1.0 INTRODUCTION

This independent laboratory validation (ILV) study is required by the U.S. EPA under the Guidelines for Environmental Chemistry Method and Associated Independent Laboratory Validations OCSPP No. 850.6100 (U.S. EPA, 2012), Residue Analytical Methods OCSPP No. 860.1340 (U.S. EPA, 1996a), and OCSPP 860.1000 (U.S. EPA, 1996b). This study also satisfies the OECD guidance document ENV/JM/MONO(2007)17 (OECD, 2007), SANCO/3029/99 rev. 4 (EC, 2000), and SANCO/825/00 rev. 8.1 (EC, 2010). The purpose of this study was to confirm that the original analytical method, developed by one laboratory, can be independently validated by a second laboratory. The analytical method used for this ILV is "Analytical Method for the Determination of Diuron, Linuron, and Metabolites in Water by LC-MS/MS" (Reichert, 2006). The analytical method was validated by fortification of two water types with diuron and metabolites, DCPMU, and mCPDMU, at the limit of quantification (LOQ, 0.0500 µg/L) and 10X LOQ (0.500 µg/L) concentration levels.

The study was initiated on 28 December 2017, 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 from 8 to 28 May 2018 at Smithers Viscient (SMV), located in Wareham, Massachusetts. All original raw data, the protocol, and the original 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 Diuron and Metabolites in Water by LC-MS/MS" (Appendix 1).

2.2 Test and Reference Substances

The test substance, diuron, was received on 9 August 2017 from MRI Global, Kansas City, Missouri. The following information was provided:

Name:	Diuron
Synonyms:	Diuron (DPX-14740-235), Diuron TC, DCMU, Karmex, Direx, Diuron II Technical
Lot No.:	E95957-67
CAS No.:	330-54-1
Purity:	99.5 ± 0.8% (Certificate of Analysis, Appendix 2)
Expiration Date:	18 January 2019

Upon receipt at Smithers Viscient, the test substance (SMV No. 9038) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, IN-15654-012, was received on 7 September 2017 from MRI Global, Kansas City, Missouri. The following information was provided:

Name:	IN-15654-012
Synonyms:	DCPMU; N-(3,4-Dichlorophenyl)-N'-methylurea; 1-(3,4-Dichlorophenyl)-3-methylurea; 1-Methyl-3-(3,4-dichlorophenyl)urea; 3,4-DCPMU; 3-(3,4-Dichlorophenyl)-1-methylurea; Monomethyldiuron; N-(3,4-Dichlorophenyl)-N'-methylurea; N-Demethoxylinuron; N-Methyl-N'-(3,4-dichlorophenyl)urea
Batch/Lot No.:	5882-103
CAS No.:	3567-62-2
Purity:	99.9% (Certificate of Analysis, Appendix 2)
Expiration Date:	29 January 2019

Upon receipt at Smithers Viscient, the test substance (SMV No. 9086) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

The test substance, IN-12894-007, was received on 7 September 2017 from MRI Global, Kansas City, Missouri. The following information was provided:

Name:	IN-12894-007
Synonym:	mCPDMU
Batch/Lot No.:	GF802546
CAS No.:	587-34-8
Purity:	98.9% (Certificate of Analysis, Appendix 2)
Expiration Date:	30 October 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 9085) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

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

2.3 Reagents

1.	Acetonitrile:	EMD, reagent grade
2.	Formic acid:	BDH, reagent grade
3.	Methanol:	EMD, reagent grade
4.	Purified reagent water:	prepared from a Millipore Milli-Q Direct 8 system (meeting ASTM Type II requirements)

