Analytical method for tebuconazole in soil

Reports:	ECM: EPA MRID No. 50755101. Navarro, F. 2018. Environmental Chemistry Method: Validation of the Analytical Method for the Determination of Tebuconazole in Sediment/Soil Matrices by LC-MS/MS. Smithers Viscient Study No.: 14162.6114. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc., King of Prussia, Pennsylvania; 61 pages. Final report issued December 20, 2018. ILV: EPA MRID No. 50768801. Cashmore, A. 2019. Tebuconazole – Independent Laboratory Validation in Soil. Study No.: 3202239. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and sponsored and submitted by Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc., King of Prussia, Pennsylvania, and Pyxis Regulatory Consulting, Inc., Gig Harbor, Washington; 67 pages. Final report issued January 30, 2019.
Document No.:	MRIDs 50755101 & 50768801
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR 160) Good Laboratory Practice (GLP) standards, which are compatible with OECD Principles of GLP (p. 3 of MRID 50755101). Signed and dated No 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 accordance with USEPA FIFRA (40 CFR 160), UK, and OECD GLP standards, except for the soil characterization (p. 3; Appendix 3, p. 53 of MRID 50768801). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-6; Appendix 3, p. 53).
Classification:	This analytical method is classified as acceptable. 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.
PC Code:	128997
EFED Final Reviewer:	Andrew Shelby, Physical Scientist **Signature:** **Date:** 3/23/2021
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist Signature: Lisa Muto Date: 05/23/2019
	Mary Samuel, M.S., Environmental Scientist Signature: Mary Samuel

Date: 05/23/2019

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 Laboratory Project No.: 14162.6114, is designed for the quantitative determination of tebuconazole in soil at the LOQ of 50.0 µg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for tebuconazole. The ECM and ILV validated the method using two characterized soil matrices; different soil for each validation. 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 tebuconazole terrestrial field dissipation studies were submitted. The ILV validated the ECM method for the quantitation and confirmation analyses of tebuconazole in two soil matrices was validated in the first trial with insignificant modifications to the analytical instruments and parameters. All ECM and ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for tebuconazole.

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						
Tebuconazole	50755101	50768801		Soil[1,2]	20/12/2018	Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc.	LC/MS/MS	50.0 µg/kg

1 In the ECM, loamy sand soil (Soil #1; Smithers Viscient Batch No.: 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic carbon) and loamy sand soil (Soil #2; Smithers Viscient Batch No.: 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 7.9% organic carbon) were obtained from Rochester, Massachusetts (pp. 11-12 of MRID 50755101). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota, using USDA soil texture classification.

2 In the ILV, Newhaven silt loam soil (sample code CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water, pH 5.4 in 0.01M $CaCl_2$; 3.2% organic carbon) obtained from Newhaven, Derbyshire, United Kingdom, and Refesol 01-A sandy loam soil (sample code CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water, pH 5.3 in 0.01M $CaCl_2$; 0.9% organic carbon) obtained from Schmallenberg, Northrhine-Westphalia, Germany, were used (p. 13; Appendix 2, pp. 51-52 of MRID 50768801). Soil characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, using USDA soil texture classification.

I. Principle of the Method

The soil sample (5.00 g dry weight) was fortified with 0.250 mL of 1000 or 10000 µg/L tebuconazole fortification solution into a 50-mL centrifuge tube (pp. 15-17 of MRID 50755101). The soil sample was extracted two times with 20.0 mL each acetonitrile by shaking (150 rpm for 30 minutes), centrifugation (3000 rpm for 10 minutes), and decanting supernatant. The volume of the combined extracts was adjusted to 50.0 mL with acetonitrile and mixed well. Samples were further diluted in the calibration range with acetonitrile:purified reagent water (20:80, v:v) followed by centrifugation (13000 rpm for 5 minutes). Aliquots of the samples were analyzed by LC/MS/MS.

Samples were analyzed for tebuconazole using a Shimadzu LC-20AD HPLC system coupled to an AB MDS Sciex API 4000 mass spectrometer with AB MDS Sciex ESI Turbo V source (pp. 11, 17-18 of MRID 50755101). The LC/MS conditions consisted of a Waters XBridge C18 BEH column (50 x 2.1 mm, 2.5 µm particle size; column temperature 40°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.00-0.50 min. 80.0:20.0, 3.00-3.50 min. 0.00:100, 3.51-5.00 min. 80.0:20.0] and MS/MS detection with MRM (source temperature 500°C) and positive ESI ionization. Injection volume was 50.0 µL for soil #1 and 20.0 µL for soil #2. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 308.2→70.2 and 308.2→125.2 for tebuconazole. Retention time was *ca.* 2.8 minutes.

