Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Dichloropropene Degradate

MRID #: 445365-01

Matrix: Soil

Analysis: GC/MS

GRM.: 94.18
EFFECTIVE: March 15, 1995
SUPERSEDES: New

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

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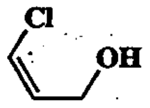
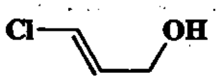
A. Scope

This method is applicable for the quantitation of residues of the 1,3-dichloropropene metabolites, *cis*- and *trans*-3-chloroallyl alcohol (CAAL) in soil. The method was validated over the concentration range 0.42 ng/g to 2.1 µg/g with a limit of quantitation of 0.42 ng/g.



cis-3-Chloroallyl Alcohol
CAS 4643-05-4



trans-3-Chloroallyl Alcohol
CAS 4643-06-5

B. Principle

CAAL residues in soil are extracted with 0.01 N hydrochloric acid and the acid extract is purified by passing through an ion-exchange solid phase extraction (SPE) column. CAAL residues are partitioned from the acid extract into methyl-*t*-butyl ether (MTBE). The MTBE is dried and purified by passing over anhydrous magnesium sulfate and through a silica gel SPE column. Hexane is added and the sample is concentrated using a Snyder distillation column. The sample is further concentrated under nitrogen and brought to a final volume of 1 mL with hexane. CAAL residues in hexane are derivatized with isobutyl chloroformate in the presence of pyridine to their corresponding *cis*- and *trans*-3-chloroallyl isobutyl carbonates (CAIBC) and analyzed by capillary gas chromatography with mass selective detection (GC/MSD). Soils indicating levels of CAAL above approximately 40 ng/g are diluted 100-fold with hexane and reanalyzed.

C. Safety Precautions

1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS; LITERATURE, AND OTHER RELATED DATA. Safety information on non-DowElanco products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.
2. Acetone, hexane, methanol, MTBE and 1-propanol are flammable and should be used in well-ventilated areas away from ignition sources.
3. *cis*- and *trans*-3-Chloroallyl alcohol are corrosive and lachrymators. It is imperative that proper eye and personal protection equipment be used when handling these compounds. Handling of neat material should be carried out in a fume hood.
4. Isobutyl chloroformate is highly toxic, irritating to eyes, respiratory system and skin. It is imperative that proper eye and personal protection equipment be used when handling this reagent. Handling of neat material should be carried out in a fume hood.

D. Equipment (Note N.1.)

1. Automatic sampler, Model 7673, Hewlett-Packard, Wilmington, DE 19808.
2. Balance, analytical, Model AE200, Mettler Instrument Corporation, Hightstown, NJ 08520.
3. Balance, pan, Model BB2440, Mettler Instrument Corporation.
4. Centrifuge, with rotor to accomodate 8-mL vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.
5. Centrifuge, with rotor to accomodate 2-oz bottles, Model CU-5000, International Equipment Company.
6. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549.
7. Gas chromatograph, Model 5890 Series II, Hewlett-Packard.
8. Heater, dry bath incubator, catalog number 11-718-2, Fisher Scientific, Pittsburgh, PA 15219.
9. Heater, dry bath incubator (aluminum) heating block, catalog number 11-718-16, Fisher Scientific.
10. Hot plate, Thermolyne extra-capacity hotplate, catalog number 11-496-5A, Fisher Scientific.
11. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.
12. Mass selective detector data system, Model G1034B, Hewlett-Packard.

13. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.
14. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.
15. Vacuum manifold box, Model spe-21, J.T. Baker, Inc., Phillipsburg, NJ 08865.
16. Vial crimper, catalog number 8710-0979, Hewlett-Packard, Wilmington, DE 19808.
17. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.
18. Water purification system, Model Milli-Q UV Plus, Millipore Corporation, Milford, MA 01757.

E. <u>Glassware and Materials</u> (Note N.1.)

1. Bottle, 2 oz, round, wide-mouth, clear, with PTFE-lined screw caps, catalog number 03-320-11C, Fisher Scientific, Pittsburgh, PA 15219.
2. Column, capillary gas chromatography, Durabond-17 liquid phase, 20 m x 0.18 mm i.d., 0.3 µm film thickness, catalog number 121-1723, J&W Scientific, Folsom, CA 95630.
3. Column, silica gel SPE, catalog number 7086-07, J.T. Baker, Inc.
4. Column, strong anion-exchange (quaternary amine) SPE, catalog number 7091-03, J.T. Baker, Inc.
5. Column adapter, PTFE, catalog number 120-1100, Jones Chromatography, Inc., Lakewood, CO 80228.
6. Column inlet liner, deactivated, catalog number 5181-3315, Hewlett-Packard.
7. Column reservoir, 25 mL, catalog number 71213-1011, Varian Sample Preparation Products, Harbor City, CA 90710.
8. Erlenmeyer flask, 50 mL, 19/22 joint, catalog number 296510-0050, Kontes, Vineland, NJ 08360.
9. Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869. (Note N.2.)
10. Filter, moisture, catalog number 7971, Chrompack, Inc. (Note N.2.)
11. Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note N.2.)
12. Gas, helium, 99.995% purity, Airco, Murray Hill, NJ 07974.
13. Gas, nitrogen, 99.99% purity, Airco.
14. Microdispenser, 10 µL and 25 µL, Drummond Dialamatic Microdispenser, catalog numbers 300210 and 300225, Drummond Scientific Company, Broomall, PA 19008.

15. Microdispenser replacement bore, 10 µL and 25 µL, catalog numbers 300210G and 300225G, Drummond Scientific Company.
16. Micro Snyder distilling column, 19/22 joint, catalog number 569001-0319, Kontes.
17. Prefilter, glass fiber Acrodisc, catalog number 09-730-195, Fisher Scientific.
18. Syringe, 100 and 500 µL capacity, catalog numbers 80600 and 80800, Hamilton Co., Reno, NV89520.
19. Vial, 8 mL, with PTFE-lined screw cap, catalog number B7800-3, National Scientific Company, Lawrenceville, GA 30243.
20. Vial, 45 mL, with PTFE-lined screw cap, catalog number 60958A-11, Kimble Glass, Vineland, NJ 08360.
21. Vial, autosampler, 2 mL, catalog number C4011-1, National Scientific Company.
22. Vial seal, catalog number C4011-1A, National Scientific Company.

F. <u>Reagents and Chemicals</u> (Note N.1.)

1. <u>Reagents</u>

   a. Acetone, Optima grade, catalog number A929-4, Fisher Scientific, Pittsburgh, PA 15219.
   b. Hexane, Optima grade, catalog number H303-4, Fisher Scientific.
   c. Hydrochloric acid, 0.1 N, ACS reagent grade, certified concentration, catalog number SA54-4, Fisher Scientific.
   d. Isobutyl chloroformate, 98%, catalog number 17798-9, Aldrich Chemical Company, Milwaukee, WI 53233.
   e. Magnesium sulfate (anhydrous), Certified, catalog number M65-500, Fisher Scientific.
   f. Methanol, HPLC grade, catalog number A452-4, Fisher Scientific.
   g. Methyl-*t*-butyl ether, HPLC grade, catalog number E127-4, Fisher Scientific.
   h. 1-Propanol, 99.5+%, HPLC grade, catalog number 29,328-8, Aldrich Chemical Company.
   i. Pyridine, HPLC grade, catalog number 27040-7, Aldrich Chemical Company.
   j. Sodium chloride, ACS reagent grade, catalog number S271-1, Fisher Scientific.
   k. Sodium sulfate (anhydrous), certified ACS grade, catalog number S421-500, Fisher Scientific.

k. Standards

(1) *cis*-3-Chloroallyl alcohol

The *cis*-CAAL standard, AGR164303, Lot Number GHC 0083-27, with a purity of 95.1% was used in this study (1).

(2) *trans*-3-Chloroallyl alcohol

The *trans*-CAAL standard, AGR159855, Lot Number GHC-2-12-119, with a purity of 94.8% was used in this study (2).

Obtain from Test Substance Coordinator, DowElanco, Indianapolis, IN 46268-1053.

