# 1.0 EXECUTIVE SUMMARY

ALS Laboratory Group – Environmental Division (formerly Enviro-Test Laboratories) performed an independent laboratory validation (ILV) of Syngenta Analytical Method T013656-05: Determination of Difenoconazole and its Metabolites CGA-205375, CGA-142856 and CGA-71019 in Soil, Using Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry. The soil analytical set consisted of one reagent blank, two control samples, five control samples fortified at the limit of quantitation (LOQ) of 1 ng/g, and five control samples fortified at 10X LOQ, 10 ng/g.

The method was successfully validated for the analyte at the LOQ and 10X LOQ concentration levels in soil. The mean recoveries, relative standard deviations (RSDs) and 95% confidence intervals were reported for each matrix at each fortification level. In addition, the statistics for the overall (LOQ and 10X LOQ) results for each matrix were reported.

The average recoveries at each fortification level were between 70% and 120% of the fortified theoretical concentrations for soil. In addition, the relative standard deviations of replicate measurements were less than 20% for each fortification level/matrix.

# 2.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Syngenta Analytical Method No. T013656-05 as performed by ALS Laboratory Group - Environmental Division (formerly Enviro-Test Laboratories) for the determination of Difenoconazole in soil using high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC/MS/MS), etc. [Ref. 1].

This study was conducted to satisfy guideline requirements described in the harmonized guidelines from the OPPTS, "Public Draft" - Data Reporting for Environmental Chemistry Methods, OPPTS 850.7100 [Ref. 2]. In addition, this report also satisfies the requirements outlined in the US EPA FIFRA Pesticide Assessment Guidelines for Subdivisions N, E, and K, and addenda for Data Reporting Guideline for Environmental Methods [Ref. 3].

# 3.0 MATERIALS AND METHODS

## 3.1 Test and Reference Substances

The test/reference substances were shipped from Syngenta Crop Protection, Inc., Greensboro, North Carolina to Enviro-Test Laboratories. CGA-142856 and CGA-169374 were received on June 18, 2004. CGA-71019 was received on March 9, 2005. CGA-205375 was received on January 12, 2006.

The following test/reference substances were used:

| | |
|---|---|
| Syngenta Code: | CGA-169374 |
| Common Name: | Difenoconazole |
| CAS Name: | 1H-1,2,4-Triazole, 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]- |
| CAS Registry No.: | 119446-68-3 |
| Reference No.: | 593-1669 |
| Purity: | 96.0% |
| Expiration Date: | April 2006 |
| Storage Conditions: | Freezer (<-20°C) |

| | |
|---|---|
| Syngenta Code: | CGA-205375 |
| CAS Name: | 1H-1,2,4-Triazole-1-ethanol, alpha-[2-chloro-4-(4-chlorophenoxy)phenyl]- |
| CAS Registry No.: | 117018-19-6 |
| Reference No.: | NV-XXVIII-45 |
| Purity: | 97.4% |
| Expiration Date: | January 2008 |
| Storage Conditions: | Freezer (<-20°C) |

| | |
|---|---|
| Syngenta Code: | CGA-71019 |
| CAS Name: | 1H-1,2,4-Triazole |
| CAS Registry No.: | 288-88-0 |
| Reference No.: | WFH-IV-5 |
| Purity: | 99.2% |
| Expiration Date: | March 2007 |
| Storage Conditions: | Freezer (<-20°C) |

| | |
|---|---|
| Syngenta Code: | CGA-142856 |
| CAS Name: | 1H-1,2,4-Triazole-1-acetic acid |
| CAS Registry No.: | 28711-29-7 |
| Reference No.: | GAN-VI-84-111 |
| Purity: | 99.0% |
| Expiration Date: | March 2006 |
| Storage Conditions: | Freezer (<-20°C) |

The Analytical and Product Chemistry Department of Syngenta Crop Protection, Inc., Greensboro, North Carolina maintains the characterization and stability data for the test/reference substances.

NOTE: On receipt of the neat test substances at Enviro-Test Laboratories on June 18, 2004, March 9, 2005, and January 12, 2006, they were logged in and stored at -20°C.

