



1525 Fulton Avenue  
Sacramento, California 95825

TEL: 916-481-3141  
FAX: 916-481-2959

E-MAIL: morseslab@morselabs.com

February 1, 2001

Morse Laboratories, Inc. Method Modifications to ICI Americas, Inc. Report No. WRC 89-51,  
"Determination by Gas Chromatography of Captan and Tetrahydrophthalimide Residues in Raw  
and Processed Agricultural Crops" June 1, 1989

Reasons for Modifications (specific for oily crop matrices):

1. To change the LOQ for both captan and tetrahydrophthalimide (THPI) from 0.05 ppm to 0.02 ppm.
2. To take an aliquot of sample extract, rather than the whole sample, through the cleanup steps.
3. To identify the solvent evaporation apparatus.
4. To incorporate a GPC cleanup.
5. To alter the silica gel cleanup column due to non-availability of the specific Nuchar activated carbon component.
6. To change instrumentation conditions for 1) captan to GC with FPD-S detection employing a Rtx-200 GC column and 2) THPI to GC with MSD detection employing a Rtx-50 GC column.
7. To describe the calculations that will be used.

Modifications to Section:

II. MATERIALS/METHODS

A. Apparatus (add or replace with the following):

1. Gas Chromatographs. Captan: Hewlett-Packard (HP) gas chromatograph Model 6890 equipped with a flame photometric detector in the sulfur mode, an HP6890 Autosampler and an HP G2070AA ChemStation.

Modifications to WRC 89-51  
February 1, 2001  
Page 2

THPI: Hewlett-Packard (HP) gas chromatograph Model 5890A equipped with a HP 5970B mass selective detector, a HP 6890 autosampler and a HP G1701AA MS ChemStation.

2. GC Columns. Captan: 15 M × 0.53 mm i.d. fused silica column crossbonded with 1.0 μm film thickness Rtx-200 (manufacturer: Restek). THPI: 15 M × 0.25 mm i.d. fused silica column crossbonded with 0.25 μm film thickness Rtx-50 (manufacturer: Restek).
3. Evaporator: Rotary evaporator equipped with a Dewar condenser
4. Evaporation flasks: 125 and 500 mL flat bottom, glass
5. Gel Permeation Chromatograph (GPC): O.I. Analytical, Autoprep 1000, column packed with 70 g Envirobeads<sup>®</sup> S-X3 Select.
6. Graduated Mixing Cylinders: Glass, 500 mL.
7. Separatory Funnels: Glass, 250 mL
8. Ultrasonic Bath: Branson Model 2210 (VWR Scientific, Bridgeport, NJ)
9. Assorted laboratory glassware

B. Reagents (add or replace with the following):

1. Solvents. Add toluene, pesticide grade
2. Captan and Tetrahydrophthalimide (THPI). Analytical grade. Lot and purity to be documented in the raw data.
3. Calibration and Fortification Solutions. Prepare calibration solutions in toluene from the working fortification solutions at the typical concentrations of:

Captan: 0.2, 0.15, 0.1 and 0.06 μg/mL

THPI: 0.8, 0.4, 0.2 and 0.06 μg/mL

Modifications to WRC 89-51  
February 1, 2001  
Page 3

C. Analytical Procedure

1. Extraction

Extract the samples as indicated using 3 × 100 mL of ethyl acetate. Collect all filtrates in a 500 mL mixing cylinder. Using ethyl acetate, bring volume to 300 mL and invert to mix.

b. Oily Crops

Using a rotary evaporator at  $\leq 40^{\circ}\text{C}$ , evaporate a 150 mL aliquot (representing 10g of sample) of the ethyl acetate extract in a 500 mL evaporation flask. Conduct partition as directed using a 250 mL separatory funnel. After partition, evaporate combined acetonitrile extracts to near dryness in a 125 mL evaporation flask using a rotary evaporator at  $\leq 40^{\circ}\text{C}$ . Continue evaporation to dryness using manual nitrogen blowdown. Dissolve the residue in ~3 mL of dichloromethane. Sonicate to help dissolve the solids. Transfer the extract to a test tube calibrated at 10.0 mL using 2 × 3 mL rinses. Make to a volume of 10.0 mL with dichloromethane and invert to mix. Proceed with GPC cleanup.

**GPC Cleanup:**

Conduct a GPC cleanup according to the manufacturer's directions and to specifications obtained from fractionation of captan and THPI on a 70 g Envirobead column.

Note: Of the amount of sample extract submitted to GPC cleanup (generally 10 mL), 5 mL ( $\frac{1}{2}$  of the 10 mL of sample extract) is processed by the GPC.

