# 1.0 INTRODUCTION

The purpose of this study was to validate an analytical method used to determine the content of dicloran in soil samples by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). The method was validated to quantify the concentrations of dicloran present in recovery samples prepared in sandy loam soil and loamy sand soil. The analytical method was validated with regards to accuracy, precision, specificity, linearity, limit of quantitation (LOQ), limit of detection (LOD), method detection limit (MDL), and confirmation of analyte identification.

The method was validated in sandy loam soil and loamy sand soil by fortification with dicloran at concentrations of 0.0500 (LOQ) and 0.500 (10X LOQ) mg/kg. Samples were extracted twice with acetonitrile. The recovery samples were further diluted into the calibration range with 20/80 acetonitrile/ultra-pure or purified reagent water (v/v) and/or matrix-matched control diluent (control final fraction, see Section 2.11). All samples were analyzed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

The study was initiated on 18 April 2019, the day the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental portion of the validation was conducted from 6 June to 20 August 2019 at Smithers, located in Wareham, Massachusetts. At the study closure, all original raw data (original protocol and amendments, correspondence, all study data, study documentation), and the final report will be sent for archival to: Attention: Maria Jauregui, Gowan Company, 370 South Main Street, Yuma, Arizona 85364.

# 2.0 MATERIALS AND METHODS

## 2.1 Protocol

Procedures used in this study followed those described in the Smithers Viscient protocol entitled "Environmental Chemistry Method: Validation of the Analytical Method for the Determination of Dicloran in Soil by LC-MS/MS" (Appendix 1). The study was conducted under Good

Laboratory Practice (GLP) regulations and principles as described in 40 CFR 160 (U.S. EPA, 1989) and the OECD principles on GLP (OECD, 1998), and followed the SANCO/3029/99 rev 4 guidance document (EC, 2000) and OCSPP 850.6100 guideline (U.S. EPA, 2012).

## 2.2 Test and Reference Substances

### 2.2.1 Test Substance

The test substance, dicloran technical, was received on 2 April 2019 from EPL Archives, Inc., Sterling, Virginia. The following information was provided:

| | |
|---|---|
| Name: | Dicloran technical |
| Synonym(s): | BOTRAN technical; 2,6-dichloro-4-nitroaniline |
| Lot No.: | 20130605 |
| CAS No.: | 99-30-9 |
| Purity: | 98.9% (Certificate of Analysis, Appendix 2) |
| Recertification Date: | 19 April 2021 |

Upon receipt at Smithers Viscient, the dicloran technical (SMV No. 9932) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the dicloran technical. This sample of dicloran technical was used to prepare recovery samples during testing.

### 2.2.2 Reference Substance

The reference substance, dichloran PESTANAL, was received on 11 April 2019 from EPL Archives, Inc., Sterling, Virginia. The following information was provided:

| | |
|---|---|
| Name: | Dichloran PESTANAL |
| Synonym: | Dichloran |
| Batch No.: | SZBF103XV |
| CAS No.: | 99-30-9 |
| Purity: | 99.6% (Certificate of Analysis, Appendix 2) |
| Expiry Date: | 13 April 2020 |

Upon receipt at Smithers Viscient, the dichloran PESTANAL (SMV No. 9945) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the dichloran PESTANAL. This sample of dichloran PESTANAL was used to prepare calibration standards during testing.

Determination of stability and characterization, verification of the test and reference substance identities, maintenance of records on the test and reference substances, and archival of a sample of the test and reference substances are the responsibility of the Study Sponsor.

## 2.3 Reagents

| | | |
|---|---|---|
| 1. | Acetonitrile: | EMD, reagent grade |
| 2. | Ultra-pure reagent water: | Fisher, reagent grade |
| 3. | Methanol: | EMD reagent grade |
| 4. | Acetone: | EMD, reagent grade |
| 5. | 0.1% Formic acid in water: | Fisher and Honeywell, reagent grade |
| 6. | 0.1% Formic acid in acetonitrile: | Honeywell, reagent grade |
| 7. | Purified reagent water: | Prepared from a Millipore MilliQ Direct 8 water purification system (meets ASTM Type II requirements) |

