Determination of NEMACUR®, NEMACUR Sulfoxide and NEMACUR Sulfone in Water and Soil Samples from the Georgia Runoff Study, Study Number NE222401

1.0 Introduction

The Miles Environmental Fate Analytical Laboratory analyzed water and soil samples to determine the concentrations of NEMACUR, NEMACUR sulfoxide and NEMACUR sulfone. Ninety one water samples including twelve field spikes and forty one soil samples were analyzed. This analytical report is in conjunction with the NEMACUR Runoff Study, Study Number NE222401 performed by Miles' Ecological Effects Group.

The samples for the study were received between 03/02/90 and 04/19/90. The analyses of the samples were completed in July 1990. All samples were shipped in coolers containing dry ice, and were received frozen. The samples were placed in a walk-in freezer after receiving until analysis.

2.0 Method

The method for the water analysis was based on the report titled "Gas Chromatographic Method for the Determination of Fenamiphos, Fenamiphos Sulfoxide, and Fenamiphos Sulfone in Water" submitted to Mobay Corporation by Alpine West Laboratories in Provo, Utah. The limit of determination for each compound in this study is 0.2 µg/L.

The method for the soil analysis was based on the report titled "Fenamiphos Analytical Method for Soil" submitted to Mobay Corporation by Alpine West Laboratories in Provo, Utah. The limit of determination for each compound in this study is 10 µg/kg.

2.1 Preparation of the standard solutions for water analysis

1) Weigh 0.01 ± 0.0001 g each of NEMACUR analytical standard (Ref. 79R-29-146, 97.8% purity), NEMACUR sulfoxide analytical standard (Ref. 68-105-72, 93.4% purity), and NEMACUR sulfone analytical standard (Ref. 76-207-91, 98.0% purity) into a 100-mL volumetric flask. Dilute to volume with ethyl acetate and mix thoroughly. Correct the standard concentration to its absolute concentration using the standard purity.

2) Pipet 2 mL of the standard solution from Step 1 into a 100-mL volumetric flask. Dilute to volume with toluene and mix thoroughly. This is the 2-ppm standard solution.

3) Pipet 5, 2.5, and 1 mL of the standard solution from Step 2 into separate 10-mL volumetric flasks, dilute to volume with toluene and mix thoroughly. These are the 1.0, 0.5-, and 0.2-ppm standard solutions, respectively.

2.2 Preparation of the water samples

1) Allow the sample to thaw completely, then measure 500 mL of the sample using a graduated cylinder. Transfer the sample from the graduated cylinder into a 1-L separatory funnel.

2) Extract the sample with 75 mL of methylene chloride for three minutes.

3) Allow the phases to settle, then drain the organic phase through a glass funnel containing a sodium sulfate layer (pre-rinsed with methylene chloride) into a 300-mL boiling flask.

4) Repeat Step 2 and 3 two more times.

5) Rotoevaporate the methylene chloride to approximately 3 mL at 40°C and transfer the sample extract into a 1/2-oz square bottle using a disposable pipet. Rinse the boiling flask with methylene chloride, then transfer the rinses into the same bottle.

6) Evaporate the sample extract to dryness under a stream of dry nitrogen.

7) Pipet 0.5 mL of toluene into the square bottle, rotate to dissolve all residues on the inside walls of the bottle.

8) Transfer the sample solution from Step 7 into a sample vial and inject on the gas chromatograph.

A 1-ppm NEMACUR standard concentration is equivalent to 1-ppb sample residues as calculated below:

$$1\ \mu g/mL \times \frac{\frac{0.5\ mL}{(Final\ Volume)}}{\frac{500\ mL}{(Extraction\ Volume)}} = 1\ \mu g/L$$

2.3 Spiking procedure for Laboratory QA concurrent recovery

1) Weigh 0.01 ± 0.0001 g each of NEMACUR analytical standard (Ref. 79R-29-146, 97.8% purity), NEMACUR sulfoxide analytical standard (Ref. 68-105-72, 93.4% purity), and NEMACUR sulfone analytical standard (Ref. 76-207-91, 98.0% purity) into a 100-mL volumetric flask. Dilute to volume with acetonitrile and mix thoroughly.

