Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Thiram

MRID #: 447245-01

Matrix: Soil

Analysis: GC/FPD

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MORSE LABORATORIES, INC. SOP# Meth-97

Revision #2 Date 07/96

DETERMINATION OF THIRAM IN SOIL

Reason for Revision: To provide for the preparation and use of thiram standards as solutions in acetonitrile.

1.0 PRINCIPLE

Thiram present in the sample is converted to CS_2 during reaction with HCl/Stannous chloride reagent at 100°C in a sealed reaction vial. An aliquot of the headspace is injected into a gas chromatograph where the sample responses are compared to a thiram standard similarly prepared and injected. The limit of quantitation for thiram in soil is 0.02 ppm.

2.0 EQUIVALENCE STATEMENT

During the conduct of this analysis, equivalent apparatus, solvents, glassware, or techniques (such as sample concentration) may be substituted for those specified in this method, except where otherwise noted. In the event an equivalent piece of equipment or an equivalent technique is used, its use will be documented in the study records, when appropriate.

3.0 APPARATUS AND EQUIPMENT

Reaction vials - 160 mL equipped for crimp sealing with Teflon-lined septums, Pierce Chemical Co., Rockford, IL 61105, cat. #12995 (listed as 125 mL in catalog)

Crimp seals with Teflon-lined septums - Pierce Chemical Company, Rockford, IL 61105
Teflon/Silicone Disc Septums cat #12720
Aluminum seals (20 mm) cat #13214

Crimper - Wheaton Instruments, Millville, NJ, part #224303

Water Bath

Gas Chromatograph -	Microtek MT-220 or equivalent with a flame photometric detector in the sulfur mode
Gas Chromatographic Column -	6' x 1/4" o.d. x 4 mm i.d. glass column packed with 28% Pennwalt 223 + 4% KOH on 80/100 Gas Chrom R or equivalent
Gas tight syringes -	10, 50, 100, 250, 500, 1000, 2500 μL, Hamilton Co., Reno, NV
Magnetic Stir Plate with stir bars	
"Airpettor" Adjustable pipettes -	American SMI "Airpettor", Scientific Products, Sunnyvale, CA 94089 or equivalent
Pipettes:	50-200 μL volume range cat. #P5086-2 200-1000 μL volume range cat. #P5086-3
Tips:	2-200 μL cat. #P5059-37 (Baxter) 100-1000 μL cat. #P5059-801 (Baxter)

4.0 REAGENTS AND MATERIALS

Thiram:	Analytical grade
GLC Column Packing -	28% Pennwalt 223 + 4% KOH on 80/100 Gas Chrom R, Alltech Associates, Inc., Deerfield, IL 60015 or equivalent
	Note: The PT 28% Alltech 223 column is equivalent to the Pennwalt 223 column.
EDTA (tetrasodium) -	over 98% purity, Mallinckrodt, Paris, Kentucky 40361
EDTA Solution -	10% (w/v) in boiled deionized water
Hydrochloric Acid -	concentrated, J.T. Baker Chemical Co.,Phillipsburg, NJ

Stannous Chloride -	analytical grade, EM Science, Gibbstown, NJ 08027
Carbon disulfide -	reagent grade, J.T. Baker Chemical Co. Phillipsburg, NJ 08665
Acetonitrile -	Pesticide residue grade
Methanol -	nanograde, J.T. Baker Chemical Co. Phillipsburg, NJ 08665
Hexane -	nanograde, J.T. Baker Chemical Co. Phillipsburg, NJ 08665
HCl/Stannous chloride reagent -	8N:3%; weigh 3.6 g $SnCl_2.(H_2O)_2$ into a 100 mL volumetric flask. Add 64 mL concentrated HCl. QS to 100 mL with boiled deionized water

5.0 SAMPLE PREPARATION

Frozen soil is homogenized with dry ice in a Hobart Food Cutter (or equivalent) to mix thoroughly prior to analysis.

6.0 STANDARD PREPARATION

6.1 Carbon disulfide (CS_2):

1. 100 μL CS_2 is dissolved in 100 mL hexane.

2. 4 μL of the 0.1% solution is placed in a reaction flask, which is immediately sealed and heated to 100°C to allow all hexane and CS_2 to equilibrate in headspace.