## 2.4 Instrumentation and Laboratory Equipment

| | | |
|---|---|---|
| 1. | Instrument: | AB MDS Sciex 5000 mass spectrometer equipped with an ESI Turbo V ion source<br>Shimadzu SIL-20ACXR autosampler<br>Shimadzu DGU-20A5R vacuum degassers<br>Shimadzu CBM-20A communications bus<br>Shimadzu LC-20ADXR binary pumps<br>Shimadzu CTO-20AC column oven<br>Analyst 1.6 software for data acquisition |
| 2. | Balances: | Mettler Toledo Top Loader PG-2002-S;<br>Mettler Toledo XSE205DU |
| 3. | Shaker table: | VWR Standard Analog 3500STD |
| 4. | Centrifuge: | Thermo Scientific Sorvall Legend XFR; Eppendorf 5417 C |
| 5. | Moisture balance: | Mettler Toledo HB43-S |

| | | |
|---|---|---|
| 6. | Laboratory equipment: | Positive displacement pipets, graduated cylinders, volumetric flasks, disposable glass pipets, stir bars, stir plates, vortex mixer, 50-mL centrifuge tubes, clear vials with snap caps, amber vials with crimp caps, sonicator, and amber glass bottles with Teflon-lined caps |

Other equipment or instrumentation may be used in future testing but may require optimization to achieve the desired separation and sensitivity.

## 2.5 Test Matrixes

The matrixes used during this method validation were sandy loam soil and loamy sand soil.

Characterization of the sandy loam soil and loamy sand soil was performed by Agvise Laboratories, Northwood, North Dakota.

| Parameter | Sandy Loam Soil | Loamy Sand Soil |
|---|---|---|
| Smithers Viscient Batch No.: | 24Oct18Soil-A | 041917B |
| Collection location: | Grand Forks, ND | Rochester, AA |
| Percent organic matter: | 3.7% | 13.5% |
| USDA textural class: | Sandy loam | Loamy sand |
| Particle size distribution: | 64% sand<br>17% silt<br>19% clay | 83% sand<br>16% silt<br>1% clay |
| pH (1/1 matrix/water ratio): | 6.6 | 6.6 |
| Percent water holding capacity (at 1/3 bar): | 23.6% | 31.1% |
| Bulk Density (gm/cc): | 1.05 | 0.96 |

## 2.6 Preparation of Liquid Reagent Solutions

The volumes listed in this section were those used during the validation. For future testing, the actual volumes used may be scaled up or down as necessary.

A 20/80 acetonitrile/ultra-pure or purified reagent water (v/v) liquid reagent solution was typically prepared by combining 100 mL of acetonitrile and 400 mL of ultra-pure or purified reagent water. The solution was mixed well using a stir bar and stir plate for 5 minutes.

A 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v) autosampler needle wash solution was typically prepared by combining 1500 mL of acetonitrile, 1500 mL of methanol, and 2000 mL of purified reagent water. The solution was mixed well before use.

## 2.7 Preparation of Stock Solutions

The volumes and masses listed in this section were those used during each separate validation. For future testing, the actual volumes and masses used may be scaled up or down as necessary.

Primary stock solutions were typically prepared as described in the table below:

| Primary Stock ID | Amount Weighed (g), Net Weight | Amount Weighed (g), as Active Ingredient | Stock Solvent | Final Volume (mL) | Primary Stock Concentration (mg/L) | Primary Stock Use |
|---|---|---|---|---|---|---|
| **Test Substances** | | | | | | |
| 9932-1AK | 0.0507 | 0.0502 | Acetone | 50.0 | 1000 | Sub-stock solution |
| 9932-1M | 0.5057 | 0.5001 | Acetone | 50.0 | 10,000 | Sub-stock solution |
| **Reference Substance** | | | | | | |
| 9945-2A | 0.0502 | 0.0500 | Acetonitrile | 50.0 | 1000 | Secondary stock solution |

A secondary stock solution was typically prepared as described in the table below:

| Fortifying Stock ID | Fortifying Stock Concentration (mg/L) | Volume of Fortification (mL) | Final Volume (mL) | Stock Solvent | Stock ID | Stock Concentration (mg/L) | Stock Use |
|---|---|---|---|---|---|---|---|
| **Reference Substance** | | | | | | | |
| 9945-2A | 1000 | 0.500 | 50.0 | Acetonitrile | 99452A-1 | 10.0 | Sub-stock solution |