2) Pipet 1 mL of solution from Step 1 into a 100-mL volumetric flask, dilute to volume with acetonitrile and mix thoroughly.

3) Pipet 0.5 mL of solution from Step 2 into 500-mL of control water to produce a 1 ppb spike.

2.3 Spiking procedure for Laboratory QA concurrent recovery (continued)

4) Pipet 5 mL of solution from Step 2 into 500-mL of control water to produce a 10 ppb spike.

5) Extract, concentrate and analyze the spiked samples with the same method used for the field samples.

2.4 Preparation of the standard solutions for soil analysis

1) Weigh 0.01 ± 0.0001 g each of NEMACUR analytical standard (Ref. 79R-29-146, 97.8% purity), NEMACUR sulfoxide analytical standard (Ref. 68-105-72, 93.4% purity), and NEMACUR sulfone analytical standard (Ref. 76-207-91, 98.0% purity) into a 100-mL volumetric flask. Dilute to volume with ethyl acetate and mix thoroughly.

2) Pipet 10 mL of the standard solution from Step 1 into a 100-mL volumetric flask, dilute to volume with ethyl acetate and mix thoroughly. This is a 10-ppm standard solution.

3) Pipet 2- and 1-mL aliquots of the standard solution from Step 2 into separate 10-mL volumetric flasks, dilute to volume with ethyl acetate and mix thoroughly. These are the 2- and 1-ppm standard solutions, respectively.

4) Pipet 5- and 2-mL aliquots of the standard solution from Step 2 into separate 100-mL volumetric flasks, dilute to volume with ethyl acetate and mix thoroughly. These are the 0.5- and 0.2-ppm standard solutions, respectively.

5) Transfer the standard solutions into sample vials for analysis.

2.5 Preparation of the soil samples

1) Allow the samples to thaw completely, then weigh 50 g of sieved sample into a 500-mL boiling flask. If the moisture content in the sample is less than 10 percent, pipet 5 mL of HPLC-grade water to the sample before proceeding. Extract three times with 75 mL of methylene chloride by shaking for three minutes after each solvent addition. After each extraction, permit the soil to settle. Decant the organic layer through a glass funnel containing approximately 10 g of sodium sulfate supported by glasswool and into a separate boiling flask.

2) Evaporate the methylene chloride to approximately 5 mL using a roto evaporator. Transfer the soil extract using a disposable pipet into a 15-mm i.d. x 250-mm long, with 250-mL reservoir, Kontes chromatographic column packed with Florisil prepared as follows:

a) A plug of glass wool to hold the packing in the column.

b) Add 1/2 teaspoon of anhydrous sodium sulfate into the column containing five centimeters of 2.5% acetone in benzene (2.5% acetone/benzene).

2.5 Preparation of the soil samples (continued)

c) Weigh 3 g of 2.5% water deactivated Florisil. Make a slurry using 2.5% acetone/benzene, then wash the Florisil into the column with 2.5% acetone/benzene. Leave five centimeters of 2.5% acetone/benzene on the top of the Florisil.

d) Gently add 1/2 teaspoon of anhydrous sodium sulfate into the column, then rinse the column with 15 mL of 2.5% acetone/benzene and adjust the solvent level to one centimeter above the upper sodium sulfate layer.

Chromatographic Column Cleanup

3) Preparation of the column must precede the sample transfer. Allow the sample extract to flow into the column until the liquid level is at the top layer of the column packing. Rinse the boiling flask twice with 10 mL of 2.5% acetone/benzene; transfer each rinse into the column. Place the boiling flask under the column and adjust the solvent level to the top layer of the column packing.

4) Remove the boiling flask containing all eluates and discard. Place a clean 250-mL boiling flask under the chromatographic column, then add 100 mL of 90% acetone/benzene to the column.

5) Allow all the solvent to pass through the column one drop at a time.

