]
Mocap	5.0
Monitor	1.0
Nemacur	0.1
OMPA	0.75[d]
Orthene	10.0
Parathion	5.0[b]
Phosalone	40.0[b]
Phosdrin	5.0
Phosphamidon	0.5
Ronnel	10.0
Ruelene	1.0[b]
Supracide	6.0
Systox	12.0[c]
TEPP	5.0
Terbacil	1.0
Tetradifon	100.0
Thimet	5.0[a]
Torak	1.5
Trifluralin	1.0
Trithion	5.0
Zinophos	5.0
Zytron	5.0

[a] Ppm relative to a 50-g sample size. [b] Interferes with standard GC method but can be separated using a 40 cm × 3 mm i.d. glass column packed with 6% QF-1 coated on 80-100 mesh Gas Chrom Q. [c] Can be separated from Metasystox-R at 180 °C. [d] Interference eliminated by using flame photometric detector (sulfur mode).

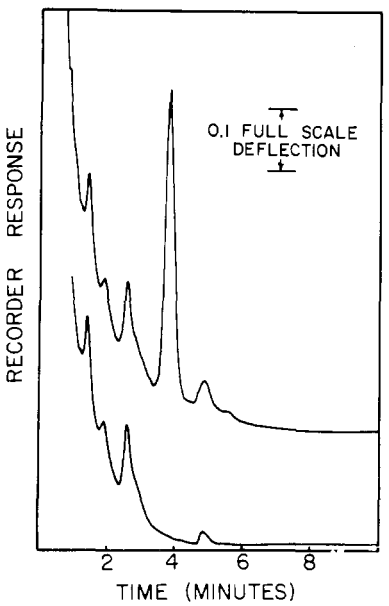

Figure 1. Gas chromatograms of walnut meat control (lower curve) and walnut meat fortified with 0.1 ppm of Metasystox-R (upper curve).

The slurry filtration (solution coating) technique described by Supina (1974) was used to prepare the gas chromatographic column packing. A 1:1 mixture of acetone and hexane was used to dissolve the DC-200, QF-1 mixture. This coating procedure was by far the best method for consistently preparing a packing which would allow Metasystox-R sulfone to be chromatographed without tailing or adsorption. The empty glass columns were also treated with a 5% solution of dimethyldichlorosilane in toluene and then flushed with toluene and methanol and dried before packing. After packing, new columns were purged with carrier gas at room temperature to remove oxygen, the exit end capped and the column conditioned with no flow for 8 h at 250 °C. Then, after normal flow conditioning at operating conditions overnight, the columns generally would allow 5 ng of Metasystox-R sulfone to yield one-half to full-scale recorder response with <1% noise.

A standard curve was run to determine linearity of response in the gas chromatograph for Metasystox-R sulfone. Response was linear over at least a 100-fold range up to 80 ng injected. Samples containing residues in excess of this amount in the injection volume should be diluted and re-injected to ensure that response falls within the linear portion of the response curve.

If 0.1 sq in. is considered the smallest area which can be accurately measured with a polar planimeter, the level of sensitivity is determined by the amount of Metasystox-R sulfone necessary to produce this area. In general, 0.1 ppm (10 ng) of standard produces a peak of 1 sq in. or better, indicating the sensitivity of the method to be approximately 0.01 ppm. If the criteria of 2 × the noise level is selected as the limiting factor, sensitivity would be somewhat better.

To determine the specificity of the method for Metasystox-R in the presence of other pest control chemicals, an interference study was run. Only phosphorus-containing chemicals were tested because of the phosphorus-specific nature of the alkali-flame detector. All organophosphorus chemicals currently registered for use on the above mentioned crops as reported in the U.S. Federal Register and listed in Table II were tested for interference with the analysis procedure. One-tenth part per million of Metasystox-R could readily be analyzed in the presence of any of these chemicals at their maximum registered level.

The method described above has been used extensively for the analysis of field residue and animal-feeding study samples. The various extraction procedures which were described include all of the modifications which have been found necessary to prepare any sample which has been analyzed for Metasystox-R in this laboratory and in various contract laboratories during the past nine years.

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