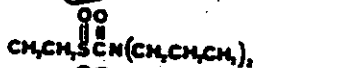
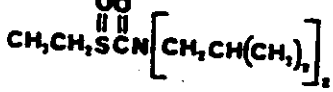
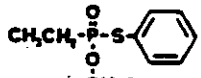
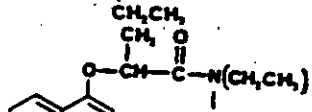
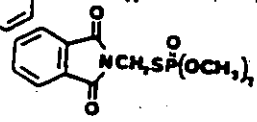
GAS CHROMATOGRAPHIC DETERMINATION OF EPTC SULFOXIDE, BUTYLATE SULFOXIDE, S-METHYL MOLINATE, FONOFOS OXON, DESETHYL NAPROPAMIDE, AND PHOSMET OXON RESIDUES IN SOIL

I. SUMMARY/INTRODUCTION

This method is intended for determining S-methyl molinate, EPTC sulfoxide, butylate sulfoxide, fonofos oxon, desethyl napropamide, and phosmet oxon residues in soils at levels of 0.01 ppm to 0.50 ppm. All the analytes are metabolites of active ingredients of registered compounds. The table below gives the analyte, the active ingredient of which it is a metabolite, and the chemical name and structure of the analyte.

Analyte	Ingredient	Chemical Name	Structure
S-methyl Molinate	Molinate	S-methyl hexahydro-1H-azepine-1-carbothioate	
EPTC Sulfoxide	EPTC	S-ethyl dipropylthiocarbamate sulfoxide	
Butylate Sulfoxide	Butylate	S-ethyl diisobutylthiocarbamate sulfoxide	
Fonofos Oxon	Fonofos	O-ethyl S-phenyl ethylphosphonothioate	
Desethyl Napropamide	Napropamide	N-ethyl-2-(1-naphthalenyloxy)propionamide	
Phosmet Oxon	Phosmet	N-(mercaptomethyl) phthalimide S-(O,O-dimethylphosphorothioate)	

S-Methyl molinate, EPTC sulfoxide, butylate sulfoxide, fonofos oxon, desethyl napropamide, and phosmet oxon are extracted directly from soil with water and toluene. The toluene extract is analyzed for S-methyl molinate, EPTC sulfoxide, butylate sulfoxide, fonofos oxon, desethyl napropamide, and phosmet oxon by capillary gas chromatography with nitrogen-specific detection.

II. MATERIALS/METHODS

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment with equivalent performance specifications and reagents of comparable purity can be used.

A. Apparatus

1. Gas Chromatograph. Hewlett-Packard Model 5880A, equipped with on-column injector inlet, Hewlett-Packard Model 7673A automatic

sampler, nitrogen-phosphorus detector, and electronic integrator or data acquisition system. Any chromatographic system giving equivalent performance can be used.

2. Chromatographic Column. J & W DB-1 (crosslinked methyl silicone), 15 m x 0.53 mm x 1.5 µm thickness, or equivalent.

3. Glass Bottles. Four-ounce, wide mouth bottles with aluminum foil lined caps.

4. Syringe. 10, 100, and 500 microliter capacities, Hamilton 701N, 710N, 750N or equivalent.

5. Reciprocating Shaker. Eberbach Corporation, model 6010 or equivalent.

6. Centrifuge. IEC International, model C1582 or equivalent.

B. Reagents

1. Solvents. Toluene, Acetone, Nanograde® or equivalent.

2. NaCl, Anhydrous Na_2SO_4. Reagent grade.

3. S-Methyl Molinate, EPTC Sulfoxide, Butylate Sulfoxide, Fonofos Oxon, Desethyl Napropamide, and Phosmet Oxon. Analytical reference-standards S-methyl molinate, EPTC sulfoxide, butylate sulfoxide, fonofos oxon, desethyl napropamide, and phosmet oxon. Available from ICI Americas Inc., 1200 So. 47th Street, Box 4023, Richmond, CA 94804-0023, Attention: Environmental Sciences Department Manager.