6) Evaporate the solvent mixture to a volume of approximately 2 mL then transfer the solution into a 1/2-oz glass bottle. Rinse the boiling flask two times with 2 mL of acetone, transfer the rinses into the bottle. Wrap the sample bottle with aluminum foil to exclude light. Evaporate the solvents using a nitrogen stream, then pipet 1 mL of ethyl acetate into the glass bottle. Rotate to dissolve the residue inside the bottle.

A 1-ppm NEMACUR standard concentration is equivalent to a 20-ppb sample residue as calculated below:

$$1\ \mu g/mL \times \frac{\text{Final Volume (1 mL)}}{\text{Sample Weight (50 g)}} = 0.02\ \mu g/g = 20\ ppb$$

2.6 Spiking procedure for a 10- and a 100-ppb concurrent recoveries (Laboratory QA Samples)

1) Weigh 0.01 ± 0.0001 g each of NEMACUR, NEMACUR sulfoxide and NEMACUR sulfone into the same 100-mL volumetric flask, dilute to volume with ethyl acetate and mix thoroughly (100 ppm solution).

2) Pipet 1 mL of solution from Step 1 into a 100-mL volumetric flask, dilute to volume with ethyl acetate and mix thoroughly. This is the 1-ppm spiking solution.

2.6 Spiking procedure for a 10- and a 100-ppb concurrent recoveries (Laboratory QA Samples) (continued)

3) Pipet 0.5 and 5 mL of the spiking solution into separate boiling flasks containing 50 g of control soil to produce 10- and 100-ppb spikes, respectively.

4) Extract, concentrate and analyze the spiked samples with the same method used for the field samples.

2.7 Instrument

use a DB-225 (15m x 0.25mm, 0.25 µm film)

Gas chromatograph, Varian 3400, or equivalent, equipped with a nitrogen-phosphorus detector and a 7-m long SB 25 % Cyanopropyl capillary column with a 100 µm i.d. and a 0.25-µ film thickness. no longer available

The following parameters were set on the chromatograph for both water and soil analyses:

Detector temperature, °C:	300
Injection port temp., °C:	250
Injection volume, µL :	2

Temperature parameters:

initial °C	100
hold time, min	1

Program 1 : rate, 30 °C/min; final temp. 210 °C; hold time 10 min

Program 2 : rate, 30 °C/min; final temp. 250 °C; hold time 15 min

Flow rate:

Air	= 170 mL/min
He (carrier gas)	= 2 mL/min
Hydrogen	= 4 mL/min
Nitrogen (make-up)	= 26 mL/min

Inject the standard solution to establish a calibration curve, then the sample solutions. Typical standard and sample scans are shown in Figure 1, 2, 3, 4, 5, and 6.

2.8 Calculation

1) Use least squares curve fitting to generate the "best" line which can be used to calculate the corresponding concentration for a given peak area or peak height. Enter the standard responses as variable X, and the respective standard concentration as variable Y.

2) Determine the concentration (Cppm) corresponding to each sample peak response using the intercept and slope from the calculation in Step 1.

   Cppm = (Slope of Regression Curve X Sample Area) + Intercept of Curve

3) Calculate the amount of NEMACUR, Sulfoxide and Sulfone in the water sample:

$$\text{NEMACUR, } \mu g/L = \frac{\text{Cppm X 1000}}{\text{Volume Extracted (mL)}} \text{ X 0.5 X DF*}$$

$$\text{NEMACUR sulfoxide, } \mu g/L = \frac{\text{Cppm X 1000}}{\text{Volume Extracted (mL)}} \text{ X 0.5 X DF*}$$

$$\text{NEMACUR sulfone, } \mu g/L = \frac{\text{Cppm X 1000}}{\text{Volume Extracted (mL)}} \text{ X 0.5 X DF*}$$

* DF = Dilution Factor, if applicable

EXAMPLE OF NEMACUR CALCULATION FOR A WATER SAMPLE, ID # GA-103

Standard Concentration mg/L	Average Standard Area	Average Sample Area
0.198	7702	16944
0.494	18210	
0.988	36773	
1.976	73076	

Cppm = (0.0000247 X 16944) + 0.0371346 = 0.4563
Dilution Factor = 100
Volume Extracted (mL) = 500

$$\text{NEMACUR, } \mu g/L = \frac{0.4563 \times 1000}{500} \times 0.5 \times 100 = 45.6$$

