

## 2.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Bayer Analytical Method CC-001-S14-01, "Independent Laboratory Validation (ILV) of Bayer Method CC-001-S14-01 for the Determination of Residues of Cyclanilide and its Metabolite 2,4-Dichloroaniline in Soil and Cyclanilide in Water by LC-MS/MS" performed by ADPEN Laboratories, Inc. The analytical method submitted by Bayer is presented in Appendix 2.

This study was designed to satisfy harmonized guideline requirements described in US EPA Test Guidelines OCSP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (Reference 2), OPPTS 850.7100 (Reference 3), and OPPTS (OCSP) 860.1340(c) (6) (Reference 4). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (Reference 5).

## 3.0 MATERIALS AND METHODS

### 3.1 Reference Substances

The following reference substances were obtained from Bayer CropScience:

**Standard name:** Cyclanilide

Standard Number: K-1751

CAS name: 1-[[[(2,4-dichlorophenyl)amino]carbonyl]cyclopropanecarboxylic acid

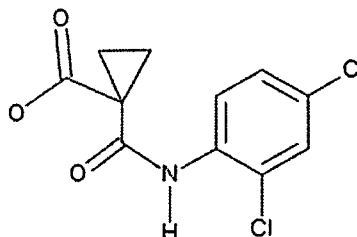
CAS number: 113136-77-9

Ref. Substance Lot: 0625200303

GLP purity: 98.9%

Expiration date: August 9, 2016

Storage conditions: Refrigerator



**Standard name:** 2,4-Dichloroaniline

Standard Number: K-2092

CAS name: 2,4-Dichlorobenzeneamine

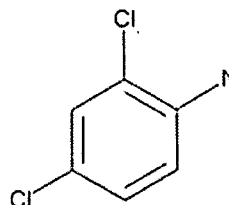
CAS number: 554-00-7

Ref. Substance Lot: 0716200415

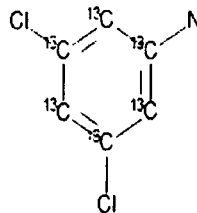
GLP purity: 99.2%

Expiration date: November 16, 2016

Storage conditions: Freezer



**Standard name:** 3,5-Dichloroaniline-<sup>13</sup>C<sub>6</sub>  
**Standard Number:** K-1575  
**CAS name:** N/A  
**CAS number:** N/A  
**Ref. Substance Lot:** 0303200601  
**GLP purity:** 98.5%  
**Expiration date:** December 14, 2015  
**Storage conditions:** Freezer



The reference substances used in this study were obtained from Bayer and received at ADPEN on April 1, 2014. Reference standards were stored as directed on their respective certificate of analysis. Upon receipt, the reference standard for cyclanilide was stored in refrigerator E-119, and the reference standards for 2,4-dichloroaniline (2,4-DCA) and 3,5-dichloroaniline-<sup>13</sup>C<sub>6</sub> were stored in freezer E-119. Refrigerator and freezer E-119 typically operate at  $1.7 \pm 1$  °C and  $-22.8 \pm 1$  °C, respectively.

The certificates of analysis are presented in Appendix 3. All fortification and calibration solutions made from the reference substances were stored according to the method.

### 3.2 Test System

The test systems used in this study were soil and water sample. The control samples were provided by Bayer and stored frozen prior to analysis. The following table is a description of the samples.

MATRIX	SAMPLE ID	SAMPLE DESCRIPTION
Soil	MESXN008CA-S001	1 kg soil
Water	061113-W	1 L pond water

Samples were logged into the Laboratory Information Management System (LIMS) and assigned a unique laboratory code, which is cross-referenced to the sample number on raw data and detailed residue reports. During the course of this study, the sample was stored in freezer E-16, which operates at a temperature of  $-21 \pm 1$  °C.

### 3.3 Analytical Procedures

Analytical Method CC-001-S14-01 was independently validated, as written and submitted by Bayer (Appendix 2). The apparatus and reagents were used as outlined in the analytical method with equivalent apparatus or reagents substituted as necessary.

#### 3.3.1 Fortifications

Untreated control soil samples were fortified at LOQ (5 ppb) and 10× LOQ (50 ppb) using the appropriate fortification standard concentrations as per the method. Fortifications were prepared as described below.

