

**Analytical method for triadimefon in soil**

**Reports:** ECM: EPA MRID No.: 51029802. Smith, R.J. 2019. Validation of an Environmental Chemistry Method for the Determination of Triadimefon in Soil by LC-MS/MS. Report prepared by Smithers (formerly Smithers Viscient), Wareham, Massachusetts, and sponsored and submitted by ChemStarr, LLC, Irvine, California; 68 pages. Smithers Viscient Study No.: 14181.6107. Final report issued October 7, 2019.

ILV: EPA MRID No.: 51198006. Cashmore, A., and O. Idialu. 2020. Independent Laboratory Validation of Analytical Method 14181.6107 for the Determination of Triadimefon in Soil. Report prepared by Smithers ERS Limited, North Yorkshire, United Kingdom, monitored by Pyxis Regulatory Consulting, Inc., Gig Harbor, Washington, and sponsored and submitted by ChemStarr, LLC, Irvine, California; 68 pages. Study No.: 3202453. Final report issued July 9, 2020.

**Document No.:** MRIDs 51029802 & 51198006

**Guideline:** 850.6100


**Statements:** ECM: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160), as accepted by OECD GLP (1998; p. 3 of MRID 51029802). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement.

ILV: The study was conducted in compliance with United Kingdom (1999) GLP standards, as amended by GLP (2004) and OECD GLP (1998), as well as the United Kingdom Department of Health (p. 3; Appendix 6, p. 68 of MRID 51198006). The study was suitable for submission to US FDA, USEPA, and Japanese regulatory authorities. Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5). An Authenticity statement was also included with the GLP and Quality Assurance statements (pp. 3-4).


**Classification:** This analytical method is classified as **acceptable**. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. The ECM adjustment for test material purity caused the ECM LOQ and 10×LOQ fortifications to differ slightly from the ILV LOQ and 10×LOQ fortifications. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method.

**PC Code:** 109901


**EFED Primary Reviewer:** Megan Guevara, Physical Scientist

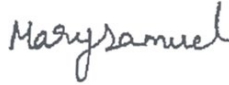
Signature:   
Date: 6/29/2021

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Date: 1/27/2021

**CDM/CSS-** Lisa Muto, M.S.,

Signature: 

<b>Dynamac JV</b>	Environmental Scientist	Date:	09/15/2020
<b>Reviewers:</b>	Mary Samuel, M.S., Environmental Scientist	Signature:	
		Date:	09/15/2020

*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. 14181.6107, is designed for the quantitative determination of triadimefon in soil at the stated LOQ of 0.0530 mg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for triadimefon. The ECM Protocol originally stated that the LOQ was 0.05 mg/kg, and the ILV was based on the original ECM Protocol. In a Protocol Amendment, the ECM test material purity was corrected which caused the adjusted ECM LOQ. Since the ILV LOQ was slightly less than the ECM LOQ, the ECM LOQ and 10×LOQ was considered to be supported by the ILV performance data at the ILV LOQ and 10×LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for triadimefon in the tested soil matrices (0.0530 mg/kg).

The ECM validated the method using characterized sandy loam and loamy sand soil matrices; the ILV validated the method using characterized sandy loam and silt loam soil matrices. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method and if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies since no triadimefon terrestrial field dissipation studies were submitted. The ILV validated the method for triadimefon in the first trial for surface soil with insignificant modifications to the analytical parameters. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for triadimefon in test soil matrices.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Triadimefon	51029802 <sup>1</sup>	51198006 <sup>2</sup>		Soil	07/10/2019	ChemStarr, LLC	LC/MS/MS	0.0530 mg/kg (ECM)  0.05 mg/kg (ILV) <sup>3</sup>

1 In the ECM, sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13 of MRID 51029802). The soils were characterized by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, sandy loam soil (RefeSol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl<sub>2</sub>; 0.9% organic carbon) from Schmallenberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl<sub>2</sub>; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 53-54 of MRID 51198006). The soils were characterized by Fraunhofer IME and Smithers Viscient (ERS) Ltd., United Kingdom.