2.4 Equipment

1.	Instruments:	AB Sciex API 4000 mass spectrometer equipped with an AB MDS Sciex ESI Turbo V source Shimadzu LC-20AD binary pumps Shimadzu DGU-20A3 vacuum degasser Shimadzu DGU-20A5R vacuum degasser Shimadzu SIL-20ACHT autosampler Shimadzu CTO-20AC column oven Shimadzu CBM-20A communications bus Analyst version 1.6.3 software for data acquisition
2.	SPE Column:	Supelchem LC-18
3.	Laboratory equipment:	Volumetric flasks, disposable glass pipets, positive displacement pipets, stir bars, stir plates, vortexers, sonicator, 20-mL disposable glass vials, autosampler vials, and amber glass bottles with Teflon-lined caps

2.5 Test Systems

The test systems evaluated during this study were waters representative of the matrices that the method was intended to analyze. The waters used for this ILV were ground water (in-house well water) and surface water (Weweantic River, West Wareham, Massachusetts, Lot No. 17Oct16Wat-A-3). The in-house well water is unadulterated water from a 100-meter bedrock well which is considered soft with a typical hardness of <160 mg (as $CaCO_3$). The surface water was determined have a pH of 6.9 and a dissolved oxygen concentration of 9.3 mg/L (Agvise Laboratories, Northwood, North Dakota).

2.6 Liquid Reagent and Mobile Phase Solution Preparation

An 80/20 methanol/purified reagent water (v/v) liquid reagent solution was typically prepared by combining 40.0 mL of methanol and 10.0 mL of purified reagent water. The solution was mixed well using a stir bar and stir plate for five minutes.

A 30/70 methanol/purified reagent water (v/v) with 0.2% formic acid liquid reagent solution was typically prepared by combining 75.0 mL of methanol and 175 mL of purified reagent water

within a secondary container. A 0.500 mL portion of formic acid was measured using a disposable glass pipet and was transferred to the secondary container with the methanol and purified reagent water. The solution was mixed well using a stir bar and stir plate for five minutes.

A 0.2% formic acid in purified reagent water mobile phase solution was typically prepared by adding 4.00 mL of formic acid to 2000 mL of purified reagent water. The solution was mixed well using a stir bar and stir plate for five minutes, then degassed under vacuum with sonication for ten minutes.

A 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v) autosampler needle wash solution was typically prepared by combining 1200 mL of acetonitrile, 1200 mL of methanol, and 1600 mL of purified reagent water. The solution was mixed well before use.

2.7 Preparation of Stock Solutions

Primary stock solutions were prepared as described in the table below.

Primary Stock ID	Analyte	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
9038AL	Diuron	0.0503	0.0500	Methanol	50.0	1000	Sub-stock solutions
9085D	mCPDMU	0.0508	0.0502		50.0	1000	
9086B	DCPMU	0.0503	0.0502		50.0	1000	

Sub-stock solutions were 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
9038AL	1000	1.00	10.0	Methanol	MIX STK 1	100	Sub-stock solution
9085D	1000	1.00					
9086B	1000	1.00					
MIX STK 1	100	0.0200	20.0	30/70 methanol/purified reagent water (v/v) with 0.2% formic acid	MIX STK 2	0 100	Calibration standards and 10X LOQ recovery samples
MIX STK 2	0.100	2.00	10.0		MIX STK 3	0.0200	LOQ recovery samples

All primary 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

Standards were prepared in 30/70 methanol/purified reagent water (v/v) with 0.2% formic acid using the 0.100 mg/L sub-stock solution according to the table below. Following fortification, each solution was vortex-mixed for 15 seconds prior to being submitted for analysis.