Matrix	Concentration (ng/ $\mu$ L)	Aliquot Volume (mL)	Dilution Volume (mL)	Final Concentration (ng/ $\mu$ L)	Replicates
Soil	5.0	5	50	0.5	5
	84.36/84.72	2.963/2.951	50	5.0	5

Untreated control water samples were fortified at LOQ (0.5 ppb) and 10 $\times$  LOQ (5 ppb) using the appropriate fortification standard concentrations as per the method. Fortifications were prepared as described below.

Matrix	Concentration (ng/ $\mu$ L)	Aliquot Volume (mL)	Dilution Volume (mL)	Final Concentration (ng/ $\mu$ L)	Replicates
Water	0.5	5	50	0.05	5
	5.0	5	50	0.5	5

### 3.3.2 Extraction Procedure for Soil

1. A 20-g soil sample was transferred into a glass jar.
2. Recovery samples were fortified at the appropriate level.
3. Approximately 40 mL of water/methanol (50:50) was added to the sample and shaken for approximately 10 minutes.
4. Sample was centrifuged at 3000 rpm for 5 minutes and the aqueous layer was decanted into a stoppered glass flask.
5. Extraction steps 3 and 4 were repeated with the additional step of adding 160  $\mu$ L of the isotopic internal standard solution (5  $\mu$ g/mL).
6. Sample was centrifuged at 3000 rpm for 5 minutes and the aqueous layer was decanted into the stoppered glass flask from step 4.
7. Combined extracts were mixed well and a 10-mL aliquot was transferred into a round bottom flask.
8. The 10-mL aliquot was evaporated to approximately 5 mL using a rotary evaporator set at 40  $^{\circ}$ C.
9. Extracts were diluted, if necessary, with HPLC water and vialled for analysis.

### 3.3.3 Extraction Procedure for Water

1. A 20-g water sample was transferred into a 25 mL volumetric flask.
2. Recovery samples were fortified at the appropriate level.
3. To the sample, 2 mL of methanol, 50  $\mu$ L of formic acid, and 100  $\mu$ L of internal standard (5  $\mu$ g/mL) were added. Sample was diluted to 25 mL with HPLC water.
4. An aliquot was transferred to a LC vial for analysis.

### 3.3.4 Modifications

The following were modifications made to the analytical method:

1. A dilution of the final volume was necessary to resolve matrix interferences.

### 3.4 Instrument Operating Parameters

Chromatography System:	Agilent 1290 UPLC			
Analytical Column:	Phenomenex Luna C18(2)-HST, 2.0 x 50 mm, 2.5 $\mu$ m			
Column Temperature:	60 $^{\circ}$ C			
Injection Volume:	10.0 $\mu$ L			
Run Time:	9 minutes			
Mobile Phase A:	0.3% Formic Acid in HPLC Water			
Mobile Phase B:	Acetonitrile			
Gradient:	Time (min.)	Flow Rate (mL/min)	A (%)	B (%)
	0.0	0.40	90	10
	4.0	0.40	50	50
	5.0	0.40	50	50
	6.0	0.40	10	90
	7.0	0.40	10	90
	7.1	0.40	90	10
	9.0	0.40	90	10

Detection System:	Agilent 6490 Triple Quad				
Gas Flow:	14 L/min				
Temperature:	150 $^{\circ}$ C				
Nebulizer (psi):	45				
Sheath Gas Heater:	300				
Sheath Gas Flow:	12				
Capillary (V):	3000				
V Charging:	1500				
<b>MRM Conditions</b>	<b>Cyclanilide</b>		<b>2,4-DCA</b>		<b>3,5-DCA-<sup>13</sup>C<sub>6</sub></b>
Retention Time (minutes)	4.5	4.5	4.1	4.1	4.0
Transition:	Quantitation	Confirmatory	Quantitation	Confirmatory	Internal Standard
MS1:	272	272	162	162	168
MS2:	160	228	99	126	133
Ionization:	ESI	ESI	ESI	ESI	ESI
Dwell Time:	100	100	100	100	10
Frag (V):	380	380	380	380	380
Collision Energy (V):	20	8	28	16	20
Cell Acc (V):	7	7	7	7	7
Polarity:	Negative	Negative	Positive	Positive	Positive

### 3.5 Data Acquisition

Peak integration and peak area count quantitation were performed by Agilent MassHunter (version B.04.01) data handling software. A best-fit, linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of the analyte. The square of correlation coefficients ( $R^2$ ) for the calibration curves for each analytical set was greater than 0.99.