Sub-stock solutions were typically prepared as described in the table below:

| Fortifying Stock ID | Fortifying Stock Concentration (mg/L) | Volume of Fortification (mL) | Final Volume (mL) | Stock Solvent | Stock ID | Stock Concentration (mg/L) | Stock Use |
|---|---|---|---|---|---|---|---|
| **Test Substances** | | | | | | | |
| 9932-1AK-3 | 1000 | 0.100 | 10.0 | Acetonitrile | Tech Stk 1 | 10.0 | Sub-stock solution and 10X LOQ recovery samples (sandy loam) |
| Tech Stk 1 | 10.0 | 1.00 | 10.0 | Acetonitrile | Tech Stk 2 | 1.00 | LOQ recovery samples (sandy loam) |
| 9932-1M | 10,000 | 0.0200 | 20.0 | Acetonitrile | Tech Stk 3 | 10.0 | Sub-stock solution and 10X LOQ recovery samples (loamy sand) |
| Tech Stk 3 | 10.0 | 1.00 | 10.0 | Acetonitrile | Tech Stk 4 | 1.00 | LOQ recovery samples (loamy sand) |
| **Reference Substances** | | | | | | | |
| 9945-2A-1 | 10.0 | 1.00 | 10.0 | Acetonitrile | Ana Stk 1 | 1.00 | Sub-stock solution and high-level calibration standards |
| Ana Stk 1 | 1.00 | 1.00 | 10.0 | Acetonitrile | Ana Stk 2 | 0.100 | Low-level calibration standards and matrix effects investigation samples |

All primary and secondary stock solutions were stored refrigerated (2 to 8 °C) in amber glass bottles fitted with Teflon-lined caps. Sub-stock solutions were prepared fresh on the day of use and discarded after use.

## 2.8 Preparation of Calibration Standards

Solvent-based calibration standards used in the quantitation of sandy loam soil samples were prepared in 20/80 acetonitrile/ultra-pure or purified reagent water (v/v) by dosing with the 0.100 and 1.00 mg/L sub-stock solutions to yield concentrations of 0.500, 0.750, 1.00, 2.00, 3.00, 4.00, and 5.00 µg/L.

Matrix-matched calibration standards used in the quantitation of loamy sand soil samples were prepared in control final fraction (see Section 2.11) by dosing with the 0.100 and 1.00 mg/L sub-stock solutions to yield concentrations of 0.500, 0.750, 1.00, 2.00, 3.00, 4.00, and 5.00 µg/L.

## 2.9 Matrix Effect Investigation

The effects of matrix enhancement or suppression were evaluated through the assessment of matrix-matched and solvent-based calibration standards in the following manner. Calibration standards used to assess possible matrix effects were prepared in triplicate. One set was prepared in control final fraction (see Section 2.11) and a second set was prepared in 20/80 acetonitrile/purified reagent water (v/v) by fortifying with the 0.100 mg/L sub-stock solution to yield a concentration of 1.00 µg/L. The preparation procedure for each separate matrix is outlined in the tables below.

**Sandy loam soil validation**

| Sample ID | Sample Type | Stock Concentration (mg/L) | Fortification Volume (mL) | Final Volume (mL) | Fortified Concentration (µg/L) |
|---|---|---|---|---|---|
| SL-MM-Std D, E, & F | Matrix-matched calibration standard | 0.100 | 0.100 | 10.0[a] | 1.00 |
| SL-Sol-Std D, E, & F | Solvent-based calibration standard | 0.100 | 0.100 | 10.0[b] | 1.00 |

[a] Diluted with control final fraction 12791-6320-42
[b] Diluted with 20/80 acetonitrile/ultra-pure reagent water (v/v)

**Loamy sand soil validation**

| Sample ID | Sample Type | Stock Concentration (mg/L) | Fortification Volume (mL) | Final Volume (mL) | Fortified Concentration (µg/L) |
|---|---|---|---|---|---|
| L-MM-Std G, H, & I | Matrix-matched calibration standard | 0.100 | 0.100 | 10.0[a] | 1.00 |
| L-Sol-Std G, H, & I | Solvent-based calibration standard | 0.100 | 0.100 | 10.0[b] | 1.00 |

[a] Diluted with control final fraction 12791-6320-29
[b] Diluted with 20/80 acetonitrile/purified reagent water (v/v)

## 2.10 Sample Fortification and Preparation

The recovery samples were prepared in two different matrixes (sandy loam soil and loamy sand soil) by fortification with stock solutions of dicloran at concentrations of 0.0500 (LOQ) and 0.500 (10X LOQ) mg/kg. Recovery samples for both matrixes were prepared separately ("de novo") at these concentrations. Five replicates were produced for each concentration level. Two samples of each matrix were left unfortified to serve as controls and were diluted in the same fashion as the LOQ concentration recovery samples. In addition, one reagent blank was prepared for each sample set and processed in the same manner as the control samples. The preparation procedure for each separate matrix is outlined in the tables below.

