1. DESCRIPTION OF ANALYTICAL METHOD

Method Identification Number: GRM 94.18

Title of Method: Determination of Residues of *cis*- and *trans*-3-Chloroallyl Alcohol in Soil by Capillary Gas Chromatography with Mass Selective Detection

Scope of Method: This method is applicable for the quantitation of residues of *cis*- and *trans*-3-chloroallyl alcohol in soil by capillary gas chromatography/mass spectrometry (GC/MS) following derivatization. The validated lower limit of quantitation (LOQ) is 0.42 ng/g as described by the Sponsor.

Identification of test substance used: Name:	*cis*-3-Chloroallyl Alcohol
TSN Number:	AGR164303
% Purity:	95.1%
Reference:	FA & PC 950175
Date:	December 11, 1995
Re-certification Date:	December 11, 1997

Identification of test substance used: Name:	*trans*-3-Chloroallyl Alcohol
TSN Number:	AGR159855
% Purity:	96.9%
Reference:	FA & PC 950174
Date:	December 11, 1995
Re-certification Date:	December 11, 1997

METHOD OUTLINE

RESIDUE DETERMINATION METHOD: GRM 94.18

INDEPENDENT LABORATORY VALIDATION OF METHOD GRM 94.18 - DETERMINATION OF RESIDUES OF *CIS*- AND *TRANS*-3-CHLOROALLYL ALCOHOL IN SOIL BY CAPILLARY GAS CHROMATOGRAPHY WITH MASS SELECTIVE DETECTION

Rinse all reusable glassware with 0.01 N HCl followed by acetone prior to use.

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Weigh 10.0 g of control soil into 45-mL labelled glass vials.

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Fortify soil as appropriate.

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Add 15.0 mL of 0.01 N HCl to each vial and seal with a PTFE-lined cap. Vortex the samples briefly and place in a ultrasonic bath for 10-15 seconds. Shake the samples for at least 30 minutes at approximately 180 excursions/minute.

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Centrifuge samples for 10 minutes at 2500 rpm.

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Carefully decant the extracts into clean 45-mL vials.

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Extract soil again with 15.0 mL of 0.01 N HCl, vortexing, sonicating, shaking for at least 30 minutes, and centrifuging at 2500 rpm for 10 minutes.

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Combine the extracts.

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Place an ion-exchange (quaternary amine) SPE column on the vacuum manifold box. Attach a glass fiber Acrodisc (prefilter) to the top of the column using a SPE column adapter. Attach a 25-mL reservoir to the prefilter.

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Rinse the column and reservoir with approximately 5 mL of methanol (do not allow column bed to dry). Condition with approximately 5 mL deionized water (do not allow the column bed to dry).

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Transfer the extract to the reservoir. With the aid of vacuum, pull the extract through the column at a flow rate of approximately 2 mL/minute, collecting the eluent in a 45-mL vial.

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METHOD OUTLINE (Continued)

Rinse the sample vial with 3 mL of deionized water and transfer the rinse to the reservoir. Pass through SPE column at approximately 2 mL/minute, collecting the eluent in the same 45-mL vial. Rinse the sample vial with a second 3 mL of deionized water (allow the first 3 mL rinse to pass through the column before adding the second rinse).

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Transfer the combined eluents to a 2-oz glass bottle. Rinse the 45-mL vial with approximately 2 mL of deionized water and add to the bottle.

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Add 10 μL of 1-propanol, approximately 15 g of NaCl, 15 mL of MTBE and seal bottle with a PTFE-lined cap. Shake for 15 minutes at approximately 180 excursions/minute. Centrifuge for 3 minutes at 1000 rpm.

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Place a silica gel SPE column on the vacuum manifold box. Add approximately 2 g of magnesium sulfate (anhydrous) to the SPE column. Attach a 25 mL reservoir to the top of the column using a SPE column adapter. Wash (condition) the column with 10 mL of MTBE, pulling the MTBE through the column with the aid of vacuum.

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Transfer the MTBE layer (top layer - from previous extraction) to the silica gel SPE column. Pull the sample through the column at a flow rate of approximately 2 mL/minute, collecting the MTBE in a 45-mL vial.

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Add another 15 mL of MTBE to the vial (containing the aqueous phase), shake for 5 minutes and centrifuge for 3 minutes at 1000 rpm. Transfer the MTBE layer to the silica gel SPE column. With the aid of vacuum, pull the MTBE through the column at a flow rate of approximately 2 mL/minute, collecting the eluent in the 45-mL vial.

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Add approximately 5 mL of MTBE to the reservoir and pull through the column at approximately 2 mL/min. collecting the eluent in the 45-mL vial.

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Quantitatively transfer the MTBE to a 50-mL Erlenmeyer flask, rinsing the 45-mL vial with approximately 2 mL MTBE.

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Add approximately 3 mL of hexane and approximately 0.1 g of sodium sulfate (anhydrous) to the flask. Attach a Snyder column to the flask. In a fume hood, heat the flask on a hot plate to a steady boil.

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Allow the sample to concentrate to near dryness (**do not allow sample to go to complete dryness**).

