

Analytical method for triallate and its metabolite TCPSA in soil

Reports: ECM: EPA MRID No.: 50432502. Wu, X. 2016. Validation of the Analytical Method for the Determination of Triallate and TCPSA in Soil Matrices by LC-MS/MS. Report prepared by Smithers Viscient, Wareham Massachusetts, and sponsored and submitted by Gowan Company, Yuma, Arizona; 113 pages. Smithers Viscient Study No. 12791.6266. Final report issued January 16, 2017.

ILV: EPA MRID No. 50352203. MacGregor, J.A., E.S. Bodle, and R.L. VanHoven. 2017. INDEPENDENT LABORATORY VALIDATION OF A METHOD FOR THE DETERMINATION OF TRIALLATE AND TCPSA IN SOIL MATRICES BY LC/MS/MS. Report prepared by Wildlife International (now doing business as EAG), Easton, Maryland, sponsored and submitted by Gowan Company, Yuma, Arizona; 99 pages. Project No. 334C-135. Final report issued July 21, 2017.

Document No.: MRIDs 50432502 & 50352203

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR 160) and OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 50432502). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). Authenticity statement was included with the QA statement (p. 4).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50352203). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was not included.

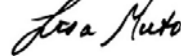
Classification: This analytical method is classified as **Unacceptable**. The LOD was not reported in the ILV and linearity data was not provided to assess the accuracy of the recovery data. ILV performance data was not satisfactory for triallate in one of the two soils. ILV linearity was not satisfactory for triallate and TCPSA. The LOQ is greater than the lowest toxicological level of concern.

PC Code: 078802

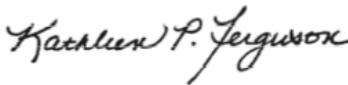
**Final EPA
Reviewer:** A'ja Duncan,
Chemist

Signature:
Date: 9/10/18

**CDM/CSS-
Dynamac JV
Reviewers:** Lisa Muto,
Environmental Scientist

Signature: 
Date: 6/21/18

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 
Date: 6/21/18

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Smithers Viscient Study No. 12791.6266, is designed for the quantitative determination of triallate and its metabolite TCPA in soil at the LOQ of 50.0 µg/kg using LC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in soil (10 µg/kg; USEPA, 2014) for the two analytes. The ECM and ILV validated the method using different characterized clay loam and loamy sand soil matrices; however, the USDA soil texture calculator used by the reviewer did not validate the reported soil texture classifications. ILV staff validated the ECM method for the quantitation and confirmation analyses of triallate and TCPA in two soil matrices in the first trial with insignificant modifications to the analytical method and the adjustment of the centrifuge speed for the clay loam soil. All ECM and ILV data regarding repeatability, accuracy, precision, and specificity were satisfactory for both analytes, except for the repeatability of triallate in one of the ILV test soils. ILV linearity was unsatisfactory for triallate and TCPA. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Triallate	50432502	50352203		Soil ^{1,2}	16/01/2017	Gowan Company	LC/MS/MS	50.0 µg/kg
TCPA								

1 In the ECM, clay loam soil (SMV Lot No. 091215AS-DU-L 0-6"; 44% sand 26% silt 30% clay, pH 5.4 in 1:1 soil:water ratio, 5.2% organic matter) was obtained from Eastern, North Dakota, and loamy sand soil (SMV Lot No. 012616A; 78% sand 18% silt 4% clay, pH 6.8 in 1:1 soil:water ratio, 4.9% organic matter, obtained from Rochester, Massachusetts, were used (USDA soil texture classification not specified). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, clay loam soil (MCL 0-6"; 26% sand 42% silt 32% clay, pH 5.9 in 1:1 soil:water ratio, 4.7% organic matter Walkley-Black) and loamy sand soil (PD SOIL-PF 0-6"; 80% sand 14% silt 6% clay, pH 6.2 in 1:1 soil:water ratio, 0.88% organic matter) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Samples (5.00 g dry weight) were transferred to 50-mL Nalgene centrifuge tubes and fortified, as necessary, with mixed fortification solutions of triallate and TCPSA in acetonitrile (pp. 16-17, 20-23 of MRID 50432502). The sample was extracted twice with 20.0 mL with acetonitrile:purified reagent water (50:50, v:v). After centrifugation (3000 rpm for 10 minutes), the volume of the combined extracts was adjusted to 50.0 mL with acetonitrile:purified reagent water (50:50, v:v) and mixed well. The samples for triallate analysis were further diluted into the calibration standard range with acetonitrile:purified reagent water (50:50, v:v); samples for TCPSA analysis were further diluted into the calibration standard range with acetonitrile. All samples were then centrifuged at 13,000 rpm for 5 minutes; the supernatant was transferred to HPLC vials and analyzed by LC/MS/MS.