In the ILV, the ECM was performed as written, except for insignificant modifications of analytical instruments and parameters (pp. 14, 16-19 of MRID 50768801). A Shimadzu Nexera series HPLC System coupled to an AB Sciex API 5000 Triple Quadrupole mass spectrometer was used. The LC/MS conditions were the same as those of the ECM, except that injection volume was 50.0 µL for both soils. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 308.4→70.0 and 308.4→125.0 for tebuconazole. Retention time was *ca.* 2.8 minutes.

The Limit of Quantification (LOQ) was 50.0 µg/kg for tebuconazole in soil in the ECM and ILV (pp. 19-21 of MRID 50755101; pp. 22-23 of MRID 50768801). In the ECM, the Limit of Detection (LOD) was calculated as 6.71-7.66 µg/kg for soil #1 and 11.4-22.8 µg/kg for soil #2. In the ILV, the LOD was calculated as 10.6-12.8 µg/kg for Newhaven soil and 4.46-6.39 µg/kg for RefeSol 01-A soil. The Method Detection Limit (MDL) was calculated to be 16.7 µg/kg in the ECM and ILV.

II. Recovery Findings

ECM (MRID 50755101): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of tebuconazole in two soil matrices at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ; p. 21; Tables 1-4, pp. 28-31). Performance data (recovery results) from primary and confirmatory analyses were comparable. The loamy sand soil (Soil #1; Smithers Viscient Batch No.: 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic carbon) and loamy sand soil (Soil

#2; Smithers Viscient Batch No.: 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 7.9% organic carbon) were obtained from Rochester, Massachusetts (pp. 11-12). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota, using USDA soil texture classification.

ILV (MRID 50768801): Mean recoveries and RSDs were within guideline requirements for analysis of tebuconazole in two soil matrices at fortification levels of 50.0 µg/kg (LOQ) and 50 µg/kg (10×LOQ; Tables 1-4, pp. 27-30). Performance data (recovery results) from primary and confirmatory analyses were comparable. Newhaven silt loam soil (sample code CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water, pH 5.4 in 0.01M $CaCl_2$; 3.2% organic carbon) obtained from Newhaven, Derbyshire, United Kingdom, and Refesol 01-A sandy loam soil (sample code CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water, pH 5.3 in 0.01M $CaCl_2$; 0.9% organic carbon) obtained from Schmallenberg, Northrhine-Westphalia, Germany, were used (p. 13; Appendix 2, pp. 51-52). Soil characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, using USDA soil texture classification. The ECM method for the quantitation and confirmation analyses of tebuconazole in two soil matrices was validated in the first trial with insignificant modifications to the analytical instruments and parameters (pp. 14, 16-19, 23).

Table 2. Initial Validation Method Recoveries for Tebuconazole in Soil[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil #1					
	Quantitation ion					
Tebuconazole	50.0 (LOQ)	5	91.8-100	95.8	3.64	3.80
	500	5	86.3-96.7	91.8	4.46	4.86
	Confirmation ion					
Tebuconazole	50.0 (LOQ)	5	82.9-99.1	93.7	6.73	7.18
	500	5	86.1-96.5	92.4	3.88	4.20
	Loamy Sand Soil #2					
	Quantitation ion					
Tebuconazole	50.0 (LOQ)	5	87.8-96.9	93.1	3.40	3.65
	500	5	87.4-90.4	89.0	1.23	1.39
	Confirmation ion					
Tebuconazole	50.0 (LOQ)	5	93.5-109	99.8	6.11	6.13
	500	5	85.8-91.5	89.0	2.05	2.30

Data (uncorrected recovery results, p. 20) were obtained from p. 21; Tables 1-4, pp. 28-31 of MRID 50755101.

1 The loamy sand soil (Soil #1; Smithers Viscient Batch No.: 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic carbon) and loamy sand soil (Soil #2; Smithers Viscient Batch No.: 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 7.9% organic carbon) were obtained from Rochester, Massachusetts (pp. 11-12). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota, using USDA soil texture classification.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 308.2→70.2 and 308.2→125.2 for tebuconazole.