Statistical treatment of the data including the calculation of percent recovery, means, and standard deviations were calculated within LIMS and reported using Microsoft® Office Excel spreadsheets. Example calculations are presented in Appendix 4.

## 7.0 REFERENCES

1. Netzband, Derek. Bayer Method CC-001-S14-01, "An Analytical Method for the Determination of Residues of Cyclanilide and its Metabolite 2,4-Dichloroaniline in Soil and Cyclanilide in Water Using LC-MS/MS". Bayer CropScience. April 19, 2013.
2. United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, 712-C-001 (OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation), January, 2012.
3. United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, 712-C-96-348 (OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods), April, 1996.
4. United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, 712-C-96-174 (OCSPP) 860.1340, Residue Chemistry Test Guidelines, August 1996.
5. FIFRA Good Laboratory Practice Standards. *Code of Federal Regulations*, Section 160, Title 40, 1982; *Fed. Regist.* 1989, 48, 53946 ff.
6. Dean, R.B. and W.J. Dixon, 1951. *Analytical Chemistry* 23:636. American Chemical Society, Washington DC.
7. Engineering Statistics Handbook.  
<http://www.itl.nist.gov/div898/handbook/eda/section3/eda35h1.htm>. (Date accessed March 5, 2014). Section 1.3.5.17.1.Grubb's Test for Outliers.

**TABLE 1. Soil Fortification Levels**

Lab Code	Fortification Level (ppb)	Number of Samples
14042288B-MB-1	Method Blank	1
140403005-002B 140403005-002C	Control	2
Recovery1-1, Recovery1-2, Recovery1-3, Recovery1-4, Recovery1-5	5	5
Recovery2-1, Recovery2-2, Recovery2-3, Recovery2-4, Recovery2-5	50	5

**TABLE 2. Water Fortification Levels**

Lab Code	Fortification Level (ppb)	Number of Samples
14042289-MB-1	Method Blank	1
140403005-001B 140403005-001C	Control	2
Recovery1-1, Recovery1-2, Recovery1-3, Recovery1-4, Recovery1-5	0.5	5
Recovery2-1, Recovery2-2, Recovery2-3, Recovery2-4, Recovery2-5	5	5



### Flow Diagram of the Analytical Procedure for Soil

Weigh 20 g of soil into a glass jar and fortify the recovery samples



Add approximately 40 mL of water:methanol (50:50) and shake for 10 minutes



Centrifuge samples at 3000 rpm for 5 min and repeat extraction with an additional 40 mL of water:methanol (50:50)



Add 160  $\mu$ L of internal standard by pipet and shake



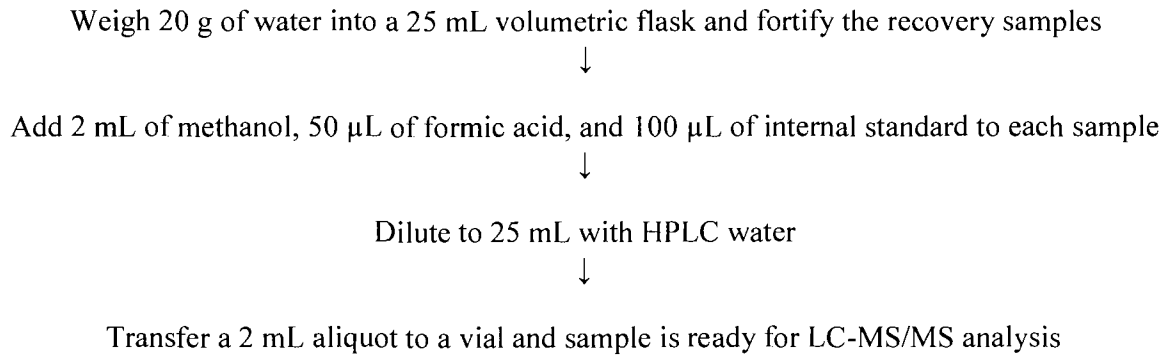
Centrifuge the sample at 3000 rpm for 5 minutes



Mix the combined extracts and transfer a 10 mL aliquot to a vial.



Evaporate to 5 mL, dilute, and sample is now ready for LC-MS/MS analysis

**Flow Diagram of the Analytical Procedure for Water**

**APPENDIX 2. Bayer Analytical Method CC-001-S14-01**

**Bayer CropScience**

CC-001-S14-01

**1. Principle**

The residues of cyclanilide and its metabolite 2,4-dichloroaniline in soil are determined by shaking a 20 gram soil sample twice with methanol/water, amending with an internal standard, centrifuging and an aliquot evaporated to reduce volume with analysis by LC/MS/MS.

