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Determination of Captan and THPI
Residues in Soil
Chevron Method No. RM-1S-A

File No. 740.01/Captan
Date: February 25, 1987

Morse Laboratories, Inc. Modification (3/26/87) for Captan Task Force

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INTRODUCTION:

Captan [Cis-N-(trichloromethythio)-4-cyclohexene-1,2-dicarboximide] is a fungicide with a wide spectrum activity. 4-Cyclohexene-1,2-dicarboximide (or tetrahydrophthalimide hereafter referred to as THPI) has been shown to be a principal metabolite of captan.

This analytical method determines both the parent compound captan, and the metabolite, THPI. Briefly, it involves extractions with acetone and acid-methanol, and partitioning captan and THPI into dichloromethane. Cleanup and separation of captan and THPI are accomplished using a nuchar:silica gel column. Captan is analyzed using the halogen detector while THPI is analyzed using the nitrogen detector.

REAGENTS:

Acetone - nanograde
Ethyl acetate - nanograde
Methanol - nanograde
Dichloromethane - nanograde
Phosphoric acid - 85%, AR
Nuchar - S-N, Westvaco, Covington, VA
pH 11 Water (pH 10 buffer adjusted to pH 11 with 1N NaOH)
Silica gel - Chromatographic grade, 100-200 mesh, Davison Chemical
Sodium chloride - AR
Sodium Sulfate - anhydrous, reagent grade
Acidic Methanol - 1:1 (v/v) 0.5% Phosphoric acid:Methanol
Captan Reference standard - Stauffer Chemical Lot No. 115-2 (spiking standards in acetone)
THPI Reference standard - Chevron Chemical Co. Lot No. 4898-28 (spiking standards in acetone)

SPECIAL EQUIPMENT: (Note: Use of plastic equipment should be avoided.)

Omni-Mixer with adapter for use with Mason jars.
Rotary vacuum evaporators equipped with 40C water bath for evaporation steps.
Liquid chromatographic columns - 25 x 400 mm equipped with Teflon stopcocks
Centrifuge
Centrifuge bottles, glass, 250 ml capacity
Whatman No. 541 Filter paper, fluted, 18.5 cm.
500 ml separatory funnels

SPECIAL EQUIPMENT: (continued)

Gas Chromatograph (Varian 2100 or equivalent) equipped with a Coulson electrolytic conductivity detector in the halogen mode and the following parameters for captan analysis:

Column A: 3% SP 2401 on Supelcoport, 100/120 mesh, 2' x 1/4" od glass column.

Column B: 10% DC-200 on Chromosorb WHP, 80/100 mesh, 4' x 1/4" od glass column.

	Column A	Column B
Carrier gas (H_2) flow rate:	90 ml/min.	90 ml/min.
Pyrolysis temperature:	810°C	810°C
Oven temperature:	155°C	205°C
Injector temperature:	240°C	240°C
Detector temperature:	—	—
Retention time:	2.4 min.	2.4 min.

Gas Chromatograph (Varian 2100 or equivalent) equipped with Coulson electrolytic conductivity detector in the nitrogen mode and the following parameters for THPI analysis:

Column A: 5% EG SS-X on Gas Chrom Q, 100/120 mesh, 3' x 1/4" od glass column.

Column B: 20% OV-11 on Chrom WHP, 100/120 mesh, 2' x 1/4" od glass column.

Column C: 1% HiEff 8BP on Gas Chrom Q, 100/120 mesh, 2' x 1/4" od glass column.

	Column A	Column B	Column C
Carrier gas (H_2) flow rate:	130 ml/min.	120 ml/min.	140 ml/min.
Pyrolysis temperature:	810°C	810°C	810°C
Oven temperature:	180°C	160°C	145°C
Injector temperature:	240°C	240°C	240°C
Detector temperature:	—	—	—
Retention time:	2.4 min.	2.5 min.	2.4 min.

EXTRACTION:

Transfer a 50 gram sample of soil to a pint Mason jar. (Fortify an untreated sample for recovery purposes with 1.0 ml of an acetone solution containing 5 ug/ml captan plus 1.0 ml of an acetone solution containing 5 ug/ml THPI. If levels are known, fortify soil at the anticipated levels. Evaporate the acetone by means of a stream of air.) Add 150 ml acetone and blend on an Omni-mixer for 5 minutes. (Note: If thorough agitation is not obtained during

EXTRACTION: (continued)

the blending step, additional acetone may be required.) Filter the extract through filter paper into a round bottom flask. Repeat the extraction and filtration step two more times using 100 ml acetone each time.

After the third acetone extraction, extract the soil with 75 ml acidic methanol by blending on an Omni-mixer for 5 minutes. Transfer to centrifuge bottle and centrifuge for 10 minutes at about 2000 rpm. Decant supernatant into an Erlenmeyer flask. Transfer soil back to extraction jar and repeat extraction and centrifugation using 75 ml acidic methanol. Combine the acidic methanol extracts.

Evaporate the acetone extract to dryness. Dissolve the residue in 75 ml of dichloromethane and transfer to a 500 ml separatory funnel. Save the round bottom flask.

Rinse the round bottom flask with the acidic methanol extract and transfer to the separatory funnel. Add 100 ml deionized water and 10 ml of saturated sodium chloride solution.

Dichloromethane Partition:

Shake the mixture vigorously for 1 minute. Filter the dichloromethane extract through sodium sulfate (prewashed with dichloromethane). Extract the aqueous mixture two more times using 75 ml dichloromethane each time. Combine the dichloromethane extracts containing the captan and THPI. Evaporate to 10 ml for nuchar:silica gel column cleanup and separation of captan and THPI.