Collect the appropriate fraction, as determined during the fractionation evaluation, from the GPC in a 125 mL evaporation flask. Concentrate the extract using a rotary evaporator at  $\leq 40^{\circ}\text{C}$  to approximately 10 mL. Proceed with Column Cleanup and Separation.

2. Column Cleanup and Separation

Prepare column with 15 g of silica gel only (do not incorporate Nuchar). After evaporation to dryness for both captan and THPI, add 1.0 mL of toluene to the flask, stopper, then swirl and/or sonicate to dissolve the residue. Transfer the concentrate to a 13 × 100 mm screw cap test tube and submit to GC analysis. 1 mL = 5.0 g

Note: Omit pH 11 cleanup for the THPI extracts.

Modifications to WRC 89-51  
February 1, 2001  
Page 4

D. Instrumentation

1. Operating Conditions.

**Note:** The column and conditions have been satisfactory for the matrices being analyzed. The specific column packing/coating, carrier gas, column temperature and flow rate listed are typical conditions for this analysis. Alternate columns may be used depending on the need to resolve analyte and/or interfering responses. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

Captan:

Instrument: Hewlett-Packard (HP) gas chromatograph Model 6890 Series II equipped with a flame photometric detector in the sulfur mode, an HP6890 Autosampler and an HPG2070AA ChemStation.

Inlet design: "Purged/packed" recommended. "Capillary split-splitless" is **not** recommended. When set up to simulate a purged packed inlet, a capillary split-splitless inlet is often operated with the purge valve off over the course of the injection which, with megabore columns, tends to cause non-reproducible injections as well as non-linearity.

GC analytical column: 15 M × 0.53 mm i.d. fused silica column crossbonded with 1.0 μm film thickness Rtx-200

GC guard column: 3 M × 0.53 mm i.d. nonpolar dimethyl deactivated fused (Manufacturer: Restek)

Inlet liner: 2 mm glass sleeve lightly packed with fused silica wool

Injection volume: 5 μL

Temps: Injector: 190°C

Detector: 200°C

Modifications to WRC 89-51  
February 1, 2001  
Page 5

Column: Initial: 130°C  
Rate: 25°C/min.  
Final: 230°C and hold for 2.00  
minutes.

Helium carrier  
gas flow: 20 mL/min.

Helium make-  
up gas flow: 70 mL/min.

Retention  
time: ~3.6 minutes

Run time: ~10 minutes

THPI:

Instrument: Hewlett-Packard (HP) gas chromatograph Model 5890A  
equipped with a HP 5970B mass selective detector, a HP  
6890 autosampler, and a HP G1701AA MS ChemStation.

Column: 15 M × 0.25 mm i.d. fused silica column crossbonded with  
0.25 μm film thickness Rtx-50

Inlet liner: 4 mm i.d. gooseneck splitless liner packed with Carbo Frit™  
(manufacturer: Restek)

Injection  
Volume: 2 μL

Carrier Gas: Helium

Column Head  
Pressure: 8 psi

Purge Flow  
Timing: on at 0.80 minutes

Tuning: Prior to analysis, the instrument is tuned manually for ions  
m/e 131 and 219.

Ion  
Monitored: THPI m/e 151.

Modifications to WRC 89-51  
February 1, 2001  
Page 6

Dwell Time: 45 msec

Temperatures:

Injector: 240°C

GC/MSD

Transfer line: 300°C

Column:

Initial: 100°C, hold 1.00 minute

Rate: 20°C/minute

Final: 300°C, hold 2.00 minutes

Retention

Time: approximately 4.7 minutes

## 2. Analysis of Extracts

For each analyte, prepare a four-point standard curve by injecting constant volumes of applicable analyte standard solutions. Use constant volume injections for sample extracts as well. Sample responses greater than those produced by the highest concentration of applicable standard in the standard curve require dilution and reinjection. Inject a curve check standard every 4-5 sample injections.

### G. Calculations

#### Captan:

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on non-linear regression. The data points for captan concentration and peak response are obtained from the chromatograms. All standards injected for the standard curve and their corresponding peak responses are entered into the program.

