1.0 INTRODUCTION

This independent laboratory validation (ILV) study is required by the U.S. EPA under Guideline for Environmental Chemistry Methods No. 850.6100 (U.S. EPA, 2012) and No. 850.7100 (U.S. EPA, 1996) to confirm that the original analytical method, developed by one laboratory, can be independently validated by a second laboratory. This analytical method was validated by fortification of soil with dichlobenil and 2,6-dichlorobenzamide (BAM) at the limit of quantification (LOQ) and 10X LOQ (0.010 mg/kg and 0.100 mg/kg for dichlobenil and 0.0050 mg/kg and 0.050 mg/kg for BAM) concentration levels.

The study was initiated on 15 January 2016, 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 ILV study was conducted on 18 January to 8 March 2016 at Smithers Viscient (SMV), located in Wareham, Massachusetts. All original raw data, study protocol, and the final report produced during this study are stored in Smithers Viscient's archives at the above location.

2.0 MATERIALS AND METHODS

2.1 Study Protocol

This study was performed following the Smithers Viscient protocol entitled "Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Dichlobenil and its Metabolite 2,6-Dichlorobenzamide in Soil" (Appendix 1). The methods described in this protocol meet the requirements specified in the OCSPP Guideline 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (U.S. EPA, 2012) and OSCPP Guideline 850.7100: Data Reporting for Environmental Chemistry Methods (U.S. EPA, 1996).

2.2 Test Substances and Internal Standard

2.2.1 Test Substances

The test substance, dichlobenil, was received on 22 December 2015 from MacDermid Agricultural Canada Company, Guelph, Ontario, Canada. The following information was provided:

Name:	dichlobenil
Synonym:	2,6-dichlorobenzonitrile
Lot No.:	2757-105-RRG
CAS No.:	1194-65-6
Purity, %:	99.8 (±0.05) (Certificates of Analysis, Appendix 2)
Expiration Date:	30 September 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8002) was stored in a freezer in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, BAM, was received on 22 December 2015 from MacDermid Agricultural Canada Company, Guelph, Ontario, Canada. The following information was provided:

Name:	BAM
Synonym:	2,6-dichlorobenzamide
Lot No.:	2757-103-RRG
CAS No.:	2008-58-4
Purity, %:	99.7 (±0.2; Certificates of Analysis, Appendix 2)
Expiration Date:	30 September 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8003) was stored in a freezer in the original container. Concentrations were adjusted for the purity of the test substance.

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

2.2.2 Internal Standard

The internal standard (IS), 4-chlorobenzonitrile, was received on 13 January 2016 from Sigma-Aldrich, Inc., Milwaukee, Wisconsin. The following information was provided:

Name:	4-chlorobenzonitrile
Batch No.:	STBD5757V
CAS No.:	623-03-0
Purity:	> 99.9%
Expiration Date:	13 January 2017

Upon receipt at Smithers Viscient, the internal standard (SMV No. 8040) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were not adjusted for the purity of the internal standard.

2.3 Reagents

1.	Purified reagent water:	prepared from a Millipore Milli-Q® Direct 8 system (meeting ASTM Type II requirements)
2.	Ethyl acetate:	EMD, reagent grade
3.	Methanol:	EMD, reagent grade
4.	Acetone:	EMD, reagent grade
5.	Hexanes:	EMD, reagent grade
6.	Ammonium chloride:	Fisher, reagent grade
7.	Anhydrous sodium sulfate:	EMD, reagent grade
8.	Ammonium hydroxide:	JT Baker, reagent grade
9.	Sodium chloride:	Fisher, reagent grade

2.4 Equipment

1.	Instruments:	Agilent Model 6890 gas chromatograph equipped with mass selective detector series 5973 Agilent Model 7683 autosampler Agilent Model 7683 injector and ChemStation Version A.01.00 software for data aquisition
2.	Balance:	Mettler Toledo AG285, Mettler Toledo XSE205DU, Mettler PJ-3000
3.	Moisture balance:	Mettler Toledo HB43-S
4.	Processing equipment:	SPEX Geno Grinder 2010
5.	Laboratory equipment:	volumetric flasks, disposable glass pipets, positive displacement pipets, Titan 0.2 µm nylon filters, pH indicator paper, SPEX SamplePrep grinding cylinders, WISP vials, Whatman GF8 glass fiber filters, Reeve Angel fluted filter paper, graduated cylinders, stir bars, stir plates, sonicators, vortexers, shaker tables, Buchner funnels, 50-mL polypropylene centrifuge tubes, side-arm flasks, separatory funnels, round-bottom flasks, rotary evaporator, 15-mL glass conical tubes, Phenomenex Strata alumina-N SPE cartridges, amber autosampler vials, amber Wheaton bottles, and amber glass bottles with Teflon®-lined caps