3 The reported ILV LOQ differed slightly from the reported ECM LOQ (p. 10 of MRID 51029802; p. 22 of MRID 51198006). The difference was due to an ECM adjustment for test material purity (Appendix 1, p. 62 of MRID 51029802; see Reviewer's Comment #1).

## I. Principle of the Method

Soil samples (5 g dry wt.) were fortified (0.25 mL of 1.00 or 10.0 mg/L fortification solution) and extracted twice with 20 mL with acetonitrile via sonication for 10 minutes, shaking on a shaker table for 30 minutes (at 250 rpm), and centrifugation at 3000 rpm for 10 minutes (pp. 17-18 of MRID 51029802). The volume of the combined supernatants was adjusted to 50 mL with acetonitrile. The LOQ samples (0.05 mL aliquot) were diluted 2000xs with acetonitrile:purified reagent soil (20:80, v:v). The 10×LOQ samples (0.02 mL aliquot) were diluted 10,000xs with acetonitrile:purified reagent soil (20:80, v:v). An aliquot was taken for LC/MS/MS analysis.

Samples were analyzed for triadimefon using a Shimadzu LC-20AD HPLC coupled with an AB MDS Sciex 5000 MS with an ESI Turbo V ion source operated in the positive ion mode with multiple reaction monitoring (MRM; pp. 12, 18-19 of MRID 51029802). The following LC conditions were used: Waters XBridge BEH C18 column (2.1 mm x 50 mm, 2.5 μm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.50 min. 80.0:20.0, 4.00-5.00 min. 0.00:100.0, 5.10-6.00 min. 80.0:20.0] and injection volume of 100.0 μL. MS source temperature was 650°C. Two ion pair transitions were monitored for triadimefon (quantitation and confirmation, respectively):  $m/z$  294.3→197.1 and  $m/z$  294.3→69.1. Reported retention time was *ca.* 3.6 minutes for triadimefon.

The ILV performed the ECM methods as written, except for insignificant modifications to the analytical parameters (pp. 13-18; Appendix 3, pp. 59-61 of MRID 51198006). The LOQ and 10×LOQ fortification concentrations differed slightly from those of the ECM, due to an ECM adjustment for test material purity (Appendix 1, p. 62 of MRID 51029802; see Reviewer's Comment #1). Samples were analyzed for triadimefon using Nexera series HPLC coupled with an AB Sciex API 5000 Triple Quadrupole LC/MS/MS. The LC/MS/MS parameters were similar to those of the ECM. The following LC conditions were used: Waters XBridge BEH C18 column (2.1 mm x 50 mm, 2.5 μm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0-0.5 min. 80:20, 4.0-5.0 min. 0:100, 5.1-6 min. 80:20] and injection volume of 50 μL. MS source temperature was 500°C. Two ion pair transitions were monitored for triadimefon (quantitation and confirmation, respectively):  $m/z$  294.1→197.6 and  $m/z$  294.1→69.1. These ion transitions were similar to those of the ECM. Reported retention time was *ca.* 3.0 minutes for triadimefon. The ILV noted that the LC column could not be modified. The ILV modifications did not warrant an updated ECM.

The Limit of Quantification (LOQ) for triadimefon in soil was 0.0530 mg/kg in the ECM and 0.05 mg/kg in the ILV (pp. 10, 20-25 of MRID 51029802; pp. 10, 19-24 of MRID 51198006). In the ECM, the Limit of Detection (LOD) for triadimefon was calculated as 0.004 mg/kg for sandy loam soil and 0.003 mg/kg for loamy sand soil. In the ILV, the LOD for triadimefon was calculated as 0.00314-0.00555 mg/kg for sandy loam soil and 0.00108-0.00210 mg/kg for silt loam soil. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