Samples were analyzed for both analytes using an Agilent 1200 HPLC system coupled to an AB Sciex API 5000 mass spectrometer with an ESI Turbo V source (pp. 24-27 of MRID 50432502). The LC/MS conditions for triallate consisted of a XBridge C18 column (2.1 x 50 mm, 2.5- μ m; column temperature 40°C), a mobile phase of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid [percent A:B (v:v) at 0.00-0.50 min. 75:25, 4.00-6.00 min. 0:100, 6.10-7.50 min. 75:25] and MS/MS detection in positive ion mode (ionization temperature 500°C). Injection volume was 100 μ L. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 304.1 \rightarrow 86.1 and m/z 304.1 \rightarrow 142.8 for triallate. Retention times were 3.85-3.86 minutes for triallate in both matrices. The LC/MS conditions for primary analysis of TCPSA consisted of an Atlantis® HILIC silica column (3.0 x 100 mm, 3- μ m; column temperature 40°C), a isocratic mobile phase at 10:90, v:v, of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. MS/MS detection was conducted in negative ion mode (ionization temperature 500°C). Injection volume was 50 μ L. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 224.8 \rightarrow 79.8 and m/z 224.8 \rightarrow 186.9 for TCPSA. Retention times were 3.94-3.95 minutes for TCPSA in both matrices.

In the ILV, the ECM was performed as written, except for the adjustment of the centrifuge speed from 3000 to 4500 rpm for the clay loam soil (due to the color of the extract) and a few minor modifications of analytical instruments and parameters (pp. 14-16; Tables 1-2, pp. 22-23; Appendix VI, p. 98 of MRID 50352203). An Agilent 1200 HPLC System coupled to an AB Sciex API 5000 Turbo-V Ion Spray mass spectrometer was used. The LC/MS conditions were generally the same. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 304.1 \rightarrow 85.8 and m/z 304.1 \rightarrow 142.8 for triallate, and m/z 224.8 \rightarrow 79.8 and m/z 224.8 \rightarrow 186.9 for TCPSA. Retention times were *ca.* 5.8 minutes for triallate and *ca.* 4.0 minutes for TCPSA. No significant modifications were made by the ILV.

The Limit of Quantification (LOQ) was 50.0 μ g/kg for triallate and TCPSA in soil in the ECM and ILV (pp. 12, 27-28, 33-36 of MRID 50432502; p. 11 of MRID 50352203). The Limit of Detection (LOD) was 0.275-2.95 μ g/kg for triallate and 1.37-1.88 μ g/kg for TCPSA in the soil matrices in the ECM; the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 50432502): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of triallate and its metabolite TCPSA in two soil matrices at fortification levels of 50.0 $\mu\text{g/kg}$ (LOQ) and 500 $\mu\text{g/kg}$ (10 \times LOQ; Tables 1-8, pp. 39-46). Performance data (recovery results) from primary and confirmatory analyses were comparable. Clay loam soil (SMV Lot No. 091215AS-DU-L 0-6"; 44% sand 26% silt 30% clay, pH 5.4 in 1:1 soil:water ratio, 5.2% organic matter Walkley-Black) was obtained from Eastern, North Dakota, and loamy sand soil (SMV Lot No. 012616A; 78% sand 18% silt 4% clay, pH 6.8 in 1:1 soil:water ratio, 4.9% organic matter Walkley-Black), obtained from Rochester, Massachusetts, were used (USDA soil texture classification not specified; p. 15). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50352203): Mean recoveries and RSDs were within guideline requirements for analysis of triallate and its metabolite TCPSA in two soil matrices at fortification levels of 50.0 $\mu\text{g/kg}$ (LOQ) and 500 $\mu\text{g/kg}$ (10 \times LOQ), except for the quantitation ion analysis of triallate in loamy sand soil at the LOQ (mean 127%; Tables 3-10, pp. 24-31). Performance data (recovery results) from primary and confirmatory analyses were comparable. Clay loam soil (MCL 0-6"; 26% sand 42% silt 32% clay, pH 5.9 in 1:1 soil:water ratio, 4.7% organic matter Walkley-Black) and loamy sand soil (PD SOIL-PF 0-6"; 80% sand 14% silt 6% clay, pH 6.2 in 1:1 soil:water ratio, 0.88% organic matter Walkley-Black) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 12; Appendices IV-V, pp. 90-92). The ECM method for the quantitation and confirmation analyses of triallate and TCPSA in two soil matrices was validated in the first trial with insignificant modifications to the analytical instruments and parameters and the adjustment of the centrifuge speed for the clay loam soil (pp. 11, 15-16; Appendix VI, pp. 97-98).

