I. INTRODUCTION/SUMMARY

A. Scope

This method is used for the determination of CGA-362622 (Chemical Abstracts Registry (CAS) Number: 199119-58-9) and its degradates CGA-53052, CGA-368732, CGA-368733, NOA-436664, and CGA-382997 in soil. The compounds are separated by high performance liquid chromatography (HPLC) and detected by mass spectrometry (LC/MS/MS). The limit of detection by LC/MS/MS (smallest standard amount injected during the chromatographic run) is 0.0125 ng injected for all analytes. The limit of quantification (LOQ) (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for LC/MS/MS analyses is 0.5 ppb for all analytes in soil.

The results presented in this method amendment are for revalidation experiments of analytical method AG-692[1] with some modifications made to the extraction procedure. Low recoveries of analytes CGA-368733 and NOA-436664 were observed using method AG-692 as written when analyzing samples from a terrestrial field dissipation study being conducted in Louisiana. Subsequent method development experiments indicated a need to perform a more rigorous extraction. The experiments also indicated that the 0-6" soil layer needed a slightly different extraction procedure than soils of depths greater than 6".

The only modifications made to AG-692 involve the nature and sequence of the extraction solvents. All other aspects of the subsequent sample cleanup remain the same. The HPLC gradient has been slightly modified to lessen the analysis time. All changes will be noted in this method amendment. Sections that are unchanged from AG-692 will only reference the original method. All data and chromatograms from these additional validation studies will be presented.

B. Principle

A 20-gram subsample of soil is extracted three times with a series of extraction solvents (once with 70% (v/v) methanol/water and twice with 70% (v/v) methanol/water, 2% in ammonium hydroxide for 0-6" soil layers, and three times with 70% (v/v) methanol/water, 2% in ammonium hydroxide for soils of depths greater than 6") at room temperature using mechanical agitation. The sample is centrifuged and filtered with extracts combined. The methanol content is removed by rotary evaporation. The aqueous is passed through a SAX SPE column

attached piggy-back style to a ENV SPE column. The SAX SPE column, which retains soil matrix and does not retain the analytes, is detached and discarded. The analytes are eluted from the ENV SPE using acetonitrile. The samples are adjusted to the desired final volume and organic content. LC/MS/MS is used for analysis of the samples.

II. MATERIALS AND METHODS

A. Apparatus

The apparatus used in these additional method validation studies are the same as used and described in analytical method AG-692.

B. Reagents and Analytical Standards

The reagents and analytical standards used in these additional method validation studies are the same as used and described in analytical method AG-692.

C. Safety and Health

Whereas most of the chemicals used and analyzed for in this method have not been completely characterized, general laboratory safety is advised (e.g., safety glasses, gloves, etc. should be used). Acetic acid is an irritant and should be used in a well-ventilated area (i.e., a fume hood).

D. Analytical Procedure

Note: All glassware, including the polyallomer bottles used for soil extraction should be thoroughly cleaned and rinsed with acetonitrile or methanol prior to use. The analysis system is very sensitive and may detect contamination from previous samples if all glassware and extraction bottles are not properly cleaned prior to each use.

1.0 Soil Moisture Determination

The procedure used for soil moisture determination in these additional method validation studies is the same as used and described in analytical method AG-692.

2.0 Soil

Modifications made to the soil extraction/cleanup procedure are indicated in bold type.

Soil characterization data for the soils used in these experiments are presented in Table 1.

(Note: Samples must be homogenized prior to analysis using suitable sample preparation techniques.)

2.1 Weigh and record 20 ± 0.1 g of soil sample into a plastic extraction bottle.

2.2 Sample fortification, if required for this particular sample, is to be done at this time (refer to Section II.K.2.0).

2.3 Add 75 mL of the soil extraction solvent **(70% methanol/water for 0-6" soil samples, 70% methanol/water, 2% in ammonium hydroxide for soil depths >6")** to the sample.

2.4 **Shake the bottle vigorously for 5-10 seconds to break up soil clumps.** Place the sample on an orbital shaker and agitate the sample at high speed for thirty minutes at room temperature.

2.5 Centrifuge the sample at approximately 9,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.6 Decant the sample extract through filter paper into a 500-mL round bottom flask.

2.7 Repeat the extraction adding 75 mL of extraction solvent **(70% methanol/water, 2% in ammonium hydroxide)** to the sample and repeat Steps 2.4 through 2.6, adding the second extract to the first contained in the round bottom flask from Step 2.6.

2.8 Repeat the extraction adding 75 mL of extraction solvent (70% methanol/water, 2% in ammonium hydroxide) to the sample and repeat Steps 2.4

through 2.6, adding the third extract to the round bottom flask from Step 2.6. When all of the extract has passed through the filter paper, rinse the filter paper with approximately 10 mL of methanol, using a squirt bottle, prior to initiating the rotary evaporation step.