## II. Recovery Findings

ECM (MRID 51029802): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis of triadimefon at fortification levels of 0.0530 mg/kg (LOQ) and 0.530 mg/kg (10 $\times$ LOQ) in two soil matrices (Tables 1-4, pp. 29-32). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13). The soils were characterized by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 51198006): Mean recoveries and RSDs were within guidelines for analysis of triadimefon at fortification levels of 0.05 mg/kg (LOQ) and 0.5 mg/kg (10 $\times$ LOQ) in one soil matrix (Tables 1-4, pp. 28-31). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The sandy loam soil (RefeSol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl<sub>2</sub>; 0.9% organic carbon) from Schmallenberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl<sub>2</sub>; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 53-54). The soils were characterized by Fraunhofer IME and Smithers Viscient (ERS) Ltd., United Kingdom. The method for triadimefon in soil was validated in the first trial with insignificant modifications to the analytical parameters (pp. 10, 21-25; Appendix 5, p. 67). The LOQ and 10 $\times$ LOQ fortification concentrations differed slightly from those of the ECM, due to an ECM adjustment for test material purity (Appendix 1, p. 62 of MRID 51029802; see Reviewer's Comment #1).

**Table 2. Initial Validation Method Recoveries for Triadimefon in Soil<sup>1,2</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>Sandy Loam Soil</b>						
Quantitation ion transition						
Triadimefon	0.0530 (LOQ)	5	89.5-96.1	92.5	2.49	2.69
	0.530	5	91.2-102	94.7	4.17	4.41
Confirmation ion transition						
Triadimefon	0.0530 (LOQ)	5	90.1-97.7	93.6	3.08	3.29
	0.530	5	91.3-98.7	93.6	2.94	3.15
<b>Loamy Sand Soil</b>						
Quantitation ion transition						
Triadimefon	0.0530 (LOQ)	5	83.5-92.2	88.0	3.20	3.64
	0.530	5	86.1-94.5	90.8	3.42	3.77
Confirmation ion transition						
Triadimefon	0.0530 (LOQ)	5	83.5-92.5	89.0	3.98	4.47
	0.530	5	87.6-94.2	91.0	2.69	2.95

Data (uncorrected recovery results; p. 21) were obtained from Tables 1-4, pp. 29-32 of MRID 51029802.

1 The sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The the soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for triadimefon (quantitation and confirmation, respectively):  $m/z$  294.3→197.1 and  $m/z$  294.3→69.1.

**Table 3. Independent Validation Method Recoveries for Triadimefon in Soil<sup>1,2,3</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Sandy Loam Soil</b>						
Quantitation ion transition						
Triadimefon	0.05 (LOQ)	5	96.8-103	98.4	2.40	2.44
	0.5	5	106-113	108	2.82	2.60
Confirmation ion transition						
Triadimefon	0.05 (LOQ)	5	94.0-104	99.1	3.93	3.97
	0.5	5	106-112	108	2.42	2.23
<b>Silt Loam Soil</b>						
Quantitation ion transition						
Triadimefon	0.05 (LOQ)	5	88.2-96.4	92.4	3.31	3.59
	0.5	5	94.0-98.8	96.0	1.90	1.98
Confirmation ion transition						
Triadimefon	0.05 (LOQ)	5	90.0-95.4	91.5	2.29	2.50
	0.5	5	94.0-100	96.7	2.74	2.84

Data (uncorrected recovery results; p. 19) were obtained from Tables 1-4, pp. 28-31 of MRID 51198006.

1 The sandy loam soil (RefeSol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl<sub>2</sub>; 0.9% organic carbon) from Schmallenberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl<sub>2</sub>; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 53-54). The soils were characterized by Fraunhofer IME and Smithers Viscient (ERS) ltd., United Kingdom. The the soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for triadimefon (quantitation and confirmation, respectively):  $m/z$  294.1→197.6 and  $m/z$  294.1→69.1. These ion transitions were similar to those of the ECM.