Table 2. Initial Validation Method Recoveries for Triallate and TCPSA in Soil^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) ²
Clay Loam Soil						
Quantitation ion						
Triallate	50.0 (LOQ)	5	94.0-101	97.5	2.61	2.67
	500	5	81.3-112	99.2	15.3	15.5
TCPSA	50.0 (LOQ)	5	81.1-87.3	83.6	2.31	2.77
	500	5	96.7-110	102	5.20	5.11
Confirmation ion						
Triallate	50.0 (LOQ)	5	97.3-105	102	3.16	3.10
	500	5	81.8-115	101	16.6	16.4
TCPSA	50.0 (LOQ)	5	71.7-92.4	83.3	7.80	9.37
	500	5	94.6-106	101	4.26	4.24
Loamy Sand Soil						
Quantitation ion						
Triallate	50.0 (LOQ)	5	98.2-103	100	1.75	1.74
	500	5	93.7-106	101	4.97	4.90
TCPSA	50.0 (LOQ)	5	72.3-83.4	75.8	4.60	6.07
	500	5	101-114	108	4.97	4.59
Confirmation ion						
Triallate	50.0 (LOQ)	5	95.1-100	97.6	1.93	1.98
	500	5	96.6-104	102	2.87	2.83
TCPSA	50.0 (LOQ)	5	74.7-86.7	79.2	4.55	5.75
	500	5	111-116	114	2.30	2.03

Data (uncorrected recovery results, p. 28) were obtained from Tables 1-8, pp. 39-46 of MRID 50432502.

1 The clay loam soil (SMV Lot No. 091215AS-DU-L 0-6"; 44% sand 26% silt 30% clay, pH 5.4 in 1:1 soil:water ratio, 5.2% organic matter Walkley-Black) was obtained from Eastern, North Dakota, and loamy sand soil (SMV Lot No. 012616A; 78% sand 18% silt 4% clay, pH 6.8 in 1:1 soil:water ratio, 4.9% organic matter Walkley-Black), obtained from Rochester, Massachusetts, were used (USDA soil texture classification not specified; p. 15). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 304.1→86.1 and m/z 304.1→142.8 for triallate, and m/z 224.8→79.8 and m/z 224.8→186.9 for TCPSA.

Table 3. Independent Validation Method Recoveries for Triallate and TCPSA in Soil^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) ²
Loamy Sand Soil						
Quantitation ion						
Triallate	50.0 (LOQ)	5	117-143	127	9.88	7.80
	500	5	110-119	113	3.54	3.13
TCPSA	50.0 (LOQ)	5	91.5-106	100	6.90	6.89
	500	5	74.6-98.6	85.7	10.1	11.8
Confirmation ion or analysis						
Triallate	50.0 (LOQ)	5	109-133	120	8.64	7.20
	500	5	100-118	108	6.72	6.22
TCPSA	50.0 (LOQ)	5	88.7-94.6	92.2	2.27	2.46
	500	5	77.8-92.5	85.6	7.24	8.46
Clay Loam Soil						
Quantitation ion						
Triallate	50.0 (LOQ)	5	101-113	108	4.34	4.02
	500	5	84.8-102	92.8	7.05	7.60
TCPSA	50.0 (LOQ)	5	75.3-94.4	83.6	8.08	9.67
	500	5	72.5-89.0	82.9	6.85	8.25
Confirmation ion or analysis						
Triallate	50.0 (LOQ)	5	109-123	115	5.93	5.16
	500	5	85.9-109	97.8	8.27	8.46
TCPSA	50.0 (LOQ)	5	75.1-84.7	77.9	4.02	5.16
	500	5	79.7-90.0	84.5	3.92	4.64

Data (uncorrected recovery results, pp. 16-18) were obtained from Tables 3-10, pp. 24-31 of MRID 50352203.