**Sandy loam soil recovery samples**

| Sample ID 12791-6320- | Sample Type | Stock Concentration (mg/L) | Fortification Volume (mL) | Dry Weight (g) | Wet Weight (g) | Fortified Concentration (mg/kg) |
|---|---|---|---|---|---|---|
| 40 | Reagent Blank | NA[a] | NA | NA | NA | 0.00 |
| 41 & 42 | Control | NA | NA | 5.00 | 5.88 | 0.00 |
| 43, 44, 45, 46, & 47 | LOQ | 1.00 | 0.250 | 5.00 | 5.88 | 0.0500 |
| 48, 49, 50, 51, & 52 | 10X LOQ | 10.0 | 0.250 | 5.00 | 5.88 | 0.500 |

[a] NA = Not Applicable

**Loamy sand soil recovery samples**

| Sample ID 12791-6320- | Sample Type | Stock Concentration (mg/L) | Fortification Volume (mL) | Dry Weight (g) | Wet Weight (g) | Fortified Concentration (mg/kg) |
|---|---|---|---|---|---|---|
| 27 | Reagent Blank | NA[a] | NA | NA | NA | 0.00 |
| 28 & 29 | Control | NA | NA | 5.00 | 7.54 | 0.00 |
| 30, 31, 32, 33, & 34 | LOQ | 1.00 | 0.250 | 5.00 | 7.54 | 0.0500 |
| 35, 36, 37, 38, & 39 | 10X LOQ | 10.0 | 0.250 | 5.00 | 7.54 | 0.500 |

[a] NA = Not Applicable

## 2.11 Extraction of Samples

Samples were extracted twice with the extraction solvent, acetonitrile. A 20-mL aliquot of acetonitrile was added to each soil recovery sample (5.00 g dry weight) and they were sonicated 10 minutes and placed on a shaker table for 30 minutes at 250 rpm. Samples were then centrifuged at 3000 rpm for 10 minutes and the extracts were transferred to 50-mL volumetric flasks. The extraction and centrifugation procedures were repeated one more time with an additional 20-mL aliquot of acetonitrile. The extracts were combined, taken to volume (50 mL) with acetonitrile, and mixed well. The recovery sample extracts were further diluted into the calibration standard range with 20/80 acetonitrile/ultra-pure or purified reagent water (v/v). Loamy sand soil samples at the high concentration were additionally diluted using control final fraction (see Section 2.11). Following dilution, the samples were centrifuged at 13,000 rpm for 5 minutes. Raw extracts were re-diluted with 20/80 acetonitrile/purified reagent water (v/v). The extraction and dilution procedures for each separate matrix is outlined in the tables below.

**Sandy loam soil recovery samples**

| Sample ID 12791-6320- | Sample Type | Nominal Concentration (mg/kg) | Dry Weight (g) | Extract Volume[a] (mL) | Final Volume[a] (mL) | Sample Volume (mL) | Final Volume[b] (mL) | Dilution Factor |
|---|---|---|---|---|---|---|---|---|
| 40 | Reagent Blank | 0.00 | 5.00 | 20.0 | 50.0 | 2.00 | 10.0 | 50.0 |
| 41 | Control | 0.00 | 5.00 | 20.0 | 50.0 | 2.00 | 10.0 | 50.0 |
| 42 | Control | 0.00 | 5.00 | 20.0 | 50.0 | 10.0 | 50.0[c] | 50.0 |
| 43, 44, 45, 46, & 47 | LOQ | 0.0500 | 5.00 | 20.0 | 50.0 | 2.00 | 10.0 | 50.0 |
| 48, 49, 50, 51, & 52 | 10X LOQ | 0.500 | 5.00 | 20.0 | 50.0 | 0.400 | 10.0 | 250 |

[a] Extraction solvent: acetonitrile
[b] Dilution solvent: 20/80 acetonitrile/purified reagent water (v/v)
[c] Volume increased to prepare matrix-matched calibration standards to assess matrix effects.

