ABSTRACT

This report presents the validation of the extraction of METASYSTOX-R and its oxidized metabolite METASYSTOX-R Sulfone from soil (Mobay Method No. 53204) coupled with Mobay preliminary method development studies for the separation of these compounds from soil extracts as performed by the staff members of Colorado Analytical Research & Development Corporation. The Mobay Corporation Biochemistry Study Specification is attached to this report (Appendix 1).

INTRODUCTION

The purpose of the study was to develop an analytical method for the determination of METASYSTOX-R and METASYSTOX-R Sulfone in soil at a screening level of 0.01 part per million using capillary gas chromatographic analysis. Mobay Method 53204 describes the determination of residues of METASYSTOX-R and its metabolite in plant, animal tissues and soil (Appendix 2). This report presents development of an analytical method based on Mobay Method 53204 and its validation on two California soils.

MATERIALS AND METHODS

APPARATUS

1.1 Bottle, Nalgene, 500 ml

1.2 Centrifuge

1.3 Filter paper, Whatman #1, 7 cm

1.4 Flask, filtering, 500 ml

1.5 Flask, round bottom, 100 ml, 250 ml, 500 ml

1.6 Funnel, Büchner, 7 cm

1.7 Funnel, long stem, 100 ml

1.8 Funnel, separatory, 250 ml

1.9 Orbit shaker, Lab Line or equivalent

1.10 Rotary evaporator, Büchi or equivalent

1.11 Sample vials, GC autosampler

REAGENTS

2.1 Acetone, distilled in glass

2.2 Chloroform, distilled in glass

2.3 Hexane, distilled in glass

2.4 Magnesium sulfate, reagent grade

2.5 Potassium permanganate, reagent grade

2.6 Sep Pak, silica gel, Waters, part No. 51900

2.7 Sodium chloride, reagent grade

2.8 Sodium sulfate, anhydrous, reagent grade

2.9 Water, deionized

SOILS

The method was validated for two sandy loam soils (Mobay Sample Numbers: 1001, 1002, 1003, 1004, 1005, 1006, 1007, 2001, 2002, 2003, 2004, 2005, 2006 and 2007) both supplied by Mobay Corporation. Characteristics of these soils are presented in Appendix 3.

STANDARDS

METASYSTOX-R (95.4% purity) and METASYSTOX-R Sulfone (96.3% purity) analytical standards were supplied by Mobay Corporation.

ANALYTICAL METHODOLOGY

3.1 Extraction

3.1.1 Weigh 25 grams of a well-homogenized, stone-free soil sample into a 500 ml Nalgene screw cap bottle. Add 250 ml of 10% (v/v) water/acetone. Cap the bottle securely.

3.1.2 Place the bottle on an orbital shaker and shake for 30 minutes at 2500 rpm.

3.1.3 Vacuum filter the sample through Whatman #1, 7 cm filter paper into a 500 ml filtering flask.

3.1.4 Wash the soil filter cake with 25 ml of 10% (v/v) water/acetone.

3.2 Partition

3.2.1 Transfer the combined extracts from steps 3.1.3 and 3.1.4 to a 500 ml round bottom flask.

3.2.2 Add 25 ml of water and remove the acetone by vacuum rotary evaporation (bath temperature 35°C).

3.2.3 Transfer the aqueous sample (approximately 50 ml) to a 250 ml separatory funnel and partition once with 150 ml of hexane using a 1 minute shake. Allow the layers to separate and discard the hexane.

3.2.4 Add 5 ml of saturated sodium chloride to the aqueous phase from step 3.2.3 and partition three times with 50 ml portions of chloroform using 30 second shakes. Drain each chloroform partition sample through a bed of anhydrous sodium sulfate into a 250 ml round bottom flask. After the last chloroform partition sample has been drained through the anhydrous sodium sulfate bed, rinse the bed with 25 ml of chloroform. Discard the water phase.

3.3 Silica Gel Sep Pak

3.3.1 Evaporate the chloroform just to dryness using a vacuum rotary evaporator (bath temperature 35°C). Blow off residual chloroform with a stream of nitrogen.