2. Prepared Solutions

a. 0.01 N Hydrochloric acid solution

Pipet 100 mL of 0.1 N hydrochloric acid into a 1000-mL volumetric flask and dilute to volume with deionized water.

G. Preparation of Standards

All solutions prepared in Section G should be stored in amber bottles and sealed with PTFE-lined caps.

1. Preparation of *cis*- and *trans*-CAAL Stock Solutions

cis- and *trans*-CAAL are volatile liquids and pose some difficulty in weighing to a specific value. The following procedure is meant as a guideline to stress that the analyte be accurately weighed and recorded to four significant figures. Based upon an average density of 1.17 g/mL (3), a 100-µL syringe was used to deliver 86 µL of *cis*- or *trans*-CAAL in the preparation of stock solutions.

a. Tare a 100-mL volumetric flask and scintered glass stopper. Deliver 86 µL of *cis*-CAAL to the flask and stopper the flask. Weigh and record the amount of *cis*-CAAL in the flask. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

b. Tare a 100-mL volumetric flask and scintered glass stopper. Deliver 86 µL of *trans*-CAAL to the flask and stopper the flask. Weigh and record the amount of *trans*-CAAL in the flask. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

2. Preparation of *cis*- and *trans*-CAAL Spiking Solutions

a. Transfer 1.0 mL of each of the stock solutions in Sections G.1.a. and G.1.b. to a 100-mL volumetric flask and bring to volume with acetone to obtain an initial solution of 10.0 µg/mL for each *cis*- and *trans*-CAAL. This solution is used for preparation of spiking solutions.

b. Solutions for spiking soil samples are prepared by diluting the initial solution from Section G.2.a. with acetone as follows:

Aliquot of 10.0 µg/mL Soln. mL	Final Soln. Volume mL	Spiking Soln. Final Conc. ng/mL	Equivalent Sample Conc.[a] ng/g
0.100	250	4.00	0.400
0.100	100	10.0	1.00
0.500	250	20.0	2.00
1.00	250	40.0	4.00
1.00	100	100.	10.00
2.00	100	200.	20.00

[a] The equivalent sample concentration is based on fortifying a 10-g soil sample with 1.0 mL of spiking solution.

c. Fortification of soils at levels above 20 ng/g are performed by adding the appropriate aliquot of the 10.0 µg/mL solution from Step G.2.a. directly to the soil as follows:

Aliquot of 10.0 µg/mL mL	Equivalent Sample Conc.[a] ng/g
0.100	100.0
0.500	500.0
2.00	2000.0

[a] The equivalent sample concentration is based on fortifying a 10-g soil sample with the appropriate aliquot of the 10.0 µg/mL solution.

3. Preparation of *cis*- and *trans*-CAAL Calibration Solutions

a. Transfer 1.0 mL of each of the stock solutions in Sections G.1.a. and G.1.b. to a 100-mL volumetric flask and bring to volume with hexane to obtain an initial solution of 10.0 µg/mL for each *cis*- and *trans*-CAAL. This solution is used for preparation of calibration solutions.

b. Solutions for calibration are prepared by diluting the initial solution from Section G.3.a. with hexane as follows:

Aliquot of 10.0 µg/mL Soln. mL	Final Soln. Volume mL	Soln. Final Conc. ng/mL	Equivalent Sample Conc.[a] ng/g
0.050	250	2.00	0.20
0.100	250	4.00	0.40
0.500	250	20.0	2.0
1.00	100	100.	10.0
2.00	100	200.	20.0
4.00	100	400.	40.0

[a] The equivalent sample concentration is based on the concentration of a 10-g soil extract to a final volume of 1.0 mL.

c. Calibration standards are prepared for capillary gas chromatography/mass spectrometry as described in Steps I.1.z. through I.1.ff.

H. Gas Chromatography/Mass Spectrometry

1. Column

Install the splitless column inlet liner (Section E.6.) and the capillary column (Section E.2.) in the split/splitless injection port of the GC/MSD following the manufacturer's recommended procedure.

2. Typical Operating Conditions

Instrumentation:	Hewlett-Packard Model 5890 (II) Gas Chromatograph Hewlett-Packard Model 5971A Mass Selective Detector Hewlett-Packard Model G1034B Data System Software
Column:	J&W Scientific fused silica capillary Durabond-17 liquid phase 20 m x 0.18 mm i.d. 0.3 μm film thickness
Temperatures:	
Column	65 °C for 1.0 min 65 °C to 150 °C at 5 °C/min, 0 min hold at 150 °C 150 °C to 260 °C at 20 °C/min, 0 min hold at 260 °C
Injector	230 °C
Interface	280 °C
Carrier Gas:	helium
Head Pressure	approximately 100 kPa
Linear Velocity	approximately 40 cm/sec at an oven temperature of 130 °C
Injection Mode:	splitless
Purge Delay	0.7 min
Splitter Flow	50 mL/min
Septum Purge	1.0 mL/min
Injection Volume:	2 μL
Detector:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune (Note N.3.)
Electron Multiplier	approximately 1412 volts (tune voltage plus 200)
Ions Monitored:	
cis-CAIBC	*m/z* 136 (quantitation) and *m/z* 75 (confirmation)
trans-CAIBC	*m/z* 136 (quantitation) and *m/z* 75 (confirmation)
Dwell Time	100 msec

Mass spectra of *cis*- and *trans*-CAIBC are shown in Figures 1 and 2, respectively. The nominal *m/z* 136 quantitation ion results from loss of 2-methyl-2-propene (mass 56). Both the quantitation and the nominal *m/z* 75 confirmation ions retain the chlorine functionality of CAAL.

3. Calibration Curves

Typical calibration curves for the determination of *cis*- and *trans*-CAAL in soil are shown in Figures 3 and 4, respectively.

4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 0.42 ng/g recovery sample for *cis*- and *trans*-CAAL in soil are shown in Figures 5-10.

I. Determination of Recovery of *cis*- and *trans*-CAAL from Soil

1. Preparation of Recovery Samples

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

b. For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding the specified aliquots of the appropriate spiking solutions (Section G.2.b. and G.2.c.) in acetone to obtain concentrations ranging from 0.40 to 2000 ng/g. A reagent blank containing no soil should be carried through the method with the samples. To minimize the potential for cross contamination, equipment used to process samples and reusable glassware should be thoroughly rinsed with 0.01 N hydrochloric acid followed by acetone and allowed to dry prior to use.

c. Add 15.0 mL of 0.01 N hydrochloric acid to each sample vial and seal with a PTFE-lined cap.

d. Vortex the samples briefly and sonicate for 10-15 seconds.

e. Shake the samples for a minimum of 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.

f. Centrifuge each sample for 10 minutes at 2500 rpm.

g. Carefully decant each extract to a clean 45-mL vial.

h. Extract each sample a second time by repeating Steps I.1.c., d. and f. Combine the extracts by decanting to the vial in Step I.1.g.

i. The samples are then purified using the following ion-exchange SPE procedure (Note N.4.):

(1) Place an ion-exchange SPE column (Section E.4.) on the vacuum manifold box.

(2) Attach a prefilter (Section E.17.) to the top of the column using an SPE column adapter (Section E.5.).

(3) Attach a 25-mL reservoir (Section E.7.) to the prefilter.

(4) Rinse the reservoir, prefilter and SPE column with approximately 5 mL of methanol. (Do not allow the column bed to dry.)

(5) Condition the SPE column with approximately 5 mL of deionized water. (Do not allow the column bed to dry.)

(6) Transfer the sample solution from Step I.1.h. to the reservoir and, with the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min. Collect the eluent in a 45-mL vial.

(7) Rinse the sample vial with 3 mL of deionized water and transfer the rinse to the reservoir after the original sample load has completely passed through the column. With the aid of vacuum, pull the rinse through the column at a flow rate of approximately 2 mL/min. Collect and combine the eluents in the 45-mL vial from Step I.1.i.(6).

