INTRODUCTION

EAG Laboratories performed an independent laboratory validation (ILV) of validation of a method for the determination of residues of Triallate and TCPSA in soil by LC/MS/MS. The protocol for this study titled "Independent Laboratory Validation of a Method for the Determination of Triallate and TCPSA in Soil by LC/MS/MS" is presented in Appendix I. The Sponsor provided analytical method report entitled; "Validation of the Analytical Method for the Determination of Triallate and TCPSA in Soil Matrices by LC/MS/MS" for independent validation (relevant portion) is presented in Appendix II.

This study was performed to satisfy regulatory requirements for independent laboratory validation of methods as set forth by the U.S. Environmental Protection Agency Series 860 - Residue Chemistry Test Guidelines, OCSPP 850.6100, *Environmental Chemistry Methods and Associated Independent Laboratory Validation* (1) and U.S. Environmental Protection Agency, 1996. Pesticide Regulation (PR) Notice 96-1: Notice to Manufacturers, Formulators, Producers and Registrants of Pesticides Products, *Tolerance Enforcement Methods - Independent Laboratory Validation By Petitioner* (2). The study was performed at the EAG Laboratories analytical chemistry facility in Easton, Maryland. The experimental portion of the study was conducted between June 06 and June 10, 2017. Raw data and a copy of the final report are archived at the EAG Laboratories-Easton site under project number 334C-135.

PURPOSE

This study was conducted to fulfill EPA requirements set forth in guideline OCSPP 850.6100 and PR Notice 96-1. This study provides validation data demonstrating that an independent researcher could reproduce the results of the analytical method with minimal contact with the method developers.

EXPERIMENTAL DESIGN

Two soil types (loamy sand and clay loam) were fortified with Triallate and TCPSA at two concentrations and analyzed according to the method supplied by the Sponsor. The LOQ for each analyte was set at 50.0 µg/kg. The higher concentration was ten-fold the LOQ, i.e., 500 µg/kg. Reagent and matrix blanks (controls) were analyzed concurrently to evaluate potential analytical interferences.

MATERIALS AND METHODS

Untreated Control Soils (Loamy Sand/Clay Loam) - Origin

Loamy sand (soil #2) and clay loam (soil #1) control matrices used for this study were both originally obtained from Agvise Laboratories located in Northwood, ND. The soils were stored under refrigerated conditions in the dark following receipt and when not in use. The soils were characterized prior to use by Agvise Laboratories and these results are presented in Appendices IV and V. The moisture content (percent soil) for use in calculating initial sample concentration on a dry weight basis as in provided methodology were calculated by EAG prior to use for each soil type.

Analytical Reference Substances

A reference substance Triallate was received from Smithers on July 25, 2016 and was assigned the EAG Laboratories-Easton Identification Number 13901. The material was a liquid and was identified on the label as Tri-allate; Lot# 5125900; Purity 99.5%; Expiration Date 06/17/2018. This reference substance was stored under ambient conditions. A certificate of analysis is presented in Appendix III.

A reference substance of TCPSA was received from Gowan on March 29, 2017 and was assigned the EAG Laboratories-Easton Identification Number 13701. The material was a solid and was identified on the label as TCPSA; Lot# SP15-106-1-1; Purity 99.8%; Expiration Date 06/16/2018. This reference substance was stored under ambient conditions. A certificate of analysis is presented in Appendix III.

The reference substances above were used to prepare separate primary analytical stocks and subsequently various combined secondary fortification/calibration stocks and standards.

Preparation of Primary Analytical Stocks and Secondary Combined Fortification Stocks and Calibration Standards

A primary stock solution of the Triallate reference standard was prepared by weighing a 50.3 mg aliquot into a beaker. The reference material was transferred to a 50-mL volumetric flask, dissolved and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 1000 µg/mL (corrected for purity). This Triallate primary stock was diluted in acetonitrile to prepare and intermediate stock at 100 µg/mL.

A primary stock solution of TCPSA reference standard was prepared by weighing a 10.02 mg aliquot onto a piece of weigh paper. The reference material was transferred to a 100-mL volumetric flask, dissolved and adjusted to final volume using acetonitrile: HPLC grade water (1:1, v/v) to yield a final nominal stock concentration of 100 µg/mL (corrected for purity).

Combined secondary fortification stocks of Triallate and TCPSA analytes were prepared at 10.0, 1.00, 0.0100, and 0.00100 µg/mL in acetonitrile solvent from the above 100 µg/mL stocks as shown below:

Stock Conc. (µg/mL)	Aliquot (mL)	Final Volume (mL)	Combined Standard Conc. (µg/mL)
100 (Triallate)	1.00	10.0	10.0
100 (TCPSA)	1.00	--	--
10.0 (combined)	1.00	10.0	1.00
1.00 (combined)	0.100	10.0	0.0100
0.0100 (combined)	1.00	10.0	0.00100

All solutions were prepared using volumetric flasks and gas-tight syringes and were stored under refrigerated conditions when not in use.