1 The clay loam soil (MCL 0-6"; 26% sand 42% silt 32% clay, pH 5.9 in 1:1 soil:water ratio, 4.7% organic matter Walkley-Black) and loamy sand soil (PD SOIL-PF 0-6"; 80% sand 14% silt 6% clay, pH 6.2 in 1:1 soil:water ratio, 0.88% organic matter Walkley-Black) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 12; Appendices IV-V, pp. 90-92).

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 304.1→85.8 and m/z 304.1→142.8 for triallate, and m/z 224.8→79.8 and m/z 224.8→186.9 for TCPSA.

III. Method Characteristics

The LOQ was 50.0 µg/kg for triallate and TCPSA in soil in the ECM and ILV (pp. 12, 27-28, 33-36 of MRID 50432502; p. 11 of MRID 50352203). In the ECM, the LOQ was defined as the lowest fortification level, and blank values should not be >30% of the LOQ; no calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM. In the ILV, the LOQ was reported from the ECM without justification. The LOD was 0.275-2.95 µg/kg for triallate and 1.37-1.88 µg/kg for TCPSA in the soil matrices in the ECM; the LOD was not reported in the ILV.; the LOD was not reported in the ILV. The LOD was calculated in the ECM using the following equation:

$$\text{LOD} = (3 \times (\text{SN}_{\text{ctl}}) / (\text{Resp}_{\text{LS}}) \times \text{Conc}_{\text{LS}}$$

Where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), Resp_{LS} is the mean response in height of the two low calibration standards, and Conc_{LS} is the concentration of the low calibration standard.

Table 4. Method Characteristics

Analyte ¹			Triallate	TCPSA
Limit of Quantitation (LOQ)	ECM		50.0 µg/kg	
	ILV			
Limit of Detection (LOD)	ECM	Clay loam	2.09 µg/kg (Q) 1.46 µg/kg (C)	1.37 µg/kg (Q) 1.40 µg/kg (C)
		Loamy sand	2.95 µg/kg (Q) 0.275 µg/kg (C)	1.43 µg/kg (Q) 1.88 µg/kg (C)
	ILV		Not reported	
	Linearity (calibration curve r ² and concentration range)	ECM	Clay loam	r ² = 0.9974 (Q) r ² = 0.9970 (C)
Loamy sand			r ² = 0.9994 (Q) r ² = 0.9998 (C)	r ² = 0.9960 (C- LOQ) r ² = 0.9970 (C- 10×LOQ)
ILV ¹		Clay loam	Not reported	
		Loamy sand	r ² = 0.9937 (Q)	r ² = 0.9848 (Q)
Range			0.005-0.500 µg/L	
Repeatable	ECM ²		Yes at LOQ and 10×LOQ (two characterized soil matrices).	
	ILV ^{3,4}		Yes at 10×LOQ (two characterized soil matrices). Yes at LOQ in clay loam soil, but No at LOQ in loamy sand soil (Q mean 127%).	Yes at LOQ and 10×LOQ (two characterized soil matrices).
Reproducible			Yes at LOQ and 10×LOQ in one soil matrix.	Yes at LOQ and 10×LOQ in two soil matrices.
Specific	ECM		Yes, matrix interferences were < 5% of the LOQ (based on peak area).	Yes, no matrix interferences were observed; however, a minor contaminant peak interfered with the LOQ peak integration and attenuation.
	ILV		Only quantitation analysis chromatograms were provided.	

Analyte ¹		Triallate	TCPSA
		Yes, matrix interferences were <i>ca.</i> 8% of the LOQ (based on peak area).	Yes, matrix interferences were <i>ca.</i> 20% of the LOQ (based on peak area).