(8) Repeat Step I.1.i.(7) with a second 3 mL rinse of the sample vial, allow the first rinse to pass through the column before adding the second rinse to the reservoir. With the aid of vacuum, pull the rinse through the column. Collect and combine the eluents in the 45-mL vial from Step I.1.i.(6).

j. 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.

k. Add 10 µL 1-propanol, approximately 15 g of sodium chloride, 15 mL of MTBE and seal the bottle with a PTFE-lined cap. (The addition of 1-propanol is critical to reduce evaporative losses of CAAL.)

l. Shake the sample for 15 minutes on a reciprocating shaker at approximately 180 excursions/minute.

m. Centrifuge the bottle for 3 minutes at 1000 rpm.

n. The samples are then dried and purified using the following silica gel SPE procedure (Note N.4.):

(1) Place a silica gel SPE column (Section E.3.) on the vacuum manifold box.

(2) Add approximately 2 g of magnesium sulfate (anhydrous) to the SPE column.

(3) Attach a 25-mL reservoir to the top of the column using an SPE column adapter.

(4) Wash the SPE column by adding approximately 10 mL of MTBE to the reservoir and, with the aid of vacuum, pull the MTBE through the column. Discard the column wash.

(5) Transfer the MTBE layer (top layer) of the sample solution from Step I.1.m. to the reservoir and, with the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min. Collect the MTBE in a 45-mL vial.

(6) Add 15 mL of MTBE to the sample bottle, shake for 5 minutes and repeat Steps I.1.m. and I.1.n.(5).

(7) After the two MTBE extracts have passed through the column, add approximately 5 mL of MTBE to the reservoir and, with the aid of vacuum, pull the MTBE through the column at a flow rate of approximately 2 mL/min. Collect and combine the eluents in the 45-mL vial from Step I.1.n.(5).

o. Quantitatively transfer the MTBE in the 45-mL vial to a 50-mL Erlenmeyer flask (Section E.8.). Rinse the 45-mL vial with approximately 2 mL of MTBE and add to flask.

p. Add approximately 3 mL of hexane and approximately 0.1 g of sodium sulfate (anhydrous) to the flask. (The sodium sulfate eliminates the need for boiling chips.)

q. Attach a Snyder column (Section E.16.) to the flask.

r. In a fume hood, heat the flask on a hot plate (Section D.9.) to a steady boil.

s. Allow the sample to concentrate to near dryness. Significant loss of CAAL will occur if the flask goes to dryness.

t. 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 ambient temperature.

u. Remove the Snyder column from the flask 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.

v. Concentrate the sample at ambient temperature on an N-Evap 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 CAAL will occur.

w. 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.

x. Add approximately 0.1 g of anhydrous sodium sulfate.

y. Add 25 µL of pyridine and 25 µL of isobutyl chloroformate, seal the vial with a PTFE-lined cap, and vortex and sonicate the samples for 5 seconds.

z. Transfer 1.0 mL of each of the calibration standards in Section G.3.b. to 8-mL vials and derivatize following Step I.1.y.

aa. Heat the samples and standards in an aluminum block (Section D.7. and D.8.) at 70 °C for 15 minutes.

bb. Remove the vials from the aluminum block and allow the derivatized samples and standards to cool to ambient temperature.

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

dd. Centrifuge the vials for 5 minutes at 2500 rpm.

ee. Transfer the top hexane layer to a 2-mL autosampler vial and seal the vial with a cap and crimper.

ff. Analyze the samples and calibration standards by GC/MSD as described in Section H.

gg. Samples showing levels of CAAL above approximately 40 ng/g are diluted 100-fold as follows:

(1) Transfer 100 µL of the derivatized sample from Step I.1.gg. to a 10-mL volumetric flask and dilute to mark with hexane.

(2) Transfer approximately 1 mL of the diluted sample to a 2-mL autosampler vial and seal the vial with a cap and crimper. (No rederivatization is required.)

(3) Reanalyze the sample by GC/MSD as described in Section H.

2. <u>Calculation of Percent Recovery</u>

a. Determine the *m/z* 75 and 136 response areas for both *cis*- and *trans*-CAIBC in the calibration standards.

b. For each standard, calculate the *cis*- and *trans*-CAAL confirmation ratios. The average confirmation ratio for all calibration standards will be used to confirm the presence of the respective CAAL in the soil samples.

For example, using the data for *cis*-CAIBC from Figure 5:

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

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

$$\text{Confirmation Ratio} = \frac{1225}{3952}$$

$$\text{Confirmation Ratio} = 0.3100$$

Positive confirmation of the presence of *cis*- or *trans*-CAAL is indicated when the confirmation ratio for the samples is in the range of ±20% of the average found for the standards.

c. Prepare *cis*- and *trans*-CAAL standard curves by plotting the equivalent soil sample concentration (ng/g) on the abscissa (x-axis) and the *cis*- and *trans*-CAIBC *m/z* 136 peak area on the ordinate (y-axis) as shown in Figures 3 and 4, respectively. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (4) with the *trans*-CAAL data from Figure 4:

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

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

$$\underset{(\text{ng/g})}{\textit{trans}\text{-CAAL Conc.}} = \left(\frac{\textit{trans}\text{-CAIBC peak area}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\underset{(\text{ng/g})}{\textit{trans}\text{-CAAL Conc.}} = \left(\frac{\textit{trans}\text{-CAIBC peak area}}{3225.7}\right)^{1/1.0220}$$

d. Determine the net concentration in each recovery sample by first subtracting the average *cis*- and *trans*-CAIBC *m/z* 136 peak area in the control sample from that of the recovery sample. Substitute *m/z* 136 the peak area obtained into the above equation and solve for the concentration.

For example, using the *trans*-CAAL data from Figures 9 and 10:

$$\underset{(\text{ng/g})}{\textit{trans}\text{-CAAL Conc.}} = \left(\frac{\text{net }\textit{trans}\text{-CAIBC peak area}}{3225.7}\right)^{1/1.0220}$$

$$\underset{(\text{ng/g})}{\textit{trans}\text{-CAAL Conc.}} = \left(\frac{1235 - 0}{3225.7}\right)^{1/1.0220}$$

$$\textit{trans}\text{-CAAL Conc.} = 0.3909 \text{ ng/g}$$

e. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

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

$$\text{Recovery} = \frac{0.3909 \text{ ng/g}}{0.4156 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 94\%$$

The average recovery of all the recovery samples in a given sample set can be used to correct individual sample results for method efficiency.

J. Determination of *cis*- and *trans*-CAAL in Soil

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.

2. Prepare standard calibration curves for *cis*- and *trans*-CAAL and determine the percentage recoveries as described in Section I.2.

3. Determine the concentration of *cis*- and *trans*-CAAL in each treated sample by substituting the *cis*- and *trans*-CAIBC *m/z* 136 peak area obtained into the respective equations for the standard calibration curve, and calculate the uncorrected residue result.

   For example, using the *cis*-CAAL data from Figures 3 and 7, the uncorrected concentration is calculated as follows:

$$\underset{(\text{ng/g})}{\textit{cis}\text{-CAAL Conc.}} = \left(\frac{\textit{cis}\text{-CAIBC peak area}}{3162.7}\right)^{1/1.0237}$$

$$\underset{(\text{ng/g})}{\textit{cis}\text{-CAAL Conc.}} = \left(\frac{1130}{3162.7}\right)^{1/1.0237}$$

$$\textit{cis}\text{-CAAL Conc.} = 0.3659\ \text{ng/g}$$

4. To correct for method recovery, the following procedure is used:

   a. Determine the *cis*-CAAC concentrations in the soil samples as described in Section J.3. Calculate the average percent recovery from recovery samples in the set (Table I).

   b. Determine the corrected analyte concentration in the soil samples as follows:

$$\underset{(\text{corrected ng/g})}{\textit{cis}\text{-CAAL Conc.}} = \underset{(\text{ng/g})}{\textit{cis}\text{-CAAL Conc.}} \times \frac{100}{\%\ \text{Recovery}}$$

$$\underset{(\text{corrected ng/g})}{\textit{cis}\text{-CAAL Conc.}} = 0.3659\ \text{ng/g} \times \frac{100}{86}$$

$$\underset{(\text{corrected})}{\textit{cis}\text{-CAAL Conc.}} = 0.4255\ \text{ng/g}$$

K. Determination of Soil Moisture

1. Weigh 10.00 g of soil in an aluminum or glass container.

2. Place the sample in an oven at approximately 130 °C and allow to dry for a minimum of 16 hours.

3. Remove the sample from the oven, place in a desiccator until the sample has cooled to ambient temperature, and then re-weigh.