Fortifying Stock ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)	Standard Concentration (µg/L)	Sample ID
MIX STK 2	0.100	0.0600	20.0	0.300	Std 1
		0.100	20.0	0.500	Std 2
		0.200	20.0	1.00	Std 3
		0.500	20.0	2.50	Std 4
		1.00	20.0	5.00	Std 5
		2.00	20.0	10.0	Std 6
		3.00	20.0	15.0	Std 7

2.9 Sample Fortification and Preparation

2.9.1 Ground Water

Twelve replicates were established, each with a 20.0-mL aliquot of ground water in a 20-mL disposable glass vial. Five replicates were dosed with the 0.0200 mg/L sub-stock solution and five replicates were dosed with the 0.100 mg/L sub-stock solution to obtain concentrations of 0.0500 (LOQ) and 0.500 µg/L (10X LOQ), respectively. Two replicates were left unfortified to serve as controls. An additional sample was prepared using only reagents as a reagent blank, free of test materials and matrix. The dosing procedure is detailed in the following table.

Sample ID 14134-6112-	Sample Type	Stock ID	Fortifying Stock Concentration (mg/L)	Fortification Volume (mL)	Sample Volume (mL)	Nominal Concentration (µg/L)
01	Reagent Blank	NA[a]	NA	NA	NA[b]	0.00
02 & 03	Control	NA	NA	NA	20.0	0.00
04, 05, 06, 07, & 08	LOQ	MIX STK 3	0.0200	0.0500	20.0	0.0500
09, 10, 11, 12, & 13	10X LOQ	MIX STK 2	0.100	0.100	20.0	0.500

[a] NA = Not Applicable
[b] Only processing reagents used for the reagent blank

2.9.2 Surface Water

Twelve replicates were established, each with a 20.0-mL aliquot of surface water in a 20-mL disposable glass vials with PTFE-lined caps. Five replicates were dosed with the 0.0200 mg/L sub-stock solution and five replicates were dosed with the 0.100 mg/L sub-stock solution to obtain concentrations of 0.0500 (LOQ) and 0.500 µg/L (10X LOQ), respectively. Two replicates were left unfortified to serve as controls. An additional sample was prepared using only reagents as a reagent blank, free of test materials and matrix. The dosing procedure is detailed in the following table.

Sample ID 14134-6112-	Sample Type	Stock ID	Fortifying Stock Concentration (mg/L)	Fortification Volume (mL)	Sample Volume (mL)	Nominal Concentration (µg/L)
14	Reagent Blank	NA[a]	NA	NA	NA[b]	0.00
15 & 16	Control	NA	NA	NA	20.0	0.00
17, 18, 19, 20, & 21	LOQ	MIX STK 3	0.0200	0.0500	20.0	0.0500
22, 23, 24, 25, & 26	10X LOQ	MIX STK 2	0.100	0.100	20.0	0.500

[a] NA = Not Applicable
[b] Only processing reagents used for the reagent blank

2.10 Extraction of Fortified Recovery Samples

LC-18 SPE columns (500 mg, 3 mL) were conditioned by rinsing with two column volumes of methanol followed by two column volumes of purified reagent water. The columns were not allowed to dry. A 0.200-mL aliquot of methanol was added to each fortified recovery sample, followed by thorough mixing for 15 seconds. Each sample was then loaded onto the SPE columns and allowed to flow through under vacuum at a flow rate of 1 mL/min; this eluent was discarded. The sampling vessels and columns were rinsed twice with 2.0-mL aliquots of purified reagent water under vacuum at a flow rate no greater than 1 mL/min; this rinsate was discarded. The SPE columns were dried under vacuum for approximately 10 minutes. A 0.5-mL aliquot of 80/20 methanol/purified reagent water (v/v) was transferred to each column and columns were allowed to incubate and elute via gravity. The sampling vessels were then eluted with 2.0 mL of 80/20 methanol/purified reagent water (v/v) into graduated glass conical vials. The samples were concentrated to approximately 1 mL under a gentle stream of nitrogen using an N-Evap at 25 °C. If necessary, the reagent blank, control, and LOQ samples were adjusted to a final volume of 1.00 mL with methanol. Each 10X LOQ sample was diluted to 10.0 mL with methanol. The sample processing is summarized in the tables below.