On January 11, 2006, 1107 µg/mL stock standard solution of CGA-142856 was prepared from the neat reference substance. On January 11, 2006, 1127 µg/mL stock standard solution of CGA-71019 was prepared from the neat reference substance. On January 16, 2006, 1284 µg/mL stock standard solution of CGA-205375 was prepared from the neat reference substance. On January 16, 2006, 1114 µg/mL stock standard solution of CGA-169374 (difenoconazole) was prepared from the neat reference substance. These stock solutions were used in the preparation of fortification and instrument calibration solutions. All standard solutions were prepared as per the method. Fortification standard solutions used for the ILV were prepared on January 16, 2006 and calibration working standard solutions were prepared on January 18, 2006 by serial dilution from standard solutions prepared on January 18, 2006. These fortification and calibration standard solutions were stored in a freezer at -20°C when not in use.

### 3.2 Soil Samples

The control soil sample used in this ILV was a pre-application North Dakota control soil sample from the 0-6" depth obtained from a soil dissipation study (Syngenta Study T002983-03). The sample was assigned an Enviro-Test Laboratories sample number. The soil had been previously characterized by Agvise as part of the soil dissipation study. Complete characterization results are presented in Appendix 3.

### 3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in Method T013656-05 (Section 2.0, Materials and Apparatus, and Appendices 1 and 2). Identical or equivalent apparatus and materials were used.

## 4.0 METHOD AND METHOD MODIFICATIONS

### 4.1 Modifications

1. Table 5, Difenoconazole and CGA-205375 HPLC gradient, extended final LC step from 10 to 12 minutes. More equilibration time was needed for consistent retention times.
2. Table 7, Dansyl triazole HPLC gradient, extended final LC step from 10 to 13 minutes. More equilibration time was needed for consistent retention times.
3. Table 7, Dansyl triazole autosampler conditions, the injection volume was reduced from 50 to 25 µL. Sensitivity was sufficient at 25 µL.

4. Table 9, CGA-142856 autosampler conditions, the injection volume was reduced from 50 to 25 µL. Sensitivity was sufficient at 25 µL.
5. Table 9, CGA-142856 HPLC gradient, maximum flow rates were reduced from 1.0 to 0.8 mL/min. Problems were encountered with excessive column back pressure due to high flow rate. Final run time was increased to compensate for reduced flow rate and allow for equilibration between runs.

## 4.2 Sample Validation Sets, Fortification and Extraction Procedure

Sample validation sets:

The method was validated successfully on the first extraction and analysis attempt of soil. The analytical set consisted of 13 samples: one reagent blank, two control samples, five control samples fortified at the LOQ (1 ppb) and five control samples fortified at 10X LOQ (10 ppb). Twelve 10 g aliquots of soil were used as samples for the set, plus a reagent blank sample of extraction solvent (acetonitrile/0.3% formic acid in water 70:30).

Fortification:

Samples were fortified in the round-bottomed flasks prior to addition of extraction solvent.

Extraction and Workup:

1. Weigh 10 g of soil into a 250-mL round-bottomed flask.
2. Fortify recovery samples with known amounts of difenoconazole, CGA-205375, CGA-71019 and CGA-142856.
3. Add 100 mL of extraction solution (acetonitrile/0.3% formic acid in water 70:30), reflux for 1 hour.
4. Allow to cool to room temperature, pour 45 mL of extract into a 50-mL centrifuge tube.
5. Centrifuge to clarify extract, 3500 rpm for 5 minutes.
6. For difenoconazole/ CGA-205375 analysis, transfer 0.50 mL of extract from step 5 to an autosampler vial, add 0.50 mL of water and mix. Store in a freezer for LC/MS/MS analysis.
7. For CGA-71019 analysis:
   a. Transfer 1.0 mL of extract from step 5 to a 15 mL screw cap culture tube.
   b. Add 1 mL of 0.1 M sodium bicarbonate, 20 µL of 10% ammonium hydroxide, 100 µL of 10% EDTA and 100 µL of 50 mM dansyl chloride in acetone to the extract.
   c. Cap and vortex a few seconds, heat at 40°C for 30 minutes. Protect from light with foil.
   d. Remove from heating and allow to cool 10 minutes.
   e. Add 2 mL of dichloromethane, cap and vortex 30 seconds.
   f. Add 5 mL of water and mix, centrifuge at 1000 rpm for a minute.
   g. Transfer the lower layer to a 4 mL vial using a Pasteur pipette.
   h. Evaporate to dryness with a nitrogen evaporator.
   i. Re-dissolve extract in 1.0 mL of pH 11 water/acetonitrile 60:40 and sonicate.
   j. Transfer to an autosampler vial for LC/MS/MS analysis. (Store in freezer)