4) Calculate the amount of NEMACUR, Sulfoxide and Sulfone in the soil sample:

$$\text{NEMACUR, mg/kg} = \frac{\text{Cppm}}{\text{Sample Weight (g)}} \text{ X DF*}$$

$$\text{NEMACUR sulfoxide, mg/kg} = \frac{\text{Cppm}}{\text{Sample Weight (g)}} \text{ X DF*}$$

2.8 Calculation (continued)

$$\text{NEMACUR sulfone, mg/kg} = \frac{\text{Cppm}}{\text{Sample Weight (g)}} \times \text{DF*}$$

EXAMPLE OF NEMACUR CALCULATION FOR A SOIL SAMPLE, ID # GA-16

Standard Concentration mg/L	Average Standard Area	Average Sample Area
0.23	6816	58422
0.57	16749	
1.13	40354	
2.27	107095	

Cppm = (0.000019671 X 58422) + 0.208993239 = 1.358212401
Dilution Factor = 100
Sample Weight (g) = 50

$$\text{NEMACUR, mg/kg} = \frac{1.358212401}{50} \times 100 = 2.72$$

2.9 Bromide Determination

Ten runoff samples from Plot 2 were analyzed for bromide by Ion Chromatography. The samples were filtered through a Sep-Pak filter and injected against a 1-ppm bromide standard solution.

2.9.1 Preparation of the Standard Solution

a) Weigh 1.2841 g of sodium bromide (99.4 % purity) into a 1-L volumetric flask, dilute to volume with Barnstead water and mix thoroughly.

b) Pipet 1 mL from Step a) into a 100-mL volumetric flask, dilute to volume with Barnstead water.

c) Pipet 10 mL from Step b) into a 100-mL volumetric flask, dilute to volume with Barnstead water and mix thoroughly. This is the 1-ppm bromide standard solution.

Calculation

1) Weight corrected by purity:

1.2841 g X 0.994 = 1.2764 g sodium bromide.

2) Bromide concentration = 1.2764 g/L X 77.66 %* = 0.99125 g/L in the stock solution.

* (Percentage of bromide in sodium bromide molecular weight)

3) Working standard concentration = 0.991 mg/L

2.9.2 Instrument Conditions

Dionex Ion Chromatograph Model # 4000 i equipped with a conductivity detector and a HPIC - AS4A column.

Flow Rate, mL/min	1.7
Injection volume, mL	2.5
Mobile phase	1.7 mM $NaHCO_3$/1.8 mM Na_2CO_3
Regeneration solvent	0.025N H_2SO_4

2.10 Free vs. Bound Fenamiphos

Four samples (GA-85, GA-86, GA-87, GA-88) were filtered and analyzed for NEMACUR and its metabolites in water phase as well as in the sediments to determine the distribution of fenamiphos in both phases.

2.11 Determination of Total Suspended Solids (TSS)

Two water samples (GA-98 and GA-99) were submitted for Total Suspended Solids (TSS) analysis.

1) Shake the sample vigorously for one minute, then pour 100 mL of sample into a 100-mL graduated cylinder. Record the volume as V.

2) Filter the sample through a pre-weighed ($W_1 \pm 0.0001$ g) polypropylene Buchner funnel containing a glass fiber filter under vacuum.

3) Place the funnel in a 103°C oven overnight.

4) Remove the funnel from the oven and allow it to cool in a desiccator.

5) Reweigh the funnel and record as $W_2 \pm 0.0001$ g.

Calculation

$$\text{Total Suspended Solids (TSS), \%} = \frac{(W_2 - W_1) \times 100}{V\ (mL)}$$

2.12 Moisture Determination

The moisture was determined for all soil samples.

1) Weigh an aluminum pan on a tared top-loader balance, record the weight (W_1).

2) Place approximately 10 - 15 g of the sieved sample onto the aluminum pan, record the weight (W_2). Place the pan and sample into an oven at 103°C.

3) After three hours, remove the aluminum pan from the oven and place it in a desiccator. Allow the sample to cool to ambient temperature.