1.0 INTRODUCTION

Methodology from Smithers Viscient (DeVellis, 2014) was validated (20 to 21 May 2014) to quantify the concentration of Temik 15G present in independent laboratory validation (ILV) recovery samples prepared in soil on 14 July 2014. This independent laboratory validation (ILV) study is required by U.S. EPA under Guideline No. 850.6100 (U.S. EPA, 2012) to confirm that the original analytical method, developed by one group, can be independently validated by a second group with no major interaction between the two groups. This method was validated by fortification of soil with Temik 15G at concentrations of approximately 0.10 mg/kg (limit of quantitation, LOQ) and 1.0 mg/kg (10X LOQ). Recovery samples were extracted twice with 0.1% formic acid in acetonitrile and were diluted into the calibration standard curve with 20:80 acetonitrile:purified reagent water (v:v) prior to analysis. Samples were then analyzed using liquid chromatography with mass spectrometry (LC/MS/MS).

The study was initiated on 7 July 2014, 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 14 July 2014 at Smithers Viscient (SMV), located in Wareham, Massachusetts. All original raw data, the 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: Temik 15G (Aldicarb) – Validation of the Analytical Method for the Determination of a Test Substance 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 and Reference Substances

2.2.1 Test Substance

The test substance, Temik 15G, was received on 10 April 2014 from Analytical and Regulatory Chemistry, Sumter, South Carolina. The following information was provided:

Name:	Temik 15G
Lot No.:	NWTEAXV179
CAS No.:	116-06-3
Purity:	14.81% as aldicarb
Expiration Date:	7 April 2016

Upon receipt at Smithers Viscient, the test substance (SMV No. 6935) was stored at room temperature in the original container in a dark, ventilated cabinet. Concentrations were adjusted for the purity of the test substance.

2.2.2 Reference Substance

The reference substance, aldicarb, was received on 21 April 2014 from Sigma Aldrich, Allentown, Pennsylvania. The following information was provided:

Name:	aldicarb
Batch No.:	SZBC166XV
CAS No.:	116-06-3
Purity:	99.9%
Expiry Date:	14 June 2017

Upon receipt at Smithers Viscient, the reference substance (SMV No. 6962) was stored at room temperature in the original container in a dark, ventilated cabinet. Concentrations were adjusted for the purity of the reference substance.

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

2.3 Reagents

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

2.4 Equipment

1.	Instrument:	AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V ESI Ion Spray source, an Acquity Sample Manager autosampler, an Acquity Binary Solvent Manager binary pump, an Acquity Column Compartment column oven, and Analyst 1.6 software for data acquisition
2.	Balance:	Mettler PJ-3000, Mettler Toledo AG245, Sartorius Moisture Analyzer MA-45
3.	Centrifuge:	Beckman Allegra X-12
4.	Shaker table:	VWR Analog 3500 STD
5.	Laboratory equipment:	volumetric flasks, disposable glass pipets, disposable glass vials, positive displacement pipets, Nalgene centrifuge tubes, autosampler vials, and amber glass bottles with Teflon®-lined caps

2.5 Test Soil

The soil used for this ILV analysis was Rochester Sandy Loam soil (SMV Lot No. 021814, Sample ID 2014 100 ROCH LOAM) from Rochester, Massachusetts. The soil was stored refrigerated in the dark until needed for analysis. Prior to testing, soil moisture content of the soil was determined to be 27.35% using a Sartorius MA-45 moisture analyzer.

2.6 Preparation of Stock Solutions

Primary stock solutions were typically prepared from the test and reference substances as summarized below.

Primary Stock ID	Amount of Substance Weighed (g), Net Weight	Amount of Substance Weighed (g), as Active Ingredient	Final Volume (mL)	Solvent Stock	Primary Stock Concentration (mg/L)	Primary Stock Uses
Test Substance						
69351	0.3392	0.0502	50.0	DMF	1000	Secondary stocks for recovery samples
Reference Substance						
6962-2D	0.0503	0.0502	50.0	Acetonitrile	1000	Secondary stock for calibration standards

Note: The formulation granules need to be crushed, and sonicated in a sonicator for one hour to ensure dissolution in the DMF solvent.