3.3.2 Dissolve the sample from step 3.3.1 in 5 ml of 50/50 acetone/hexane and load the sample onto a silica gel Sep Pak which has previously been washed with 20 ml of hexane. Use a 100 ml round bottom flask to collect the eluant.

3.3.3 Rinse the sample container from step 3.3.2 twice with 5 ml portions of 50/50 acetone/hexane and load these onto the Sep Pak. NOTE: THIS ELUANT CONTAINS THE METASYSTOX-R SULFONE.

3.3.4 Place a fresh 100 ml round bottom flask under the Sep Pak and elute with 15 ml of acetone. NOTE: THIS ELUANT CONTAINS THE METASYSTOX-R.

3.3.5 Remove the acetone/hexane from the eluant collected in step 3.3.3 by vacuum rotary evaporation (bath temperature 35°C).

3.3.6 Bring the sample from step 3.3.5 to volume with 2.0 ml of acetone and analyze by capillary gas chromatography.

3.4 Oxidation

3.4.1 Remove the acetone from the eluant collected in step 3.3.4 by vacuum rotary evaporation (bath temperature 35°C).

3.4.2 Dissolve the residue from step 3.4.1 in 2 ml of acetone. Add 5 ml of a 20% aqueous $MgSO_4$ solution followed by the addition of 25 ml of 0.1 M aqueous $KMnO_4$ solution. Let the sample sit for 30 minutes with occasional shaking.

3.4.3 Transfer the sample from step 3.4.2 into a 250 ml separatory funnel and partition three times with 25 ml portions of chloroform using 30 second shakes. Drain each chloroform partition sample through a bed of anhydrous sodium sulfate. After the last chloroform partition sample has been drained through the anhydrous sodium sulfate bed, rinse the bed with 10 ml of chloroform. NOTE: In general, emulsions will be present in the permanganate chloroform partition samples. Centrifuging may be necessary for all three partitions. Centrifuge the samples for 5 minutes at 3000 rpm.

3.4.4 Remove the chloroform from the sample obtained in step 3.4.3 by vacuum rotary evaporation (bath temperature 35°C).

3.4.5 Bring the sample from step 3.4.4 to volume with 2.0 ml of acetone and analyze by capillary gas chromatography.

The flow diagram for the method is shown in Figure 1.

CAPILLARY GAS CHROMATOGRAPHIC ANALYSIS

The samples from steps 3.3.6 and 3.4.5 are analyzed by capillary gas chromatography using a nitrogen phosphorous specific detector. The chromatographic conditions are given in Table I.

4.1 Preparation of Standard METASYSTOX-R Sulfone

4.1.1 Weigh 10.0 mg of METASYSTOX-R Sulfone (corrected for purity) into a 100 ml volumetric flask and bring to volume with acetone. This standard solution represents 100 micrograms of METASYSTOX-R Sulfone per ml. Prepare a 1.33 microgram per ml METASYSTOX-R Sulfone gas chromatographic standard by pipetting 1.0 ml of the stock 100 microgram per ml solution into 74 ml of

acetone. This gas chromatographic standard is equivalent to 1.25 micrograms per ml of METASYSTOX-R when a 0.939 multiplication factor for the difference in the molecular weights of METASYSTOX-R to METASYSTOX-R Sulfone (246.275/262.270), METASYSTOX-R MW/METASYSTOX-R Sulfone MW) is used.

4.2 Preparation of METASYSTOX-R and METASYSTOX-R Sulfone Soil Spiking Solutions

4.2.1 Weigh 10 mg of METASYSTOX-R (corrected for purity) into a 100 ml volumetric flask. Weigh 10.0 mg of METASYSTOX-R Sulfone (corrected for purity) into the same volumetric flask. Bring to volume with acetone. This standard solution represents 100 micrograms of METASYSTOX-R and METASYSTOX-R Sulfone per ml. Serially dilute this stock solution with acetone to obtain spiking solutions containing 10.0, 2.5, 1.0 and 0.5 micrograms of METASYSTOX-R and METASYSTOX-R Sulfone per ml.