Ground water:

Sample ID 14134-6112-	Sample Type	Nominal Concentration (µg/L)	Sample Volume (mL)	Eluted Volume[a] (mL)	Final Volume[b] (mL)	Dilution Factor
01	Reagent Blank	0.00	20.0	2.50	1.00	0.0500
02 & 03	Control	0.00	20.0	2.50	1.00	0.0500
04, 05, 06, 07, & 08	LOQ	0.0500	20.0	2.50	1.00	0.0500
09, 10, 11, 12, & 13	10X LOQ	0.500	20.0	2.50	10.0	0.500

[a] Elution solvent: 80/20 methanol/purified reagent water (v/v)
[b] Dilution solvent: 100% methanol

Surface water:

Sample ID 14134-6112-	Sample Type	Nominal Concentration (µg/L)	Sample Volume (mL)	Eluted Volume[a] (mL)	Final Volume[b] (mL)	Dilution Factor
14	Reagent Blank	0.00	20.0	2.50	1.00	0.0500
15 & 16	Control	0.00	20.0	2.50	1.00	0.0500
17, 18, 19, 20, & 21	LOQ	0.0500	20.0	2.50	1.00	0.0500
22, 23, 24, 25, & 26	10X LOQ	0.500	20.0	2.50	10.0	0.500

[a] Elution solvent: 80/20 methanol/purified reagent water (v/v)
[b] Dilution solvent: 100% methanol

2.11 LC-MS/MS Instrumental Conditions

The LC-MS/MS analysis for diuron, DCPMU, and mCPDMU was conducted using the following instrumental conditions:

LC Parameters:

Column: Thermo Scientific Hypurity C8, 5 µm, 3 × 150 mm
Mobile Phase A: 0.2% formic acid in purified reagent water
Mobile Phase B: methanol
Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
0.01	0.500	70.0	30.0
10.00	0.500	10.0	90.0
15.00	0.500	1.00	99.0
15.10	0.500	1.00	99.0
17.10	0.500	70.0	30.0
23.00	0.500	70.0	30.0
23.01	0.500	70.0	30.0

Run time: 23.01 minutes
Injector Wash solvent: 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)
Column temperature: 40 °C
Sample temperature: 10 °C
Injection volume: 15.0 µL for ground water
10.0 µL for surface water (see Method Differences)

Approximate Retention Times:

Analyte	Retention Time (min.)	
	Ground water	Surface water
Diuron	7.7	7.8
DCPMU	7.7	7.7
mCPDMU	6.1	6.1

MS Parameters:

Instrument:	MDS Sciex API 4000 mass spectrometer
Ionization Mode:	Positive (+) ESI
Resolution Q1/Q3:	Unit/Unit
Ion Spray Voltage:	5000 V
Scan type:	MRM
Source Temperature:	500 ºC
Curtain Gas:	10.00
Ion Source – Gas 1 / Gas 2:	30.00 / 40.00
Collision Gas:	10.00
Collision Cell Entrance Potential:	10.00
Collision Cell Exit Potential:	6.50
Declustering Potential:	60.00

Analyte	Q1/Q3 *m/z*	Dwell Time (msec)	Collision Energy
Diuron	233.12/72.10 (Primary)	100	35.00
	233.12/46.10 (Confirmatory)	100	37.00
DCPMU	219.02/161.96 (Primary)	100	22.10
	219.02/127.06 (Confirmatory)	100	38.40
mCPDMU	199.04/72.10 (Primary)	100	35.10
	199.04/46.19 (Confirmatory)	100	29.50

2.11.1 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 two to six injections. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

2.11.2 Method Differences

The analytical method used for diuron and its metabolites in this independent laboratory validation followed the procedures described in the original method validation. The analytical method used for diuron, DCPMU, and mCPDMU in this independent laboratory validation required the following minor modifications from the original method validation.

