Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Thiophanate

MRID #: 419301-02

Matrix: Soil

Analysis: HPLC/UV

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APPENDIX D

Method of Determination of T-M
and Its Metabolites in Soil

ANALYTICAL METHOD FOR RESIDUES OF THIOPHANATE-METHYL,
METHYL-2-BENZIMIDAZOLE CARBAMATE, AND ALLOPHANATE
IN SOIL USING HIGH PERFORMANCE LIQUID
CHROMATOGRAPHY

TCM-004-1B (Hughson Location)

October 5, 1987

In this method, thiophanate-methyl (T-M), methyl-2-benzimidazole
carbamate (MBC), and allophanate are extracted from soil (Hughson
location) with aqueous methanol. After evaporating the
solvent, the analytes are extracted into chloroform. The chloroform
is then dried and evaporated and the residue reconstituted in
methanol and quantitated by HPLC.

I. REAGENTS

A. All reagents should be AR grade or better.

B. All water should be distilled or deionized.

C. HPLC grade solvents

D. The following special reagents should be prepared:

   1. 1 N HCl - dilute 83 mLs concentrated HCl to 1 liter with water.

   2. 4% ethanol in chloroform - dilute 4 mLs 95% (190 proof) ethanol to 100 mLs with chloroform (Omnisolv, Catalog No. 1054, or equivalent). Prepare daily as needed.

   3. Citric acid/phosphate buffer - dissolve 4.3 grams citric acid monohydrate and 4.2 grams sodium phosphate (dibasic) heptahydrate in water (sonication speeds dissolution) and dilute to one liter with water.
Prepare daily - buffer should be discarded if algae growth is observed.

II. EQUIPMENT

A. Class A volumetric glassware should be used for all steps requiring volumetric measurement.

B. The following special equipment is recommended:

1. Wrist action shaker

2. Rotary evaporator (Buchler PTFE-1GN or equivalent)

3. HPLC with UV detector

4. Syringe filters (Gelman ACRO LC13 ,No. 4452, or equivalent)

5. Vortex mixer (Scientific Industries Inc. Model K850-C or equivalent)

6. Ultrasonic cleaner

III. METHODOLOGY

A. Sample Preparation

Thaw samples sufficiently to allow thorough mixing and sub-sampling.

B. Sample Extraction

1. Soil

a. Weigh 100 gram sub-sample into a 500 mL Erlenmeyer flask.

b. Add 150 mLs methanol and 50 mLs water to the flask.

c. Stopper and shake at maximum speed on a wrist action shaker for 15 minutes.

d. Transfer the sample to 250 mL centrifuge bottles without rinses and centrifuge at 2.000 rpm for 15 to 20 minutes.

e. Decant the aqueous methanol into a 250 mL graduated cylinder and record the volume. Discard the pellet.

f. Transfer the solution to a clean one liter flat bottom flask with three approximately 10mL rinses of methanol, and evaporate the alcohol on a rotary evaporator (bath temperature no more than 40°C).

C. Isolation of T-M. MBC. and Allophanate Residues

1. Add 100 mg sodium acetate to aqueous as a buffer. Adjust the pH of the aqueous solution to 6.8 ± 0.1 using 1 N HCl and 1 N ammonium hydroxide.

2. Add 25 mLs 4% ethanol in chloroform to the flask. Stopper and shake ten minutes on a wrist action shaker at maximum setting. Transfer to a 500 mL separatory funnel.

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3. Allow the layers to separate (if an emulsion forms add an additional 10 mLs of the 4% ethanol in chloroform solution\ and stir well with a stick). Collect the chloroform (bottom layer) in a 500 mL flat bottom flask equipped with a glass funnel containing a plug of silanized glass wool and about 4 grams (½ a 5 mL beaker full) of anhydrous sodium sulfate.

4. Repeat Steps 2 and 3 until a total of three extractions have been completed.

5. Rinse the glass funnel with about 10 mLs of the 4% ethanol in chloroform solution.

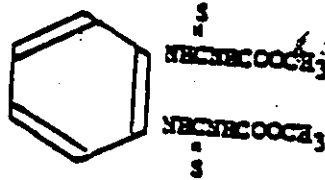6. Discard the aqueous layer.

D. Analysis of Residues

1. Evaporate the dry chloroform extracts down to about 4 to 5 mLs on a rotary evaporator (water bath <40°C). Add about 30 mLs methanol to the flask, swirl to mix, and evaporate to about 4 to 5 mLs on a rotary evaporator again.

2. Transfer the flask contents quantitatively with two approximately 2mL methanol rinses to a graduated centrifuge tube and evaporate below final volume of 1 mL with a gentle stream of nitrogen in a <40°C water bath.

3. Adjust the final volume to 1.0 mL with methanol, stopper, vortex to mix, and pass through a 0.45 micron syringe filter.

4. Inject an appropriate aliquot into the HPLC and quantitate with external standards taking into consideration the volume recovered in Step III.B.1.e.