A power curve 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 response is:

$$y = ax^b$$

where:

y = peak response

Modifications to WRC 89-51  
February 1, 2001  
Page 7

x =  $\mu\text{g/mL}$  found for peak of interest

a,b = variables dependent on data points entered

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

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

$$\text{ppm} = \mu\text{g/mL found} \times \frac{\text{final vol. (mL)}}{\text{sample wt. (g)}} \times \frac{\text{mL ext solv}}{\text{mL aliq}} \times \text{GPC dil. factor} \times \text{GC dil. factor}$$

where:

$\mu\text{g/mL found}$  =  $\mu\text{g/mL}$  of analyte found

final vol. (mL) = volume of final extract submitted to GC

sample wt. (g) = gram weight of sample extracted

mL ext. solv. = volume of extraction solvent

mL aliq. = volume of sample extract taken through acetonitrile/hexane solvent partition

GPC dil. factor = designated as "2"; represents a 5 mL aliquot from a 10 mL extract volume utilized by the GPC

GC dil. factor = dilution of sample extract required to produce an analyte response bracketed by standards

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

$$\% \text{ Rec.} = \frac{\text{ppm found in fortified control (spike)} - \text{ppm found in control}}{\text{fortification level (ppm) added}} \times 100$$

Modifications to WRC 89-51  
 February 1, 2001  
 Page 8

THPI:

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on linear regression. These regression functions are used to calculate a best fit line (from a set of standard concentrations in  $\mu\text{g/mL}$  versus peak response) and to determine concentrations of the analyte found during sample analysis from the calculated best fit line.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response
x	=	$\mu\text{g/mL}$ found for peak of interest
m	=	slope
b	=	y-intercept

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

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

$$\text{ppm} = \mu\text{g/mL found} \times \frac{\text{final vol. (mL)}}{\text{sample wt. (g)}} \times \frac{\text{mL ext solv}}{\text{mL aliq}} \times \text{GPC dil. factor} \times \text{GC dil. factor}$$

where:

$\mu\text{g/mL}$ found	=	$\mu\text{g/mL}$ of analyte found
final vol. (mL)	=	volume of final extract submitted to GC
sample wt. (g)	=	gram weight of sample extracted
mL ext. solv.	=	volume of extraction solvent
mL aliq.	=	volume of sample extract taken through acetonitrile/hexane solvent partition
GPC dil. factor	=	designated as "2"; represents a 5 mL aliquot

Modifications to WRC 89-51  
February 1, 2001  
Page 9

GPC dil. factor = designated as "2"; represents a 5 mL aliquot from a 10 mL extract volume utilized by the GPC

GC dil. factor = dilution of sample extract required to produce an analyte response bracketed by standards

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

$$\% \text{ Rec.} = \frac{\text{ppm found in fortified control (spike)} - \text{ppm found in control}}{\text{fortification level (ppm) added}} \times 100$$



1525 Fulton Avenue  
Sacramento, California 95825

TEL: 916-481-3141  
FAX: 916-481-2959

E-MAIL: morselab@morselabs.com

April 11, 2001

Morse Laboratories, Inc. Method Modifications to ICI Americas, Inc. Report No. WRC 89-51,  
"Determination by Gas Chromatography of Captan and Tetrahydrophthalimide Residues in Raw  
and Processed Agricultural Crops" June 1, 1989

Reasons for Modifications (specific for peppers: bell and non-bell):

1. To change the LOQ for both captan and tetrahydrophthalimide (THPI) from 0.05 ppm to 0.02 ppm.
2. To take an aliquot of sample extract, rather than the whole sample, through the cleanup steps.
3. To identify the solvent evaporation apparatus.
4. To incorporate a GPC cleanup.
5. To alter the silica gel cleanup column due to non-availability of the specific Nuchar activated carbon component.
6. To change instrumentation conditions for 1) captan to GC with FPD-S detection employing a Rtx-200 GC column and 2) THPI to GC with MSD detection employing a Rtx-50 GC column.
7. To describe the calculations that will be used.
8. To add keeper prior to evaporation for GPC.

Modifications to Section:

II. MATERIALS/METHODS

A. Apparatus (add or replace with the following):

1. Gas Chromatographs. Captan: Hewlett-Packard (HP) gas chromatograph Model 6890 equipped with a flame photometric detector in the sulfur mode, an HP6890 Autosampler and an HP G2070.AA ChemStation.

THPI: Hewlett-Packard (HP) gas chromatograph Model 5890A equipped with a HP 5970B mass selective detector, a HP 6890 autosampler and a HP G1701AA MS ChemStation.