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METHOD OUTLINE (Continued)

Remove the flask from the hot plate, add approximately 1 mL of hexane to the flask through the top of the Snyder column and allow the flask to equilibrate to room temperature.

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Remove the Snyder column and quantitatively transfer the sample to an 8-mL vial. Rinse the flask twice with 1 mL MTBE, transferring each rinse to the 8-mL vial.

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Concentrate the sample at room temperature on a Multivap Analytical evaporator under a **gentle** flow of nitrogen to a volume of approximately 0.5 mL. **Do not allow the volume to go significantly below 0.5 mL or loss of analyte will occur.**

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Adjust the volume to 1.0 mL with hexane by visual comparison to two 8-mL vials containing a measured volume of 1.0 mL hexane. Add approximately 0.1 g anhydrous sodium sulfate.

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Transfer 1.0 mL of each of the calibration standards into 8-mL vials. Add 25 μL of pyridine and 25 μl of isobutyl chloroformate (to samples and standards). Seal the vial with PTFE-lined cap, and vortex and sonicate the samples for 5 seconds.

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Heat the samples and standards at 70°C for 15 minutes. Remove from heat and allow to cool the room temperature.

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Add 1.0 mL of 0.1 N hydrochloric acid and vortex each vial for 5 seconds.

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Centrifuge vials for 5 minutes at 2500 rpm.

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Transfer the top hexane layer to a 2-mL autosampler vial and seal.

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Analyze by GC/MSD

2. CALCULATIONS AND STATISTICAL METHODS

Calculations:

Standard Curves: The standard curves were prepared by plotting the equivalent soil sample concentration (ng/g) on the abscissa and the *cis*- and *trans*-3-chloroallyl isobutyl carbonates (CAIBC) *m/z* 136 peak area on the ordinate. Power regression analysis was applied to the data to determine the equation with respect to the abscissa.

For example, using the data from Figure 1:

$$Y = \text{constant} \times X^{(\text{exponent})}$$

$$X = \left(\frac{Y}{\text{constant}}\right)^{1/\text{exponent}}$$

$$X = \left(\frac{Y}{471.5121}\right)^{1/1.0390}$$

Recovery Sample Concentration: The net concentration in each recovery sample was determined by first subtracting the average *cis*- and *trans*-CAIBC *m/z* 136 peak area in the control sample from that of the recovery sample.

For example, using the data from Figures 4 and 5:

$$\underset{(ng/g)}{\textit{cis}\text{-CAAL Conc.}} = \left(\frac{\text{net } \textit{cis} \text{ - CAIBC peak area}}{471.5121}\right)^{1/1.0390}$$

$$\underset{(ng/g)}{\textit{cis}\text{-CAAL Conc.}} = \left(\frac{172 - 0}{471.5121}\right)^{1/1.0390}$$

cis-CAAL Conc. = 0.3788 ng/g

Confirmation Ratios: Confirmation ratios were calculated for each calibration standard and sample analyzed. Confirmation ratios were determined for both the *cis*- and the *trans*-3-chloroallyl alcohol by dividing the peak area of the quantitation ion by the peak area of the confirmation ion.

For example, using the data from Figure 3:

$$\text{Confirmation Ratio} = \frac{\text{peak area of quantitation ion}}{\text{peak area of confirmation ion}}$$

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 136}}{\text{peak area at } m/z \text{ 75}}$$

Confirmation Ratio = $\frac{85}{447}$

Confirmation Ratio = 0.1902

Confirmation of the presence of *cis*- and *trans*-3-chloroallyl alcohol was achieved when the confirmation ratio for the samples were in the range of ± 20% of the average found for the calibration standards.

Percent Recovery: The percent recovery was determined by dividing the net analyte concentration found in the sample by the theoretical concentration added.

$$\%\ Recovery = \frac{Concentration\ Found}{Concentration\ Added}\ X\ 100$$

For example, using the data from Figure 5:

$$\%\ Recovery = \frac{0.3788\ ng/g}{0.42\ ng/g}\ X\ 100$$

% Recovery = 90%

Statistical Methods:

Average recoveries for each analyte in the matrix were calculated by dividing the sum of the percent recoveries by the total number of fortified samples.

Standard deviations for each analyte in the matrix were also determined. The standard deviation was calculated by summing the squares of the individual deviations from the average recoveries, dividing by the number of degrees of freedom, and extracting the square root of the quotient.

3. FULL DESCRIPTION OF ANALYTICAL INSTRUMENTATION USED

Instrument:	Hewlett-Packard Model 5890 Series II Gas Chromatograph (GC) equipped with an Hewlett Packard 7673A Autosampler and a Model G1034B Chemstation
Detector:	Hewlett-Packard 5971A Mass Selective Detector (MSD) in the SIM mode
Analytical Column:	J & W Scientific DB-17 Capillary Column (20 m X 0.18 mm i.d., 0.3 μm film thickness)
Temperatures:	
Column:	65°C for 1.0 minutes 65° to 150°C at 5°C/minute 150° to 260°C at 20°C/minute
Injector:	230°C
Transfer Line:	280°C
Carrier Gas:	Helium
Head Pressure:	100 kPa
Purge Time:	0.7 minute
Injection Volume:	2 μL
Injection Mode	Splitless