Analytical method for ipconazole in soil and sediment

Reports:	ECM: EPA MRID No.: 50564602. Reibach, P. 2017. Validation of the Analytical Method for the Determination of Ipconazole in Soil and Sediment by LC-MS/MS. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Kureha Corporation, Tokyo, Japan, and Kureha America Inc., Houston, Texas; 101 pages. Smithers Viscient Study No.: 11106.6616. Sponsor Protocol/Project No.: 317_2017/008. Final report issued October 31, 2017. ILV: EPA MRID No.: 50564601. Cashmore, A. 2018. Ipconazole - Independent Laboratory Validation of Analytical Method 11106.6616- for the Determination of Ipconazole in Soil and Sediment. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and sponsored and submitted by Kureha Corporation, Tokyo, Japan, and Kureha America Inc., Houston, Texas; 60 pages. Study No.: 3201882. Final report issued April 5, 2018 (p. 2).
Document No.:	MRIDs 50564602 & 50564601
Guideline:	850.6100
Statements:	ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160), as acceptable by the OECD GLP (p. 3 of MRID 50564602). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the QA statement (p. 4). The purity of the test material was determined at Huntingdon Life Sciences (Suffolk, United Kingdom) in compliance with United Kingdom Department of Health GLP standards (Appendix 2, p. 99). ILV: The study was conducted in compliance with United Kingdom Department of Health and OECD GLP standards, except for the characterization of the Speyer 5M soil test system (p. 3; Appendix 3, p. 58 of MRID 50564601). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the GLP statements (p. 3). An unsigned statement of authenticity was included (p. 5).
Classification:	This analytical method is classified as supplemental. The soil/sediment matrices did not cover the range of soils/sediments used in the aerobic soil and aquatic metabolism studies. The soil extraction method used was insufficient.
PC Code:	125618
EFED Final Reviewer:	Zoe Ruge, M.S., Physical Scientist Signature: Zoe Ruge Date: June 30, 2020
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist Signature: Lisa Muto Date: 03/20/2020 Mary Samuel, M.S., Environmental Scientist Signature: Mary Samuel Date: 03/20/2020

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

Executive Summary

The analytical method, Smithers Viscient Study No.: 11106.6616, is designed for the quantitative determination of ipconazole in soil and sediment at the stated LOQ of 50.0 µg/kg using HPLC/MS/MS. The LOQ is not greater than any known lowest toxicological level of concern in soil/sediment. Ipconazole was a mixture of cis,cis:cis, trans (cc:ct) ipconazole which were quantified separately then summed. The ECM used non-USDA characterized loamy sand soil and freshwater sediment; the ILV used a characterized sandy loam soil and silt loam sediment. The soil/sediment matrices did not cover the range of soils/sediments used in the aerobic soil and aquatic metabolism and terrestrial field dissipation studies. Submitted aerobic soil metabolism studies used sandy loam, sandy clay loam, silt loam, and clay loam soils. The submitted aerobic aquatic metabolism study used sandy clay loam and loamy sand sediments. No anaerobic metabolism studies nor terrestrial field dissipation studies were submitted for ipconazole. ILV validated the method for ipconazole in soil and sediment with the first trial as written, except for insignificant analytical instrument and equipment modifications. For the sediment validation, samples were re-injected after dilution errors were corrected. No updated ECM is required. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for ipconazole in the soil and sediment matrices which were tested.

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						
Ipconazole	50564602[1]	50564601[2]		Soil/ Sediment	31/10/2017	Kureha Corporation, and Kureha America Inc.	LC/MS/MS	50.0 µg/kg

1 Ipconazole was a mixture of cis/cis ipconazole (ipconazole cc) and cis/trans ipconazole (ipconazole ct). Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol; and ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol.

2 In the ECM, loamy sand soil (78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black; 1.06 g/cm^3 bulk density; 9.7 meq/100 g cation exchange capacity) obtained from Rochester, Massachusetts, and freshwater sediment (96% sand, 4% silt, 0% clay; pH 5.2 in 1:1 soil:water ratio; 2.0% organic matter – Walkley Black) obtained from Glen Charlie Pond, Wareham, Massachusetts, were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification not reported; pp. 13-14 of MRID 50564602).