4. Calculate the percent moisture on a dry weight basis as follows:

$$\text{Percent Moisture} = \frac{\text{soil moisture weight (g)}}{\text{dehydrated soil weight (g)}} \times 100$$

$$= \frac{\left(\begin{matrix}\text{soil weight}\\ \text{before drying}\end{matrix} - \begin{matrix}\text{soil weight}\\ \text{after drying}\end{matrix}\right)}{\begin{matrix}\text{soil weight}\\ \text{after drying}\end{matrix}} \times 100$$

L. Determination of Corrected *cis*- and *trans*-CAAL in Soil

1. Determine the *cis*- and *trans*-CAAL concentration in the soil samples as described in Section J.
2. Determine the soil moisture as described in Section K.
3. Determine the corrected *cis*- and *trans*-CAAL concentrations in soil samples as follows:

$$\begin{matrix}\text{CAAL}\\ \text{Corrected Conc. (ng/g)}\end{matrix} = \left(\begin{matrix}\text{CAAL}\\ \text{Conc. (ng/g)}\end{matrix}\right)\left(\frac{100}{\text{\% Recovery}}\right)\left(1 + \frac{\text{\% Moisture}}{100}\right)$$

M. Results and Discussion

1. Method Validation

a. Recovery Levels and Precision

A method validation study was conducted to determine the recovery levels and the precision of the method for *cis*- and *trans*-CAAL in soil. The results are summarized in Tables I and II.

Recovery values of *cis*-CAAL from soil samples fortified over the concentration range 0.42 ng/g to 2.1 µg/g averaged 86% with one standard deviation equal to 8% (Table I).

Recovery values of *trans*-CAAL from soil samples fortified over the concentration range 0.42 ng/g to 2.1 µg/g averaged 89% with one standard deviation equal to 7% (Table II).

b. Standard Curve Fit

The average correlation coefficient (r^2) for the power regression equations describing the detector response as a function of the standard calibration curve concentration was greater than 0.999 for both *cis*- and *trans*-CAAL.

c. Calculated Limits of Quantitation and Detection

Following established guidelines (5), the limits of quantitation (LOQ) and detection (LOD) were calculated using the standard deviation from the 0.42 ng/g recovery results. The LOQ was calculated as ten times the standard deviation (10*s*) and the LOD was calculated as three times the standard deviation (3*s*) of the results for the analysis of nine samples. The results are summarized in Tables III and IV.

For *cis*- and *trans*-CAAL, statistics support an LOQ of 0.42 and 0.36 ng/g, respectively. Results should not be quantified, however, at levels below which no recovery samples have been analyzed.

For *cis*- and *trans*-CAAL, statistics support an LOD of 0.13 and 0.11 ng/g, respectively.

2. <u>Confirmation of Residue Identity</u>

Confirmation of the presence of residues is described in Section I.2.b. For *cis*- and *trans*-CAAL, confirmation is by comparison of the retention time as well as the peak area ratios resulting from selected ion monitoring. Positive confirmation of the presence of *cis*- and *trans*-CAAL is indicated when the confirmation ratio for the sample is in the range of ±20% of the average found for the standards. If additional confirmation is required beyond that discussed in this method, the nominal *m/z* 101 ion may be monitored. High levels of naphthalene, assumed to come from use of a spray cleanser, was found to interfere with the *m/z* 75 confirmation ion for *cis*-CAIBC. No interference was observed when use of the cleanser was discontinued.

3. <u>Assay Time</u>

A typical analytical run would consist of a minimum of four standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of two fortified controls (one of which must be at the LOQ), and ten samples. This typical analytical run could be prepared in approximately 10 hours, and the chromatographic analysis take place the same evening.

There are several acceptable "stopping points" in the method, where sample preparation (Section I) may be suspended without deleterious effects on the sample analysis. These are indicated below:

a. Step I.1.l. If the samples are to be stored overnight, the bottles should be sealed with PTFE-lined caps.

b. Step I.1.n.(7) If the samples are to be stored overnight, the vials should be sealed with PTFE-lined caps.

c. Step I.1.u. If the samples are to be stored overnight, the vials should be sealed with PTFE-lined caps.

N. <u>Notes</u>

1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

2. The filters are used in the carrier gas supply lines to purify the helium entering the gas chromatograph.

3. Several tuning, or calibration, options are available for the Model 597X series of MSDs. The "Maximum Sensitivity Autotune" feature was found to consistently yield approximately 5-10 times the sensitivity compared to that of the "Standard Autotune."
4. Depending on the number of samples being prepared, one may elute CAAL from the SPE column individually, using either gravity-feed or pressurized elution, or as a group, using the vacuum manifold box.

O. References

1. Stolz, W.L., DowElanco Laboratory Notebook, B065, p 39, March 25, 1993, unpublished data of DowElanco.
2. Stolz, W.L., DowElanco Laboratory Notebook, B065, p 39, April 8, 1993, unpublished data of DowElanco.
3. *CRC Handbook of Chemistry and Physics*, 72nd Edition, Lide, D. R., Ed.: CRC: Boca Raton, Ann Arbor, Boston, 1991-1992.
4. *HP-41C/41CV Standard Applications Handbook*, Hewlett-Packard Publication No. 00041-90402, 1982, pp 42-48.
5. Keith, L.H.; Crummett, W.B.; Deegan, J.; Libby, R.A.; Taylor, J.T.; Wentler, G., "Principles of Environmental Analysis", *Anal. Chem.*, 55, 2210-2218 (1983).

Table I. Recovery of *cis*-CAAL from Soil

Sample Number	Date of Analysis	*cis*-CAAL, ng/g Added	*cis*-CAAL, ng/g Found[a]	Percent Recovery
M465 A	07-Nov-1994	0.000	0.0000	–
M465 B	07-Nov-1994	0.000	0.0000	–
M465 E	07-Nov-1994	0.000	0.0000	–
M465 A	09-Nov-1994	0.000	0.0000	–
M465 B	09-Nov-1994	0.000	0.0000	–
M465 E	09-Nov-1994	0.000	0.0000	–
M465 A	18-Nov-1994	0.0834	0.090	NA[b]
M465 E	18-Nov-1994	0.0834	0.045	NA
M465 A	07-Nov-1994	0.4172	0.3659	88
M465 A	07-Nov-1994	0.4172	0.3520	84
M465 B	07-Nov-1994	0.4172	0.3551	85
M465 B	07-Nov-1994	0.4172	0.3342	80
M465 E	07-Nov-1994	0.4172	0.3912	94
M465 E	07-Nov-1994	0.4172	0.2974	71
M465 A	09-Nov-1994	0.4172	0.4043	97
M465 B	09-Nov-1994	0.4172	0.3596	86
M465 E	09-Nov-1994	0.4172	0.4426	106
M465 A	09-Nov-1994	1.043	1.027	98
M465 B	09-Nov-1994	1.043	0.9468	91
M465 A	07-Nov-1994	2.086	1.723	83
M465 B	07-Nov-1994	2.086	1.567	75
M465 E	09-Nov-1994	10.43	8.019	77
M465 A	09-Nov-1994	10.43	9.098	87
M465 E	07-Nov-1994	20.86	18.77	90
M465 A	07-Nov-1994	20.86	19.67	94
M465 B	11-Nov-1994	104.3	80.34	77
M465 E	11-Nov-1994	104.3	81.50	78
M465 B	09-Nov-1994	521.5	407.4	78
M465 E	09-Nov-1994	521.5	438.3	84
M465 A	09-Nov-1994	2086	1736	83
M465 B	09-Nov-1994	2086	1921	92
			$\bar{x}$ =	86
			s =	8
			n =	23

---

[a] Fortifications greater than 40 ng/g were diluted 100-fold after derivatization.

[b] NA = not applicable. The residue was below the 0.40 ng/g LOQ.