The residues of cyclanilide in water are determined by amending a sample with an internal standard, with analysis by LC/MS/MS.

Quantification is based on a comparison of peak areas with those of known standards. Two sets of MRM transitions are shown, one for quantitation and the second for confirmatory purposes.

The method limit of quantitation (LOQ) for cyclanilide and its metabolite 2,4-dichloroaniline is 5ng/g in soil and 0.5ng/g for cyclanilide in water.

**2. Apparatus**

Use as a guide; equivalent apparatus may be substituted.

VWR Pyrex® Brand volumetric pipets, glass class A (Assorted Volumes)  
Eppendorf Reference and Repeat pipettes and tips  
VWR Pyrex® Brand volumetric flasks, glass class A (Assorted Volumes)  
VWR Pyrex® Brand mixing cylinders, glass class A (Assorted Volumes)  
VWR Pyrex® Brand disposable Pasteur pipets (Cat. No.: 53283-910 & 53283-914)  
BD Falcon 50mL conical centrifuge tubes (Cat. No.: 352070)  
National Scientific LC vials, Snap-Its (Cat. No.: C4011-5)  
National Scientific LC vial Snap-It Seals, (Cat. No.: C4011-55)  
Phenomenex® Luna 2.5µm C18(2)-HST 50x2mm (Cat 00B-4446-B0)  
ABSciex 5500 (LC/MS/MS) equipped with an electrospray interface, Shimadzu 20ADXR  
HPLC pumps (2), CBM 20A communications bus module, CTO-20 A column oven and  
a CTC autosampler, and data collection/processing software (Analyst 1.6.2)  
TurboVap evaporator (Zymark Corporation, Model LV)  
Centrifuge  
Mechanical Shaker  
Various general laboratory glassware and utensils

**3. Reagents**

Use as a guide; equivalents or different manufactures (brands) may be substituted.

Water (HPLC Grade or Millipore)  
Methanol (HPLC Grade)  
Acetonitrile (HPLC Grade)  
Formic acid (HPLC Grade)  
50/50(v/v) Water/Methanol. Combine 500mL water and 500mL Methanol. Mix well.  
85/15(v/v) Water/Methanol. Combine 850mL water and 150mL Methanol. Mix well.  
0.3% formic acid in water. Add 3mL of formic acid to 1L of water. Mix well.

**Bayer CropScience**

CC-001-S14-01

**4. Preparation of Analytical Standards**

**NOTE:** The following procedure is an example description of how standard solutions may be prepared. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in a freezer when not in use. Solutions should be allowed to warm to room temperature prior to use.

**4.1 Primary Stock Standard Solutions**

Prepare individual 100µg/mL stock solutions of cyclanilide and 2,4-dichloroaniline by transferring 0.0100 grams of each analyte to separate 100mL volumetric flasks. Dilute to volume with acetonitrile and mix well. Store frozen when not in use.

**NOTE:** Corrections for standard purities should be applied when expressing standard concentrations.

**4.2 Mixed Standard Solutions**

Prepare a mixed 5µg/mL solutions of cyclanilide and 2,4-dichloroaniline by taking a 5.0mL aliquot of the 100µg/mL stock solutions and diluting to 100mL with acetonitrile. Refrigerate when not in use.

Prepare a mixed 500ng/mL solutions of cyclanilide and 2,4-dichloroaniline by taking a 10.0mL aliquot of the 5µg/mL mixed solution and diluting to 100mL with acetonitrile. Refrigerate when not in use.

Prepare a mixed 50ng/mL solution of cyclanilide and 2,4-dichloroaniline by taking a 10.0mL aliquot of the 500ng/mL mixed solution and diluting to 100mL with acetonitrile. Store refrigerated when not in use.

**4.3 Internal Standard Solution**

Prepare an individual 100µg/mL stock solution of 3,5-dichloroaniline-<sup>13</sup>C<sub>6</sub> by transferring 0.005 grams of the analyte into a 50mL volumetric flasks. Dilute to volume with acetonitrile and mix well. Store frozen when not in use.

Prepare an individual 5µg/mL solution of 3,5-dichloroaniline-<sup>13</sup>C<sub>6</sub> by taking a 5.0mL aliquot of the 100µg/mL mixed solution and diluting to 100mL with acetonitrile. Refrigerate when not in use.