2.9 Remove methanol from the sample until only aqueous remains via rotary evaporation with a water bath temperature of approximately 35 °C. Add water, if necessary, to prevent the sample from going dry. There should be a minimum aqueous volume of approximately 25 mL.

2.10 **Add 10 mL of 0.1 M ammonium carbonate to basify the sample.** If the sample is cloudy with suspended particulates, transfer the sample to a centrifugable plastic bottle, using several mL of 0.1% ammonium carbonate to rinse the round bottom flask, adding this rinsate to the bottle. Centrifuge the sample at approximately 9,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.11 Pass the sample through a Varian SAX SPE column attached piggy-back style to a Varian ENV SPE. Discard the eluate. (The SPE columns are preconditioned by passing 10 mL of methanol and then 10 mL of 0.1 M ammonium carbonate through each column. Add 2-3 mL of 0.1 M ammonium carbonate to the lower ENV column just prior to adding the sample to the top SAX column. Do not permit the lower ENV column to go dry while the top SAX column still contains sample. Add 0.1 M ammonium carbonate to the lower ENV column, as needed, to prevent it from going dry.)

2.12 Add approximately 10 mL of 0.1 M ammonium carbonate to the round bottom flask from Step 2.9 and swirl to dissolve any residues still on the glass. Pass through both SPE columns. Discard the eluate. Pass an additional 3-4 mL of 0.1 M ammonium carbonate through both SPE columns. Discard the eluate.

2.13 Disconnect the SAX column and discard. Rinse the ENV column with approximately 3 mL of 0.1 M ammonium carbonate, followed by 6 mL of water.

2.14 Apply vacuum to the ENV column for approximately 30 seconds to remove aqueous trapped in the void spaces of the column.

2.15 Elute the analytes into a pre-calibrated 50-mL concentration tube using 10 mL of acetonitrile. (The concentration tube is calibrated by pipetting the desired final volume of sample diluent into the tube and then marking the meniscus with a marking pen. In these studies, a final sample volume of 5 mL was used for control samples and those fortified at the method LOQ of 0.5 ppb.) Add 0.2 mL of ethylene glycol to each concentration tube prior to rotary evaporation.

2.16 Remove the organic content from the sample via rotary evaporation with a water bath temperature of approximately 35 °C.

2.17 Add 1.0 mL of acetonitrile to the sample. Adjust the final volume to the calibration mark using purified water. The sample may be further diluted with sample diluent, if necessary. The sample should be stored refrigerated (<5°C) until the time of analysis. Samples should be stored frozen for long term storage (> 2 weeks).

2.18 Analyze the sample using LC/MS/MS.

E. Instrumentation

1.0 Description and Operating Conditions: HPLC

See Table 2 for a description of the reversed phase HPLC system and chromatographic conditions used for the analysis. The HPLC gradient has been slightly modified to lessen the overall analysis time.

2.0 Description and Operating Conditions: LC/MS/MS

CGA-362622, CGA-53052, CGA-382997, CGA-368732, CGA-368733, and NOA-436664 are monitored as positive ions. Triple quadrupole analysis (MS/MS) of the unique precurser/product ion pair is suggested to achieve the low LOQ of 0.5 ppb for all analytes. See Table 3 for a description of the mass spectrometer instrumentation and operating conditions.

3.0 Description and Operating Conditions: LC/MS/MS Turboionspray Interface

The optimized values for the turboionspray interface may vary with time and may need to be periodically re-optimized. The turboionspray split flow is adjusted so that a small wet spot is visible on the orifice plate at the initial mobile phase gradient composition. Typical turboionspray operating conditions are described in Table 3.

4.0 Calibration and Standardization: LC/MS/MS

Calibration and standardization procedure are the same as detailed in method AG-692. Typical precurser/product ion pairs that are monitored for each analyte are presented in Table 4.

F. Interferences

1.0 There are no known interferences originating from the sample cleanup procedure. However, interferences can originate from impure chemicals, solvents, contaminated glassware, and the HPLC water supply.

G. Confirmatory Techniques

1.0 No confirmatory analysis procedure is included in this method. This method employs highly specific LC/MS/MS for the detection mode, coupled with the characteristic retention time observed for the analyte on the appropriate HPLC column.

H. Time Required

1.0 The sample extraction and cleanup procedure can be completed for a set of ten samples in an eight-hour working day.

2.0 Each HPLC analysis requires approximately 21 minutes.

I. Modifications and Potential Problems

See modifications and potential problems detailed in method AG-692.

J. Preparation of Standard Solutions

The procedures for preparation of standard solutions are detailed in method AG-692.