Table II. Recovery of *trans*-CAAL from Soil

Sample Number	Date of Analysis	*trans*-CAAL, ng/g Added	*trans*-CAAL, ng/g Found[a]	Percent Recovery
M465 A	07-Nov-1994	0.000	0.0000	--
M465 B	07-Nov-1994	0.000	0.0000	--
M465 E	07-Nov-1994	0.000	0.0000	--
M465 A	09-Nov-1994	0.000	0.0000	--
M465 B	09-Nov-1994	0.000	0.0000	--
M465 E	09-Nov-1994	0.000	0.0000	--
M465 A	18-Nov-1994	0.0831	0.093	NA[b]
M465 E	18-Nov-1994	0.0831	0.051	NA
M465 A	07-Nov-1994	0.4156	0.3729	90
M465 A	07-Nov-1994	0.4156	0.3850	93
M465 B	07-Nov-1994	0.4156	0.3332	80
M465 B	07-Nov-1994	0.4156	0.3596	87
M465 E	07-Nov-1994	0.4156	0.3909	94
M465 E	07-Nov-1994	0.4156	0.3154	76
M465 A	09-Nov-1994	0.4156	0.3977	96
M465 B	09-Nov-1994	0.4156	0.3720	90
M465 E	09-Nov-1994	0.4156	0.4383	105
M465 A	09-Nov-1994	1.039	0.9905	95
M465 B	09-Nov-1994	1.039	0.9697	93
M465 A	07-Nov-1994	2.078	1.790	86
M465 B	07-Nov-1994	2.078	1.594	77
M465 E	09-Nov-1994	10.39	8.201	79
M465 A	09-Nov-1994	10.39	9.250	89
M465 E	07-Nov-1994	20.78	19.18	92
M465 A	07-Nov-1994	20.78	20.32	98
M465 B	11-Nov-1994	103.9	85.33	82
M465 E	11-Nov-1994	103.9	87.36	84
M465 B	09-Nov-1994	519.5	422.9	81
M465 E	09-Nov-1994	519.5	455.5	88
M465 A	09-Nov-1994	2078	1778	86
M465 B	09-Nov-1994	2078	1977	95
			$\bar{x}$ =	89
			s =	7
			n =	23

---

[a] Fortifications greater than 40 ng/g were diluted 100-fold after derivatization.
[b] NA = not applicable. The residue was below the 0.40 ng/g LOQ.

Table III. Calculated Limits of Detection and Quantitation for the Determination of *cis*-CAAL in Soil

Sample Number	Date of Analysis	*cis*-CAAL, ng/g Added	*cis*-CAAL, ng/g Found
M465 A	07-Nov-1994	0.4172	0.3659
M465 A	07-Nov-1994	0.4172	0.3520
M465 B	07-Nov-1994	0.4172	0.3551
M465 B	07-Nov-1994	0.4172	0.3342
M465 E	07-Nov-1994	0.4172	0.3912
M465 E	07-Nov-1994	0.4172	0.2974
M465 A	09-Nov-1994	0.4172	0.4043
M465 B	09-Nov-1994	0.4172	0.3596
M465 E	09-Nov-1994	0.4172	0.4426
		$\bar{x}$ =	0.37
		s =	0.042
		LOD[a] ($3s$) =	0.13
		LOQ[b] ($10s$) =	0.42

[a] LOD = Limit of Detection.

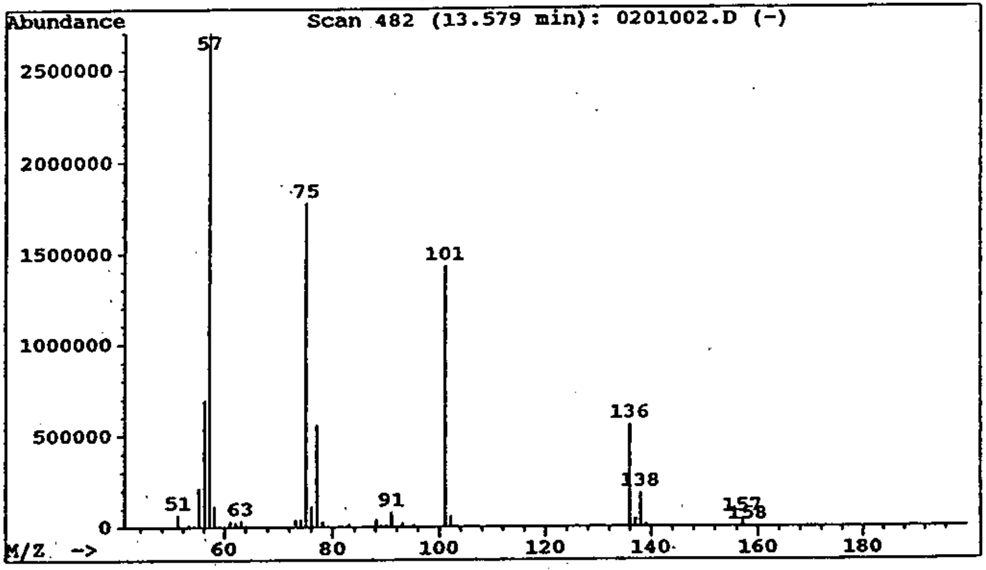
[b] LOQ = Limit of Quantitation.

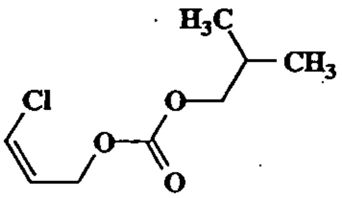
Table IV. Calculated Limits of Detection and Quantitation for the Determination of *trans*-CAAL in Soil

Sample Number	Date of Analysis	*trans*-CAAL, ng/g	
		Added	Found
M465 A	07-Nov-1994	0.4156	0.3729
M465 A	07-Nov-1994	0.4156	0.3850
M465 B	07-Nov-1994	0.4156	0.3332
M465 B	07-Nov-1994	0.4156	0.3596
M465 E	07-Nov-1994	0.4156	0.3909
M465 E	07-Nov-1994	0.4156	0.3154
M465 A	09-Nov-1994	0.4156	0.3977
M465 B	09-Nov-1994	0.4156	0.3720
M465 E	09-Nov-1994	0.4156	0.4383
		$\overline{x}$ =	0.37
		s =	0.036
		LOD[a] ($3s$) =	0.11
		LOQ[b] ($10s$) =	0.36

---

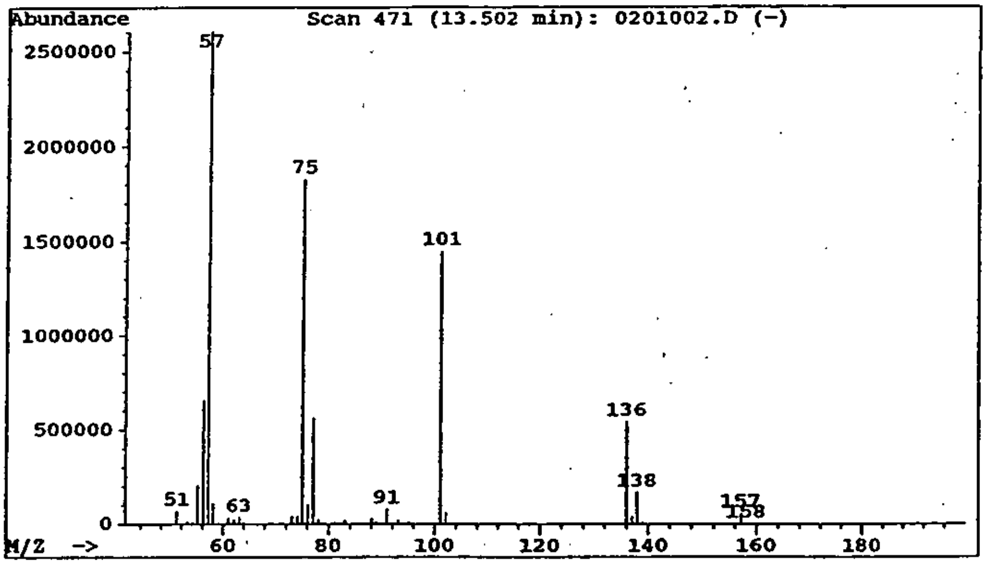
[a] LOD = Limit of Detection.
[b] LOQ = Limit of Quantitation.