Further dilutions of this mixed fortification solution may be made as needed. Note that 3,5-dichloroaniline-<sup>13</sup>C<sub>6</sub> is used as a surrogate internal standard for the cyclanilide analysis.

**Bayer CropScience**

CC-001-S14-01

**4.4 Calibration Standard Solutions**

Prepare working calibration solutions as described below and dilute to volume with 85/15(v/v) water/methanol.

Conc. of Native Standard Solution (µg/mL)	Aliquot Native Standard Solution Taken (mL)	Conc. of Internal Standard Solution (µg/mL)	Aliquot Internal Standard Solution Taken (mL)	Dilution Volume (mL)	Native Conc in Calibration Solution (ng/mL)	Internal Standard Conc in Calibration Solution (ng/mL)
5.0	0.5	5.0	0.2	100	25	10
5.0	0.2	5.0	0.2	100	10	10
0.5	1.0	5.0	0.2	100	5	10
0.5	0.5	5.0	0.2	100	2.5	10
0.5	0.2	5.0	0.2	100	1.0	10
0.5	0.1	5.0	0.2	100	0.5	10
0.5	0.05	5.0	0.2	100	0.25	10

Additional calibration solutions may be prepared as required. Refrigerate when not in use.

**5. Extraction****5.1 Soil Analysis**

1. Transfer 20±0.01 grams of soil into a suitably sized glass jar.
2. Fortify the recovery samples at the desired fortification level using the individual standard solutions (see Section 4.2 Mixed Standard Solutions). For fortifications at the method LOQ add, by pipet, 0.2mL of the 500ng/mL mixed standard solution.
3. Add ≈40mL of water/methanol 50/50 (v/v) to all samples and shake for ~10 minutes.
4. Centrifuge the sample at ~3000rpm for ~5 minutes. Decant the aqueous layer into a stoppered glass container.
5. Repeat the extraction by adding an additional ≈40mL of water/methanol 50/50 to the soil extract and shake for ~10 minutes. Add, by pipet, 80µL of the 5µg/mL isotopic internal standard solution. Stopper and shake well.

**Bayer CropScience**

CC-001-S14-01

6. Centrifuge the sample at ~3000rpm for ~5 minutes. Decant the aqueous layer into a stoppered glass container used in step 4. Dilute the contents to ~80mL with methanol and mix well.
7. Transfer a 10mL aliquot into a suitable container and evaporate the contents to ~5mL using a TurboVap set at 35°C.
8. Transfer ~2mL to a LC vial, cap and analyze the contents by LC/MS/MS.

**5.2 Water Analysis**

1. Transfer 20±0.1mL of water into a suitably sized glass jar.
2. Fortify the recovery samples at the desired fortification level using the individual standard solutions (see Section 4.2 Mixed Standard Solutions). For fortifications at the method LOQ add, by pipet, 0.2mL of the 50ng/mL mixed standard solution.
3. Add 2mL of methanol, 50uL of formic acid and 50uL of the 5ug/mL isotopic internal standard to each sample. Dilute to 25mL with deionized water.
4. Transfer an aliquot to a LC vial and analyze the contents by LC/MS/MS.

**6. Analysis****6.1 Sample Analysis**

Step 1. Using the recommended procedures listed below; analyze an aliquot of the 0.25, 0.5, 1.0, 2.5, 5, 10 and 25ng/mL standard solutions. (these are calibration solution analyses).

Step 2. Analyze an aliquot of each analytical sample from Section 5.1 Step 8 or Section 5.2.

Step 3. Again analyze an aliquot of the 0.25, 0.5, 1.0, 2.5, 5, 10 and 25ng/mL calibration standard solutions.

**6.2 LC/MS/MS Standard Calibration and Residue Calculations**

Standardize the LC/MS/MS response under the conditions outlined in Appendix 1 by injecting an aliquot of each LC/MS/MS calibration solution interspersed with samples.

The residues of cyclanilide and its metabolite 2,4-dichloroaniline are quantified using internal standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by linear regression of the standard peak area versus the standard concentrations in ng/mL using ABSciex Analyst Software (Version 1.6.2), a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to

**Bayer CropScience**

CC-001-S14-01

calculate the regression coefficients M and B, respectively called slope and intercept, for each analytical set.