K. Methods of Calculation

1.0 Determination of Residues in Samples

Same as detailed in method AG-692.

2.0 Determination of Residues in Fortified Samples

Same as detailed in method AG-692.

3.0 Calculations

Calculations may be performed by computer program or manually as follows (soil concentrations are based on their wet weight):

3.1 Calculate the analyte concentration (in ppb, wet weight) for field samples from equation (1):

$$\text{(1) ppb analyte (wet weight)} = \frac{\text{ng analyte found}}{\text{g sample injected}} \text{ X } \frac{1}{R}$$

where R is the recovery factor expressed in decimal form (i.e., 0.8 = 80%) and is calculated from equation (4), and the chemical purity of the analytical standard has been accounted for in the preparation of the standard solutions. The use of the recovery correction factor "1/R" is left to the discretion of the study director.

The mass of sample injected is calculated from equations (3) and (4), respectively.

The residue found may also be expressed on a dry weight basis by equation (2).

$$(2)\ \text{ppb analyte (dry weight)} = \frac{\text{ppb analyte (wet weight)}}{1-m}$$

Where "m" is the moisture percent of the soil sample expressed in decimal form (i.e., 15% soil moisture = 0.15).

The grams of sample injected for soil is calculated from equation (3).

$$(3)\ \text{g soil injected} = g \times \frac{V_i}{V_f}$$

where, g is the grams of soil (wet weight) used, V_i is the volume (mL) injected onto the HPLC column, and V_f is the final volume (mL) of the cleaned-up sample (from Step II.D.2.15).

The recovery factor, expressed as a percentage (R%), is calculated from fortification experiments and is presented in equation (4).

$$(4)\ R\% = \frac{\text{ppb analyte found} - \text{ppb analyte (control)}}{\text{ppb analyte added}} \times 100\%$$

The amount (ppb) of analyte found is calculated from equation (5).

$$(5)\ \text{ppb analyte found} = \frac{\text{ng analyte found}}{\text{g sample injected}}$$

Residues of degradates found in test samples may also be expressed as parent equivalents by multiplying the amount found by the ratio of the molecular weight of CGA-362622 to that of the metabolite (equation (6)).

$$(6)\ \text{ppb CGA-362622 equiv.} = \text{ppb metabolite} \times \frac{MW(p)}{MW(m)}$$

where MW(p) is the average molecular weight of CGA-362622 (437.3) and MW(m) is the average molecular weight of the metabolite, 155.2 for CGA-53052, 256.2 for CGA-382997, 373.3 for

CGA-368732, 330.3 for CGA-368733, and 316.2 for NOA-436664.

3.2 The accuracy of the method is determined by the average recovery of the analytes fortified into the test substrate. The precision is estimated by the relative standard deviation of the determined concentration.

3.3 The confidence limits at 95% were determined for each fortification level in each substrate using equation (7)

$$(7)\quad \text{C.L.}(95\%) = X \pm \frac{ts}{\sqrt{n}}$$

where "X" is the mean recovery, "s" is the standard deviation, "n" is the number of samples, and "t" is the "t-test" value for (n-1) degrees of freedom. In this report, value of 3.182 was used for "t" with 3 degrees of freedom (for 4 samples), a value of 2.776 was used for 4 degrees of freedom (for 5 samples), a value of 2.179 was used for 12 degrees of freedom (for 13 samples), and a value of 2.145 was used for 14 degrees of freedom (for 15 samples), respectively, for the 95% confidence limit.

TABLE 2. HPLC SYSTEM AND OPERATING CONDITIONS

Instrumentation:

Perkin-Elmer Series 200 Gradient Pump with Series 200 Vacuum Degasser
Perkin-Elmer Series 200 Autosampler with Peltier Sample Cooling Tray
Eppendorf Model CH-30 Column Heater

Operating Conditions:

Peltier Cooling Tray Temperature: 15°C
Column Heater: 30°C
Injection Volume: 25 µL
Mobile Phase Flow Rate: 0.6 mL/min
Column: MetaChem MetaSil AQ (MetaChem Technologies, Inc., #0530-150X030, 15-cm x 3.0 mm, dp = 5 µm, equipped with a MetaChem MetaGaurd 2.0 mm MetaSil AQ guard column (#0530-MG2)Upchurch (#A-318) pre-column filter (0.5 µm)
Mobile Phase A: 0.1% (v/v) acetic acid in acetonitrile
Mobile Phase B: 0.1% (v/v) acetic acid in purified water

Mobile Phase Gradient Program:

Time Duration (min.)	% A	% B	
-1 (equil.)	10	90	
0.0 (inject)	10	90	
10.0	70	30	linear ramp
0.1	100	0	linear ramp
3.0	100	0	
0.5	10	90	linear ramp
6.0	10	90	

Total Run Time: 20.6 min.