- Mass spectrometer parameters were optimized for sensitivity and linearity, as necessary. This includes any and all parameters where necessary including the model of mass spectrometer used.
- The samples were not filtered through a 0.2 µm PTFE syringe filter. These were not used in order to mitigate possible losses during the extraction.
- The calibration curve for the groundwater validation was truncated by removing the highest two standards. This was done because analyte response was approaching the maximum detection limit. Since all samples were diluted to the third calibration standard (1.00 µg/L), the two highest standards were unnecessary and, therefore, have no effect on quantitation.
- The injection volume was lowered to 10 µL for the surface water validation. This was done in order to decrease the signal, as it was saturating the instrument detector.

The method differences discussed above were not considered to be significant changes and did not impact the performance of the original method.

2.12 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 70.0 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. 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 diuron and metabolites which might interfere with the quantitation of the analytes. Interferences with peak areas that are less than 50% of the LOD are not considered significant. Linearity of the method was determined by the correlation coefficient (r), y-intercept, and slope of the regression line. A linear regression was used for the LC-MS/MS analysis. The calibration curves were evaluated based on the correlation coefficient and the recoveries of the calibration standards. The signal response data should have an intercept close to zero and a correlation coefficient (r) not less than 0.995. The precision of the method at the LOQ was reported in terms of the coefficient of variation of the observed recovery values.

2.13 Limit of Quantitation (LOQ)

The method was validated at the Limit of Quantitation (LOQ). This was defined as the lowest fortification level. Blank values (reagent blanks and untreated control samples) did not exceed 50% of the LOD, with the exception of Diuron during the groundwater validation (See Protocol Deviation).

2.14 Limit of Detection (LOD) and Method Detection Limit (MDL)

The Limit of Detection (LOD) was calculated using three times the signal-to-noise (S/N) ratio of the control samples. Representative calculations for the LOD can be found in Section 3.0.

The Method Detection Limit (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.

2.15 Communications

Communications occurred with the Study Monitor to discuss items including: approval of the protocol and method, challenges in regards to calibration curves, and the results of the first attempt of the ILV. A complete summary list of communications is provided in Appendix 3.

2.16 Time Required for Analysis

This ILV study included the validation of two water matrices, ground and surface water. Each water matrix validation included one set of samples used for LC-MS/MS analysis. Each set of samples consisted of ten fortified, two unfortified samples, one reagent blank, and seven calibration standards (20 samples total). A single analyst completed one set of 20 samples in one working day (8 hours) with LC-MS/MS analysis performed overnight (16 hours) per sample set.

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. Recoveries were not corrected for residues found in the untreated control samples. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

(1) $y = mx + b$

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

(3) $A = DC \times DF$

where:

x	=	analyte concentration (µg/L)
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 (µg/L) in the sample
DF	=	dilution factor (final volume of the sample divided by the original sample volume)
A	=	analytical result (µg/L), concentration in the original sample

The method detection limit (MDL) is defined as the lowest concentration that can be detected by this method in test solution samples. The MDL is calculated (Equation 4) based on the concentration of the low calibration standard and the dilution factor of the control samples.

$$(4) \quad MDL = MDL_{LCAL} \times DF_{CNTL}$$

where:

MDL_{LCAL}	=	lowest concentration calibration standard (0.300 µg/L)
DF_{CNTL}	=	dilution factor of the control samples (smallest dilution factor used, 0.0500)
MDL	=	method detection limit reported for the analysis (0.300 µg/L × 0.0500 = 0.0150 µg/L)

The Instrument LOD was calculated using the following equation:

$$(5) \quad LOD = (3xSN_{ctl})/Resp_{LS} \text{ x } Conc_{LS}$$

where:

SN_{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 (0.300 µg/L)
$Conc_{LS}$	=	Concentration of the low calibration standard (µg/L)
LOD	=	Instrument Limit of Detection for the analysis (µg/L)

The Overall Method LOD was calculated using the following equation:

$$(6) \quad LOD_{Overall} = LOD \times DF_{Ctl}$$

where:

LOD = Limit of Detection calculated from signal to noise ratio (µg/L)
DF_{Ctl} = Dilution factor for control sample
$LOD_{Overall}$ = Overall Method Limit of Detection

PROTOCOL DEVIATION

The protocol states, “The limit of detection (LOD) will be established by evaluating the signal-to-noise (S/N) ratio from samples of known concentration and blank samples to establish the lowest level at which the analyte can be reliably detected. A S/N ratio of 3/1 is generally considered the minimum acceptable ratio for reliable detection.” The protocol also states, “Interferences with peak areas that are less than 50% at the limit of detection (LOD) are not considered significant.” The controls for the diuron groundwater ILV contain peaks that place the LOD (three times the signal-to-noise ratio), above the MDL, and lowest calibration standard. The LOD, therefore, was not established according to the protocol since the MDL, and lowest calibration standard concentration fall below the calculated LOD. This had no significant impact on the study because the LOQ samples and lowest calibration standards were unaffected by what appears to be contamination during processing and the protocol acceptance criteria were met. The peaks in the control samples are less than 30% of the LOQ samples, and they are below the MDL. The surface water validation did not have analyte peaks in the controls for diuron, and the LODs fell within the acceptable range. Surface water is typically a more complex matrix and it is expected that interferences are more likely, thus contributing to higher noise during the analysis. The LODs for DCPMU and mCPDMU were very low in both matrices and this event was isolated to diuron analysis in groundwater.

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APPENDIX 1 - STUDY PROTOCOL

Study No.: 14134.6112

Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Diuron and Metabolites in Water by LC-MS/MS

1.0 INTRODUCTION

The purpose of this study is to confirm that an analytical method, developed by one group, can be independently validated by a second group in the absence of major interaction between the two. This study is required by EPA under Guideline OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation [EPA 712-C-001], Guideline OCSPP 850.7100: Data Reporting for Environmental Chemistry Methods [EPA 712-C-96-348], and Guideline OCSPP 860.1340: Residue Analytical Method [EPA 712–C–96–174], as well as satisfies OECD guidance document ENV/JM/MONO(2007)17, EC guidance documents SANCO/3029/99 REV 4(2000) and SANCO/825/00 REV 8.1(2010). Independent laboratories are allowed to analyze three sample sets in order to validate the method as written. A complete set of samples should consist of, at a minimum, a reagent blank, two un-spiked matrix control samples, five matrix control samples fortified at the limit of quantification (LOQ), and five matrix control samples fortified at 10X LOQ for each distinct matrix. A complete set may include more than thirteen samples depending on the number of reagents, and un-fortified and fortified control matrix samples. It may be necessary, however, to divide a complete set into two subsets for efficient handling. Each subset should contain a reagent blank, two un-fortified matrix control samples, and five matrix control samples fortified at the LOQ or 10X LOQ.

If the performance data on the first set of samples at any of the required spiking levels is unsuccessful, the independent laboratory may contact the registrant to clarify the directions given in the method. Any contact with the registrant or developers during the method validation must be documented in writing in the final report submitted by the independent laboratory. If the independent laboratory cannot generate performance data that is similar to the registrant's or developers' after the second set of spiked samples, the independent laboratory may contact the registrant to further clarify the directions given in the method. If the independent laboratory cannot generate performance data that is similar to the registrant's or developers' after the third set, the method should be failed, and a report will be sent to the registrant explaining why the method failed. The registrant should then decide whether to repeat the independent laboratory validation at another laboratory, further develop the method, or withdraw it. This ILV trial will be conducted under FIFRA Good Laboratory Practice (GLP) standards as specified in 40 CFR part 160. A maximum of three sample sets are used by an independent laboratory to validate the method as written. A successful ILV trial will require adequate results on at least one complete set of samples on a given matrix.