For the analysis of Triallate (both primary quantitation and secondary confirmation analyses – Xbridge C18 Column) combined working calibration standards ranging in concentration from 0.00500 to 0.500 µg/L were prepared in acetonitrile: HPLC grade water (1:1, v/v) from the combined secondary fortification stocks above as shown below:

Combined Secondary Fortification Stock Concentration (µg/mL)	Aliquot (mL)	Final Volume (mL)	Combined Calibration STD Conc. (µg/L)
0.0100	0.500	10.0	0.500
0.0100	0.250	10.0	0.250
0.0100	0.100	10.0	0.100
0.0100	0.0500	10.0	0.0500
0.0100	0.0250	10.0	0.0250
0.00100	0.100	10.0	0.0100
0.00100	0.0500	10.0	0.00500

For the analysis of TCPSA (both primary quantitation and secondary confirmation analyses – HILIC column) combined working calibration standards ranging in concentration from 0.00500 to 0.500 µg/L were prepared in 100% acetonitrile from the combined secondary fortification stocks above as shown below:

Combined Secondary Fortification Stock Concentration (µg/mL)	Aliquot (mL)	Final Volume (mL)	Combined Calibration STD Conc. (µg/L)
0.0100	0.500	10.0	0.500
0.0100	0.250	10.0	0.250
0.0100	0.100	10.0	0.100
0.0100	0.0500	10.0	0.0500
0.0100	0.0250	10.0	0.0250
0.00100	0.100	10.0	0.0100
0.00100	0.0500	10.0	0.00500

The combined calibration standard solutions stored under refrigerated conditions when not in use. All solutions were prepared using volumetric flasks and gas-tight syringes.

Analytical Method – Soil

The analytical method developed for soil matrices and provided for validation for this Independent Laboratory Validation (ILV) study utilized an initial shaker table extraction. Following the initial extraction, the method divided into two separate analyses for Triallate and TCPSA analytes, with analyte specific dilution solvents and chromatographic conditions. The assignments of each analyte to its associated dilution solvent, and analytical column are summarized below:

Analyte/Analysis:	Dilution Solvent	Column
Triallate(quantitation/confirmation)	Acetonitrile: HPLC grade water (1:1, v/v)	XBridge C18
TCPSA(quantitation/confirmation)	100% Acetonitrile	Atlantis HILIC

Final quantitation of samples was performed utilizing High Performance Liquid Chromatography with tandem mass spectrometric detection (HPLC/MS/MS).

Fortification of Recovery Samples

For each matrix validated, one reagent blank, two unfortified matrix blanks, five fortified control matrix samples at the LOQ, and five fortified control matrix samples at 10X the LOQ were prepared in each soil type as shown below:

Loamy sand/clay loam soils Fortification

Analyte (s)	Nominal Concentration (µg/kg)	Fortification Volume (mL)	Sample Weight (g)	Combined Stock Conc. (µg/mL)
Triallate/TCPSA	50.0 (LOQ)	0.250	5.00	1.00
	500 (10X LOQ)	0.250	5.00	10.0

All fortified samples were prepared with combined fortification solutions that were prepared compensating for the purity of the reference materials. Therefore, all concentration levels, expressed in µg/L, are equivalent to the expression as µg/L active ingredient (µg/L a.i.).

Processing and Analysis of Triallate and TCPSA in Soil

For analysis, 5.00 gram aliquots of each soil type were weighed into twelve individually labeled 50-mL polypropylene centrifuge tubes, five of which were fortified at the LOQ (50.0 µg/kg) and five at 10x the LOQ (500 µg/kg) with combined secondary fortification stocks of the reference substances prepared as described above. A single reagent blank consisting of all reagents except matrix, and the two matrix blanks of unfortified control matrix and carried through the methodology for each matrix validation. All samples were subsequently analyzed by the provided methodology in Appendix II. Slight adjustments in the HPLC/MS/MS source optimization parameters were utilized and were considered to be equivalent values related to inherent differences in instrumental performance and not a limitation of the methodology. Since specific details of the method are presented in Appendix II, a more general description is provided here.