The standards were fit to the linear equation:  $Y = MX + B$

where:  $X$  is the concentration of the reference standard in ng/mL  
 $M$  is the calibration line slope  
 $B$  is the calibration line intercept  
 $Y$  is the native peak area

The calibration points are weighted  $1/x$  to provide better fit near the limit of detection.

After regression coefficients were calculated, the residue in parts per billion was determined. The parts per billion (ppb) of cyclanilide and 2,4-dichloroaniline in soil was calculated using the following equation,

$$\text{residue found (ppb)} = \frac{Y-B}{M} \times D$$

$$\text{Dilution Factor (D)} = \frac{\text{Initial volume (V1)}}{\text{Initial sample wt. (W)}} \times \frac{\text{Final volume (V3)}}{\text{Aliquot (V2)}}$$

For soil:             $W = 20\text{g}$   
                           $V1 = 80\text{mL}$   
                           $V2 = 10\text{mL}$   
                           $V3 = 5\text{mL}$

For water:            $W = 20\text{mL}$   
                           $V1 = 25\text{mL}$

Analyst software was used to calculate the residues of cyclanilide and 2,4-dichloroaniline in ppb for each sample and the percent recovery for the fortified samples.

**6.3 Fortification Experiments**

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation.

$$\text{Recovery (\%)} = \frac{(R-S)}{T} \times 100$$

Where:  $R =$         *ppb of target analyte found in fortified sample*  
 $S =$         *ppb of target analyte found in control sample*  
 $T =$         *theoretical ppb in fortified sample*



**Bayer CropScience**

CC-001-S14-01

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ of 5ng/g for soil and 0.5ng/mL for water or other appropriate level with fortification solutions. Calculate the residue for the control (S) and fortified control (R) samples.

**Bayer CropScience**

CC-001-S14-01

**Appendix 1 Instrument Conditions for Cyclanilide and Its Metabolite  
2,4-dichloroaniline**

Equipment with equivalent or better sensitivity and performance may be substituted.

**LC/MS/MS Parameters**

**NOTE:** Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Therefore, the given LC/MS/MS parameters listed below are guidelines and may be modified. These parameters should be optimized for the instrument and column actually used. Also, instrument parameters and mobile phase may be adjusted to improve separation from any observed interfering peaks.

The following conditions were used on an ABSciex API 5500 LC/MS/MS system.

**HPLC Parameters**

Pumps Used: Two Shimadzu LC-20ADXR pumps with a Shimadzu CBM-20A communications bus module and a Shimadzu CTO20A oven

Autosampler CTC PAL

Column Temperature: 60°C

Injection Volume: 5uL

Column: Manufacturer: Phenomenex®  
Type: Luna 2.5µm C18(2)-HST  
Particle Size: 2.5µm  
Diameter: 2.0 mm  
Length: 50 mm

Mobile Phase A: 0.3% formic acid in water

Mobile Phase B: Acetonitrile

**HPLC gradient program:**

Time (min.)	Module	Flow Rate (mL/min)	A(%)	B(%)
0.0	Pumps	0.40	90	10
5.0	Pumps	0.40	10	90
6.0	Pumps	0.40	10	90
6.1	Pumps	0.40	90	10
9.0	System Controller	Stop		

**Diverter valve program :**

Time (min.)	Position
0.0	Waste
2.0	Source
5.0	Waste

Bayer CropScience

CC-001-S14-01

**Mass Spectrometer Instrument Conditions**

The method is set up with two separate periods, the first with positive ionization for DCA analysis and the second with negative ionization for cyclanilide analysis

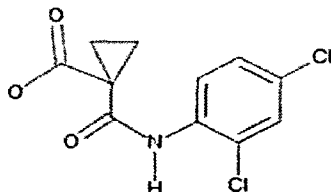
Component:	Cyclanilide	Cyclanilide	2,4-DCA	2,4-DCA	3,5-DCA- <sup>13</sup> C <sub>6</sub>
Retention Time	4.5 minutes	4.5 minutes	3.9 minutes	3.9 minutes	4.0 minutes
Transition	Quantitation	Confirmation	Quantitation	Confirmation	Internal Standard
Parent Ion	272	272	162	162	168
Product Ion	160	228	126	99	133
Ionization Mode	ESI	ESI	ESI	ESI	ESI
Polarity	-	-	+	+	+
Dwell Time (ms)	50	50	25	25	25
Resolution Q1/Q3	Unit/Unit	Unit/Unit	Unit/Low	Unit/Low	Unit/Low
Declustering Potential (DP)	-35	-35	66	66	70
Entrance Potential (EP)	-10	-10	10	10	6
Collision Energy (CE)	-28	-16	27	27	30
Collision Cell Exit Potential (CXP)	-19	-21	12	12	7
Curtain Gas (CUR)	25	25	25	25	25
Collision Gas (CAD)	10	10	10	10	10
Ion Source Gas 1 (GS1)	40	40	40	40	40
Ion Source Gas 2 (GS2)	65	65	65	65	65
Source Temp (TEM)	750	750	750	750	750
Interface Heater (IHE)	Off	Off	Off	Off	Off
Ion Transfer Voltage (IS)	-4500	-4500	5500	5500	5500