3 In the ILV, sandy loam soil (Speyer 5M, CS 31/17; 59% sand, 30% silt, 11% clay; pH 7.3 in 0.01M $CaCl_2$; pH 8.3 in water; 1.0% organic carbon; 15.7 meq/100 g cation exchange capacity) and silt loam sediment (Calwich Abbey, CS 55/17; 29% sand, 57% silt, 14% clay; pH 7.3 in 0.01M $CaCl_2$; pH 7.7 in water; 4.7% organic carbon; 17.4 meq/100 g cation exchange capacity) were used in the study (USDA Soil Texture Classification; pp. 13-14; Appendix 2, pp. 56-57 of MRID 50564601). The soils were characterized by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, and Lufa Speyer.

I. Principle of the Method

Loamy sand soil (5.00 g dry wt.) in 50-mL centrifuge tubes was fortified at a low concentration with 0.25 mL of 0.928 mg/L:0.072 mg/L (ipconazole cc:ct) or at a high concentration with 9.28 mg/L:0.72 mg/L (ipconazole cc:ct) fortification solutions to achieve nominal concentrations of 46.4:3.6 and 464:36 µg/kg, respectively (pp. 18-20 of MRID 50564602). Freshwater soil (sediment; 5.00 g dry wt.) in 50-mL centrifuge tubes was fortified with 0.25 mL of 0.929 mg/L:0.073 mg/L (ipconazole cc:ct) or 9.29 mg/L:0.73 mg/L (ipconazole cc:ct) fortification solutions to achieve nominal concentrations of 46.5:3.7 and 465:37 µg/kg, respectively. The soil samples were extracted twice using 20 mL of acetonitrile:purified reagent water:formic acid (90:10:0.1, v:v:v) with shaking (150 rpm) for 30 minutes. The sample was centrifuged for 10 minutes at 3000 rpm. The volume of the combined extracts was adjusted to 50 mL with acetonitrile:purified reagent water:formic acid (90:10:0.1, v:v:v) and mixed well. Further dilutions into the calibration standard range were performed with methanol:purified reagent water (50:50, v:v). Loamy sand soil extracts were centrifuged at 13000 rpm for 5 minutes using low-binding centrifuge tubes; sediment samples were not centrifuged. Samples were transferred to autosampler vials prior to LC-MS/MS analysis. Further dilutions with methanol:purified reagent water (50:50, v:v) were performed, if necessary.

Samples are analyzed using an MDS Sciex 4000 Q Trap mass spectrometer coupled with a Shimadzu LC-20AD HPLC (pp. 12, 21-22 of MRID 50564602). The following LC conditions were used: Waters XBridge C18 column (2.1 mm x 100 mm, 3.5 µm; column temperature 40°C), gradient mobile phase of A) 0.1% formic acid in reagent grade water and B) 0.1% formic acid in acetonitrile [time, percent A:B; 0.01-4.00 min. 50.0:50.0, 7.00 min. 30.0:70.0, 7.10-9.00 min. 0.00:100, 9.10 min 50.0:50.0], injection volume of 25.0 µL, MS/MS with Electrospray Ionization (ESI) source in positive polarity (source temperature 500°C). Two ion pair transitions were monitored for ipconazole (quantitation and confirmation, respectively): *m/z* 334.5→70.2 and *m/z* 336.2→70.2. Retention times were 5.07-5.08 minutes for ipconazole cc and 4.76-4.77 for ipconazole ct in loamy sand soil and 4.68-4.69 minutes for ipconazole cc and 4.37-4.38 for ipconazole ct in freshwater sediment.

The ILV performed the ECM method for ipconazole in soil and sediment as written, except for insignificant analytical instrument and equipment modifications (pp. 14-18, 21 of MRID 50564601). The LC/MS/MS instrument was an AB Sciex API 500 Triple Quadrupole mass spectrometer coupled with a HPLC System. The LC conditions were the same as those of the ECM, except that the injection volume was 10 µL. Two ion pair transitions were monitored for ipconazole (quantitation and confirmation, respectively): *m/z* 334→70 and *m/z* 336→70. Retention times were *ca*. 3.9 minutes for ipconazole cc and *ca*. 3.7 for ipconazole ct.