### III. Method Characteristics

The LOQ for triadimefon in soil was 0.0530 mg/kg in the ECM and 0.05 mg/kg in the ILV (pp. 10, 20-25 of MRID 51029802; pp. 10, 19-24 of MRID 51198006). In the ECM and ILV, the LOQ was defined as the lowest fortification level validated. Also, in the ECM, the LOQ was defined as the level which the blank values did not exceed 30% of the LOQ. In the ECM, the LOD was calculated as 0.004 mg/kg for sandy loam soil and 0.003 mg/kg for loamy sand soil from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation:

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

Where, LOD is the limit of detection of the analysis,  $N_{\text{ctl}}$  is the mean signal to noise in height of the control samples (or blanks),  $\text{Resp}_{\text{LS}}$  is the mean response in height of the two low calibration standards,  $\text{Conc}_{\text{LS}}$  is the concentration of the low calibration standard, and  $\text{DF}_{\text{CNTL}}$  is the dilution factor of the control samples (2000 mL/g).

The LOD for triadimefon in soil was estimated in the ILV as 0.00314-0.00555 mg/kg for sandy loam soil and 0.00108-0.00210 mg/kg for silt loam soil at 3 x baseline noise for the primary and confirmatory transitions.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.



**Table 4. Method Characteristics in Soil**

		<b>Triadimefon</b>
Limit of Quantitation (LOQ)*	ECM	0.0530 mg/kg
	ILV	0.05 mg/kg <sup>1</sup>
Limit of Detection (LOD)	ECM (calc)	0.004 mg/kg (SL) 0.003 mg/kg (LS)
	ILV (calc)	0.00314-0.00555 mg/kg (SL) 0.00108-0.00210 mg/kg (SIL)
Linearity (calibration curve r and concentration range)	ECM <sup>2</sup>	r = 1.000 (Q & C, SL & LS)
		0.00530-0.265 ng/mL
	ILV	r = 0.9998 (Q & C, SL) r = 0.9996 (Q, SIL) r = 0.9997 (C, SIL)
		0.005-0.3 ng/mL
Repeatable	ECM <sup>3</sup>	Yes at LOQ (0.0530 mg/kg) and 10×LOQ (0.5300 mg/kg) (two characterized soil matrices)
	ILV <sup>4,5</sup>	Yes at LOQ (0.05 mg/kg) and 10×LOQ (0.5 mg/kg) (two characterized soil matrices)
Reproducible <sup>6</sup>		Yes for 0.0530 mg/kg (LLMV)* and 0.5300 mg/kg in soil matrices
Specific	ECM	Yes, matrix interferences were <5% of the LOQ (based on peak area). Minor peak tailing was observed.
	ILV	Yes, no matrix interferences were observed.

Data were obtained from pp. 10, 20-25 (LOQ/LOD); Tables 1-4, pp. 29-32 (recovery results); p. 23; Figures 11-14, pp. 47-50 (calibration curves); Figures 1-10, pp. 37-46 (chromatograms) of MRID 51029802; pp. 10, 19-24 (LOQ/LOD); Tables 1-4, pp. 28-31 (recovery results); Figures 1-2, pp. 35-36; Figures 15-16, pp. 43-44 (calibration curves); Figures 3-28, pp. 37-50 (chromatograms) of MRID 51198006. Q = quantitation ion transition; C = confirmation ion transition; SL = Sand Loam Soil; LS = Loamy Sand Soil; SIL = Silt Loam Soil.

\* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 The reported ILV LOQ differed slightly from the reported ECM LOQ (p. 10 of MRID 51029802; pp. 10, 22 of MRID 51198006). The difference was due to an ECM adjustment for test material purity (Appendix 1, p. 62 of MRID 51029802; see Reviewer's Comment #1).

2 ECM correlation coefficients (r) were reviewer-calculated based on r<sup>2</sup> values reported in the study report (p. 23; Figures 11-14, pp. 47-50 of MRID 51029802; DER Attachment 2). Matrix effects were insignificant and solvent-based calibration standards were used in the ECM and ILV (pp. 24-25 of MRID 51029802; pp. 23-25 of MRID 51198006).