Twenty milliliter (20 mL) volumes of acetonitrile: HPLC grade water (1:1, v/v) extraction solvent were added to each soil sample in tubes above. The samples tubes were placed on a shaker table apparatus for 30 minutes at a setting of approximately 150 RPM. Following the shaking period the samples were centrifuged at 3000 RPM setting for 10 minutes and the supernatants transferred to 50-mL class A volumetric flasks. The extraction and centrifugation procedures were repeated. The supernatants were combined in their respective 50-mL volumetric flasks, and brought to 50.0 mL final volumes using the extraction solvent.

For Triallate analyses (both primary quantitation and secondary confirmation analysis using the XBridge C18 column), aliquots of the blanks and LOQ sample extracts were volumetrically diluted further 0.100 mL to 10.0 mL final volume using acetonitrile: HPLC grade water extraction/dilution solvent. For the 10X LOQ sample extracts, aliquots were volumetrically diluted 0.0250 mL to 25.0 mL final volumes using the same dilution solvent. The final dilutions were mix well.

For TCPSA analyses (both primary quantitation and secondary confirmation analysis using the HILIC column), aliquots of the blank, LOQ, and 10X LOQ sample extracts were diluted using the same dilution scheme as Triallate analyses, however using 100% acetonitrile as the dilution solvent. The final dilutions were mixed well.

Aliquots of each final sample dilution were transferred to low-binding micro-centrifuge tubes and centrifuged at approximately 13,000 RPM for approximately 5 minutes and aliquots of each of the final supernatants were transferred to auto-sampler vials and submitted for HPLC/MS/MS analysis. The remainders of each initial sample extracts were transferred to 20-mL glass vials and stored under refrigerated conditions.

Quantitation of Triallate and TCPSA by HPLC/MS/MS

An Agilent Technologies Model 1200 Infinity Series High Performance Liquid Chromatograph connected to an AB /MDS Sciex API 5000 Mass Spectrometric Detector (HPLC/MS/MS) was used to analyze samples. An acidified (0.1% formic acid) acetonitrile: water gradient elution profile was used for the analysis of Triallate and an isocratic elution profile was used for the analysis of TCPSA.

Quantitation analysis was performed using the responses of the primary ion transitions for each analyte and associated analytical column specific method. Confirmation analysis was performed using the response of a secondary confirmation ion for each analyte. The ion transitions monitored and specific analytical columns used for each are summarized below:

Analyte	Primary(Quantitation)/ Column	Secondary(Confirmation)/ Column
Triallate	304.1→85.8 amu XBridge BEH C18	304.1→142.8 amu/ XBridge BEH C18
TCPSA	224.8→79.8 amu/ Atlantis HILIC Silica	222.8→186.9 amu Atlantis HILIC Silica

Specific details of the HPLC/MS/MS instrumentation and operational parameters for each analyte are presented in Tables 1 and 2.

Example Calculations

For each analysis set, a regression equation was derived from the chromatographic peak area responses of the analytes determined in calibration standard solutions versus the respective nominal concentrations of the standards. Standard curves were generated by plotting this function with analyte concentration (µg/L) on the abscissa and the respective analyte peak area response on the ordinate. The applied regression was weighted 1/x with respect to concentration and expressed as a linear regression as follows:

$$y = mx + b$$

Where: Y = peak area
m = slope
b = Y-intercept
x = analyte concentration

Concentrations of analytes in the samples (primary quantitation and secondary confirmation analyses) were determined by substituting peak area responses of the samples into the re-arranged weighted (1/x) regression equation as follows:

$$\text{Analyte Concentration} = \frac{\text{Peak area - (Y-intercept)}}{\text{Slope}}$$

Using the data from the loamy sand method validation sample 334C-135-S2VMAS-5-1, 50.0 µg/kg shown below, the Triallate analytical result and percent recovery were calculated as follows using the software algorithms of Analyst version 1.6 of the AB /MDS Sciex API 5000 mass spectrometer system in full precision mode. Note: manual calculations shown here may differ slightly than reported.

Where:

Peak area = 10663
Y-intercept = 396.216
Slope = 175367

The concentration of Triallate at instrument was determined by substituting the resulting analyte peak area response into the above equation. Using the values above, the concentration in the final sample dilution was calculated as:

$$\text{Concentration at instrument (µg/L): } = \frac{10663 - (396.216)}{175367}$$

Concentration at instrument (µg/L): = 0.0585

The residue concentration (µg/kg) for Triallate in the fortified soil recovery sample was determined as the product of the at instrument solution concentration determined above and the overall dilution factor as follows:

$$\text{Concentration in µg/kg} = \text{Triallate Concentration at Instrument x } \frac{\text{(Final Volume)}}{\text{(Initial Volume)}} \text{ x DF}$$

Where: Initial Volume= 5.00 g
Final Volume = 50.0 mL
Secondary Dilution (DF) = 100