8. For CGA-142856 analysis:
   a. Transfer 20 mL of extract from step 5 to a 40-mL screw cap culture tube.
   b. Add 400 µL of concentrated formic acid, mix.
   c. Transfer to a cation exchange column (Appendix 1, section 2.3), drain under vacuum at 2 mL/min, discard eluate.
   d. Stop flow 1-2 mm above bed.
   e. Rinse culture tube with 5 mL of water, add to cation exchange column and elute through, discarding eluate. Stop flow 1-2 mm above bed.
   f. Remove column from vacuum and place in a 125 mL round-bottomed flask.
   g. Add 20 mL of methanol:concentrated ammonium hydroxide (75:25) to the column.
   h. Elute through column, collect eluate in flask.
   i. Evaporate extract to dryness on a rotary evaporator.
   j. Re-dissolve in 4.0 mL of acetonitrile/0.3% formic acid in water 50:50, sonicate.
   k. Transfer 1 mL to an autosampler vial for LC/MS/MS analysis.

## 4.3 LC/MS/MS Instrumentation

The following instrumentation was used for each of the three analyses:

Mass Spectrometer: PE-Applied Biosystems Sciex API 3000 LC/MS/MS system

HPLC: PE Series 200 Micropumps

Autoinjector: Perkin Elmer Series 200

Data System: Apple Power Mac G3 running Applied Biosystems

MassChrom 1.1.2 software

The HPLC operating parameters for difenoconazole and CGA-205375 are presented in Table 5. The MS/MS operating parameters for difenoconazole and CGA-205375 are presented in Table 6. The HPLC operating parameters for CGA-71019 are presented in Table 7. The MS/MS operating parameters for CGA-71019 are presented in Table 8. The HPLC operating parameters for CGA-142856 are presented in Table 9. The MS/MS operating parameters for CGA-142856 are presented in Table 10.

## 4.4 Data Acquisition and Reporting

Peak integration and quantitation were performed by MacQuan version 1.6. Analyte quantitation was achieved by external calibration. The MS detector response (peak area) versus the standard concentration was used to generate calibration curves for the analytes. Best-fit weighted 1/x linear regression equations for the curves were derived and these equations were used to calculate the concentration of analyte in the samples. The correlation coefficient for the calibration curve for each analytical set was >0.990. Recovery results were computed for each sample. The equations used for quantitation are presented in Appendix 4.

## 5.3 Potential Interferences

Some soils contain backgrounds of CGA-71019 and/or CGA-142856 above LOQ, however the soil used for this ILV showed no detectable residues of any of the analytes.

## 5.4 Critical Steps

The dansyl derivative of CGA-71019 is unstable. Extracts must be kept in the freezer pending analysis.

## 5.5 Time Required for Analysis

One set of approximately 12 samples can be extracted in the lab and prepared for the instrument within 12 hours. Time required for analysis is three separate days, due to the fact each analysis requires unique HPLC conditions.

The method worked well as written. The CGA-142856 extracts were re-injected at the request of the study monitor, due to the analyte peak initially eluting on tailing edge of an area of unstable baseline. A freshly prepared mobile phase retained the CGA-142856 slightly longer, and was no longer in the unstable area.

Although the original method used both API-3000 and API-4000 instrumentation, the API-4000 instrument was not available for sample analysis during the time period this study was conducted. The API-3000 was sufficiently sensitive to analyse the LOQ samples. Gradients and injection volumes were also modified slightly in some instrumental methods to optimize chromatography and to reduce column backpressure. See section 4.1 and Table 5, Table 7, and Table 9 for specific instrumental modifications.

**TABLE 5. HPLC SYTEM FOR DIFENOCONAZOLE AND CGA-205375 ANALYSIS**

| | |
|---|---|
| **HPLC Instrumentation/Model** | Perkin Elmer Series 200 Micro Pumps |
| **HPLC Autosampler/Model** | Perkin Elmer Series 200 Autosampler |
| **Column** | Keystone Aquasil C18, 3µm, 150 mm x 3.0 mm i.d., with guard cartridge. |
| **Column oven temperature** | 40°C |
| **Injection volume** | 25 µL |
| **Sample compartment temperature control** | Ambient |
| **Mobile phase** | Solvent 1 = 0.2% formic acid in HPLC grade water<br>Solvent 2 = HPLC grade acetonitrile |