2.5 Test System

The test system evaluated during this study was a soil representative of the type of matrix this method was intended to analyze. The soil used for this ILV analysis was Rochester sandy loam (SMV Lot Nos. 093015 and 011116). Prior to testing, moisture content of the soil was determined to be 8.61% (SMV Lot No. 093015) and 9.82% (SMV Lot No. 011116) using a Mettler Toledo HB43-S moisture balance.

2.6 Preparation of Stock Solutions

Primary stock solutions were typically prepared as described in the table below. All volumes and masses may be scaled up or down as necessary.

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Final Volume (mL)	Solvent Stock	Primary Stock Concentration (mg/L)	Primary Stock Uses
8002C	0.0502	0.0501	50.0	Ethyl acetate	1000	Secondary stock solution
8003B	0.0502	0.0500	50.0		1000	Secondary stock solution
8040A	0.0250	0.0250	25.0		1000	Secondary stock solution

Secondary 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)	Solvent Stock	Final Stock Concentration (mg/L)	Stock ID	Stock Uses
8002C	1000	0.500	50.0	Ethyl acetate	10.0	8002C-2	Sub-stock solution and 10X LOQ recovery samples
8003B	1000	0.500	50.0		10.0	8003B-2	Sub-stock solution
8040A	1000	5.00	50.0		100	8040A-2	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)	Solvent Stock	Final Stock Concentration (mg/L)	Stock ID	Stock Uses
8002C-2	10.0	1.00	10.0	Hexane	1.00	DBN Stk 1	Calibration standards and LOQ recovery samples
8003B-2	10.0	5.00	10.0	Ethyl acetate	5.00	BAM Stk 1	10X LOQ recovery samples
		1.00	10.0		1.00	BAM Stk 2	Calibration standards
		0.500	10.0		0.500	BAM Stk 3	Calibration standards and LOQ recovery samples
8040A-2	100	0.250	50.0		0.500	IS Stk 1	Calibration standards and recovery samples

All primary and secondary stock solutions were stored in a freezer (< 0 °C) in amber glass bottles fitted with Teflon®-lined caps until use. The sub-stock solutions were prepared on the day of use and discarded after use.

2.7 Liquid Reagent Preparation

All volumes and masses may be scaled up or down as necessary.

A 50:50 acetone:hexanes (v:v) liquid reagent solution was typically prepared by combining 500 mL of acetone and 500 mL of hexanes. The solution was mixed using a stir bar and stir plate for five minutes.

A 0.2% ammonium chloride liquid reagent solution was typically prepared by adding 0.50 g of ammonium chloride to 250 mL of purified reagent water. The solution was mixed using a stir bar and stir plate for five minutes.

A saturated sodium chloride liquid reagent solution was typically prepared by adding 16.06 g of sodium chloride to 50.0 mL of purified reagent water. The solution was mixed using a stir bar and stir plate for five minutes.

A 2% acetone in hexanes liquid reagent solution was typically prepared by adding 5.00 mL of acetone to 250 mL of hexanes. The solution was mixed using a stir bar and stir plate for five minutes.

A 50:50 methanol:purified reagent water (v:v) GC/MS autosampler rinse vial solution was typically prepared by combining 125 mL of methanol with 125 mL of purified reagent water.

2.8 Preparation of Calibration Standards

2.8.1 Calibration Standard for GC/MS Analysis of Dichlobenil

Standards were prepared in hexane using the sub-stock solutions according to the table below. Following fortication, each solution was vortex-mixed for 15 seconds, then standards were transferred to amber autosampler vials for analysis.