**Loamy sand soil recovery samples**

| Sample ID 12791-6320- | Sample Type | Nominal Concentration (mg/kg) | Dry Weight (g) | Extract Volume[a] (mL) | Final Volume[a] (mL) | Sample Volume (mL) | Final Volume[b] (mL) | Dilution Factor |
|---|---|---|---|---|---|---|---|---|
| 27 | Reagent Blank | 0.00 | 5.00 | 20.0 | 50.0 | 2.00 | 10.0 | 50.0 |
| 28 | Control | 0.00 | 5.00 | 20.0 | 50.0 | 2.00 | 10.0 | 50.0 |
| 29 | Control | 0.00 | 5.00 | 20.0 | 50.0 | 30.0 | 150[c] | 50.0 |
| 30, 31, 32, 33, & 34 | LOQ | 0.0500 | 5.00 | 20.0 | 50.0 | 2.00 | 10.0 | 50.0 |
| 35, 36, 37, 38, & 39 | 10X LOQ | 0.500 | 5.00 | 20.0 | 50.0 | 0.200 | 5.00[e] | 250 |

[a] Extraction solvent: acetonitrile
[b] Dilution solvent: 20/80 acetonitrile/purified reagent water (v/v)
[c] Volume increased to prepare matrix-matched calibration standards to assess matrix effects and prepare matrix-matched calibration standards and dilution of 10X LOQ samples.
[e] Dilution solvent: matrix-matched control loamy sand diluent (control final fraction)

## 2.12 Analysis

### 2.12.1 Instrumental Conditions

The LC-MS/MS analysis was conducted utilizing the following instrumental conditions:

**LC parameters:**

| | |
|---|---|
| Column: | Phenomenex Kinetex, 2.6 µm phenyl-hexyl, 3 × 50 mm |
| Mobile Phase A: | 0.1% formic acid in water |
| Mobile Phase B: | 0.1% formic acid in acetonitrile |

Gradient:

| Time (min.) | Flow rate (mL/min.) | Solvent A (%) | Solvent B (%) |
|---|---|---|---|
| 0.01 | 0.500 | 80 | 20 |
| 0.50 | 0.500 | 80 | 20 |
| 3.00 | 0.500 | 0.0 | 100 |
| 4.00 | 0.500 | 0.0 | 100 |
| 4.10 | 0.500 | 80 | 20 |
| 5.50 | 0.500 | 80 | 20 |

| | |
|---|---|
| Run Time: | 5.5 minutes |
| Autosampler Wash Solvent: | 30/30/40 acetonitrile/methanol/reagent grade water (v/v/v) |
| Column Temperature: | 40 °C |
| Sample Temperature: | 10 °C |
| Injection Volume: | 100 µL |
| Retention Time: | approximately 3.0 minutes |

**MS parameters:**

| | |
|---|---|
| Instrument: | AB MDS Sciex 5000 mass spectrometer |
| Ionization Mode: | Positive (+) ESI |
| Ion Spray Voltage: | 5500 V |
| Scan Type: | MRM |
| Dwell Time: | 200 milliseconds |
| Source Temperature: | 550 °C |
| Curtain Gas: | 20.0 |
| Ion Source – Gas 1 / Gas 2: | 60.0 / 70.0 |
| Collision Gas: | 12.0 |
| Entrance Potential: | 10.0 |
| Declustering Potential: | 40.0 |
| Resolution Q1/Q3: | Unit/Unit |

| | Primary Transition | Confirmatory Transition |
|---|---|---|
| Q1/Q3 Masses (amu): | 207.1/190.0 | 207.1/160.0 |
| Collision Energy: | 22.0 | 35.0 |
| Collision Cell Exit Potential: | 19.0 | 42.0 |

Other instrumentation may be used but may require optimization to achieve the desired separation and sensitivity. It is important to note that the parameters above have been

established for this particular instrumentation and may not be applicable for other similar equipment that may be used.