**Mobile phase program**

| Time (min) | % Solvent 1 | % Solvent 2 | Flow, mL/min | Curve* |
|---|---|---|---|---|
| 0.00 | 50 | 50 | 0.50 | --- |
| 1.00 | 50 | 50 | 0.50 | 1 |
| 4.00 | 10 | 90 | 0.50 | 1 |
| 7.00 | 10 | 90 | 0.50 | 1 |
| 7.10 | 50 | 50 | 0.50 | 1 |
| 12.00 | 50 | 50 | 0.50 | 1 |

*1 = Linear, 2 = Step change

## TABLE 6. MS/MS OPERATING PARAMETERS FOR DIFENOCONAZOLE AND CGA-205375 ANALYSIS

These validation analyses were conducted using a PE-Sciex API-3000 mass spectrometer. For these analytes, the instrument was run using the turbo-ionspray source in the positive ionization mode.

| | |
|---|---|
| Ionspray (V) | 5000 |
| Source Temp (°C) | 475 |
| Orifice (V) | 46.0 |
| Ring (V) | 130.0 |
| Q0 (V) | -10.0 |
| IQ1 (V) | -10.2 |
| ST (V) | -17.0 |
| RO1 (V) | -18.0 |
| IQ2 (V) | -20.0 |

| | |
|---|---|
| RO2 (V) | -50.0 |
| ST3 (V) | -70.0 |
| RO3 (V) | -52.0 |
| DF (V) | -200 |
| CEM (V) | 2600 |
| NEB (N2) | 12 |
| CUR (N2) | 13 |
| CAD (N2) | 9 |
| AUX (N2) (LPM) | 5.0 |

**MRM operating conditions:**

*MS/MS Transitions*

| Analyte | MW (exact) | MS/MS Transition | Dwell Time (ms) |
|---|---|---|---|
| Difenoconazole | 405.06 | 406 → 251 | 250 |
| CGA-205375 | 349.04 | 350 → 70 | 250 |

## TABLE 7. HPLC SYTEM FOR CGA-71019 (DANSYL TRIAZOLE) ANALYSIS

| | |
|---|---|
| **HPLC Instrumentation/Model** | Perkin Elmer Series 200 Micro Pumps |
| **HPLC Autosampler/Model** | Perkin Elmer Series 200 Autosampler |
| **Column** | Keystone Aquasil C18, 3µm, 150 mm x 3.0 mm i.d., with guard cartridge. |
| **Column oven temperature** | 40°C |
| **Injection volume** | 25 µL |
| **Sample compartment temperature control** | Cooled 5°C |
| **Mobile phase** | Solvent 1 = 0.2% acetic acid in HPLC grade water<br>Solvent 2 = HPLC grade acetonitrile |

**Mobile phase program**

| Time (min) | % Solvent 1 | % Solvent 2 | Flow, mL/min | Curve* |
|---|---|---|---|---|
| 0.00 | 60 | 40 | 0.50 | --- |
| 1.00 | 60 | 40 | 0.50 | 1 |
| 4.00 | 10 | 90 | 0.50 | 1 |
| 8.00 | 10 | 90 | 0.50 | 1 |
| 8.10 | 60 | 40 | 0.50 | 1 |
| 13.00 | 60 | 40 | 0.50 | 1 |

*1 = Linear, 2 = Step change

## TABLE 8. MS/MS OPERATING PARAMETERS FOR CGA-71019 (DANSYL TRIAZOLE) ANALYSIS

These validation analyses were conducted using a PE-Sciex API-3000 mass spectrometer. For these analytes, the instrument was run using the turbo-ionspray source in the positive ionization mode.

| | |
|---|---|
| Ionspray (V) | 5900 |
| Source Temp (°C) | 475 |
| Orifice (V) | 60.0 |
| Ring (V) | 220.0 |
| Q0 (V) | -10.0 |
| IQ1 (V) | -10.2 |
| ST (V) | -17.0 |
| RO1 (V) | -18.0 |
| IQ2 (V) | -18.0 |

| | |
|---|---|
| RO2 (V) | -52.0 |
| ST3 (V) | -72.0 |
| RO3 (V) | -54.0 |
| DF (V) | -400 |
| CEM (V) | 2600 |
| NEB (N2) | 12 |
| CUR (N2) | 13 |
| CAD (N2) | 8 |
| AUX (N2) (LPM) | 5.0 |

**MRM operating conditions:**

*MS/MS Transitions*

| Analyte | MW (exact) | MS/MS Transition | Dwell Time (ms) |
|---|---|---|---|
| CGA-71019 (dansyl triazole) | 302.08 | 303 → 181 | 250 |