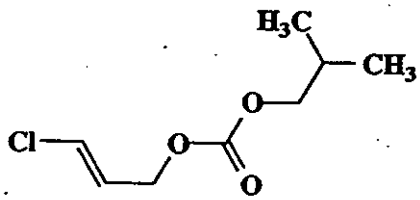




cis-CAIBC
Formula: $C_8H_{13}ClO_3$
Molecular Weight: 192

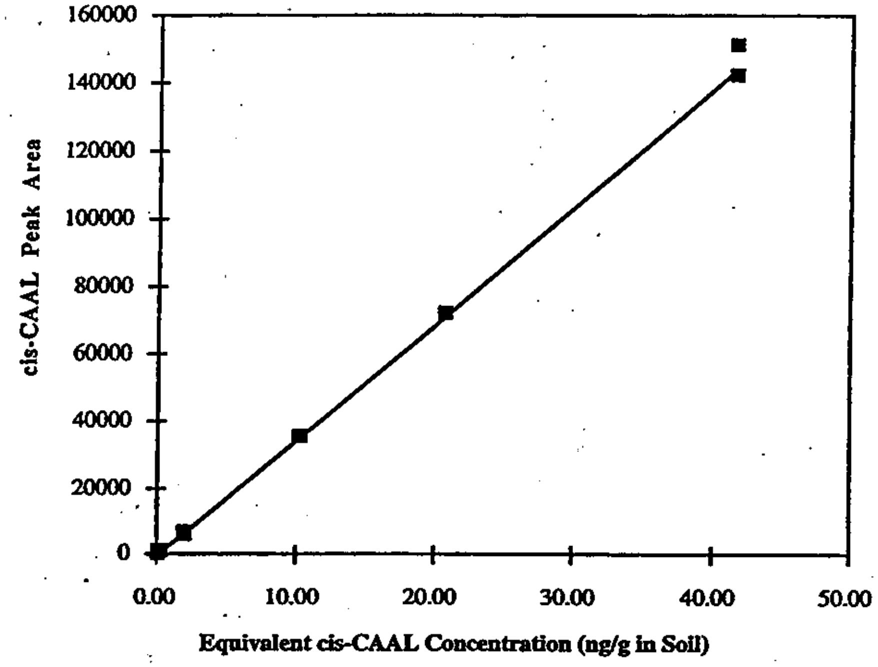
Figure 1. Mass Spectrum of *cis*-3-Chloroallyl Isobutyl Carbonate (*cis*-CAIBC)





trans-CAIBC
Formula: $C_8H_{13}ClO_3$
Molecular Weight: 192

Figure 2. Mass Spectrum of *trans*-3-Chloroallyl Isobutyl Carbonate (*trans*-CAIBC)

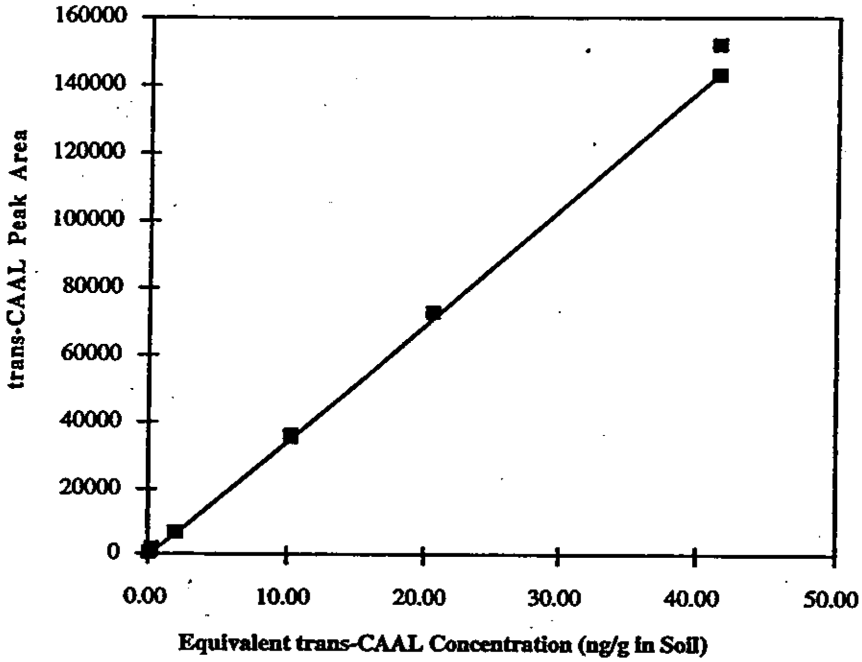


Equivalent *cis*-CAAL Conc.	*cis*-CAAL *m/z* 136 Peak Area Response	
ng/g	Start of Sequence	End of Sequence
0.2086	620	709
0.4172	1225	1335
2.086	6086	6538
10.43	34766	35249
20.86	71780	71430
41.72	142249	151135

Power Regression Equation: $X = \left[\dfrac{Y}{3162.7}\right]^{1/1.0237}$

Coefficient of Determination (r^2): 0.9994

Figure 3. Typical Calibration Curve for the Determination of *cis*-CAAL in Soil

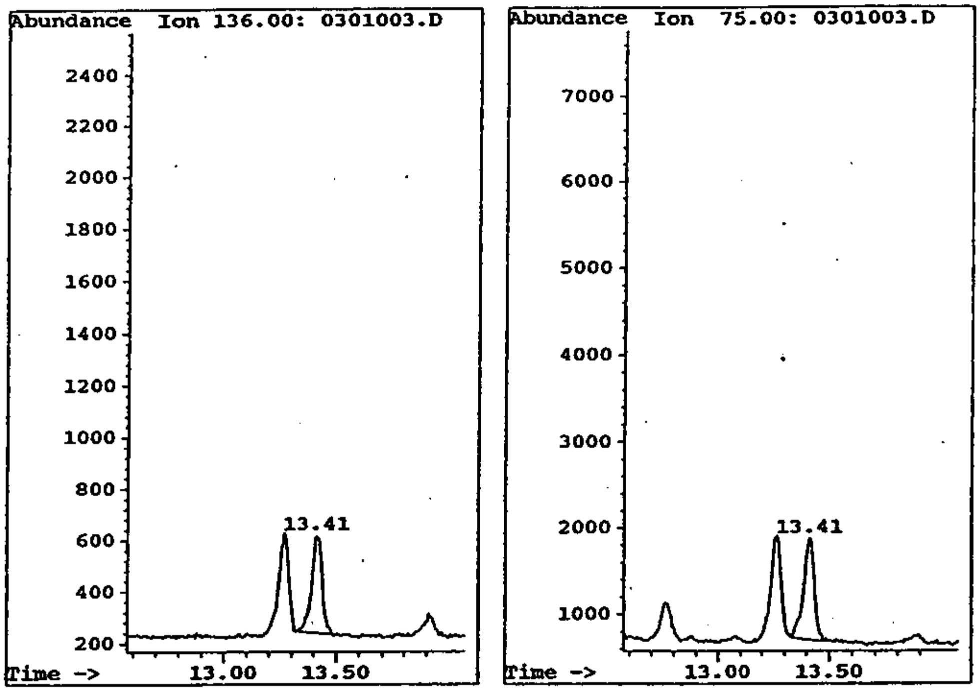


Equivalent *trans*-CAAL Conc.	*trans*-CAAL *m/z* 136 Peak Area Response	
ng/g	Start of Sequence	End of Sequence
0.2078	649	657
0.4156	1266	1419
2.078	6237	6807
10.39	34943	35325
20.78	72457	72237
41.56	142890	151802

Power Regression Equation: $X = \left[\frac{Y}{3225.7}\right]^{1/1.0220}$

Coefficient of Determination (r^2): 0.9996

Figure 4. Typical Calibration Curve for the Determination of *trans*-CAAL in Soil