### 2.12.2 Preparation of Calibration Standard Curve

Two sets of calibration standards were analyzed with each sample set. Calibration standards were interspersed among analysis of the recovery samples, every four to seven injections. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

## 2.13 Evaluation of Accuracy, Precision, Specificity, and Linearity

The accuracy was reported in terms of percent recovery of the fortified recovery samples. Recoveries of 70.0 to 110% (for the individual mean concentrations) are acceptable. The precision was reported in terms of the relative standard deviation (RSD) for the recovery samples and retention times. RSD values less than or equal to 20% were considered acceptable for the recovery samples and RSD values less than or equal to 2% were considered acceptable for the retention times. Specificity of the method was determined by examination of the control samples for peaks at the same retention times as dicloran which might interfere with the quantitation of the analytes. Linearity of the method was determined by the coefficient of determination ($r^2$), y-intercept, and slope of the regression line.

## 2.14 Limit of Quantitation (LOQ)

The method was validated at the LOQ. This was defined as the lowest fortification level. Blank values (reagent blanks and untreated control samples) did not exceed 30% of the LOQ.

## 2.15 Limit of Detection (LOD) and Method Detection Limit (MDL)

The LOD was calculated using three times the signal-to-noise value of the control samples. Representative calculations for the LOD can be found in Section 3.0.

The MDL was defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions. Representative calculations for the MDL can be found in Section 3.0.

## 3.0 CALCULATIONS

A calibration curve was constructed by plotting the analyte concentration (µg/L) of the calibration standards against the peak area of the analyte in the calibration standards. The equation of the line (equation 1) was algebraically manipulated to give equation 2. The concentration of test substance in each recovery sample was calculated using the slope and intercept from the linear regression analysis, the detector response, and the dilution factor of the recovery sample. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

$$(1) \qquad y = mx + b$$

$$(2) \qquad DC\,(x) = \frac{(y - b)}{m}$$

$$(3) \qquad A = DC \times DF$$

where:

| | | |
|---|---|---|
| x | = | analyte concentration |
| y | = | detector response (peak area) from the chromatogram |
| b | = | y-intercept from the regression analysis |
| m | = | slope from the regression analysis |
| DC (x) | = | detected concentration (mg/kg) in the sample |
| DF | = | dilution factor (final volume of the sample divided by the original sample mass, mL/g) |
| A | = | analytical result (mg/kg), concentration in the original sample |

The LOD was calculated using the following equation:

$$(4) \qquad LOD = ((3\times(N_{ctl}))/Resp_{LS}) \times Conc_{LS} \times DF_{CNTL}$$

where:

| | | |
|---|---|---|
| $N_{ctl}$ | = | mean signal-to-noise in height of the control samples (or blanks) |
| $Resp_{LS}$ | = | mean response in height of the two low calibration standards |
| $Conc_{LS}$ | = | concentration of the low calibration standard |

$DF_{CNTL}$ = dilution factor of the control samples (smallest dilution factor used, i.e., 50.0 mL/g)
LOD = limit of detection for the analysis

The MDL is defined as the lowest concentration that can be detected by this method in test solution samples. The MDL is calculated (equation 5) based on the concentration of the low calibration standard and the dilution factor of the control samples.

$$(5) \quad MDL = MDL_{LCAL} \times DF_{CNTL}$$

where:

$MDL_{LCAL}$ = lowest concentration calibration standard (0.500 µg/L)
$DF_{CNTL}$ = dilution factor of the control samples (smallest dilution factor used, i.e., 50.0 mL/g)
MDL = method detection limit reported for the analysis (0.500 µg/L × 50.0 mL/g × 1 L/1000 mL = 0.0250 µg/g or mg/kg)

12791.6320

**Environmental Chemistry Method: Validation of the Analytical Method for the Determination of Dicloran in Soil by LC-MS/MS**

## 1.0 INTRODUCTION

The purpose of this study is to validate an analytical method used to determine the content of Dicloran in two soil matrices (i.e. sandy loam and loamy sand) by LC-MS/MS. The analytical method will be validated with regards to accuracy, precision, specificity, linearity, limit of quantitation (LOQ), limit of detection (LOD), method detection limit (MDL), and confirmation of identification.

## 2.0 JUSTIFICATION OF THE TEST SYSTEM

This study is conducted to support the registration of the test substance.