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Calibration Standard Concentration (µg/L)	Sample ID
IS Stk 1	0.500	2.00	10.0	0.00	IS Blk-D
DBN Stk 1	1.00	0.0400	10.0	4.00	Std 1-D
IS Stk 1	0.500	2.00			
DBN Stk 1	1.00	0.100	10.0	10.0	Std 2-D
IS Stk 1	0.500	2.00			
DBN Stk 1	1.00	0.200	10.0	20.0	Std 3-D
IS Stk 1	0.500	2.00			
DBN Stk 1	1.00	0.500	10.0	50.0	Std 4-D
IS Stk 1	0.500	2.00			
DBN Stk 1	1.00	1.00	10.0	100	Std 5-D
IS Stk 1	0.500	2.00			
DBN Stk 1	1.00	2.00	10.0	200	Std 6-D
IS Stk 1	0.500	2.00			
DBN Stk 1	1.00	4.00	10.0	400	Std 7-D
IS Stk 1	0.500	2.00			

2.8.2 Calibration Standard for GC/MS Analysis of BAM

Standards were prepared in ethyl acetate using the sub-stock solutions according to the table below. Following fortication, each solution was vortex-mixed for 15 seconds, then standards were transferred to amber autosampler vials for analysis.

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Calibration Standard Concentration (µg/L)	Sample ID
IS Stk 1	0.500	2.00	10.0	0.00	IS Blk-B
BAM Stk 2	1.00	0.0400	10.0	4.00	Std 1-B
IS Stk 1	0.500	2.00			
BAM Stk 3	0.500	0.200	10.0	10.0	Std 2-B
IS Stk 1	0.500	2.00			
BAM Stk 3	0.500	0.500	10.0	25.0	Std 3-B
IS Stk 1	0.500	2.00			
BAM Stk 3	0.500	1.00	10.0	50.0	Std 4-B
IS Stk 1	0.500	2.00			
BAM Stk 3	0.500	2.00	10.0	100	Std 5-B
IS Stk 1	0.500	2.00			
BAM Stk 3	0.500	5.00	10.0	250	Std 6-B
IS Stk 1	0.500	2.00			
BAM Stk 2	1.00	4.00	10.0	400	Std 7-B
IS Stk 1	0.500	2.00			

2.9 Sample Fortification and Preparation

2.9.1 Sample Fortification for GC/MS Analysis of Dichlobenil

Twelve aliquots of sandy loam soil (10.0 g, dry weight) were weighed into individual 50.0-mL polypropylene centrifuge tubes. Five replicates were dosed with the 1.00 mg/L sub-stock solution and five aliquots were dosed with the 10.0 mg/L secondary stock solution to obtain concentrations of 10.0 and 100 µg/kg (ppb), respectively. Two aliquots were left unfortified to

serve as controls and an additional sample was extracted using only solvents as a reagent blank. The dosing procedure is detailed in the following table:

Sample ID	Stock ID	Fortifying Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Soil Weight (g)	Nominal Concentration (µg/kg)
Reagent Blk-3-D	NA[a]	NA	NA	NA	0.00
CTL E-D, F-D	NA	NA	NA	10.0	0.00
LOQ K-D, L-D, M-D, N-D, & O-D	DBN Stk 1	1.00	0.100	10.0	10.0
High K-D, L-D, M-D, N-D, & O-D	8002C-2	10.0	0.100	10.0	100

[a] NA = Not Applicable.

2.9.2 Sample Fortification for GC/MS Analysis of BAM

Twelve aliquots of sandy loam soil (10.0 g, dry weight) were weighed into individual 50.0-mL polypropylene centrifuge tubes. Five replicates were dosed with the 0.500 mg/L sub-stock solution and five aliquots were dosed with the 5.00 mg/L sub-stock solution to obtain concentrations of 5.00 and 50.0 µg/kg (ppb), respectively. Two aliquots were left unfortified to serve as controls and an additional sample was extracted using only solvents as a reagent blank. The dosing procedure is detailed in the following table:

Sample ID	Stock ID	Fortifying Stock Concentration (mg/L)	Fortification Volume (mL)	Dry Soil Weight (g)	Nominal Concentration (µg/kg)
Reagent Blk-B	NA[a]	NA	NA	NA	0.00
CTL A-B, B-B	NA	NA	NA	10.0	0.00
LOQ A-B, B-B, C-B, D-B, & E-B	BAM Stk 3	0.500	0.100	10.0	5.00
High A-B, B-B, C-B, D-B, & E-B	BAM Stk 1	5.00	0.100	10.0	50.0

[a] NA = Not Applicable.

2.10 Soil Extraction and Sample Processing and Dilution

2.10.1 Processing of Soil Samples for GC/MS Analysis for Dichlobenil

2.10.1.1 Extraction of Fortified Recovery Samples

Fortified recovery samples of sandy loam soil for analysis of dichlobenil were extracted by adding a 30.0-mL aliquot of 50:50 acetone:hexanes (v:v), 3.4 mL of 0.2% ammonium chloride solution, and a ceramic grinding bead to each sample before processing using a Geno Grinder for three minutes at 1500 rpm. The mixture was allowed to settle and the supernatant decanted through a GF8 glass fiber filter using a Buchner funnel into a side-arm flask. The extraction and filtration steps were repeated, with the two extracts combined into the same side-arm flask. Each resulting filter cake was subsequently rinsed with 6 mL of 50:50 acetone:hexanes (v:v).