Data were obtained from pp. 12, 27-28, 33-36 (LOQ/LOD); p. 30 (correlation coefficients); Tables 1-8, pp. 39-46 (recovery data); Figures 1-47, pp. 55-101 (chromatograms) of MRID 50432502; p. 11 (LOQ); Tables 3-10, pp. 24-31 (recovery data); Figures 1-2, pp. 32-33 (calibration curves); Figures 3-8, pp. 34-39 (chromatograms) of MRID 50352203; and DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Figures 1-2, pp. 32-33 of MRID 50352203; DER Attachment 2). Only one calibration curve per analyte was presented: the quantitation ion from the loamy sand soil analysis (soil 2, p. 12; Figures 1-2, pp. 32-33). Solvent standards were used (pp. 13-14). The reviewer limited the calculated r^2 to 4 significant figures although 7 significant figures were reported in the ECM for r.

2 In the ECM, clay loam soil (SMV Lot No. 091215AS-DU-L 0-6"; 44% sand 26% silt 30% clay, pH 5.4 in 1:1 soil:water ratio, 5.2% organic matter Walkley-Black) was obtained from Eastern, North Dakota, and loamy sand soil (SMV Lot No. 012616A; 78% sand 18% silt 4% clay, pH 6.8 in 1:1 soil:water ratio, 4.9% organic matter Walkley-Black), obtained from Rochester, Massachusetts, were used (USDA soil texture classification not specified; p. 15 of MRID 50432502). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

3 In the ILV, clay loam soil (MCL 0-6"; 26% sand 42% silt 32% clay, pH 5.9 in 1:1 soil:water ratio, 4.7% organic matter Walkley-Black) and loamy sand soil (PD SOIL-PF 0-6"; 80% sand 14% silt 6% clay, pH 6.2 in 1:1 soil:water ratio, 0.88% organic matter Walkley-Black) were obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 12; Appendices IV-V, pp. 90-92 of MRID 50352203).

4 The ILV validated the ECM method for the quantitation and confirmation analyses of triallate and TCPSA in two soil matrices in the first trial with insignificant modifications to the analytical instruments and parameters and the adjustment of the centrifuge speed for the clay loam soil (pp. 11, 15-16; Appendix VI, pp. 97-98 of MRID 50352203).

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

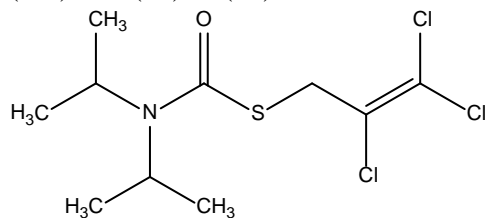
1. In the ILV, the LOD was not reported. The LOQ was reported from the ECM without justification. Also, the calibration curves and correlation coefficients were not provided for the clay loam soil sample sets. Even though solvent standards were used, all linearity data should be provided to assess the accuracy of the recovery data.
2. ILV performance data was not satisfactory for the quantitation ion analysis of triallate in loamy sand soil at the LOQ (mean 127%; Tables 3-10, pp. 24-31 of MRID 50352203). OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is $\leq 20\%$. In the communications, Jon MacGregor of the ILV proposed that the high recoveries were due to slight HPLC/MS/MS matrix associated enhancement of the loamy sand soil and the confirmation ion analysis validated the results of the quantitation ion analysis (Appendix VI, pp. 97-98). The ILV study author also noted that the results were not outside of the norm for analyses at the LOQ with varied soils.
3. In the ILV, the linearity was not satisfactory for triallate, $r^2 = 0.9937$, and TCPSA, $r^2 = 0.9848$ (Figures 1-2, pp. 32-33 of MRID 50352203; DER Attachment 2). Only one calibration curve per analyte was presented: the quantitation ion from the loamy sand soil analysis (soil 2, p. 12; Figures 1-2, pp. 32-33). Linearity is satisfactory when $r^2 \geq 0.995$.