Table 3. Independent Validation Method Recoveries for Tebuconazole in Soil[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Newhaven Silt Loam Soil					
	Quantitation ion					
Tebuconazole	50.0 (LOQ)	5	81-95	86	5.3	6.1
	500	5	93-97	95	1.8	1.9
	Confirmation ion					
Tebuconazole	50.0 (LOQ)	5	80-93	88	5.3	6.0
	500	5	93-96	95	1.3	1.4
	RefeSol 01-A Sandy Loam Soil					
	Quantitation ion					
Tebuconazole	50.0 (LOQ)	5	73-77	75	1.5	2.0
	500	5	72-80	77	3.4	4.4
	Confirmation ion					
Tebuconazole	50.0 (LOQ)	5	76-81	78	1.9	2.5
	500	5	70-81	77	4.5	5.9

Data (uncorrected recovery results, p. 20) were obtained from Tables 1-4, pp. 27-30 of MRID 50768801.

1 The Newhaven silt loam soil (sample code CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water, pH 5.4 in 0.01M $CaCl_2$; 3.2% organic carbon) obtained from Newhaven, Derbyshire, United Kingdom, and Refesol 01-A sandy loam soil (sample code CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water, pH 5.3 in 0.01M $CaCl_2$; 0.9% organic carbon) obtained from Schmallenberg, Northrhine-Westphalia, Germany, were used (p. 13; Appendix 2, pp. 51-52). Soil characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, using USDA soil texture classification.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 308.4→70.0 and 308.4→125.0 for tebuconazole. These were similar to those of the ECM.

III. Method Characteristics

The LOQ was 50.0 µg/kg for tebuconazole in soil in the ECM and ILV (pp. 19-21 of MRID 50755101; pp. 22-23 of MRID 50768801).

In the ECM and ILV, the LOD was calculated in the ECM using the following equation:

$LOD = (3x(SN_{ctl}))/(Resp_{LS}) \times Conc_{LS} \times DF_{CTRL}$

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, $Conc_{LS}$ is the concentration of the low calibration standard, and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e. 3330).

In the ECM, the LOD was calculated as 6.71-7.66 µg/kg for soil #1 and 11.4-22.8 µg/kg for soil #2. In the ILV, the LOD was calculated as 10.6-12.8 µg/kg for Newhaven soil and 4.46-6.39 µg/kg for RefeSol 01-A soil.

The MDL was calculated using the following equation:

$MDL = MDL_{LCAL} \times DF_{CTRL}$

Where, MDL is the minimum detection limit, MDL_{LCAL} is the lowest standard concentration (i.e., 0.005 µg/L), and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e. 3330).

The MDL was calculated to be 16.7 µg/kg in the ECM and ILV based upon the lowest standard concentration of 0.005 µg/L and a control dilution factor of 3333.

Table 4. Method Characteristics

Analyte			Tebuconazole
Limit of Quantitation (LOQ)	ECM		50.0 µg/kg
	ILV		
Limit of Detection (LOD)	ECM	Loamy sand #1	6.71 µg/kg (Q) 7.66 µg/kg (C)
		Loamy sand #2	11.4 µg/kg (Q) 22.8 µg/kg (C)
	ILV	Silt loam	10.6 µg/kg (Q) 12.8 µg/kg (C)
		Sandy loam	6.39 µg/kg (Q) 4.46 µg/kg (C)
Linearity (calibration curve r^2 and concentration range)	ECM	Loamy sand #1	r^2 = 1.00 (Q) r^2 = 0.999 (C)
		Loamy sand #2	r^2 = 1.00 (Q) r^2 = 0.998 (C)
	ILV[1]	Silt loam	r^2 = 0.9990 (Q) r^2 = 0.9982 (C)
		Sandy loam	r^2 = 0.9976 (Q) r^2 = 0.9978 (C)
	Range		0.00500-0.250 µg/L
Repeatable	ECM[2]		Yes at LOQ and 10×LOQ (two characterized soil matrices).
	ILV[3,4]		
Reproducible			Yes at LOQ and 10×LOQ.
Specific	ECM		Yes, matrix interferences were <10% of the LOQ (based on peak area); minor baseline noise was observed.
	ILV		

Data were obtained from p. 21 (LOQ/LOD); p. 21; Tables 1-4, pp. 28-31 (recovery data); pp. 14, 24-25 (correlation coefficients); Figures 1-10, pp. 36-45 (chromatograms); Figures 11-14, pp. 46-49 (calibration curves) of MRID 50755101; p. 22 (LOQ); Tables 1-4, pp. 27-30 (recovery data); p. 22 (linearity); Figures 1-2, p. 34; Figures 15-16, p. 41 (calibration curves); Figures 3-26, pp. 35-46 (chromatograms) of MRID 50768801; and DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 ILV correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (p. 22 of MRID 50768801; DER Attachment 2). In the ECM, matrix-matched standards were used for both soils (p. 14 of MRID 50755101). Matrix-matched standards were also used for both soils in the ILV to be consistent with the ECM even though matrix effects were found to be insignificant (<20% difference) for the test soils (p. 11 of MRID 50768801).