## TABLE 9. HPLC SYTEM FOR CGA-142856 ANALYSIS

| | |
|---|---|
| **HPLC Instrumentation/Model** | Perkin Elmer Series 200 Micro Pumps |
| **HPLC Autosampler/Model** | Perkin Elmer Series 200 Autosampler |
| **Column** | Allure PFP Propyl, 5µm, 250 mm x 3.2 mm i.d. |
| **Column oven temperature** | 40°C |
| **Injection volume** | 25 µL |
| **Sample compartment temperature control** | Ambient |
| **Mobile phase** | Solvent 1 = 20% 5 mM ammonium acetate pH 4.5 in HPLC grade acetonitrile<br>Solvent 2 = HPLC grade acetonitrile |

**Mobile phase program**

| Time (min) | % Solvent 1 | % Solvent 2 | Flow, mL/min | Curve* |
|---|---|---|---|---|
| 0.00 | 0 | 100 | 0.80 | --- |
| 2.00 | 0 | 100 | 0.80 | 1 |
| 6.00 | 100 | 0 | 0.80 | 1 |
| 8.00 | 100 | 0 | 0.80 | 1 |
| 10.00 | 0 | 100 | 0.80 | 1 |
| 15.00 | 0 | 100 | 0.80 | 1 |

*1 = Linear, 2 = Step change

## TABLE 10. MS/MS OPERATING PARAMETERS FOR CGA-142856 ANALYSIS

These validation analyses were conducted using a PE-Sciex API-3000 mass spectrometer. For these analytes, the instrument was run using the turbo-ionspray source in the negative ionization mode.

| | |
|---|---|
| Ionspray (V) | 3500 |
| Source Temp (°C) | 450 |
| Orifice (V) | -46.0 |
| Ring (V) | -160.0 |
| Q0 (V) | 10.0 |
| IQ1 (V) | 10.2 |
| ST (V) | 17.0 |
| RO1 (V) | 18.0 |
| IQ2 (V) | 20.0 |

| | |
|---|---|
| RO2 (V) | 24.0 |
| ST3 (V) | 44.0 |
| RO3 (V) | 26.0 |
| DF (V) | 100 |
| CEM (V) | 2600 |
| NEB (N2) | 12 |
| CUR (N2) | 3 |
| CAD (N2) | 9 |
| AUX (N2) (LPM) | 5.0 |

**MRM operating conditions:**

*MS/MS Transitions*

| Analyte | MW (exact) | MS/MS Transition | Dwell Time (ms) |
|---|---|---|---|
| CGA-142856 | 127.04 | 126 → 81.9 | 1000 |

## CALCULATIONS

**Calculations used in the preparation of fortification samples**

An LOQ fortification of Difenoconazole and metabolites on a 10.0 g sample was prepared by fortifying the sample with 0.100 mL of a 0.100 µg/mL (ppm) standard solution:

[(0.100 mL) (0.100 µg/mL)] / 10.0 g = 0.001 µg/g (ppm) = 1 ng/g (ppb)

A 10 X LOQ fortification of Difenoconazole and metabolites on a 10.0 g sample was prepared by fortifying the sample 0.100 mL of a 1.00 µg/mL (ppm) standard solution:

[(0.100 mL) (1.00 µg/mL)] / 10.0 g = 0.010 µg/g (ppm) = 10 ng/g (ppb)

**Calculations used in the preparation of a LOQ instrument calibration working standard**

The sample weight was 10.0 g, so the total amount of Difenoconazole in the initial extract from a LOQ (1 ng/g) fortification on a 10.0 g sample at 100% recovery was:

(10.0 g) (1 ng/g) = 10 ng

The sample was diluted to 100 mL extract volume, and a 0.5 mL aliquot was further diluted to 1.0 mL. Thus the "in-solution" concentration of Difenoconazole in the final extract from a LOQ fortification at 100% recovery was:

(10 ng / 100 mL) (0.5 mL / 1.0 mL) = 0.050 ng/mL

A LOQ (0.010 ng/mL) instrument calibration working standard was prepared by bringing 50 µL of the 0.100 ng/µL standard solution to 10.0 mL in 50:50 (v/v) ACN:water, then further diluting 1.0 mL of the 0.50 ng/mL solution to 10.0 mL. Therefore the "in solution" concentration was:

[(50 µL) (0.100 ng/µL)] / (10.0 mL) (1.0 mL/10.0 mL) = 0.050 ng/mL

This agreed with the calculated final extract "in-solution" concentration from a LOQ fortification.