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Data File  : 0301003.D
ALS Bottle : 3
Date       :  7 Nov 94    6:44 pm
Data Path  : C:\CHEMPC\DATA\T110794A\
Instrument : GC/MSD - GC serial#3126A36485

Sample Name:                  4.0 ng/ml cis- and trans-CAAl Std
Sample Info: Isobutyl Chloroformate Derivatization
Operator   : ADT

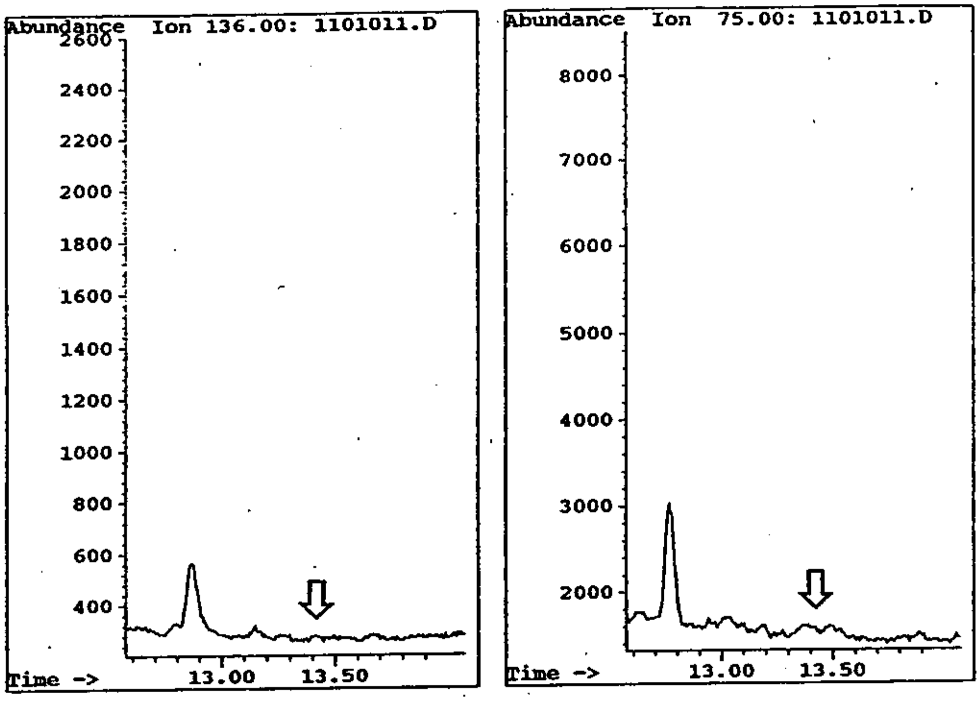

3-Chloroallyl alcohol Retention Time:     13.41
 PEAK AREA (M/Z 136) :                  1225
 PEAK AREA (M/Z 75) :                   3952

3-Chloroallyl alcohol:
 RATIO OF M/Z 136/75:                 0.3100
```

Equivalent *cis*-CAAL Concentration: 0.4172 ng/g

Average Standard Confirmation Ratio: 0.3228

Figure 5. Typical Chromatogram of a 4.172 ng/mL Standard, Equivalent to 0.4172 ng/g *cis*-CAAL in Soil



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Data File  : 1101011.D
ALS Bottle : 11
Date       :  7 Nov .94  10:20 pm
Data Path  : C:\CHEMPC\DATA\T110794A\
Instrument : GC/MSD - GC serial#3126A36485
```

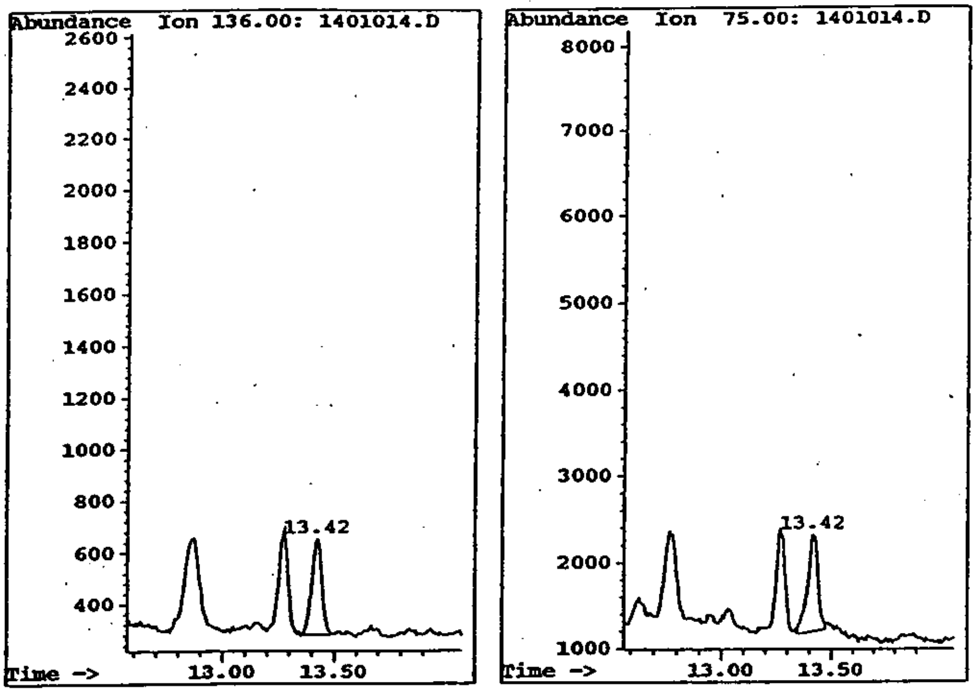
Sample Name: Soil M465A Control
Sample Info: ~~Florida Surface Water~~ ATD 11/22/94 Florida Soil
Operator : ADT

No 3-Chloroallyl alcohol Found

cis-CAAL Concentration: 0.0000 ng/g

Average Standard Confirmation Ratio: 0.3228

Figure 6. Typical Chromatogram of a Control Soil Sample for the Determination of *cis*-CAAL



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Data File   : 1401014.D
ALS Bottle  : 14
Date        :  7 Nov 94   11:41 pm
Data Path   : C:\CHEMPC\DATA\T110794A\
Instrument  : GC/MSD - GC serial#3126A36485

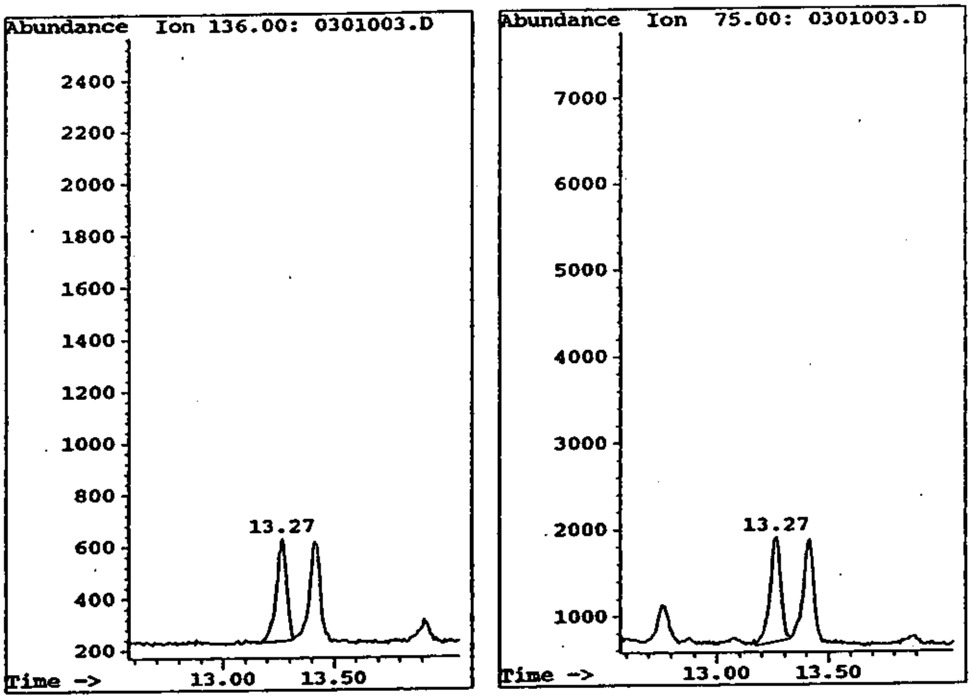
Sample Name:                    Soil M465A 0.40 Spike
Sample Info: ~~Florida Surface Water~~ Florida Soil  AT D 11/22/94
Operator   : ADT