2 In the ECM, loamy sand soil (Soil #1; Smithers Viscient Batch No.: 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic carbon) and loamy sand soil (Soil #2; Smithers Viscient Batch No.: 041917B; 83% sand, 16% silt, 1% clay; pH 6.6 in 1:1 soil:water ratio; 7.9% organic carbon) were obtained from Rochester, Massachusetts (pp. 11-12 of MRID 50755101). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota, using USDA soil texture classification.

3 In the ILV, Newhaven silt loam soil (sample code CS 17/18; 25% sand, 51% silt, 24% clay; pH 6.0 in water, pH 5.4 in 0.01M $CaCl_2$; 3.2% organic carbon) obtained from Newhaven, Derbyshire, United Kingdom, and Refesol 01-A sandy loam soil (sample code CS 30/18; 74% sand, 20% silt, 6% clay; pH 6.4 in water, pH 5.3 in 0.01M $CaCl_2$; 0.9% organic carbon) obtained from Schmallenberg, Northrhine-Westphalia, Germany, were used (p. 13; Appendix 2, pp. 51-52 of MRID 50768801). Soil characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, using USDA soil texture classification.

4 The ILV validated the ECM method for the quantitation and confirmation analyses of tebuconazole in two soil matrices was validated in the first trial with insignificant modifications to the analytical instruments and parameters (pp. 14, 16-19, 23 of MRID 50768801).

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method since the ILV soil matrices were silt loam soil (24% clay; 3.2% organic carbon) and sandy loam soil (6% clay; 0.9% organic carbon; p. 13; Appendix 2, pp. 51-52 of MRID 50768801). 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 tebuconazole terrestrial field dissipation studies were submitted**.**

2. The communications of the ILV (Smither Viscient) and Sponsor (Generic Tebuconazole DCI Task Force, c/o United Phosphorus, Inc., and Pyxis Regulatory Consulting, Inc.,) involved the exchange of the definitive protocol, successful completion of the trial, and Sponsor QC check of validation results (p. 22; Appendix 5, p. 55 of MRID 50768801). The reviewer noted that the ECM and ILV laboratories were Smithers Viscient, but the ECM was performed by the Massachusetts location while the ILV was performed by the North Yorkshire location. Reported laboratory personnel differed between the ECM and ILV (p. 5 of MRID 50755101; p. 7 of MRID 50768801).

3. The estimation of LOQ in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 19-21 of MRID 50755101; pp. 22-23 of MRID 50768801). 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 defined as the lowest level validated. The LOD was calculated in the ECM and ILV using the following equation: LOD = $(3x(SN_{ctl})/(Resp_{LS})$ x $Conc_{LS}$ x DF_{CTRL}, 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, $Conc_{LS}$ is the concentration of the low calibration standard, and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e. 3330). Detection limits should not be based on arbitrary values.

   The MDL was calculated to be 16.7 µg/kg in the ECM and ILV based upon the lowest standard concentration of 0.005 µg/L and a control dilution factor of 3333 (pp. 19-21 of MRID 50755101; pp. 22-23 of MRID 50768801).

4. In the ECM, matrix effects were found to be significant (>20% difference) for soil #1 and insignificant (<20% difference) for soil #2; matrix-matched standards were used for both soils (pp. 14, 24-25; Tables 5-8, pp. 32-35 of MRID 50755101). Matrix-matched standards were also used for both soils in the ILV to be consistent with the ECM even though matrix effects were found to be insignificant (<20% difference) for the test soils (pp. 11, 23; Tables 5-6, pp. 31-32 of MRID 50768801).

5. The time required to complete the method for one sample set was not reported in the ILV or ECM.

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.

Attachment 1: Chemical Names and Structures

Tebuconazole (HWG 1608)

IUPAC Name: (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol
CAS Name: α-[2-(4-Chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol
CAS Number: 107534-96-3
SMILES String: c1cc(Cl)ccc1CCC(O)(C(C)(C)C)Cn2ncnc2