**Calculation of Difenoconazole and metabolites concentrations**

Calculations of the difenoconazole and metabolites concentrations were performed by PE Sciex "MacQuan" software by interpolation along the curves of the best-fit weighted 1/x linear regression equations generated by the calibration standards analyzed with the sample

solution, expressed as ng/mL (with respect to the sample volume), was the independent variable. The quantitation values reported on the chromatograms and spreadsheets generated from the LC/MS/MS were calculated/printed to four decimal places. Summaries of the residue data sheets (MacQuan sheets) are presented in Appendix 1.

Difenoconazole and metabolites concentrations were calculated by the software using a linear regression equation as follows:

$Y = mX + b$

where Y = area counts for analyte
X = concentration of analyte
b = intercept constant from the linear regression
m = slope constant from the linear regression

Example calculation for Difenoconazole residues in LOQ Fortification #1 on soil, extracted January 16, 2006 and analyzed February 16, 2006, see Figure 11:

where Y = 22319
b = -757
m = 21432

$$X = \frac{Y - b}{m}$$

$$= \frac{22319 - (-757)}{21432} \times 20$$

$$= \frac{1.08\ pg}{1.250\ mg} = 0.864\ ng/g$$

Amount Injected (mg) is equal to:

$$\text{Amt. Inj.} = \left[\frac{[\text{Sample Size} \times \text{Aliquot Vol.}]}{\text{Extraction Vol.}} \times \text{Inj. Vol.}\right] \div \text{Final Vol.}$$

$$\text{Amt. Inj.} = \left[\frac{[10.0\ g \times 0.5\ mL]}{100\ mL} \times 25\ \mu L\right] \div 1.0\ mL = 1.250\ mg$$

NOTE: The intercept, slope and area count values used by the PE Sciex "MacQuan" software to calculate residues included more places to the right of the decimal point than the numbers printed out in the data packages. Therefore, the calculations cannot always be reproduced by hand beyond the first two significant figures.

**Calculation of method fortification percent recovery**

Method fortification recovery (%) =

$$\frac{\text{Residue found (ng/mL)}}{\text{Fortification concentration (ng/mL)}} \times 100\%$$

*Example calculation*

Continuing with the example in the above, Difenoconazole residues in LOQ Fortification #1 on soil, extracted January 16, 2006 and analyzed February 16, 2006, see Figure 11 in Appendix 2, and Table 1):

$$\text{Percent Recovery} = \frac{0.864 \text{ ng/g}}{1 \text{ ng/g}} \times 100\%$$

$$= 86\%$$

**Calculation of standard deviation (s) and relative standard deviation (RSD) for a mean**

Standard deviations for the average percent recoveries were calculated and expressed as an absolute percent value. Relative standard deviations were calculated as follows:

$$\text{RSD} = \frac{\text{standard deviation}}{\text{mean}} \times 100\%$$

*Example calculation*

Using the five replicates of the Difenoconazole LOQ Fortifications at 0.010 ng/mL in ground water, reported in Table 1, where the rounded mean recovery was 79% and the rounded standard deviation was 6.1%:

$$\text{RSD} = \frac{\pm 5.9\%}{79\%} \times 100\%$$

$$= \pm 7.3\%$$

**Calculation of 95% confidence interval for a mean**

$$95\%\ \text{confidence interval} = \text{mean} \pm \frac{t \times \text{standard deviation}}{\sqrt{n}}$$

where n = number of measurements
t = student t variate for n-1 degrees of freedom at the 95% confidence interval (2.776 for 4 degrees of freedom and 2.262 for 9 degrees of freedom). See table C.3, page 267, Quality Assurance of Chemical Measurements, John Keenan Taylor, Lewis Publishers, Inc., 1987

*Example calculation*
Using the five replicates of the Difenoconazole LOQ fortifications at 1 ng/g in soil, reported in Table 1, where the mean recovery was 79% and the rounded standard deviation was 5.9%:

$$95\%\ \text{confidence interval} = 79\% \pm \frac{(2.776)\,(5.9\%)}{\sqrt{5}}$$

$$= 79\% \pm 7.3\%$$

Mean percent recoveries, standard deviations, relative standard deviations, and 95% confidence intervals were calculated using an Excel 97 spreadsheet. Results were rounded off for reporting purposes but not during calculations.