**Bayer CropScience**

CC-001-S14-01

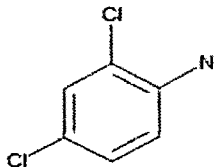
**Appendix 2 Structures**

Chemical Name: Cydanilide  
(Parent Molecule)



CAS Name: 1-[[[(2,4-Dichlorophenyl)amino]carbonyl]cyclopropane  
carboxylic acid  
CAS Number: 113136-77-9  
Molecular Formula: C<sub>11</sub> H<sub>9</sub> Cl<sub>2</sub> N O<sub>3</sub>  
Molecular Weight: 274.1

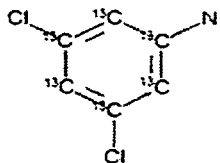
Chemical Name: 2,4-Dichloroaniline  
(Metabolite)



CAS Name: 2,4-Dichlorobenzeneamine  
CAS Number: 554-00-7  
Molecular Formula: C<sub>6</sub> H<sub>5</sub> Cl<sub>2</sub> N  
Molecular Weight: 162.2

**Bayer CropScience****CC-001-S14-01**

Chemical Name: 3,5-Dichloroaniline-<sup>13</sup>C<sub>6</sub>  
(Metabolite)



CAS Name: 3,5-Dichlorobenzeneamine-<sup>13</sup>C<sub>6</sub>  
CAS Number: 554-00-7  
Molecular Formula: C<sub>6</sub> H<sub>5</sub> Cl<sub>2</sub> N  
Molecular Weight: 168.0

#### APPENDIX 4. Example Calculations

Residue results are calculated by comparison to the standard curves obtained from a linear regression analysis of the data found by the data system. The equation for the fit of the standard curve was used to calculate intercept and slope of the linear regression curve. The intercept and the slope were used in the equation used for quantitation. LIMS was used to calculate the ppb, percent recovery, and all other data and reported in Microsoft® Excel spread sheets. The following equations were used for quantitation:

The following equations were used for the quantitation of residues of cyclanilide and 2,4-dichloroaniline:

The calculation below was performed within Agilent MassHunter:

$$1. \text{ Calibration curve: } y = mx + b \quad \text{Solving for } x: \quad x = \frac{y - b}{m}$$

Where,

- m = slope
- b = y intercept
- x = Analyte found (pg)
- y = Peak Area

The calculations below were performed within Microsoft Excel:

$$b) \quad \text{amount of sample injected} = \frac{\text{injection size (mL)}}{\text{final volume (mL)}} \times \text{sample weight (g)}$$

$$c) \quad \text{ppb} = \frac{\text{ng found}}{\text{g injected}}$$

$$d) \quad \text{Percent recovery} = \frac{(\text{ppbin the sample} - \text{ppbin the control})}{\text{ppbadded}} \times 100$$

As an example, calculations to obtain the percent recovery in a control soil sample fortified with Cyclanilide in sample number 14042289-Recovery1-1 (WO-14042289). The calculations are shown below:

$$a) \quad \text{ng found} = \frac{1317 - (-0.005729)}{4.310877} = 0.004045 \text{ ng}$$

$$b) \quad \text{amount of sample injected} = \frac{0.01 \text{ mL}}{25 \text{ mL}} \times 20.03 \text{ g} = 0.008012 \text{ g}$$

$$c) \quad \text{ppb} = \frac{0.004045 \text{ ng}}{0.008012 \text{ g}} = 0.505 \text{ ppb}$$

$$d) \quad \text{Percent recovery} = \frac{(0.505 \text{ ppb} - 0.000 \text{ ppb})}{0.500 \text{ ppb}} \times 100 = 101\%$$