3 In the ECM, sandy loam soil (SMV Batch No. 24Oct18Soil-A; 64% sand, 17% silt, 19% clay; pH 6.6 in 1:1 soil:water ratio; 3.7% organic matter) collected from Grand Forks, North Dakota, and loamy sand soil (SMV Batch No. 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 13.5% organic matter) collected from Rochester, Massachusetts, were used in the study (USDA soil texture classification; p. 13 of MRID 51029802). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The the soil texture was verified by the reviewer using USDA-NRCS technical support tools.

4 In the ILV, sandy loam soil (RefeSol 01-A; ID: CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water; pH 5.3 in 0.01M CaCl<sub>2</sub>; 0.9% organic carbon) from Schmallenberg, Northrhine-Westphalia, Germany, and silt loam soil (Newhaven; ID: CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water; pH 5.4 in 0.01M CaCl<sub>2</sub>; 3.2% organic carbon) from Newhaven, Derbyshire, United Kingdom, were sourced by Smithers ERS and used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 53-54 of MRID 51198006). The soils were characterized by Fraunhofer IME and Smithers Viscient (ERS) Ltd., United Kingdom.

5 The ILV validated the method for triadimefon in the first trial with insignificant modifications to the analytical parameters (pp. 10, 21-25; Appendix 5, p. 67 of MRID 51198006). The ILV modifications did not warrant an updated ECM. The LOQ and 10×LOQ fortification concentrations differed slightly from those of the ECM.

6 Since the ILV LOQ was slightly less than the ECM LOQ, the ECM LOQ and 10×LOQ was considered to be supported by the ILV performance data at the ILV LOQ and 10×LOQ.

#### IV. Method Deficiencies and Reviewer's Comments

1. The ECM Protocol originally stated that the LOQ and 10×LOQ validation fortifications were to be 0.050 mg/kg and 0.50 mg/kg and the test material purity was 93.0% (Appendix 1, pp. 60-63 of MRID 51029802). In order to prepare the stock solutions for the LOQ and 10×LOQ validation fortifications, 0.0539 g was weighed out which, based on the known purity, would have been equivalent to 0.05 g a.i. yielding a 1000 mg a.i./L primary stock solution concentration (p. 14). However, since the test material purity was subsequently corrected to be 98.4%, the 0.0539 g of test material was corrected as equivalent to 0.0530 g a.i. yielding a 1060 mg a.i./L primary stock solution concentration (p. 14; Appendix 1, pp. 60-63). This ECM adjustment for test material purity caused the ECM LOQ and 10×LOQ fortifications to differ slightly from the ILV LOQ and 10×LOQ fortifications.
2. Since the ILV LOQ was slightly less than the ECM LOQ, the ECM LOQ and 10×LOQ was considered to be supported by the ILV performance data at the ILV LOQ and 10×LOQ.
3. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 10, 20-25 of MRID 51029802; pp. 10, 19-24 of MRID 51198006). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for triadimefon in the tested soil matrices (0.0530 mg/kg).
4. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. In the ILV, sandy loam soil (6% clay; 0.9% organic carbon) and silt loam soil (24% clay; 3.2% organic carbon) were used in the study (p. 12; Appendix 2, pp. 53-54 of MRID 51198006). In the ECM, sandy loam soil (19% clay; 3.7% organic matter) and loamy sand soil (1% clay; 13.5% organic matter) were used in the study (p. 13 of MRID 51029802). OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Additionally, it could not be determined if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies since no triadimefon terrestrial field dissipation studies were submitted. Relevant submitted triadimefon studies included the following: MRID 51029801 (photodegradation in soil; loam, 22% clay, 0.84% organic carbon; Appendix 3, p. 87 of MRID 51029801); MRID 51198002 (aerobic soil metabolism; loam, 22% clay, 0.84% organic carbon; loam, 18% clay, 2.2% organic carbon; sandy loam, 8% clay, 2.3% organic carbon; p. 21; Appendix 3, pp. 118-122 of MRID 51198002); MRID 51198003 (aerobic aquatic metabolism, silt loam, 13% clay, 3.3% organic carbon; sand, 3% clay, 0.57% organic carbon; p. 22; Appendix 3, pp. 126-128 of MRID 51198003); and MRID 51198004 (anaerobic aquatic metabolism, silt loam, 13% clay, 4.0% organic carbon; sand, 3% clay, 0.59% organic carbon; p. 22; Appendix 3, pp. 126-128 of MRID 51198004).
5. The communications between the ILV study authors (Angela Cashmore and Ofure Idialu, Smithers ERS Limited) and ILV Study Monitor (Janelle Kay, Pyxis Regulatory Consulting,