The method validations described in this protocol are designed to conform to EPA guideline OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation and SANCO/3029/99 rev.4: Guidance for generating and reporting methods of analysis in support of pre-registration data. The study will be conducted under Good Laboratory Practices (GLP) regulations and principles as described in 40CFR160 and as accepted by OECD principles of GLP (OECD, 1998).

## 3.0 TEST SUBSTANCE

### 3.1 Test Substance

Upon arrival at Smithers Viscient, the test substances (and the reference substances) will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test and reference substances will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

Each test and reference substance will be given a unique sample ID number and stored under the conditions specified by the Sponsor or manufacturer. The following information should be provided by the Study Sponsor, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

### 3.2 Test Matrices

The soil matrices used for the method validation will be two type of soils (i.e. sandy loam and loamy sand). Prior to testing, soil moisture content will be determined using a moisture analyzer. Soil characterization data such as % sand, silt clay, bulk density and % organic matter will be determined Agvise Laboratories, Northwood, ND. All documentation relating to the preparation, storage and handling will be maintained by Smithers Viscient.

### 3.3 Reagents

Highly pure reagents will be used throughout the study. The actual reagent grade will be depending on the manufacturer's designation. Generally these reagents will have grades, such as high purity solvent, ACS grade, or Select. The reagents used are recorded along with test chemical information at the time of preparation.

## 4.0 VALIDATION DESIGN

The test design will consist of two soil matrices (sandy loam and loamy sand) fortified with each test substance at two concentrations with five replications at the target LOQ and five replicates at 10x LOQ level for each matrix. The procedural blank will be reagent blank without matrix. The control matrix for the validation will be untreated matrix representing sandy loam or loamy sand soil. The validation study levels (approximate concentrations) for each test substance are:

| | | |
|---|---|---|
| 1. | Procedural blank-reagent blank | 0.0 mg/kg |
| 2. | Matrix blank-control matrix | 0.0 mg/kg |
| 3. | Control matrix fortified at LOQ | 0.050 mg/kg |
| 4. | Control matrix fortified at 10 x LOQ | 0.50 mg/kg |

### 4.1 Accuracy and Precision

The accuracy of the analytical method will be determined by applying the method to five samples at the LOQ and five samples at 10X LOQ for each test substance. Accuracy will be reported as the mean recovery at each fortification level. Mean recoveries in the range 70 – 110% of nominal concentrations of the target analyte in the fortified samples will be considered acceptable.

The precision will be calculated for the fortified samples in terms of the relative standard deviation (RSD or coefficient of variation (CV)) calculated for the retention time, peak area based quantitation (i.e., mg/kg), and the observed recovery values at each fortification level (n = 5 per level). The retention time should have a RSD of less than or equal to 2%. The RSD of the peak area based quantitation (i.e., mg/kg) should be less than or equal to 20% per level. The RSD of the recovery values should be less than or equal to 20% per level as well.

### 4.2 Specificity

The specificity of the method will be determined by applying the method to the appropriate number of reagent blank (n=1) and control matrix samples (n=2). Chromatograms will be obtained for the control samples and examined for peaks that might interfere with the quantitation of the analyte(s) peak of interest. Peaks attributable to the test substance(s) should be sufficiently resolved from any peaks found in the samples of control matrix to enable quantification. Blank values (including procedural blanks and untreated samples) should not exceed 30% of the LOQ. If this is exceeded, detailed justification is required. Unequivocal identification of the target analyte will be achieved by LC-MS/MS primary and confirmatory analysis.

### 4.3 Regression Analysis

Quantitative analysis will be achieved with the aid of a calibration curve. The calibration curve will be constructed using a minimum of five analytical standards and will extend over a range appropriate to the lowest and highest nominal concentrations of the target analyte in relevant analytical solutions ± at least 20%.

The calibration data will be subjected to regression analysis; a plot of analyte concentration versus detector response will be included in the report along with the correlation coefficient (r) and the equation describing the curve. The linearity of the detector response will be assessed according to the strength of the correlation coefficient: this should be ≥ 0.995 (or coefficient of determination, $r^2 \geq 0.990$). If non-linear calibration is used an explanation will be provided.

### 4.4 Limits of Quantitation (LOQ)

The method will be validated at the limit of quantitation (LOQ). This will be defined as the lowest fortification level. Blank values (reagent blanks and untreated control samples) should not exceed 30% of the LOQ. If this is exceeded, it will be discussed with the Sponsor and detailed justification provided.