In the ECM and ILV, the Limit of Quantification (LOQ) was 50.0 µg/kg for ipconazole in soil/sediment matrices (pp. 23-25, 28-29 of MRID 50564602; pp. 20, 23 of MRID 50564601). In the ECM, the LOQ was specified for each isomer of the ipconazole as 46.4 µg/kg for ipconazole cc and 3.6 µg/kg for ipconazole ct in loamy sand soil and 46.5 µg/kg for ipconazole cc and 3.7 µg/kg for ipconazole ct in freshwater sediment. The Limit of Detection (LOD) values were 0.607-1.01 µg/kg for loamy sand soil and 1.32-2.52 µg/kg for freshwater sediment in the ECM for total ipconazole; values not calculated in the ILV. For each isomer of ipconazole, the ECM LOD values were 0.283-0.755 µg/kg for ipconazole cc and 0.258-0.324 µg/kg for ipconazole ct in loamy sand

soil and 0.942-1.42 µg/kg for ipconazole cc and 0.376-1.10 µg/kg for ipconazole ct in freshwater sediment. In the ILV, LOD values were 0.791-1.57 µg/kg for ipconazole cc and 0.677-1.37 µg/kg for ipconazole ct in the soil/sediment matrices.

II. Recovery Findings

ECM (MRID 50564602): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of ipconazole at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ) in one soil matrix and one sediment matrix (Tables 1-4, pp. 32-35). Results were based on total ipconazole, ipconazole cc summed with ipconazole ct. Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable. Loamy sand soil (78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black; 1.06 g/cm^3 bulk density; 9.7 meq/100 g cation exchange capacity) obtained from Rochester, Massachusetts, and freshwater sediment (96% sand, 4% silt, 0% clay; pH 5.2 in 1:1 soil:water ratio; 2.0% organic matter – Walkley Black) obtained from Glen Charlie Pond, Wareham, Massachusetts, were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification not reported).

ILV (MRID 50564601): Mean recoveries and RSDs were within guidelines for analysis of ipconazole at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ) in one soil matrix and one sediment matrix (Tables 1-4, pp. 27-30; DER Attachment 2). Results were based on total ipconazole, ipconazole cc summed with ipconazole ct. Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable. Means, standard deviations, and RSDs for the LOQ recoveries of the soil were reviewer-calculated using all five recovery values since the study author excluded one recovery value considered to be an outlier, using Grubb's test with a significance of 0.05. The study author proposed that the unusually high recovery was due to contamination of the dilution flask. The sandy loam soil (Speyer 5M, CS 31/17; 59% sand, 30% silt, 11% clay; pH 7.3 in 0.01M $CaCl_2$; pH 8.3 in water; 1.0% organic carbon; 15.7 meq/100 g cation exchange capacity) and silt loam sediment (Calwich Abbey, CS 55/17; 29% sand, 57% silt, 14% clay; pH 7.3 in 0.01M $CaCl_2$; pH 7.7 in water; 4.7% organic carbon; 17.4 meq/100 g cation exchange capacity) were used in the study (USDA Soil Texture Classification; pp. 13-14; Appendix 2, pp. 56-57). The soils were characterized by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, and Lufa Speyer. The method was validated for ipconazole in soil and sediment with the first trial as written, except for insignificant analytical instrument and equipment modifications (pp. 11, 14-18, 21; Appendix 4, p. 59). For the sediment validation, samples were re-injected after dilution errors were corrected. No updated ECM is required.

Table 2. Initial Validation Method Recoveries for Ipconazole in Soil and Sedment[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil[3]					
	Quantitation ion transition					
Ipconazole	50.0 (LOQ)	5	92.0-94.2	93.1	0.877	0.942
	500	5	93.8-96.0	94.7	0.986	1.04
	Confirmation ion transition					
Ipconazole	50.0 (LOQ)	5	89.3-98.1	93.3	3.54	3.79
	500	5	94.0-97.4	95.9	1.60	1.66
	Freshwater Sediment[3]					
	Quantitation ion transition					
Ipconazole	50.0 (LOQ)	5	102-104	103	0.624	0.607
	500	5	102-108	104	2.64	2.54
	Confirmation ion transition					
Ipconazole	50.0 (LOQ)	5	100-106	103	2.55	2.48
	500	5	103-110	105	3.14	2.99

Data (uncorrected recovery results; pp. 23-24) were obtained from Tables 1-4, pp. 32-35 of MRID 50564602.