4. Calibration and Fortification Solution.

To prepare a stock solution weigh to the 4th decimal place a convenient quantity, e.g. 50 mg, of analytical reference standard of known purity into a suitably sized bottle. Calculate the weight of solvent to add, based on the weight of reference standard taken, the purity of the reference standard, the density of the solvent, and the desired solution concentration, typically 1000 µg/mL, as follows:

$$S = \frac{W \times P \times D}{A}$$

where S = the weight of solvent to add (g),

W = the weight of primary standard taken (mg std),

P = the purity of the primary standard (mg a.i./mg std),

D = the density of the solvent (g/mL),

and A = the desired solution concentration (mg a.i./mL solvent)

Add the calculated weight of the appropriate solvent to the bottle, close the bottle with a polyseal cap, and mix thoroughly to dissolve the primary standard. Use toluene (D = 0.867 g/mL) for calibration solutions, and acetone (D = 0.792 g/mL) for fortification solutions.

To prepare working calibration solutions, dilute the stock calibration solution by weight with toluene to give solutions that contain 1.0, 0.1, and 0.01 µg/mL of each analyte to be determined or other concentrations as required.

Dilute the stock fortification solution by weight with acetone to give solutions that contain 10 µg/mL of each analyte to be determined, or other concentrations as required.

As discussed in Section III.A below, an analyte may exhibit an enhanced response in sample matrix, as demonstrated by high recoveries from fortified control samples. In such cases, the calibration solutions may be prepared in sample extract solution to compensate for the response enhancement. Prepare calibration solutions in the sample matrix by either of two methods: 1) evaporate the toluene from a known volume of working calibration solution and take the residue up to the original volume with extract from an untreated control sample, or 2) add, via a syringe, the required amount of stock calibration solution to a known volume of extract from an untreated control sample. The amount added must be small enough relative to the extract volume that dilution is insignificant. The later method is preferred if the analyte is volatile.

C. Analytical Procedure

1. Extraction

Weigh 40.0 g of thoroughly-mixed soil sample into a 4-oz wide mouth bottle. Add 40 mL of distilled water, 10 g of NaCl, and 40 mL of toluene. Cap the bottle with an aluminum foil-lined lid and shake it on the reciprocating shaker for 2 hours. Centrifuge for 10-20 minutes at 2000 rpm to aid the separation of the phases. Alternatively, use any convenient weight of soil, 20 g or more, and extract with water and toluene in a soil:water:toluene w:v:v ratio of 1:½-1:1; confirm the validity of the extraction method by analysis of fortified control samples. Remove the top (toluene) phase for analysis. Dry stored extracts with anhydrous Na_2SO_4.

The validity of the method must be confirmed by analysis of appropriate control and fortified samples with each set of samples analyzed. If method validation recoveries are adequate without added NaCl, the use of NaCl is not required. Similarly, if chromatographic sensitivity and reproduceability are adequate with split or splitless injection modes, on-column injection is not required.

2. Fortification

Analyze unfortified and fortified control samples with each set of treated samples to demonstrate method recovery according to the Quality Assurance SOP. For example, for 40-g samples, weigh 40 g of untreated control soil into a 4-oz wide-mouth bottle. Add 0.040 mL of the 10 µg/mL acetone fortification solution to produce a fortification level of 0.01 ppm, or add 20 µL of the 1000 µg/mL acetone fortification solution to produce a fortification level of 0.5 ppm. Add water, NaCl, and toluene and extract as above. If a different weight of soil is analyzed, use that weight and adjust the volume or concentration of fortification solution to give the desired analyte concentration. Extract using the same amounts of water, salt (if required), and toluene as for the treated samples.

D. Instrumentation

1. Operating Conditions

Follow the manufacturer's instructions for operation of the gas chromatograph and nitrogen-selective detector. Use these parameters for the analyses or other operating conditions that achieve equivalent sensitivity, reproducibility, and resolution.

Inlet	On-column injection
Oven initial temp.	100°C
Initial time	0.05 min
Temp. programming rate	25°C/min
Oven final time	9 min
Oven final temperature	260°C
Injector temperature	OFF
Detector temperature	300°C
Carrier gas	Helium
Carrier gas pressure	3 psi
Carrier gas flow	12 mL/min
Injection size	3 µL
Quantitation	Peak height (external standard)
Makeup-gas, helium	22.5 mL/min
Air	140 mL/min
Hydrogen	4 mL/min

Under the above conditions the elution times of the analytes range from 2.5 to 5.7 minutes. See Figure 1 for typical chromatograms.

2. Calibration

The gas chromatograph is calibrated using the analyte calibration solutions specified in section II.B.3. Chromatographic sensitivity is established by analysis of the 0.01 µg/mL calibration solution. Quantitation of residues at levels above the detection limit is done by an external standard procedure in which peak heights or areas of analyte peaks in sample extracts are compared to corresponding peak heights or areas of analyte peaks in calibration solutions. See Section G, below, for details of calculational methods.