2. GC Columns. Captan: 15 M × 0.53 mm i.d. fused silica column crossbonded with 1.0 μm film thickness Rtx-200 (manufacturer: Restek).  
THPI: 15 M × 0.25 mm i.d. fused silica column crossbonded with 0.25 μm film thickness Rtx-50 (manufacturer: Restek).
3. Evaporator: Rotary evaporator equipped with a Dewar condenser
4. Evaporation flasks: 500 mL flat bottom, glass
5. Gel Permeation Chromatograph (GPC): O.I. Analytical, Autoprep 1000, column packed with 70 g Envirobeads<sup>®</sup> S-X3 Select.
6. Graduated Mixing Cylinders: Glass, 500 mL
7. Separatory Funnels: Glass, 250 mL
8. Ultrasonic Bath: Branson Model 2210 (VWR Scientific, Bridgeport, NJ)
9. Assorted laboratory glassware

B. Reagents (add or replace with the following):

1. Solvents. Add toluene, pesticide grade
2. Captan and Tetrahydrophthalimide (THPI). Analytical grade. Lot and purity to be documented in the raw data.
3. Calibration and Fortification Solutions. Prepare calibration solutions in toluene from the working fortification solutions at the typical concentrations of:

Captan: 0.2, 0.15, 0.1 and 0.06 μg/mL

THPI: 0.8, 0.4, 0.2 and 0.06 μg/mL

C. Analytical Procedure

1. Extraction

Extract the samples as indicated using  $3 \times 100$  mL of ethyl acetate. Collect all filtrates in a 500 mL mixing cylinder. Using ethyl acetate, bring volume to 300 mL and invert to mix.

a. Non-oily Crops

Take a 150 mL aliquot (representing 10 g of sample) of the ethyl acetate extract and conduct wash as directed using a 250 mL separatory funnel, and using only 25 mL of 1% aqueous phosphoric acid per wash. After washes, pass ethyl acetate through anhydrous sodium sulfate and into a 500 mL round bottom flask. Add 3 drops of keeper (10% decanol in acetone) and evaporate the ethyl acetate extract to near dryness using a rotary evaporator at  $\leq 40$  °C. Continue evaporation to dryness using manual nitrogen blowdown. Dissolve the residue in  $\sim 3$  mL of dichloromethane. Sonicate to help dissolve the solids. Transfer the extract to a test tube calibrated at 10.0 mL using  $2 \times 3$  mL dichloromethane rinses. Make to a volume of 10.0 mL with dichloromethane and invert to mix. Proceed with GPC cleanup.

**GPC Cleanup:**

Conduct a GPC cleanup according to the manufacturer's directions and to specifications obtained from fractionation of captan and THPI on a 70 g Envirobead column.

Note: Of the amount of sample extract submitted to GPC cleanup (generally 10 mL), 5 mL ( $\frac{1}{2}$  of the 10 mL of sample extract) is processed by the GPC.

Collect the appropriate fraction, as determined during the fractionation evaluation, from the GPC in a 125 mL evaporation flask. Concentrate the extract using a rotary evaporator at  $\leq 40$ °C to approximately 10 mL. Proceed with Column Cleanup and Separation.

2. Column Cleanup and Separation

Prepare column with 15 g of silica gel only (do not incorporate Nuchar). After evaporation to dryness for both captan and THPI, add 1.0 mL of toluene to the flask, stopper, then swirl and/or sonicate to dissolve the

residue. Transfer the concentrate to a 13 × 100 mm screw cap test tube and submit to GC analysis. 1 mL = 5.0 g  
Note: Omit pH 11 cleanup for the THPI extracts.

D. Instrumentation

1. Operating Conditions.

Note: The column and conditions have been satisfactory for the matrices being analyzed. The specific column packing/coating, carrier gas, column temperature and flow rate listed are typical conditions for this analysis. Alternate columns may be used depending on the need to resolve analyte and/or interfering responses. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

Caption:

Instrument: Hewlett-Packard (HP) gas chromatograph Model 6890 Series II equipped with a flame photometric detector in the sulfur mode, an HP6890 Autosampler and an HPG2070AA ChemStation.

Inlet design: "Purged/packed" recommended. "Capillary split-splitless" is **not** recommended. When set up to simulate a purged packed inlet, a capillary split-splitless inlet is often operated with the purge valve off over the course of the injection which, with megabore columns, tends to cause non-reproducible injections as well as non-linearity.

GC analytical

column: 15 M × 0.53 mm i.d. fused silica column crossbonded with 1.0 μm film thickness Rtx-200

GC guard

column: 3 M × 0.53 mm i.d. nonpolar dimethyl deactivated fused (Manufacturer: Restek)

Inlet liner: 2 mm glass sleeve lightly packed with fused silica wool

Injection

volume: 5 μL

Temps: Injector: 190°C

Detector: 200°C

Column: Initial: 130°C  
Rate: 25°C/min.  
Final: 230°C and hold for 2.00  
minutes.