3-Chloroallyl alcohol Retention Time:      13.42
 PEAK AREA (M/Z 136) :                  1130
 PEAK AREA (M/Z 75) :                   3664

3-Chloroallyl alcohol:
 RATIO OF M/Z 136/75:                 0.3084
```

cis-CAAL Concentration: 0.3659 ng/g
Recovery: 88%
Average Standard Confirmation Ratio: 0.3228

Figure 7. Typical Chromatogram of a Control Soil Sample Fortified with 0.4172 ng/g *cis*-CAAL



```
Data File   : 0301003.D
ALS Bottle  : 3
Date        :  7 Nov 94   6:44 pm
Data Path   : C:\CHEMPC\DATA\T110794A\
Instrument  : GC/MSD - GC serial#3126A36485

Sample Name:                  4.0 ng/ml cis- and trans-CAAl Std
Sample Info: Isobutyl Chloroformate Derivatization
Operator    : ADT

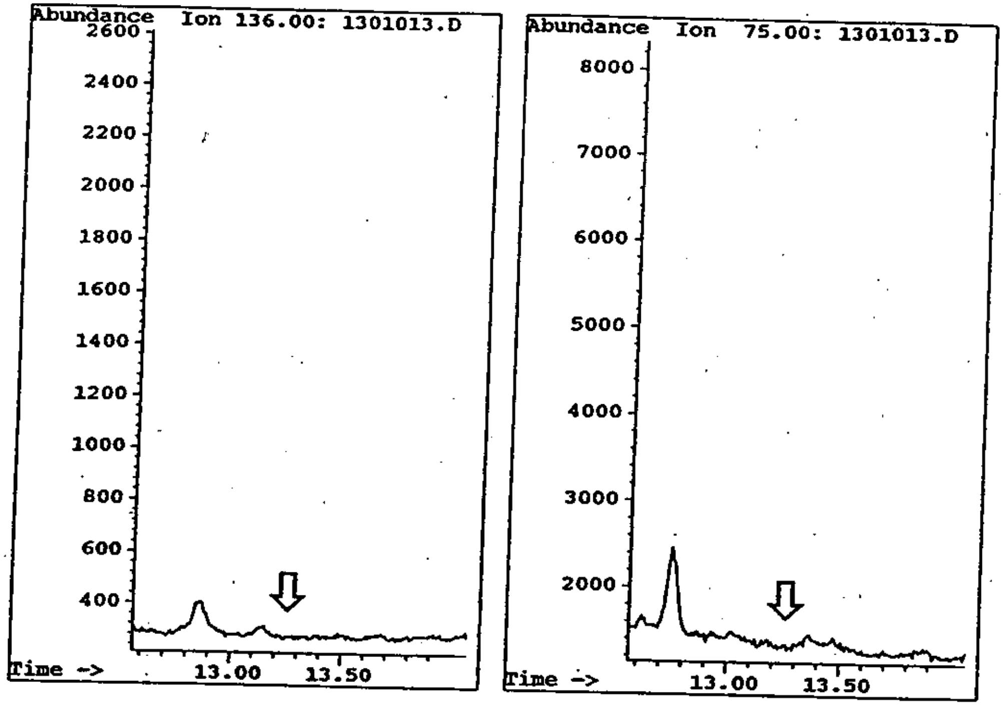
3-Chloroallyl alcohol Retention Time:      13.27
 PEAK AREA (M/Z 136) :                 1266
 PEAK AREA (M/Z 75) :                  4067

3-Chloroallyl alcohol:
 RATIO OF M/Z 136/75:                0.3113
```

Equivalent *trans*-CAAL Concentration: 0.4156 ng/g

Average Standard Confirmation Ratio: 0.3199

Figure 8. Typical Chromatogram of a 4.156 ng/mL Standard, Equivalent to 0.4156 ng/g *trans*-CAAL in Soil



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Data File   : 1301013.D
ALS Bottle  : 13
Date        :  7 Nov'94  11:14 pm
Data Path   : C:\CHEMPC\DATA\T110794A\
Instrument  : GC/MSD - GC serial#3126A36485

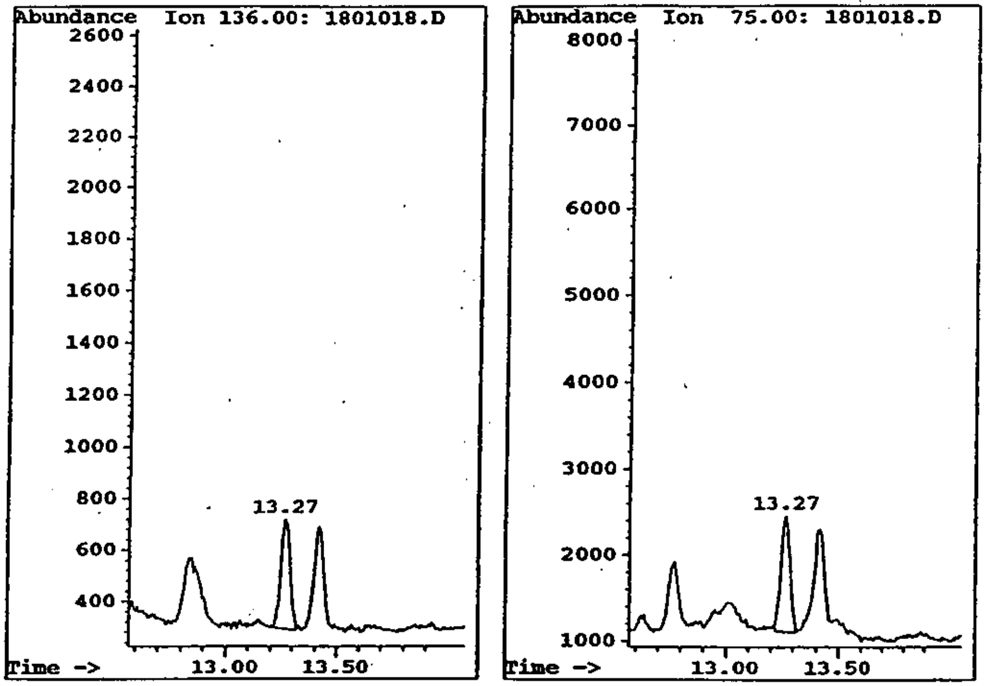
Sample Name:                    Soil M465E Control
Sample Info: ~~Florida Surface Water~~ Florida Soil  AT 11/22/94
Operator   : ADT

No 3-Chloroallyl alcohol Found
```

trans-CAAL Concentration: 0.0000 ng/g

Average Standard Confirmation Ratio: 0.3199

Figure 9. Typical Chromatogram of a Control Soil Sample for the Determination of *trans*-CAAL



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Data File   : 1801018.D
ALS Bottle  : 18
Date        :  8 Nov 94    1:29 am
Data Path   : C:\CHEMPC\DATA\T110794A\
Instrument  : GC/MSD - GC serial#3126A36485

Sample Name:                       Soil M465E 0.40 Spike
Sample Info: ~~Florida Surface Water~~ Florida Soil ATD 11/23/94
Operator    : ADT

3-Chloroallyl alcohol Retention Time:      13.27
 PEAK AREA (M/Z 136) :                 1235
 PEAK AREA (M/Z 75) :                  3763

3-Chloroallyl alcohol:
 RATIO OF M/Z 136/75:                0.3282
```

trans-CAAL Concentration:	0.3909 ng/g
Recovery:	94%
Average Standard Confirmation Ratio:	0.3199

Figure 10. Typical Chromatogram of a Control Soil Sample Fortified with 0.4156 ng/g *trans*-CAAL