3. Analysis of Extracts

Inject the sample extracts using the same conditions used for calibration. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of retention times (within 0.03 minutes) with those of the calibration chromatograms. If the response of a peak identified as an analyte exceeds that of the highest calibration solution, dilute the sample extract until its response is within the calibrated range, or extend the calibration range by injection of calibration solutions at higher concentration. Reinject the calibration solution after every two to four sample injections and recalibrate as needed. Reinject the calibration solution at completion of the sample analysis.

E. Interferences

No clean-up is required when this procedure is utilized as described. However, extractives from soil occasionally contribute peaks with retention times near those of an analyte. Satisfactory resolution can usually be achieved with appropriate oven temperature manipulations or column choice. Appendix A shows typical chromatograms. Analyze extracts of samples from untreated plots to demonstrate the absence of interferences from sample matrices, solvents, or labware. Typically, the active ingredient or parent compound may be present in any sample analyzed for a metabolite. Always confirm that the active ingredient and the metabolite do not co-elute under the conditions of analysis.

F. Confirmatory Techniques

Unexpected positive results, as in untreated control or pre-application samples, should be confirmed by other means, preferably by GC/MS, mass selective detection, or use of a second capillary column of different polarity.

G. Calculations

Calculations are done in one of two ways. If the response is linear, a factor can be calculated as described in 1 below. If the response is non-linear, or if the analyst prefers, the analyte responses over a range of calibration solution concentrations can be fit to a linear or an exponential curve, and a factor can then be calculated as in 2 below for each point on the curve that corresponds to an analyte esponse in an injection of sample extract.

1. Linear Response, Direct Calculation of Factor

a. Calibration Factors for Linear Response

F = the response factor for the analyte (ppm per electronic unit), calculated as follows:

$$F = \frac{C}{P \times S}$$

where C = the concentration of analyte in the calibration solution (µg/mL)

S = the amount of initial sample represented by each milliliter of final extract solution injected (g/mL)

P = the peak area or height (electronic units) of the analyte peak in the chromatogram of the calibration solution

Averaged response factors for multiple injections of calibration solutions and for more than one concentration of calibration solution can be used as appropriate in the calculation of the concentration of the analyte in the sample, as described below.

b Analyte in Sample

The concentration of the analyte in the original sample is calculated using an external standard method as follows:

$$ppm = F \times R$$

where ppm = the amount of analyte in the soil in parts per million

R = the peak area or height (electronic units) of the analyte peak in the chromatogram of the sample extract

and F = the response factor for the analyte (ppm per electronic unit), calculated as described above

Note for the above external standard calculations, equal volumes of both the extract and the calibration solutions are injected.

2. Curve Fit for Linear or Non-Linear Response

If the instrumental response to injections of calibration solutions is reproducible and either linear or exponentially non-linear, a concentration-response curve can be used for sample quantitation. Any valid curve-fitting program can be used. Input the concentration and response for each injection of calibration

solution. The program will generate the formula for the corresponding linear or exponential curve. From the formula, determine the calculated concentration for each injection of calibration solution as described below. The calculated and actual concentrations should agree within 10 % relative; that is, the ratio of the actual to the calculated concentration should be between .9 and 1.1. If the agreement is adequate, calculate the concentration of analyte in the sample, and corresponding response factor as follows:

a. Linear Response:

The formula will be of form $Y = mX + b$, where

Y = the concentration of the analyte, ppm,

X = the analyte response, peak height or area units,

and

m and b = constants calculated by the curve-fit program.

Since the analyte concentration should be zero if the response is zero, the constant b should be zero if there are no systematic errors in the analysis. However, it is not necessary for b to be zero for the calculational method to be valid, as long as calibration solution responses are reproducible and the calculated concentrations of the calibration solutions are within 10 % of the actual concentrations.

For each sample injection, determine Y by using the response, X, in the formula.

Calculate the response factor, F, from the formula:

$F = Y/X$

Note that this factor should be the same for any point on a linear curve which passes through the intercept; b = 0.

b. Exponential non-linear response:

The curve will be of form $Y = aX^b$, where

Y = the concentration of the analyte, ppm,

X = the analyte response, peak height or area units,

and

a and b = constants calculated by the curve-fit program.

For each sample injection, determine Y by using the response, X, in the formula.

Calculate the response factor, F, from the formula:

$$F = Y/X$$

The response factor will be different for each point on the curve.