Helium carrier  
gas flow: 20 mL/min.

Helium make-  
up gas flow: 70 mL/min.

Retention  
time: ~3.6 minutes

Run time: ~10 minutes

THPI:

Instrument: Hewlett-Packard (HP) gas chromatograph Model 5890A  
equipped with a HP 5970B mass selective detector, a HP  
6890 autosampler, and a HP G1701AA MS ChemStation.

Column: 15 M × 0.25 mm i.d. fused silica column crossbonded with  
0.25 μm film thickness Rtx-50

Inlet liner: 4 mm i.d. gooseneck splitless liner packed with Carbo Frit™  
(manufacturer: Restek)

Injection  
Volume: 2 μL

Carrier Gas: Helium

Column Head  
Pressure: 8 psi

Purge Flow  
Timing: on at 0.80 minutes

Tuning: Prior to analysis, the instrument is tuned manually for ions  
m/e 131 and 219.

Ion  
Monitored: THPI m/e 151.

Dwell Time: 45 msec

Temperatures:

Injector: 240°C

GC/MSD

Transfer line: 300°C

Column:

Initial: 100°C, hold 1.00 minute

Rate: 20°C/minute

Final: 300°C, hold 2.00 minutes

Retention

Time: approximately 4.7 minutes

## 2. Analysis of Extracts

For each analyte, prepare a four-point standard curve by injecting constant volumes of applicable analyte standard solutions. Use constant volume injections for sample extracts as well. Sample responses greater than those produced by the highest concentration of applicable standard in the standard curve require dilution and reinjection. Inject a curve check standard every 4-5 sample injections.

### G. Calculations

#### Captan:

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on non-linear regression. The data points for captan concentration and peak response are obtained from the chromatograms. All standards injected for the standard curve and their corresponding peak responses are entered into the program.

A power curve 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 response is:

$$y = ax^b$$

where:

- y = peak response  
x =  $\mu\text{g/mL}$  found for peak of interest  
a,b = variables dependent on data points entered

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

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

$$\text{ppm} = \mu\text{g/mL found} \times \frac{\text{final vol. (mL)}}{\text{sample wt. (g)}} \times \frac{\text{mL ext solv}}{\text{mL aliq}} \times \text{GPC dil. factor} \times \text{GC dil. factor}$$

where:

- $\mu\text{g/mL found}$  =  $\mu\text{g/mL}$  of analyte found  
final vol. (mL) = volume of final extract submitted to GC  
sample wt. (g) = gram weight of sample extracted  
mL ext. solv. = volume of extraction solvent  
mL aliq. = volume of sample extract taken through acetonitrile/hexane solvent partition  
GPC dil. factor = designated as "2"; represents a 5 mL aliquot from a 10 mL extract volume utilized by the GPC  
GC dil. factor = dilution of sample extract required to produce an analyte response bracketed by standards

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

$$\% \text{ Rec.} = \frac{\text{ppm found in fortified control (spike)} - \text{ppm found in control}}{\text{fortification level (ppm) added}} \times 100$$

THPI:

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on linear regression. These regression functions are used to calculate a best fit line (from a set of standard concentrations in  $\mu\text{g/mL}$  versus peak response) and to determine concentrations of the analyte found during sample analysis from the calculated best fit line.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response
x	=	$\mu\text{g/mL}$ found for peak of interest
m	=	slope
b	=	y-intercept

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

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

$$\text{ppm} = \mu\text{g/mL found} \times \frac{\text{final vol. (mL)}}{\text{sample wt. (g)}} \times \frac{\text{mL ext solv}}{\text{mL aliq}} \times \text{GPC dil. factor} \times \text{GC dil. factor}$$

where:

$\mu\text{g/mL found}$	=	$\mu\text{g/mL}$ of analyte found
final vol. (mL)	=	volume of final extract submitted to GC

sample wt. (g)	=	gram weight of sample extracted
mL ext. solv.	=	volume of extraction solvent
mL aliq.	=	volume of sample extract taken through acetonitrile/hexane solvent partition
GPC dil. factor	=	designated as "2"; represents a 5 mL aliquot from a 10 mL extract volume utilized by the GPC
GC dil. factor	=	dilution of sample extract required to produce an analyte response bracketed by standards

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

$$\% \text{ Rec.} = \frac{\text{ppm found in fortified control (spike)} - \text{ppm found in control}}{\text{fortification level (ppm) added}} \times 100$$