Secondary stock solutions were prepared from the primary stock solutions as summarized 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
Test Substance							
69351	1000	0.500	50.0	Acetonitrile	10.0	69351-2	LOQ- and high-level recovery samples
Reference Substance							
6962-2D	1000	0.500	50.0	Acetonitrile	10.0	6962-2D-2	Sub-stock solutions for calibration standards

Sub-stock solutions were prepared as summarized in the table below.

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Solvent Stock	Final Stock Concentration (µg/L)	Stock ID	Stock Uses
Reference Substance							
6292-2D-2	10.0	0.100	10.0	Acetonitrile	100	Stk 1	Calibration standards

Primary and secondary stock solutions were stored refrigerated 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 Reagent Solution and Mobile Phase Preparation

A 0.1% formic acid in purified reagent water (v:v) mobile phase solution was typically prepared by adding 2.00 mL of concentrated formic acid to 2000 mL of purified reagent water and mixed well. The mobile phase was degassed under vacuum with sonication for 10 minutes.

A 0.1% formic acid acetonitrile (v:v) liquid reagent solution and was typically prepared by adding 0.800 mL of concentrated formic acid to 800 mL of acetonitrile and mixed well. The liquid reagent was mixed using a stir bar and stir plate for five minutes. A separate mobile phase solution was also prepared and degassed under vacuum with sonication for 10 minutes.

A 20:80 acetonitrile:purified reagent water (v:v) liquid reagent solution was typically prepared by combining 400 mL of purified reagent water with 100 mL of acetonitrile. The solution was mixed using a stir bar and stir plate for five minutes.

2.8 Preparation of Calibration Standards

Calibration standards were prepared in 20:80 acetonitrile:purified reagent water (v:v) by fortifying with the 100 µg/L sub-stock solution as described in the following table.

Fortifying Stock ID	Fortifying Stock Concentration (µg/L)	Volume of Fortification (mL)	Final Volume (mL)	Calibration Standard Concentration (µg/L)
Stk 1	100	0.0500	100	0.0500
	100	0.0500	50.0	0.100
	100	0.0500	10.0	0.500
	100	0.100	10.0	1.00
	100	0.150	10.0	1.50
	100	0.200	10.0	2.00

2.9 Sample Fortification and Preparation

All soil recovery samples (5.00 g dry weight) were weighed into individual 50.0-mL Nalgene® centrifuge tubes. Five replicates of each concentration were dosed with the 10.0 mg/L secondary stock solution at 0.100 and 1.00 mg/kg (dry weight). The dosing procedure is detailed in the following tables:

Sample ID	Stock ID	Fortifying Stock Concentration (mg/L)	Fortification Volume (mL)	Dry weight (g)	Nominal Concentration (mg/kg)
Control A & B	NA[a]	NA	NA	5.00	0.00
LOQ A, B, C, D & E	6935I-2	10.0	0.0500	5.00	0.100
High A, B, C, D & E	6935I-2	10.0	0.500	5.00	1.00

[a] NA = Not Applicable

Two additional 5.00 g samples were prepared and left unfortified to serve as controls. One additional sample was extracted using only extraction solvents to serve as the reagent blank.

2.10 Soil Extraction and Dilution

A 20.0-mL aliquot of 0.1% formic acid in acetonitrile was added to the soil recovery samples (5 g dry weight). The samples were placed on an orbital shaker table for 30 minutes at 150 rpm. The samples were then centrifuged at 3000 rpm for 10 minutes and the extracts were transferred to labeled 50.0-mL volumetric flasks. The extraction and centrifugation procedure was repeated with an additional 20.0-mL aliquot of 0.1% formic acid in acetonitrile. The second extract was combined with the first in the appropriate volumetric flasks and taken to a final volume of 50.0 mL with 0.1% formic acid in acetonitrile. Samples were further diluted into the calibration standard range with 20:80 acetonitrile:purified reagent water (v:v). The extraction and dilution procedures are detailed below.

Sample ID	Fortified Concentration (mg/kg)	Dry weight (g)	Extract Volume[a] (mL)	Final Volume[a] (mL)	Secondary Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Control A & B	0.00	5.00	20.0	50.0	0.500	10.0	200
LOQ A, B, C, D & E	0.100	5.00	20.0	50.0	0.500	10.0	200
High A, B, C, D & E	1.00	5.00	20.0	50.0	0.125	10.0	800

[a] Extracted and diluted with 0.1% formic acid in acetonitrile.
[b] Dilution solvent: 20:80 acetonitrile:purified reagent water (v:v).