4.3 Preparation of Spiked Soil Samples for Recovery Determinations

4.3.1 The spiking level chosen for the recovery sample will dictate which standard solution, prepared in Step 4.2, is used for the recovery sample. It is desirable to keep the spiking volume within 1.0 - 2.0 ml. The 25 gm soil aliquot is spiked immediately after weighing and prior to the addition of the extracting solvent. The recovery samples are taken through the complete method and are run concurrently with control and treated samples.

4.4 Standardization of Gas Chromatograph

4.4.1 Prior to any gas chromatographic analysis of standards and samples, the gas chromatographic column must be "primed". Priming is accomplished by injecting two 2 microliter aliquots of a soil gas chromatographic sample and two 2 microliter aliquots of a 10.0 microgram per ml METASYSTOX-R Sulfone standard. This priming reduces the enhancement problems associated with the analysis. See the discussion section for more detailed information regarding the enhancement observed for METASYSTOX-R Sulfone.

4.4.2 Inject a 2 microliter aliquot of the 1.33 microgram per ml METASYSTOX-R Sulfone standard and adjust the attenuation of the gas chromatograph to obtain a greater or equal to 50% full scale deflection.

4.3 Determination of Sample Residues

4.3.1 Following the injection pattern of standard, sample, sample, standard, inject 2.0 microliter aliquots of the samples from steps 3.3.6 and 3.4.5 into the into the gas chromatograph. Typical chromatograms of the standard and soil samples are presented in Figure 2.

With each analytical set, a minimum of one control soil and one control soil fortified with both METASYSTOX-R and METASYSTOX-R Sulfone is required for purposes of recovery validation. The recovery samples should represent a range covering the screening level of the method, 0.01 ppm, to the highest residue found in treated samples.

CALCULATIONS

Calculation of METASYSTOX-R and METASYSTOX-R Sulfone residues in soil samples is by the following:

The 1.33 microgram per ml METASYSTOX-R Sulfone standard represents 2.66 nanograms injected from a 2 microliter aliquot. This represents 2.50 nanograms of equivalent METASYSTOX-R when corrected for the 0.939 molecular weight factor.

Determine the average integrator peak area for the 2.50 nanogram equivalent METASYSTOX-R standard.

Determine the nanograms found for soil samples from the following equations:

$$\text{nanograms METASYSTOX-R found} = \frac{\text{peak area of soil sample}}{\text{average peak area of the standard}} \times 2.5$$

$$\text{nanograms METASYSTOX-R Sulfone found} = \frac{\text{peak area of soil sample}}{\text{average peak area of the standard}} \times 2.66$$

Soil residue data are reported on a dry weight basis. Oven dry a 10 to 20 gram soil aliquot at 110°C for 16 hours, and determine the moisture content using the following equation:

$$\text{percent soil moisture} = \frac{\text{net weight of wet soil} - \text{net weight of dry soil}}{\text{net weight of wet soil}} \times 100$$

The METASYSTOX-R and METASYSTOX-R Sulfone residue values for soil samples, expressed in parts per million on a dry weight basis, are obtained from the following equation:

$$ppm = \frac{\text{nanograms METASYSTOX-R or METASYSTOX-R Sulfone found}}{\text{(milligrams equivalent soil injected)(M)}}$$

$$\text{milligrams equivalent soil injected} = \frac{\text{25 gm soil sample}}{\text{final sample volume}} \times \text{2 microliters injected}$$

M is the dry weight factor for the moisture content of the soil and is expressed as a decimal (11.84% soil moisture yields 2.96 ml of water from a 25 gram soil sample).

$$M = \frac{25.00 - 2.96}{25.00} = 0.882$$

Parts per million METASYSTOX-R Sulfone are reported as ppm equivalent METASYSTOX-R residue. The following equation accomplishes this conversion.

$$\text{ppm equivalent METASYSTOX-R} = \text{ppm METASYSTOX-R Sulfone found (dry basis)} \times 0.939$$