Note: This standard is used to locate the gas chromatographic CS_2 response the first time this analysis is attempted. Thereafter, given similar column and GC conditions, only the active ingredient standard needs to be prepared.

6.2 Thiram

Two methods are provided for the preparation of thiram standards used for fortification and instrumental analysis purposes. One produces an aqueous suspension and the other produces a solution. Both methods are suitable for the analysis of field samples and the preparation and analysis of concurrent QC fortification samples. However, **only the standard prepared as an aqueous suspension is suitable for use in the preparation of fortified samples intended for stability assessment (field or laboratory).**

6.2.1 Preparation of thiram standards as aqueous suspensions

1. Correcting for purity, weigh 10.0 mg active ingredient of a thiram analytical standard into a glass weigh boat, then transfer to a 250 mL beaker.

2. Using a 100 mL volumetric pipet, deliver 100 mL boiled deionized water (cooled) into the beaker containing the thiram standard.

3. Insert a magnetic stir bar in the beaker containing the aqueous suspension and place on a magnetic stir plate. Homogenize the thiram suspension for 10 minutes.

   Note: The resulting suspension (100 μg/mL) must be homogeneous and devoid of heavy particles. All subsequent dilutions must also be maintained in a constant suspension by stirring with a magnetic stir bar in a similar fashion as the stock suspension.

4. **While the suspension is being homogenized,** remove 10.0 mL with a graduated pipet and dilute to 100 mL with boiled deionized water.

   Note: 10 mL aliquot must be withdrawn quickly and accurately on first attempt. If the initial withdrawal overshoots or undershoots the 10 mL mark, the withdrawal procedure must be reinitiated. This produces a 10 μg/mL solution.

5. An instrumentation standard with a 0.1 μg/mL headspace concentration is prepared by adding 1.35 mL of the 10 μg/mL suspension (using an Airpettor) to a 160 mL vial.

   Note: Two 675 μL aliquots can be used in place of 1.35 mL.

6. Add 8.65 mL 10% EDTA solution and 15 mL HCl/Stannous chloride reagent to the reaction vial and immediately crimp seal.

7. React contents of vial in the same fashion as samples, as discussed later. Use this standard for gas chromatography.

Note: Based on the stability of thiram in water suspension **(<3% degradation in 30 minutes)**, all manipulations with suspension standards must be completed within this time frame.

6.2.2 Preparation of thiram standards as acetonitrile solutions

1. Correcting for purity, weigh 10.0 mg active ingredient of a thiram analytical standard into a glass weigh boat, then transfer to a 100 mL volumetric flask.

2. Bring to volume with acetonitrile and mix to dissolve. This produces a 100 μg/mL solution.

3. Transfer 10.0 mL of the 100 μg/mL solution with a graduated pipet to a 50 mL volumetric flask. Bring to volume with acetonitrile. Mix well. This produces a 20 μg/mL solution.

4. An instrumentation standard with a 0.1 μg/mL headspace concentration is prepared by adding 675 μL of the 20 μg/mL suspension to a 160 mL reaction vial.

5. Add 10.0 mL 10% EDTA solution and 15 mL HCl/Stannous chloride reagent to the reaction vial and immediately crimp seal.

6. React contents of vial in the same fashion as samples, as discussed later. Use this standard for gas chromatography.

Both standard preparations detailed above are intended for analyses with a limit of quantitation of 0.02 ppm. Instrumentation standards of differing concentrations may be prepared to enable the analysis of samples with limits of quantitation other than 0.02 ppm.

7.0 SAMPLE FORTIFICATION

1. Spike samples are fortified at the correct level by adding the appropriate volume from either the 1.0 μg/mL, 10 μg/mL, 20 μg/mL or 100 μg/mL fortification suspension or solution as applicable.

   Note: Regarding *suspension* standards, 1.0 μg/mL is only prepared if necessary. While the 10 μg/mL *suspension* is being homogenized, remove 10.0 mL and dilute to 100 mL with boiled deionized water.

2. When using *suspension* standards only pipettes or "Airpettors" should be employed to add the fortification solutions to the sample. **DO NOT USE MICROLITER SYRINGES.**

3. Place 4.00 g frozen sample into a 160 mL reaction vial.

4. Fortify the spike samples with the appropriate concentration. When using *suspensions*, aliquots are removed only **while the suspension is being actively homogenized.**

   Use volumes of no greater than 1.0mL when fortifying with *solution* standards.