### 4.5 Limits of Detection (LOD)

The Limits of Detection (LOD) will be calculated using three times the signal-to-noise value of the control samples. The method detection limit (MDL) will be set at the lowest concentration that can be detected in sample test solutions. The value is calculated based on the concentration of the low calibration standard and the dilution factor of the control samples.

### 4.6 Matrix Effects Determination

Determination of LC-MS/MS matrix effects will be evaluated through the assessment of solvent-based and matrix-matched calibration standards for both primary and confirmatory transitions. Matrix effects should be evaluated at the LOQ level for each test substance. Only if experiments clearly demonstrate that matrix effects are not significant (i.e. <20%), calibration with standards in solvent may be used.

### 4.7 Confirmatory Analyses

Unequivocal identification of the target analytes will be achieved by LC/MS-MS using a primary quantitation ion and secondary quantitation/confirmatory ion. All of the required elements need to be met for this confirmatory method with full method validation results generated for both transitions. For triple-quad MS methods, the confirmation method would be where a confirmatory (secondary) product ion will be used for quantification. The confirmatory ion analysis will also adhere to the aforementioned method specifications (Sections 4.1 - 4.6 above)

## 5.0 PROCEDURE FOR THE INDENTIFICATION OF THE TEST SYSTEM

The test system will be defined as the fortified recovery samples. The fortified recovery samples will be labeled as defined in Section 4.0 and each sample replicate will be assigned a unique identifier. Processing of fortified recovery samples will be performed at a lab station labeled with the study number.

## 6.0 CONTROL OF BIAS

Bias will be effectively controlled through techniques such as, but not limited to, preparation of replicate samples and replicate analysis.

## 7.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

## 8.0 REPORTING

The raw data generated at Smithers Viscient will be peer-reviewed and the final report will be reviewed by the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. The Quality Assurance Unit will inspect the final report to confirm that the methods, procedures, and observations are accurately and completely described, that the reported results accurately and completely reflect the raw data generated at Smithers Viscient and to confirm adherence with the study protocol. A single copy of the draft report will be submitted to the Sponsor for review. The report will be finalized according to standard operating procedures. The final report will meet the formatting requirements of EPA's PR Notice 2011-3. All reports will include, but will not be limited to reporting requirements presented in Ecological Effects Test Guidelines OCSPP 850.6100 (U.S. EPA, 2012) and SANCO/3029/99 rev.4 along with the following information:

- The report and project numbers from Smithers Viscient and Sponsor Study number (if any).
- Laboratory and site, dates of testing and personnel involved in the study, e.g. Program Coordinator (if applicable), Study Director and Principal Investigator.
- Identification of the test substance including chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, degree of purity of test substance (percent test chemical) (Sponsor supplied, if available).
- A full description of the experimental design and procedures followed and a description of the test equipment used.
- The determined accuracy, precision, specificity, linearity, LOQ, LOD and MDL, and confirmation of identification.
- The mathematical equations and statistical methods used in generating and analyzing the data as well as calculations using these equations. Tabular and graphical representations (if appropriate) of the data.
- Description of any problems experienced and how they were resolved.
- Good Laboratory Practice (GLP) Compliance Statement signed by the Study Director.

- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.
- Location of raw data and report.
- A copy of the study protocol and study amendments, if any.

## 9.0 PROTOCOL AMENDMENTS

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any.

## 10.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records and reports will comply with the U.S. Environmental Protection Agency's Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide and Rodenticide Act (40 CFR, Part 160) and as compatible with OECD Principles of Good Laboratory Practice (OECD, 1998)

## 11.0 REFERENCES

European Commission, 2000. Residues: Guidance for the generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part V, section 4) and Annex III (part A, section 5) of Directive 91/414. SANCO/3029/99 rev.4.

OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp.

U.S. EPA, 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 2011. Pesticide Registration (PR) Notice 2011-3 Standard Format for Data Submitted Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Certain Provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA). US Environmental Protection Agency Office of Pesticide Programs. November 30, 2011.

U.S. EPA, 2012. Office of Chemical Safety and Pollution Prevention. Ecological Effects Guideline, OCSPP 850.6100. Environmental Chemistry Methods and Associated Independent Laboratory Validation. EPA 712-C-001. January 2012. U.S. Environmental Protection Agency, Washington, D.C.