The purpose of this protocol is to perform an ILV for the analytical method used to determine the test substance in ground and surface water (identified in the raw data and final report). The analytical method will be validated with regards to accuracy, precision, specificity, signal response, selectivity, and limits of quantitation.

2.0 OBJECTIVE

The objective of this study is to confirm that the analytical method for Diuron, and its metabolites in ground water and surface water, developed by one group, can be independently validated by a second group in the absence of major interaction between the two.

2.1 Matrices to Analyze

The ground water consists of unadulterated water from a 100-meter bedrock well.

The surface water consists of freshwater sampled near or slightly below the surface of well aerated sections of a natural body of freshwater.

3.0 JUSTIFICATION OF THE TEST SYSTEM

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 [EPA 712-C-001], Guideline OCSPP 850.7100: Data Reporting for Environmental Chemistry Methods [EPA 712-C-96-348], and Guideline OCSPP 860.1340: Residue Analytical Method [EPA 712–C–96–174], as well as satisfies OECD guidance document ENV/JM/MONO(2007)17, EC guidance documents SANCO/3029/99 REV 4(2000) and SANCO/825/00 REV 8.1(2010).

4.0 MATERIALS

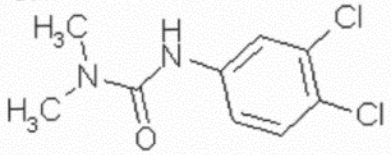
4.1 Test Substance

Upon arrival at Smithers Viscient, the test and reference substance(s) 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 substance 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 sample 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.

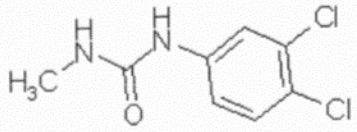
4.1.1 Test Substance Information

1. Name: Diuron
Purity: 99.5%
Batch or Lot #: E95957-67

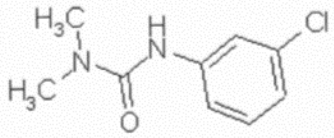


Study No.: 14134.6112

2. Name: DCPMU
Purity: 99.9%
Batch or Lot #: 5882-103



3. Name: mCPDMU
Purity: 98.9%
Batch or Lot #: GF802-546



5.0 TEST SYSTEM IDENTIFICATION

Test solution preparation will be documented on data forms which include the amount of test substance, the volume or mass of the test solution, lot, batch or other sample designation of the test substance and date the solution was prepared. Individual sample containers will be labeled with a unique ID number.

6.0 ANALYTICAL METHOD

The analytical method used for this ILV is, "Analytical Method for the Determination of Diuron, Linuron, and Metabolites in Water by LC-MS/MS (N. Reichert, DuPont-19220, completed August 4, 2006).

7.0 VALIDATION DESIGN

The standard curve will be comprised of at least five concentrations. The anticipated concentration range is 0.300-15.0 ppb. A smaller, larger, or shifted range may be necessary if achievable. The range will be documented in the study records and final report.

The limit of detection (LOD) will be established by evaluating the signal-to-noise (S/N) ratio from samples of known concentration and blank samples to establish the lowest level at which the analyte can be reliably detected. A S/N ratio of 3:1 is generally considered the minimum acceptable ratio for reliable detection.

7.1 Accuracy and Precision

The accuracy of the analytical method will be determined by applying the method to five samples of two water types (ground and surface) at the LOQ (0.0500 ppb) and five samples at 10X LOQ (0.500 ppb) for each test substance. The accuracy will be reported in terms of percent recovery and the difference between the mean determined and the theoretical value. Recoveries of 70.0 to 120% of nominal are acceptable.

The precision will be calculated for the fortified samples in terms of the standard deviation (SD) and relative standard deviation (RSD or coefficient of variation (CV)) calculated for the retention time, peak area based quantitation (i.e., µg/L), and the observed recovery values. The retention time should have a RSD of less than or equal to 2%. The RSD of the peak area based quantitation (i.e., µg/L) should be less than or equal to 20%. The RSD of the recovery values should be less than or equal to 20% as well.