Using the nominal concentration (µg/kg) from above, the concentration of Triallate in water sample was calculated as follows:

Concentration in sample (µg/kg) = 0.0585 x 10.0 x 100

Concentration in sample (µg/kg) = 58.5

The percent recovery was determined by dividing the concentration of the analyte recovered in the fortified sample by the nominal concentration added as shown below:

$$\text{Recovery (\%)} = \frac{\mu g/kg \text{ Found}}{\mu g/kg \text{ Added}} \times 100$$

For the above 50.0- µg/kg fortified sample, the percent recovery of Triallate was calculated as:

$$\text{Recovery (\%)} = \frac{58.5 \ \mu g/kg \text{ Found}}{50.0 \ \mu g/kg \text{ Added}} \times 100$$

$$\text{Recovery (\%)} = 117\%$$

The same calculation procedure was applied for the Triallate secondary confirmation analyses and TCPSA primary quantitation and secondary confirmation analyses for both soil types in this study.

Statistical Treatment of Data

Mean recoveries for each analyte for each fortification level were calculated by dividing the sum of the percent recoveries by the total number of fortified samples. The standard deviation and relative standard deviation (coefficient of variation) for the recoveries for each analyte were also determined and reported for primary quantitation and secondary confirmation analyses.

Table 1. Triallate HPLC/MS/MS Instrumentation and Operational Parameters (Quantitation and Confirmation Analysis)

Instrumentation	Agilent Technologies Model 1200 Series High Performance Liquid Chromatograph with a AB /MDS Sciex API 5000 Mass Spectrometer (HPLC/MS/MS) and Turbo-V Ion Spray Source
Analytical Column	Waters XBridge BEH C18 2.5 µm (2.1 mm x 50 mm)
Guard Column	None
Injector/Needle Wash	Flush Port – Acetonitrile: HPLC Grade Water (1:9, v/v) -10 seconds
Mobile Phases	A1: 0.1% Formic Acid in HPLC-grade water B1: 0.1% Formic Acid in Acetonitrile Gradient Elution Program: Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C) 0.00 / 75.0 / 25.0 / 350 / 40.0 0.500 / 75.0 / 25.0 / 350 / 40.0 4.00 / 0.00 / 100 / 350 / 40.0 6.00 / 0.00 / 100 / 350 / 40.0 6.10 / 75.0 / 25.0 / 350 / 40.0 7.50 / 75.0 / 25.0 / 350 / 40.0
Diverter Valve (Valco)	Time (min) / Position 0.00 / A 2.50 / B
Injection Volume	100 µL
Period 1-Experiment 1	Scan Type/Polarity: MRM/Positive: GS1 = 30, GS2 = 30, CUR = 30.0, CAD = 4, IS = 5000, TEM = 500, DP = 50, EP = 10 Primary Quantitation: (304.1/85.8 amu), CE = 25, CXP = 36 Secondary Confirmation: (304.1/142.8 amu), CE = 39, CXP = 16 Dwell Time: 500 msec for each transition
Triallate Retention Time	Approximately 5.8 minutes

Table 2. TCPSA HPLC/MS/MS Instrumentation and Operational Parameters (Quantitation and Confirmation Analysis)

Instrumentation	Agilent Technologies Model 1200 Series High Performance Liquid Chromatograph with a AB /MDS Sciex API 5000 Mass Spectrometer (HPLC/MS/MS) and Turbo-V Ion Spray Source
Analytical Column	Waters Atlantis HILIC Silica 3.0 µm (3.0 mm x 100 mm)
Guard Column	None
Injector/Needle Wash	Flush Port - Acetonitrile: HPLC Grade Water (1:9, v/v) -10 seconds
Mobile Phases	A1: 0.1% Formic Acid in HPLC-grade water B1: 0.1% Formic Acid in Acetonitrile Isocratic Elution Program: Time (min) / %A1 / %B1 / Flow Rate (µL/min) / Temp (°C) 0.00 / 10.0 / 90.0 / 200 / 40.0 5.00 / 10.0 / 90.0 / 200 / 40.0
Injection Volume	50.0 µL
Period 1-Experiment 1	Scan Type/Polarity: MRM/Negative: GS1 = 30, GS2 = 30, CUR = 30.0, CAD = 4, IS = -4500, TEM = 500, DP = -50, EP = -10 Primary Confirmation: (224.8/79.8 amu), CE = -50, CXP = -15 Secondary Confirmation: (222.8/186.9 amu), CE = -19.4, CXP = -15 Dwell Time: 800 msec for each transition
TCPSA Retention Time	Approximately 4.0 minutes