5. Add 10% EDTA solution to make a total volume of 10.0 mL (sample plus fortification solution plus 10% EDTA solution; inclusion of fortification solution volume only applies to use of *suspension* standards). **See Discussion, Section 11.0.**

6. Add 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal.

7. Treat as samples from this point on, heating the fortification at 100°C for the same time as the samples.

8.0 SAMPLE EXTRACTION

1. Place 4.00 g of frozen sample into a 160 mL reaction vial.

2. Add 10% EDTA solution to make a total volume of 10.0 mL (sample plus 10% EDTA solution). This volume must be determined for each soil type prior to analysis, based on the volume of a 4 g sample (see discussion, Section 12.0).

3. Add 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal the reaction vial.

4. Place vial in boiling waterbath for a total of 2 hours, hand-shaking vials approximately every 5 minutes for the first 30 minutes, then every 30 minutes for the remaining 1 ½ hours.

5. After reaction, maintain sample at 100°C in the waterbath during GLC analysis.

9.0 GAS CHROMATOGRAPHIC ANALYSIS

9.1 The following column and conditions are suggested. Other columns that exhibit proper analyte chromatography and resolution from interferences may be used when necessary.

Column:	6' × ¼" o.d. glass column packed with 28% Pennwalt 223 + 4% KOH on 80/100 mesh Gas Chrom R
Carrier Gas:	Nitrogen at 45 mL/min.
Temperatures:	Injector: 210 °C Detector: 160 °C Column: 125 °C isothermal
Injection Volume:	Variable. Inject 2-1000 μL of airspace from samples into GC using airtight syringes. Compare sample responses to those produced in the standard curve.
Retention Time:	~2.0 minutes

9.2 Calibration

1. Prepare a 4-point standard curve by injecting 0.4 ng through 2.5 ng (4 μL through 25 μL of a 0.1 μg/mL headspace concentration standard) of thiram.

Note: 2.5 ng may produce an off-scale response on some FPD detectors due to their inherent differences in logarithmic response. In such cases, inject an amount of standard which produces approximately a 90% full scale deflection (FSD) as the high point of the curve.

2. In any case, 0.4 ng, representing a 0.0135 ppm response when 1000 μL of sample is injected, must be included as the lowest point on the standard curve and should produce a response peak height of at least 5 mm. Identification of the peak produced by the thiram standard as CS_2 is achieved by demonstrating its retention time to be identical to that of the peak produced by an injection of CS_2 standard. This identification process needs to be conducted only if the CS_2 retention time needs to be identified (i.e., when a new GC column is employed or instrument repairs which might have affected retention time have occurred).

Compare sample responses to those produced in the standard curve.

9.3 Notes

1. Condition column overnight at 200 °C with 30 mL/minute carrier gas flow.

2. Prior to analysis, make several injections (10-100 μL) of the thiram standard (for example, 0.1 μg/mL headspace as appropriate) to sensitize the column for CS_2.

3. The column and conditions stated in the method have been satisfactory for the matrix being analyzed. The specific column packing/coating, carrier gas, column temperature and flow rate listed are typical conditions for this analysis. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

10.0 CALCULATIONS

Calculations from the instrumental analysis are conducted using a validated software application to generate a standard curve based on nonlinear regression. The data points for analyte concentration (ng) and peak response (mm) are obtained from strip chart recorder-generated chromatograms. All standards injected for the standard curve and their corresponding peak height responses are entered into the program.

A power series equation is used to determine unknown x values when given the y value. The equation that defines the curve produced when a logarithmic relationship exists between concentration and peak responses is:

$$y = ax^b$$

where:

y	=	peak height response (mm)
x	=	nanogram found for the peak of interest
a and b	=	variables dependent on data points entered

The calculations for ppm found and percent recovery (for fortified samples) are:

1. The amount of Thiram (in ppm) found in the sample is calculated according to the following equation:

$$ppm\ thiram = ng\ found \times \frac{final\ vol.\ (mL)}{sample\ wt.\ (g)} \times \frac{1}{inj.\ vol.\ (\mu L)} \times \frac{1000\ \mu L}{1\ mL} \times \frac{1\ \mu g}{1000\ ng}$$

where:

ng found	=	ng found calculated via a software program
final volume (mL)	=	headspace volume of sample-containing reaction vial (135 mL)
sample weight (g)	=	gram weight of sample
injection volume (μL)	=	μL amount of sample injected into gas chromatograph
1000 μL/mL	=	conversion factor
1 μg/1000 ng	=	conversion factor