Approximate Analyte Retention Times:

CGA-53052	5.5 min
CGA-382997	6.2 min
CGA-368733	7.6 min
NOA-436664	9.1 min
CGA-368732	9.8 min
CGA-362622	10.8 min

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS

Instrumentation:

PE Sciex API-365 Triple Quadrupole Mass Spectrometer
TurboIonSpray Liquid Introduction Interface
Instrument Control and Data Collection: Apple Macintosh Power PC Computer, Model 8500/180

Software:

Apple System 8.5

PE Sciex Software:

MassChrom v. 1.1.1
- LC2Tune v. 1.4
- Sample Control v. 1.4
- MacDAD v. 1.4
- MacQuan v. 1.6
- Multiview v. 1.4
- Bundler v. 1.4
- File Translator v. 1.6.1
- Downloader v. 1.2
- Firmware (332) v. M3L1103
- Firmware (340) v. M401100

Experiment Information:

Experiment Name: 362622 TIS-A
Scan Type: MRM
Scan Time: 0.510 sec/scan
Pause Time: 5.0 msec

Mass Range Info

	Q1 (amu)	Q3 (amu)	Dwell (msec)
Mass Range 1	156.1	100	250
Mass Range 2	257.0	176	250

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS (Continued)

Experiment Name: 362622 TIS-B
Scan Type: MRM
Scan Time: 0.62 sec/scan
Pause Time: 5.0 msec

Mass Range Info

	Q1 (amu)	Q3 (amu)	Dwell (msec)	Ramp Parameters
Mass Range 1	331.1	248	150	none
Mass Range 2	317.1	234	150	none
Mass Range 3	374.1	331	150	R02 = -22.0 OR = 16.0
Mass Range 4	438.1	182	150	OR = 16.0

Method Information

Command		Description	Time (sec)	Duration (min)	Total Time (min:sec)
Pause			150	2.5	2:30
Scan Mode:		Profile	0.51	4.5	7:00
	Threshold:	1.0 x 10 E1 cps			
	Pause:	1.0 sec			
	Expt:	362622 TIS-A			
	State:	362622 State 1			
Scan Mode:		Profile	0.62	5.0	12:00
	Threshold:	1.0 x 10 E1 cps			
	Pause:	0.0 sec			
	Expt:	362622 TIS-B			
	State:	362622 State 2			

TABLE 3. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS (Continued)

Typical State File Values

362622 State 1

Positive Ions

Parameter	Value
Gases	
NEB	6
CUR	10
CAD	5
Controls	
IS	5500
TEM	420
OR	15
RNG	85
Q0	-10
IQ1	-12
ST	-14
RO1	-12.0
IQ2	-16
RO2	-35.5
IQ3	-34.9
RO3	-45
DF	-200
CEM	1800

Q1 Resolution

Mass	Offset
30	0.038
100	0.051
1000	0.165
2000	0.280

Q3 Resolution

Mass	Offset
10	0.025
100	0.025
1000	0.120
2000	0.070

362622 State 2

Positive Ions

Parameter	Value
Gases	
NEB	6
CUR	10
CAD	5
Controls	
IS	5500
TEM	420
OR	26
RNG	110
Q0	-10
IQ1	-12
ST	-15
RO1	-11.8
IQ2	-20
RO2	-41
IQ3	-60
RO3	-46
DF	-200
CEM	1800

Q1 Resolution

Mass	Offset
30	0.038
100	0.051
1000	0.165
2000	0.280

Q3 Resolution

Mass	Offset
10	0.025
100	0.020
1000	0.100
2000	0.070

* Note: State file values will vary slightly from instrument to instrument. The values often will be changed slightly during instrument optimization procedures.

TABLE 4. TYPICAL ANALYTE MONITORING IONS: LC/MS/MS

Analyte	Exact Molecular Weight	Q1 Molecular Ion	Q3 Product Ion
CGA-362622*	437.1	438.1	182.0
CGA-53052	155.1	156.1	100.0
CGA-382997	256.0	257.0	176.0
CGA-368732	373.1	374.1	331.0
		331.1**	248.0**
CGA-368733	330.1	331.1	248.0
NOA-436664	316.1	317.1	234.0

* Chemical standard exists as a monosodium salt, which has an exact molecular weight of 459.1.

** Extensive fragmentation of this molecule occurs during ionization, forming CGA-368733, which can then be monitored based on its unique Q1/Q3 pair. In these validation experiments the 331.1/248.0 ion transition was used for quantifying CGA-368732. This ion transition exhibits better signal-to-noise and better calibration linearity than the 374.1/331.0 transition.