After filtering, the extract from each sample was transferred to separate separatory funnels, and a 60-mL aliquot of purified reagent water and 2 mL of saturated sodium chloride solution were added to each sample followed by shaking for 1 minute. The phases were allowed to separate and the hexane layer collected through a fluted filter containing 4 g of anhydrous sodium sulfate into a round-bottom flask. A 20-mL aliquot of hexanes was added to each separatory funnel (containing the aqueous layer), shaken for 1 minute, and the phases allowed to separate. The hexane layer was collected and combined with the previous hexane layer in the same round-bottom flask, through the same fluted filter containing the anhydrous sodium sulfate. The hexane extraction process was repeated twice more, for a total of three 20-mL hexane extractions.

The combined sample extracts were evaporated at ambient temperature (25 °C) using a rotary evaporator to a volume of approximately 5 mL, and transferred to 10-mL volumetric flasks. The round-bottom flask was rinsed with an additional 5 mL of hexanes and sonicated for five minutes and the rinsate was transferred to the respective volumetric flask. The sample was brought to a volume of 10 mL with hexanes and vortexed to mix.

2.10.1.2 SPE Clean-up of Fortified Recovery Samples

For the solid phase extraction (SPE) clean up, alumina neutral (1 g, 6 mL) SPE cartridges were conditioned twice with a 5-mL aliquot of hexanes prior to a 5-mL aliquot of each 10-mL sample extract loaded into each cartridge and collected into 15-mL graduated glass conical tubes. Each cartridge was eluted twice, each time with 4 mL of 2% acetone in hexanes, into the same glass conical. Each eluate was evaporated to a final volume of 2 mL under a gentle stream of nitrogen at room temperature (25 °C). A 0.500-mL aliquot of IS Stk 1 was added to each sample and the samples were sonicated well for five minutes and mixed using a vortexer for 15 seconds.

Fortified recovery samples were transferred to low-volume inserts in amber autosampler vials with crimp caps for GC/MS analysis. Samples for analysis were prepared as described in the following table.

Sample ID	Nominal Concentration (µg/kg)	Dry Soil Weight (g)	Final Volume (mL)[a]	Sample Volume (mL)	Final Volume Following Evaporation (mL)[a]	Volume of IS Stk 1 Added (mL)	Total Sample Volume (mL)[b]	Dilution Factor
Reagent Blk-3-D	0.00	NA[c]	10.0	5.00	2.00	0.500	2.50	NA
CTL E-D, F-D	0.00	10.0	10.0	5.00	2.00	0.500	2.50	0.500
LOQ K-D, L-D, M-D, N-D, & O-D	10.0	10.0	10.0	5.00	2.00	0.500	2.50	0.500
High K-D, L-D, M-D, N-D, & O-D	100	10.0	10.0	5.00	2.00	0.500	2.50	0.500

[a] Hexanes
[b] 20% Ethyl acetate (IS) and 80% hexanes
[c] NA = Not Applicable.

2.10.2 Processing of Soil Samples for GC/MS Analysis for BAM

2.10.2.1 Extraction of Fortified Recovery Samples

Fortified recovery samples of sandy loam soil for analysis of BAM were extracted by adding a 30.0-mL aliquot of 50:50 acetone:hexanes (v:v), 3.4 mL of 0.2% ammonium chloride solution, and a ceramic grinding bead before processing the samples using a Geno Grinder for three minutes at 1500 rpm. Following extraction, each sample was filtered through a GF8 glass fiber filter using a Buchner funnel into a side-arm flask. Each resulting filter cake was subsequently rinsed with 6 mL of 50:50 acetone:hexanes (v:v).

After filtering, the extract from each sample was transferred to separate separatory funnels, and a 60-mL aliquot of purified reagent water and 2 mL of saturated sodium chloride solution were added to each sample followed by shaking for 1 minute. The phases were allowed to separate and the hexane layer collected into a beaker. A 20-mL aliquot of hexanes was added to each separatory funnel (containing the aqueous layer), shaken for 1 minute, and the phases allowed to separate. The hexane layer was collected and combined with the previous hexane layer in the same beaker. The hexane extraction process was repeated twice more, for a total of three 20-mL hexane extractions. The hexane layers were discarded.