Nuchar:Silica Gel Column Cleanup and Separation of Captan and THPI:

Place a glass wool plug in the bottom of the column. Add 15 grams of the Nuchar:silica gel (5:95 w/w) mixture and cover the mixture with a glass wool plug. Wash the column with 100 ml 5% ethyl acetate in dichloromethane, followed by 2 x 25 ml dichloromethane rinses.

Dissolve the residue in 10 ml dichloromethane and transfer to the column with two 10 ml dichloromethane rinses. Allow the dichloromethane to drain to the top of the glass wool plug, then add 20 ml dichloromethane and elute. Adjust flow rate to obtain discrete drops of column eluace. Discard the dichloromethane eluates and elute captan residues with 150 ml 5% ethyl acetate in chloromethane. Replace the flask with a second round-bottom flask and elute the THPI with 150 ml 20% acetone in dichloromethane. Evaporate the eluate containing captan to dryness and proceed with the measurement for captan. Evaporate the eluate containing the THPI to dryness and proceed with pH 11 partition for THPI.

Method No. RM-1S-A with Morse Lab. modification (3/26/87)

pH 11 Partition for THPI

Dissolve the residue in the flask with 25 ml pH 11 water. Transfer the aqueous extract to a 125 ml separatory funnel. Rinse the flask with 25 ml dichloromethane, add rinsing to the separatory funnel and shake for one minute. Discard the lower organic layer. Repeat the dichloromethane cleanup one more time. Acidify the aqueous phase with 3 ml concentrated phosphoric acid and extract with 2X 25 ml dichloromethane. Filter the dichloromethane fractions through sodium sulfate into an evaporating flask. Evaporate just to dryness and proceed with THPI measurement.

MEASUREMENT:

Captan:

Dissolve residue in volume of solvent to make a concentration of 10 g/ml. Inject suitable amount of reference standard solutions (0.1, 0.4, 0.8, 1.0 ug/ml captan in hexane) into the gas chromatograph to construct a standard curve. Because captan response is not linear, a "Shipman's Curve" is used to draw line between points. Inject the same volume of each sample and standards. Determine the concentration of the sample solution by comparing the peak height of the sample with standard curve.

THPI:

Dissolve residue in volume of solvent to make a concentration of 10 g/ml. Inject a suitable volume of the reference standard solution (1.0 ug/ml THPI in benzene) to obtain a 10-15 cm peak. Inject the same volume of sample solution. Determine the concentration of the sample solution by comparing the peak height of the sample with that of the standard.

Submitted to Captan Task Force by:

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Determination of Captan and THPI Residues in Soils:
Soil Dissipation Study

Method used for the soil dissipation study:
Chevron Method No. RM-1S-A and modified by Morse Laboratories, Inc. on 3/26/87.

General Outline:

1. Preliminary extraction of a 20 gram sample with acetone and acid methanol.
2. Partition of captan and THPI from the aqueous phase into dichloromethane.
3. Cleanup and separation of captan and THPI using nuchar:silica gel column.
4. pH 11 cleanup of THPI fraction.
5. GC analysis of captan using the halogen detector.
6. GC analysis of THPI using the nitrogen detector.

Modifications (amendments):

A. For all samples:
1. Analysis of a 20 gram sample instead of a 50 gram aliquot.
2. Elimination of the pH 11 cleanup for the THPI fraction (not always indicated on report).
3. All samples were analyzed on "as is basis" and the moisture content determined for each sample.

B. Florida soils for Tomatoes:
1. Florisil column cleanup (Chevron Method No. RM-1S-A) of captan fraction prior to GC analysis of pretreatment samples only.

C. New York soils for Apples:
1. Florisil sep pak cleanup (Morse Lab. revision, July 8, 1987) on captan fraction prior to GC on earlier analyses.
2. Florisil sep pak activity in various lot numbers were found to be unreliable. A change was then made to use florisil column cleanup (Chevron RM-1S-A) instead of florisil sep paks for the cleanup of captan fractions prior to GC analysis. This cleanup was routinely done for the captan fraction cleanup of all New York soils in this study.

D. Oregon soils:
1. One some samples (Morse Lab #44131, soil samples after second & third field applications of captan) the florisil column cleanup (Chevron RM-1K-2) was found necessary for the purification of the captan fraction prior to GC analysis.

E. California soils for Tomatoes:
Except for sample fractions 12-24" soil depth, all other samples from this location required:
1. GPC column cleanup of both captan and THPI fractions.
GPC Conditions:
Instrument: GPC autoprep 1002 (Analytical Bio Chemistry Lab., Inc.)
Column: Glass with teflon fittings, having an internal diameter of 25 mm and a length of 600 mm

Column Packing: Bio Beads S-X3, 200-400 mesh, Bio Rad Laboratories
Mobile Phase: $CHCl_3$
Sample Volume: 5 ml
Flow Rate: 5 ml/min
Parameters for sample cleanup: Discard: 26 minutes
Collect: 10 minutes
Wash: 10 minutes

2. Additional florisil column cleanup (Chevron RM-1S-A) of the captan fraction prior to GC analysis.
3. The florisil minicolumn which was adapted from the florisil column cleanup (Chevron RM-1S-A) was used for a limited period during the early part of the study but was found inadequate for captan extract purification.

F. California soils for Strawberries:

During the early part of the study some samples required florisil column cleanup (Chevron RM-1S-A) prior to GC analysis of the captan fraction. These were noted on the corresponding reports.

G. Texas soils for Melons:

No modifications were necessary for this group of samples.