7.2 Specificity

The specificity of the method will be determined by applying the method to two un-fortified matrix control samples for each matrix. Chromatograms will be obtained for the control samples and examined for peaks that might interfere with the quantitation of the analyte peak of interest. Peaks attributable to test substance should be sufficiently resolved from any peaks found in the samples of control matrix to enable quantification. Interferences with peak areas that are less than 50% at the limit of detection (LOD) are not considered significant.

7.3 Signal Response

The signal response of the method will be determined by preparing a calibration curve with a minimum of five standards to encompass approximately 70.0 to 120% of the test concentration.

The calibration data will be subjected to a regression analysis; a plot of the analyte concentration versus the detector response will be included in the report along with the correlation coefficient, 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 (r^2 not less than 0.990). The responses of the standards shall be inserted into the regression equation, and a calculated concentration value calculated. This calculated value shall be within ±20% of the theoretical value. Deviations from these criteria will be addressed by reevaluating the calibration range, such that the calculated values meet these criteria.

8.0 CONTROL OF BIAS

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

9.0 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.

10.0 SAMPLE DISPOSAL

All study specimens, and/or samples collected during the study, and test materials and reference standards, etc., provided by the sponsor, client, or customer will either be returned to the originator, shipped to a third party archival facility on behalf of the Study Sponsor who will incur the costs of shipping and archival, or disposed of according to Smithers Viscient standard operating procedures.

11.0 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, the following information:

- Protocol and all amendments.
- Name, address, and telephone number of study director and other contact person for ILV laboratory.
- Description of the analytical method.
- All recovery and control values for all matrices that were obtained during all ILV trials.
- Representative chromatograms/spectra for each analyte in each matrix.
- Description of the instruments used and operating parameters.
- Description of any problems encountered and a written description of any changes or modifications that were made during the ILV.
- Any steps considered critical, i.e., steps where little variation is allowable or directions must be followed precisely.
- The number of worker-hours required to complete one set of samples.
- The number of calendar days required for one set of samples.
- Any contact between the independent laboratory and the method developers or others familiar with the method, including the reasons for the contact, any changes in the method that resulted, and the time of this communication with respect to the progress of the confirmatory trial (i.e., after the first set, during the second set, etc.).

- A statement of adherence to FIFRA GLP standards under 40 CFR160.12.
- 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, i.e., Program Coordinator (if applicable), Study Director and Principal Investigator.
- Identification of the test substance which may include chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, water solubility, vapor pressure, degree of purity of test substance (percent test chemical) (Sponsor-supplied, if available).
- The determined accuracy, precision, linearity, limit of detection, and method LOQ.
- 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 the protocol, raw data, and final report.

12.0 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.

13.0 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 accepted by OECD Principles of Good Laboratory Practice.

14.0 REFERENCES

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

European Commission, 2010. Guidance Document on Pesticide Residue Analytical Methods. SANCO/825/00 rev. 8.1. 16 November 2010.

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.

OECD, 2007. Environment Directorate. Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. OECD Series on Testing and Assessment Number 72. Guidance Document on Pesticide Residue Analytical Methods. ENV/JM/MONO(2007)17. Organization for Economic Cooperation and Development. 13 August 2007. Paris, France.

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, January 2012. OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation [EPA-712-C-001].

U.S. EPA, April 1996. OCSPP 850.7100: Data Reporting for Environmental Chemistry Methods [EPA 712-C-96-348].

U.S. EPA, August 1996. OCSPP 860.1000: Background [EPA-712-C-96-169].

U.S. EPA, August 1996. OCSPP 860.1340: Residue Analytical Method [EPA 712–C–96–174].

Reichert, N. August 2006. Analytical Method for the Determination of Diuron, Linuron, And Metabolites in Water by LC-MS/MS. DuPont-19220.