2.11 Analysis

2.11.1 Instrumental Conditions

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

LC parameters:

Column: X-Bridge™ C18, 2.5 µm, 2.1 × 50 mm
Mobile Phase A: 0.1% formic acid in purified reagent water
Mobile Phase B: 0.1% formic acid in acetonitrile
Gradient:

Time (min)	Flow rate (mL/min)	Solvent A (%)	Solvent B (%)
0.00	0.350	85.0	15.0
0.10	0.350	85.0	15.0
0.50	0.350	85.0	15.0
2.50	0.350	5.0	95.0
5.00	0.350	5.0	95.0
5.10	0.350	85.0	15.0
6.50	0.350	85.0	15.0

Injection volume: 50 µL
Column oven: 30 °C
Sample temperature: 5 °C
Retention Time: Approximately 1.8 minutes

MS parameters:

Instrument:	AB Sciex API 5000 mass spectrometer equipped with an AB Sciex Turbo V ESI Ion Spray source
Q1/Q3 Mass:	213.100/89.00 Da
Dwell Time:	200 milliseconds
Scan type:	MRM
Ion source:	ESI
Source temperature:	500 °C
Ionization mode:	Positive
Resolution Q1/Q3:	Unit/Unit
Curtain gas:	25.00
Ion source Gas 1/Gas 2:	70.00/70.00
Ion spray voltage:	5500
Collision gas:	8.00
Declustering potential:	100.00
Entrance potential:	3.00
Collision energy:	24.00
Collision cell exit potential:	11.00

2.11.2 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.

2.11.3 Method Differences

There were no method differences between the method validation (DeVellis, 2014) and this procedure.

2.12 Evaluation of Precision, Accuracy, Specificity and Linearity

The accuracy was reported in terms of percent recovery of the low- and high-level recovery samples. Recoveries of 70 to 120% of nominal were considered acceptable, with no corrections made for procedural recoveries during the study.

2.13 Communications

Communications occurred with the Sponsor Monitor to discuss items such as 1) clarification/approval of the protocol and method, 2) acquisition of analytical standard and control matrix and 3) pre-validation evaluation and method establishment including calibration curve linearity. A complete list of communications is maintained in the study raw data.

2.14 Time Required for Analysis

A normal batch of samples consists of 10 fortified and 2 unfortified samples, 1 reagent blank and 6 solvent standards (19 samples total). A single analyst completed a set of 19 samples in one working day (8 hours) with LC/MS/MS analysis performed overnight.

3.0 Calculations

A calibration curve was constructed by plotting the analyte concentration (µg/L) in the calibration standards against the peak area of the calibration standards. The equation of the line (equation 1) was algebraically manipulated to give equation 2. The concentration of the test

substance within each recovery sample was determined using the regression coefficients from the quadratic equation, the peak area of the recovery sample, and the dilution factor. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

$$(1) \qquad y = ax^2 + bx + c$$

$$(2) \qquad DC(x) = \frac{-b + \sqrt{b^2 - 4aC}}{2a}$$

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

where:

y	=	detector response (peak area) for analyte
a, b and c	=	regression constants
DC (x)	=	detected concentration (µg/L) in the sample
C	=	constant c minus the peak area; C = (c - y)
DF	=	dilution factor (the final sample volume divided by the original sample volume)
A	=	concentration of the analyte in the original sample

The limit of quantitation (LOQ) was calculated using the following equation:

$$(4)\ LOQ_{INST} = \frac{-b + \sqrt{b^2 - 4aC}}{2a}$$

$$(5)\ LOQ = LOQ_{INST} \times DF_{CTRL}$$

where:

$Area_{LS}$	=	mean detector response (peak area) of the low concentration calibration standard (two injections)
a, b, c	=	regression constants
C	=	regression constant ; C = (c - $Area_{LS}$)
LOQ_{INST}	=	limit of quantitation on the instrument
DF_{CTRL}	=	dilution factor of the control samples (smallest dilution factor used)
LOQ	=	limit of quantitation reported for the analysis