4. Performance data was not satisfactory for the ILV confirmation ion analysis of TCPSA in ground soil (mean 155%, RSD 66%; Tables 4-11, pp. 26-33 of MRID 50352203). OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is $\leq 20\%$. The reviewer did not consider this guideline deviation to be substantial since a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.
5. The reported LOQ is greater than the lowest toxicological level of concern in soil (10 $\mu\text{g/kg}$; USEPA, 2014).
6. In the ECM, the soil textures were reported as clay loam soil (44% sand 26% silt 30% clay) and loamy sand soil (78% sand 18% silt 4% clay; p. 15 of MRID 50432502). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota, but USDA soil texture classification not specified. The USDA soil texture calculator used by the reviewer classified these soils as loam and sandy loam, respectively. In the ILV, the soil textures were reported as clay loam soil (26% sand 42% silt 32% clay) and loamy sand soil (80% sand 14% silt 6% clay; USDA soil texture classification by Agvise Laboratories, Northwood, North Dakota (p. 12; Appendices IV-V, pp. 90-92 of MRID 50352203). The USDA soil texture calculator used by the reviewer classified these soils as clay and sandy loam, respectively. The reviewer investigated the soil texture of the test soils since the clay loam of the ECM had a very different particle distribution than the clay loam of the ILV.
7. The reported limit of quantification (LOQ) was determined as the lowest level of method validation (LLMV). This means that concentrations can be reliably quantified at the LOQ (i.e., LLMV), but whether lower concentrations may also be reliably quantified is uncertain (pp. 12, 27-28, 33-36 of MRID 50432502; p. 11 of MRID 50352203). In the ECM, the LOQ was defined as the lowest fortification level, and blank values should not be $>30\%$ of the LOQ; no calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM. In the ILV, the LOQ was reported from the ECM without justification. The LOD was calculated in the ECM using the following equation: $\text{LOD} = (3 \times (\text{SN}_{\text{ctl}}) / (\text{Resp}_{\text{LS}}) \times \text{Conc}_{\text{LS}}$, where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), Resp_{LS} is the mean response in height of the two low calibration standards, and Conc_{LS} is the concentration of the low calibration standard. Further work could have been done to explore the actual LOQ and LOD.
8. The reviewer notes that the HPLC column used for TCPSA analysis, Atlantis® HILIC silica column, caused challenges for the same ILV laboratory and study author when validating the water method: MRIDs 50352202 & 50423501 (see Appendix VI, pp. 109-112 of MRID 50352202). In this soil ILV report, MRID 50352203, no modifications of the analytical parameters were made, but in the water ILV report, MRID 50352202, several modifications of the analytical parameters were made, including modifying the HPLC mobile phase to be more organic (Table 3, p. 25 of MRID 50352202; Table 2, p. 23 of MIRD 50352203).
9. Matrix effects were studied in the ECM (p. 32; Tables 9-16, pp. 47-54 of MRID 50432502). Although some minor matrix effects were seen for some transitions, all values were $< \pm 20\%$, so solvent standards were used.

10. It was reported for the ILV that one sample set of 13 samples required *ca.* 2 working days (*ca.* 17 hours) including LC/MS/MS analysis time (Appendix VI, p. 97 of MRID 50352203).
11. The communications between Jon MacGregor of the ILV and the Gowan Study Monitors (Premjit Halamkar, and Adam Pilkington) were reported (Appendix VI, pp. 97-98 of MRID 50352203). The communications involved the reporting of the successful trial and the adjustment of the centrifuge speed for the clay loam soil.

V. References

- USEPA. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- USEPA. 2014. Registration Review Problem Formulation for Triallate. DP barcode 437990. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Environmental Fate and Effects Division. Memorandum to the Pesticide Re-Evaluation Division. Aug. 21, 2014.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

DER Attachment 1: Chemical Names and Structures**Triallate****IUPAC Name:** S-2,3,3-Trichloroallyl diisopropyl(thiocarbamate)**CAS Name:** S-(2,3,3-trichloro-2-propen-1-yl) N,N-bis(1-methylethyl)carbamothioate**CAS Number:** 2303-17-5**SMILES String:** CC(C)N(C(C)C)C(=O)SCC(Cl)=C(Cl)Cl**TCPSA****IUPAC Name:** Sodium 2,3,3-trichloro-2-propene-1-sulfonate**CAS Name:** Not reported**CAS Number:** Not reported**SMILES String:** Not found