Inc.) were summarized (pp. 1, 10, 25; Appendix 5, p. 67 of MRID 51198006). Reported communications included: protocol issue and the results of the first attempt of the ILV.

6. The reviewer noted that the ECM and ILV laboratories were part of the same company, Smithers (formerly Smithers Viscient) and Smithers ERS Limited, respectively (pp. 1, 5-6 of MRID 51029802; pp. 1, 6 of MRID 51198006). The laboratory location, personnel and equipment differed between the two laboratories. The only exchange of information was the ECM Method/Protocol (by the ECM study authors) provided to the ILV via the Sponsor Representative (Appendix 3, pp. 55-65 of MRID 51198006).
7. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 20-25 of MRID 51029802; pp. 10, 19-24 of MRID 51198006). In the ECM and ILV, the LOQ was defined as the lowest fortification level validated. Also, in the ECM, the LOQ was defined as the level which the blank values did not exceed 30% of the LOQ. No further justification of the LOQ was reported in the ECM or ILV. The LOD was calculated in the ECM using the following equation:  $LOD = (3 \times N_{ctl}) / (Res_{PLS}) \times Conc_{CLS} \times DF_{CNTL}$ , where, LOD is the limit of detection of the analysis,  $N_{ctl}$  is the mean noise in height of the control samples (or blanks),  $Res_{PLS}$  is the mean response in height of the two low calibration standards,  $Conc_{CLS}$  is the concentration of the low calibration standard, and  $DF_{CNTL}$  is the dilution factor of the control samples (2000 mL/g). The LOD was estimated in the ILV using the following equation: 3 x baseline noise for the primary and confirmatory transitions. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

The method detection limit (MDL) was calculated in the ECM and ILV as dependent upon the lowest concentration calibration standard and the dilution factor of the controls (pp. 21-22 of MRID 51029802; p. 22 of MRID 51198006). In the ECM, the MDL was equivalent to  $0.0053 \mu\text{g/L} \times 2000 \text{ mL/g} = 0.0106 \text{ mg/kg}$ ; in the ILV, the MDL was equivalent to  $10 \mu\text{g/kg}$  for triadimefon ( $0.005 \mu\text{g/L} \times 2000 \text{ mL/g}$ ). This MDL calculation was not in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

8. The matrix interferences were determined to be insignificant (<20%) in the ECM and ILV, and solvent-based calibration standards were used in the ECM and ILV (pp. 24-25; Tables 5-8, pp. 33-36 of MRID 51029802; pp. 23-25 of MRID 51198006).
9. The total time required to complete one set of samples was not reported in the ECM or ILV.

## **V. References**

- U.S. Environmental Protection Agency. 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.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319, and Revision 2; 1994 and 2016.

**Attachment 1: Chemical Names and Structures****Triadimefon**

**IUPAC Name:** (RS)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-one

**CAS Name:** 1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone

**CAS Number:** 43121-43-3

**SMILES String:** Clc1ccc(OCC(C)(C)C(=O)Cn2cncn2)cc1