One drop of ammonium hydroxide was added to each remaining aqueous phase and each sample was swirled to mix. The pH of each sample was measured with pH indicator paper and verified to be > 9. A 20-mL aliquot of ethyl acetate was added to each separatory funnel containing the basified aqueous layer. The samples were shaken for 1 minute and the phases allowed to separate. The ethyl acetate layer was collected through a fluted filter containing 4 g of anhydrous sodium sulfate into a round-bottom flask. The extraction and filtration steps were repeated twice, combining the ethyl acetate extracts, for a total of three 20-mL ethyl acetate extractions. The sample extract was evaporated at room temperature (25 °C) using a rotary evaporator until a volume of approximately 2-mL remained.

2.10.2.2 Clean-up of Fortified Recovery Samples

The 2-mL extract was sonicated for 5 minutes and filtered through a 0.2-µm nylon filter into a graduated glass conical tube. The round-bottom flask was rinsed with an additional 2 mL of ethyl acetate and sonicated for five minutes. The rinsate was filtered through another 0.2-µm nylon filter into the same graduated glass conical tube. The filtered extract was evaporated under a gentle stream of nitrogen at 40 °C to a final volume of 2 mL. A 0.500-mL aliquot of IS Stk 1 was added to each sample and the samples were mixed using a vortexer for 15 seconds.

Fortified recovery samples were transferred to low-volume inserts in amber autosampler vials with crimp caps for GC/MS analysis. Samples for analysis were prepared as described in the following table.

Sample ID	Nominal Concentration (µg/kg)	Dry Soil Weight (g)	Final Volume Following Evaporation (mL)[a]	Volume of IS Stk 1 Added (mL)	Total Sample Volume (mL)[a]	Dilution Factor
Reagent Blk-B	0.00	NA[b]	2.00	0.500	2.50	NA
CTL A-B, A-B	0.00	10.0	2.00	0.500	2.50	0.250
LOQ A-B, B-B, C-B, D-B, & E-B	5.00	10.0	2.00	0.500	2.50	0.250
High A-B, B-B, C-B, D-B, & E-B	50.0	10.0	2.00	0.500	2.50	0.250

[a] Ethyl Acetate
[b] NA = Not Applicable.

2.10.3 GC/MS Instrumental Conditions

The GC/MS analysis was conducted using the following instrumental conditions:

GC Parameters:

Column:	Agilent DB-17, 30 m × 0.25 mm × 0.50 µm
Temperature:	60 °C (initial) and held for 2.00 minutes

Ramps:

Rate (°C/min)	Final Temperature (°C)	Hold Time (min)
15.0	270	5.00

Run time: 21.0 minutes
Injection volume: 1.0 µL
Carrier gas/flow: Helium, constant flow of 2.0 mL/minute
Inlet mode: Splitless
Pressure: 8.32 psi
Purge time: on at 1.00 min at 1.0 mL/minute
Inlet temperature: 250 °C
Inlet liner: Double gooseneck
Inlet septum: Green thermolight
Retention time: dichlobenil

Test Substance	Approximate Retention Times (minutes)
dichlobenil	12.0
4-chlorobenzonitrile (IS)	9.8
Confirmation ion #1	12.0
Confirmation ion #2	12.0

Retention time: BAM

Test Substance	Approximate Retention Times (minutes)
BAM	15.6
4-chlorobenzonitrile (IS)	9.9
Confirmation ion #1	15.6
Confirmation ion #2	15.6

MS Parameters:
Solvent delay: 5.0 minutes
Selected ion monitoring: dichlobenil

Ion (m/z)	Dwell (msec)	Comments
171	100	Quantitation ion, dichlobenil
137	400	Quantitation ion, 4-chlorobenzonitrile (IS)
173	100	Confirmation ion #1
136	100	Confirmation ion #2

Selected ion monitoring: BAM

Ion (m/z)	Dwell (msec)	Comments
173	100	Quantitation ion, BAM
137	400	Quantitation ion, 4-chlorobenzonitrile (IS)
175	100	Confirmation ion #1
189	100	Confirmation ion #2

Temperatures: MSD Transfer Line: 280 °C
MS Quad: 150 °C
MS Source: 230 °C

2.10.4 Preparation of Calibration Standard Curve

Two sets of calibration standards were analyzed with each sample set; one set prior to analysis of the recovery samples, and the second set immediately following the analysis of the recovery samples. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

2.10.5 Method Differences

The analytical method used for BAM in this independent laboratory validation followed the procedures described in the original method validation. The analytical method used for dichlobenil in this independent laboratory validation required the following minor modifications from the original method validation.