Recovery of METASYSTOX-R and METASYSTOX-R Sulfone from control samples fortified with the analytes is calculated from the following:

$$\frac{\text{ppm found} - \text{ppm found in control soil}}{\text{ppm added}} \times 100$$

TABLE I

CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS

Instrument:	Hewlett-Packard Model 5880 Capillary Gas Chromatograph with Model 7672A or Model 7673A Automatic Sampler.
Carrier Gas:	Helium, flow adjusted to give 15 psi (1-2 cc per minute).
Makeup Gas:	Helium, 30 cc per minute.
Column:	J&W DB-5, 1.0 u, 0.32-mm I.D., 15 meters.
Injection:	Splitless.
Detector:	Nitrogen phosphorous specific.
Temperatures:	
Injector:	250°C
Detector:	275°C

Oven Program and Run Table

PROGRAM: (ANNOTATION OFF)

```
10    VALVE 5 ON
20    OVEN TEMP 60
30    OVEN TEMP EQUIB TIME 1
40    OVEN TEMP INITIAL VALUE 60
50    OVEN TEMP INITIAL TIME 1
60    OVEN TEMP PRGM RATE 30
70    OVEN TEMP FINAL VALUE 187
80    OVEN TEMP FINAL TIME 7
90    OVEN TEMP POST VALUE 187
100   OVEN TEMP POST TIME 5
110   ATTN 2↑10
120   CHART SPEED 0.1
```

(Continued on the following page)

TABLE I (continued)

CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS

```
130   %OFFSET 15
140   RUN TIME ANNOTATION OFF
150   RUN TBL ANNOTATION OFF
160   REPORT ANNOTATION OFF
170   REPORT ON
180   OVEN TEMP ANNOTATION OFF
190   DELETE RUN TBL
200   DELETE REPORT TBL
210   PEAK WIDTH 0.087
220   THRESHOLD -1
230   RUN TIME 0 VALVE 5 ON
240   RUN TIME 0.1 INTG OFF
250   RUN TIME 0.5 VALVE 5 OFF
260   RUN TIME 7.74 CHART SPEED 2
270   RUN TIME 7.75 ATTN 2↑2
280   RUN TIME 7.76 INTG ON
290   RUN TIME 7.77 RUN TIME ANNOTATION ON
300   RUN TIME 7.78 ZERO
310   RUN TIME 11.5 VALVE 5 ON
320   RUN TIME 11.51 RUN TIME ANNOTATION OFF
330   EDIT AUTO SEQ 1,1
340   SIGNAL B DEVICE# 1
350   AREA%
360   REPORT TIME 0 REJECT 0.1
```

Volume Injected: 2 ul

Retention Time: 8.84 minutes ± 0.02 minute

Figure 1

FLOW DIAGRAM FOR THE DETERMINATION OF METASYSTOX-R AND METASYSTOX-R SULFONE IN SOIL

25 gm soil sample

↓

Shake at 2500 rpm for 30 minutes with 250 ml 10% water/90% acetone

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Vacuum filter using Whatman #1 paper

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Wash filter cake with 25 ml 10% water/90% acetone

↓

Transfer total sample to a 500 ml round bottom flask

↓

Add 25 ml water

↓

Rotovap to water phase (~50 ml) at 35°C

↓

Transfer water phase to 250 ml separatory funnel

↓

Partition with 150 ml hexane

Discard hexane ←

↓

Add 5 ml saturated sodium chloride solution
Partition 3 times with 50 ml chloroform
Drain chloroform through anhydrous sodium sulfate
Rinse sodium sulfate with 25 ml chloroform

Discard water phase ←

↓

Rotovap just to dryness (blow off residual chloroform with nitrogen)

↓

Dissolve residue in 5 ml 50/50 acetone/hexane solution

↓

Prewash silica gel sep-pak with 20 ml hexane

Discard hexane rinse ←

↓

Place collection flask under sep-pak
Transfer sample to sep-pak

↓

Rinse sample container 2 times with 5 ml 50/50 acetone/hexane solution and run each rinse through sep-pak
The resultant sep-pak eluant, Eluant 1, contains METASYSTOX-R Sulfone

↓

Figure 1 (continued)