This equation simplifies to, for example:

$$mg\ sample\ injected = \frac{4.00\ g}{135\ mL} \times \mu L\ injected\ (rounded\ to\ three\ significant\ figures)$$

where: 135 mL = headspace volume of sample-containing reaction vial

2. The percent recovery for fortified control samples is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{ppm found in fortified control} - \text{ppm found in control}}{\text{ppm added}} \times 100$$

11.0 DISCUSSION

- The gas chromatographic method described herein has a limit of quantitation of 0.02 ppm for thiram.

- In order to eliminate a possibility of CS_2 contamination, reaction vials were rinsed with methanol and baked at 135°C for 45 minutes. Teflon-lined septums were rinsed with methanol and air dried. The use of rubber or plastic utensils was avoided.

  The use of Teflon-lined rubber septums is acceptable for temporary storage of rinsed, baked reaction vials.

- The volume of 4.00 g of matrix is determined by weighing 4.00 g of sample into a 25 mL graduated cylinder and adding 10 mL water. The total volume minus 10 mL is the volume of the 4 g sample. (Note: Better results may be achieved with some matrices if 20 mL of water is used in place of 10 mL.)

- Standard curves are prepared by injection of variable volumes of a single standard preparation at a concentration of 0.1 μg/mL headspace (or other headspace concentrations, as applicable).

- Samples must be kept frozen at all times until addition of extraction/reaction reagents. This includes weighing and fortification (preparation of spikes) processes. Keep samples on dry ice before and after weighing and during fortification.

- Samples must be reacted in a timely manner following addition of reaction reagents. Once reacted, the samples (now containing in the form of CS_2 any thiram that may have been present) may be stored at room temperature overnight. Simply reheat the samples the following day at 100°C for approximately 30 minutes with shaking.

- When the volume of fortification solution added to the reaction vial is less than 1.0 mL, the volume of 10% EDTA added to obtain a final headspace volume of 135 mL is not adjusted to account for the addition of the fortification solution.

  The addition of less than 1.0 mL of solution would account for at most, 0.74% of the total volume of headspace. It is felt by Morse Laboratories, Inc. that this percentage is insignificant and subtracting the fortification solution volume from the volume of 10% EDTA to be added is not necessary.

  However, because volumes of fortification solutions greater than 1.0 mL do approach significance (1.35 mL would equate to a 1.0% error), volumes ≥1.0 mL are accounted for when calculating the volume of 10% EDTA to be added to obtain a final volume headspace of 135 mL.

12.0 METHOD REFERENCES

1. JAOAC, Volume 52, Number 6, Page 1226 (1969)

2. Morse Laboratories, Inc. SOP# Meth-94, original, 06/96, "Determination of Maneb in Soil".

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Preparation of Soil Samples for Thiram

Project #: ML96-0630-UCB

Samples are to be received at Morse Laboratories, Inc. in 0-6", 6-12", 12-18", 18-24", 24-30", 30-36", 36-42" and 42-48" segments. The 0-6" segments that are received will be in cores and all other depths in sample bags (composited by field personnel). Five cores should be received for each 0-6" sample. The actual number of cores received will be recorded.

Procedure:

1. Enter ML ticket number and sample number on form ML 466. Count and record, on ML 466, the number of cores received with each sample, as applicable, and the core length/depth of the sample as received.

2. Prepare untreated samples first, then treated samples. If more than one sampling interval is being prepared at a time, samples that were collected the greatest number of days after treatment (e.g., D10) are prepared before samples collected the least number of days after treatment (e.g., D3). Record date of composite/homogenization, as applicable, on form ML 466.

3. Keep samples on dry ice or in a freezer during the preparation process. Remove soil from cores as applicable and composite samples by placing samples in pre-labeled plastic bags kept on dry ice. Remove any rocks, sticks, or debris at this time.

4. Homogenization of the composited sample is performed using a food cutter (see SOP# SP-14) in the presence of dry ice. Clean the food cutter with soapy water, followed by rinses with tap water and acetone (in that order) between all samples. Homogenized samples will be kept in properly labeled bags in the freezer until removed for analysis.