- The validated method specified that samples are to be extracted once with 50:50 acetone:hexanes (v:v) and 0.2% ammonium chloride solution using a Geno Grinder prior to filtration. In this study, an additional extraction step was added so that the samples were extracted twice with 50:50 acetone:hexanes (v:v) and 0.2% ammonium chloride solution using a Geno Grinder, allowing settling of the soil prior to filtration inbetween each extraction. The goal of this was to improve the extraction efficiency.

- The validated method specified that samples are quantified using the internal standard 4-chlorobenzonitrile. In this study, acceptable results were obtained when quantification with the use of internal standard was removed. Therefore, it is recommended to quantify results without the use of internal standard, as necessary.

2.11 Evaluation of Precision, Accuracy, Specificity, and Linearity

The accuracy was reported in terms of percent recovery of the LOQ and 10X LOQ recovery samples. Recoveries of 70 to 120% of nominal were considered acceptable, with no corrections made for procedural recoveries during the study. The precision was reported in terms of the standard deviation and relative standard deviation (RSD) for the retention time, the peak area quantitation, and the percent recovery values of the LOQ and 10X LOQ recovery samples for each analyte. The retention time should have an RSD of less than or equal to 2%. The RSD of the peak area based quantitation and of the recovery values should be less than or equal to 20%. Specificity of the method was determined by examination of the control samples for peaks at the same retention time as dichlobenil and 2,6-dichlorobenzamide (BAM) which might interfere with the quantitation of the analytes. Interferences with peak areas that are less than 50% at the limit of quantification (LOQ) are not considered significant. Linearity of the method was determined by the coefficient of determination (r^2), y-intercept, and slope of the regression line. The signal response data should have an intercept close to zero and a correlation coefficient (r) not less than 0.995 (or r^2 not less than 0.990). The precision of the method at the LOQ was reported in terms of the relative standard deviation or coefficient of variation of the observed recovery values. A power (log-log) calibration curve was used for the GC/MS analysis. The calibration curves were evaluated based on the coefficient of determination (r^2) and the recoveries of the calibration standards.

2.12 Communications

During the course of this study, numerous communications occurred among the Study Director, Study Sponsor, and Study Monitor. A summary list of communications is provided in Appendix 3.

2.13 Time Required for Analysis

The soil ILV included two sets of samples used for GC/MS analysis, one for each analyte. For GC/MS samples, each set consisted of ten fortified and two unfortified samples, one reagent blank, and seven calibration standards (20 samples total). A single analyst completed each set of 20 samples in one working day (8 hours) with GC/MS analysis performed overnight, therefore, the entire ILV test was completed in two working days.

3.0 CALCULATIONS

A calibration curve was constructed by plotting the natural logarithm (ln) of the analyte concentration (µg/L) of the calibration standards against the natural logarithm (ln) of the peak area ratio of the analyte to the internal standard 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 of the regression analysis, and the natural logarithm of the peak area and the dilution factor of the recovery sample. Equations 2, 3, and 4 were then used to calculate measured concentrations and analytical results.

(1) $\ln y = m(\ln x) + b$

(2) $\ln x = \frac{(\ln y - b)}{m}$

(3) $DC\ (x) = \text{inverse}(\ln x)$

(4) $A = DC \times DF$

where:

lnx	=	natural logarithm of sample concentration
lny	=	natural logarithm of detector response ratio
m	=	slope from regression analysis
b	=	y-intercept from regression analysis
DC (x)	=	detected concentration (µg/L) in the sample
DF	=	dilution factor (final volume of the sample divided by the original sample volume)
A	=	analytical result (µg/kg)

The method limit of detection (LOD) was calculated by evaluating the signal-to-noise (S/N) ratio from samples of a known concentration (i.e., the lowest calibration standard) and blank samples (i.e., control samples) to establish the lowest level at which the analyte can reliably be detected. A S/N ratio of 3:1 was used to determine the LOD for each analyte and for each ion monitored.

The 95% Confidence Interval (CI) was calculated for the percent of fortified values for the LOQ and 10X LOQ as follows.

$$CI = t_{df,95\%} * (s/sqrt(n))$$

Where:

$t_{df,95\%}$	=	t value (at n-1 degrees of freedom) for 95% confidence = 2.776
n	=	number of replicates
s	=	standard deviation

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