5. HPLC conditions are as follows:

   a. Column — μBondapack C18 300 x 3.9 mm or equivalent

   b. Mobile Phase — Linear gradient from 15% acetonitrile, 85% citrate/phosphate buffer to 50% acetonitrile, 50% buffer over 20 minutes

   c. Flow — 1.5 mL/min

   d. Detector — UV - 254 nm

   e. Temperature — Ambient

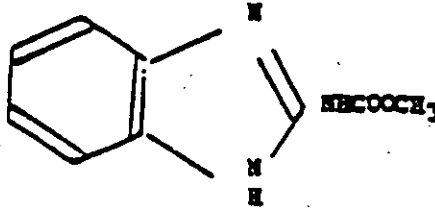E. Structural Formulas and Molecular Weights

1. T-M - M.W. 342.4

T-M = Thiophanate-methyl (TOPSIN M) = Dimethyl [(1,2-phenylene)bis(iminocarbonothioyl)]bis[carbamate]
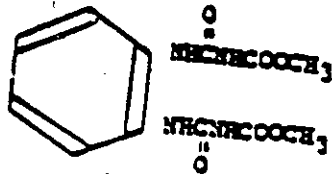

2. MBC - M.W. 191.2

MBC = Methyl-2-benzimidazole carbamate
(2-Methoxycarbonylamino-benzimidazole) - stable
transformation product (major transformation product)

3. Allophanate - M.W. 310.4

FH-432 = Dimethyl-4,4'-o-phenylenebis(allophanate) - rel-atively stable transformation product

APPROVAL

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Table D-1

Method TCM-004-18 Validation Results

Sample Description	Fortification Level (ppm) MBC/ALL/T-M	MBC		Allophanate		T-M	
		Amount Found (ppm)	Recovered (%)	Amount Found (ppm)	Recovered (%)	Amount Found (ppm)	Recovered (%)
Reagent Blank	NA	<0.050	NA	<0.050	NA	<0.050	NA
Control Soil 02	NA	<0.050	NA	<0.050	NA	<0.050	NA
SS1	0.101/0.100/0.100	0.104	103	0.113	113	0.097	97.0
SS2	0.101/0.100/0.100	0.105	104	0.118	118	0.078	78.0
SS3	0.101/0.100/0.100	0.094	93.1	0.104	104	0.057	57.0
SS1	0.504/0.499/0.499	0.533	106	0.520	104	0.423	84.8
SS2	0.504/0.499/0.499	0.508	101	0.498	99.8	0.403	80.8
SS3	0.504/0.499/0.499	0.492	97.6	0.483	96.8	0.382	76.6
SS1	2.52/2.50/2.50	2.84	113	2.39	95.6	2.08	30.4
SS2	2.52/2.50/2.50	2.28	90.5	2.29	91.6	2.14	85.6
SS3	2.52/2.50/2.50	3.76	149 (a)	2.44	97.6	2.03	81.6
SS4	2.52/2.50/2.50	2.66	100	2.60	104	2.66	106
SS5	2.52/2.50/2.50	2.96	117	2.74	110	2.73	109
Average Recovery			103		103		87.9
Standard Deviation			9.00		7.998		13.60

NA: Not Applicable

(a) Data not included in statistical analysis.

APPENDIX E

Deviations from the Method

DEVIATIONS FROM THE METHOD

The following deviations did not affect the integrity of the study:

o   Due to lack of sufficient sample, only 50 g of soil were analyzed per assay instead of the 100 g specified in the Method.

o   To validate Fisher methanol, the 0.1 ppm recovery for Analysis Set 10H was extracted with 150 mL of Fisher methanol instead of 150 mL of B&J methanol.

o   To stop the breakdown of T-M to MBC, acidified methanol was substituted for neutral methanol at the point of solvent exchange with chloroform/ethanol extract.   Samples were brought to final volume with acidified methanol. This deviation was started from set 15H.

o   To achieve better sensitivity, the following program was used on the Kratos 783 detector (from set 16H on):

	Minutes	Wavelength	Range
Step 00 time:	0.0	281	0.100
Step 01 time:	8.0	254	0.050
Step 02 time:	26.0	281	0.050
Step 03 time:	27.0	281	0.050

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APPENDIX F

Calculations

CALCULATIONS

To Calculate ppm of T-M, MBC or Allophanate

$$\frac{\dfrac{Y - B}{M} \quad \times \quad \dfrac{V_T}{V_R} \quad \times \quad V_F \quad \times \quad \dfrac{1}{D}}{W}$$

Where:

X = concentration of analyte in ppm

Y = peak height obtained from the chromatogram (mm)

B = y-intercept obtained from the linear regression of the standard curve

M = slope obtained from the linear regression of the standard curve

V_T = total volume of solution added to the sample for extraction (mL)

V_R = volume recovered after centrifuging the sample (mL)

V_F = final volume (mL)

D = dilution factor as a fraction

W = sample weight (g)

Example: 50 g of soil sample was weighed for analysis

$$X = \frac{\left(\dfrac{5.5 - (-1.06)}{1.02}\right) \times \left(\dfrac{200 \text{ mLs}}{181 \text{ mLs}}\right) \times 2 \text{ mLs} \times \dfrac{1}{\frac{1}{2}}}{50 \text{ g}} = 0.563 \text{ ppm}$$

To calculate percent recovery:

Percent recovery = 100% x $\dfrac{ppm\ found}{ppm\ added}$

Example: The control soil (50g) was weighed and fortified with 1 mL of 2.5 ug/mL standard (0.050 ppm)

Amount found = 0.048 ppm

Percent recovery = $\dfrac{0.048\ ppm}{0.050\ ppm}$ x 100% = 96.0%

To calculate percent moisture:

Percent moisture =

100% - $\dfrac{[dry\ sample + container\ wt.\ (g)] - container\ wt.\ (g)}{[wet\ sample + container\ wt.\ (g)] - container\ wt.\ (g)}$ x 100%

% solids = 100% - % moisture

$\dfrac{(\%\ solids + \%\ solids)}{2}$ = Average % solids

Dry sample weight (g) = $\dfrac{average\ \%\ solids \times sample\ weight\ (g)}{100\%}$

Calculations of dry lot concentrations:

X^1 = $\dfrac{X \times W}{dry\ sample\ weight}$

Where:

X^1 = Concentration of analyte in ppm (dry weight basis)
X = Concentration of analyte in ppm (weight received basis)
W = Initial weight of the sample in grams = 50g

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APPENDIX G

Raw Data Tables by Analysis Set.
(Set Nos. 1H through 24H)

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