1 Ipconazole was a mixture of cis/cis ipconazole (ipconazole cc) and cis/trans ipconazole (ipconazole ct). Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l- (1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol; and ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol.

2 Two ion pair transitions were monitored for ipconazole (quantitation and confirmation, respectively): *m/z* 334.5→70.2 and *m/z* 336.2→70.2.

3 The loamy sand soil (78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black; 1.06 g/cm^3 bulk density; 9.7 meq/100 g cation exchange capacity) obtained from Rochester, Massachusetts, and freshwater sediment (96% sand, 4% silt, 0% clay; pH 5.2 in 1:1 soil:water ratio; 2.0% organic matter – Walkley Black) obtained from Glen Charlie Pond, Wareham, Massachusetts, were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification not reported; pp. 13-14 of MRID 50564602). The soil texture could not be verified by the reviewer using USDA-NRCS technical support tools.

Table 3. Independent Validation Method Recoveries for Ipconazole in Soil and Sediment[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Speyer 5M Soil[3]					
	Quantitation ion transition					
Ipconazole	50.0 (LOQ)[4]	5	102-139	111	16	14
	500	5	101-105	104	1.67	1.61
	Confirmation ion transition					
Ipconazole	50.0 (LOQ)[4]	5	103-142	112	17	15
	500	5	101-103	102	1.04	1.01
	Calwich Abbey Sediment[3]					
	Quantitation ion transition					
Ipconazole	50.0 (LOQ)	5	95.7-123	105	10.5	10.0
	500	5	97.1-99.6	98.3	1.14	1.16
	Confirmation ion transition					
Ipconazole	50.0 (LOQ)	5	95.5-122	104	10.6	10.2
	500	5	96.2-99.4	98.2	1.31	1.33

Data (uncorrected recovery results; p. 19) were obtained from Tables 1-4, pp. 27-30 of MRID 50564601 and DER Attachment 2.

1 Ipconazole was a mixture of cis/cis ipconazole (ipconazole cc) and cis/trans ipconazole (ipconazole ct). Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l- (1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol; and ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol.

2 Two ion pair transitions were monitored for ipconazole (quantitation and confirmation, respectively): *m/z* 334→70 and *m/z* 336→70.

3 The sandy loam soil (Speyer 5M, CS 31/17; 59% sand, 30% silt, 11% clay; pH 7.3 in 0.01M $CaCl_2$; pH 8.3 in water; 1.0% organic carbon; 15.7 meq/100 g cation exchange capacity) and silt loam sediment (Calwich Abbey, CS 55/17; 29% sand, 57% silt, 14% clay; pH 7.3 in 0.01M $CaCl_2$; pH 7.7 in water; 4.7% organic carbon; 17.4 meq/100 g cation exchange capacity) were used in the study (USDA Soil Texture Classification; pp. 13-14; Appendix 2, pp. 56-57 of MRID 50564601). The soils were characterized by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, and Lufa Speyer. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

4 Means, standard deviations, and RSDs were reviewer-calculated using all five recovery values since the study author excluded one of the recovery values since it was considered an outlier, using Grubb's test with a significance of 0.05. The study author proposed that the unusually high recovery was due to contamination of the dilution flask.

III. Method Characteristic

In the ECM and ILV, the LOQ was 50.0 µg/kg for all analytes in soil matrices (pp. 23-25, 28-29 of MRID 50564602; pp. 20, 23 of MRID 50564601). In the ECM, the LOQ was specified for each isomer of the ipconazole as 46.4 µg/kg for ipconazole cc and 3.6 µg/kg for ipconazole ct in loamy sand soil and 46.5 µg/kg for ipconazole cc and 3.7 µg/kg for ipconazole ct in freshwater sediment. In the ECM, the LOQ was determined as the lowest fortification level and as the fortification level at which the blank values did not exceed 30%. In the ECM, the LOD was defined as three times the signal-to-noise ration of the control samples. In the ILV, the LOD was defined as three time the baseline noise of the control and sediment for ipconazole cc and ipconazole ct (primary and confirmatory). The calculated LOD values were 0.607-1.01 µg/kg for loamy sand soil and 1.32-2.52 µg/kg for freshwater sediment in the ECM for total ipconazole; values not calculated in the ILV. For each isomer of ipconazole, the ECM LOD values were 0.283-0.755 µg/kg for ipconazole cc and 0.258-0.324 µg/kg for ipconazole ct in loamy sand soil and 0.942-1.42 µg/kg for ipconazole cc and 0.376-1.10 µg/kg for ipconazole ct in freshwater sediment. In the ILV, LOD values were 0.791-1.57 µg/kg for ipconazole cc and 0.677-1.37 µg/kg for ipconazole ct in the soil/sediment matrices. In the ECM, the LOD values for ipconazole were calculated, based on the following equation:

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

Where, LOD is the limit of detection of the analysis, N_{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. 10). In the ILV, the LOD values for ipconazole were calculated, based on the following equation:

LOD = 3 x height of control baseline noise x control dilution factor x calibration standard concentration (µg/L) / height of calibration standard peak.

No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV.

Table 4. Method Characteristics Ipconazole in Soil and Sediment

Test Material[1]		Ipconazole	
Matrix		**Soil**	**Sediment**
Limit of Quantitation (LOQ)	ECM	50.0 µg/kg for ipconazole total 46.4 µg/kg for ipconazole cc 3.6 µg/kg for ipconazole ct	50.0 µg/kg for ipconazole total 46.5 µg/kg for ipconazole cc 3.7 µg/kg for ipconazole ct
	ILV	50.0 µg/kg for ipconazole total	
Limit of Detection (LOD)	ECM (calculated)	0.607 µg/kg (Q) and 1.01 µg/kg (C) for ipconazole total 0.283 µg/kg (Q) and 0.755 µg/kg (C) for ipconazole cc 0.324 µg/kg (Q) and 0.258 µg/kg (C) for ipconazole ct	1.32 µg/kg (Q) and 2.52 µg/kg (C) for ipconazole total 0.942 µg/kg (Q) and 1.42 µg/kg (C) for ipconazole cc 0.376 µg/kg (Q) and 1.10 µg/kg (C) for ipconazole ct
	ILV (calculated)	0.791 µg/kg (Q) and 1.49 µg/kg (C) for ipconazole cc 0.677 µg/kg (Q) and 1.28 µg/kg (C) for ipconazole ct (not reported for ipconazole total)	0.968 µg/kg (Q) and 1.57 µg/kg (C) for ipconazole cc 0.784 µg/kg (Q) and 1.37 µg/kg (C) for ipconazole ct (not reported for ipconazole total)
Linearity (calibration curve r and concentration range)	ECM[2]	r = 0.9997 (Q, ipconazole cc) r = 0.9998 (C, ipconazole cc) 0.0500-0.500 ng/mL r = 0.9990 (Q, ipconazole ct) r = 0.9979 (C, ipconazole ct) 0.00350-0.0350 ng/mL	r = 0.9993 (Q, ipconazole cc) r = 0.9984 (C, ipconazole cc) 0.0500-0.500 ng/mL r = 0.9988 (Q, ipconazole ct) r = 0.9982 (C, ipconazole ct) 0.00350-0.0350 ng/mL
	ILV	r = 0.9990 (Q & C, ipconazole cc) 0.0500-0.500 ng/mL r = 0.9974 (Q, ipconazole ct) r = 0.9985 (C, ipconazole ct) 0.00350-0.0350 ng/mL	r = 0.9988-0.9998 (Q, ipconazole cc) r = 0.9986-0.9997 (C, ipconazole cc) 0.0500-0.500 ng/mL r = 0.9984-0.9993 (Q, ipconazole ct) r = 0.9970-0.9984 (C, ipconazole ct) 0.00350-0.0350 ng/mL
Repeatable	ECM[3]	Yes at LOQ and 10×LOQ in two non-USDA characterized soil/sediment matrices.	
	ILV[4,5]	Yes at LOQ and 10×LOQ in two characterized soil/sediment matrices.	
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM	Yes, matrix interferences were <5% of the LOQ (based on peak area) for ipconazole cc and <10% of the LOQ (based on peak area) for ipconazole ct. Analyte peaks for ipconazole cc and ipconazole ct co-eluted, which made accurate integration and quantification difficult.	
	ILV	Yes, no matrix interferences were observed. Analyte peaks for ipconazole cc and ipconazole ct eluted near each other which affected accurate integration and quantification. Some minor peak splitting was observed for ipconazole ct in sediment at the LOQ.	

Data were obtained from pp. 23-25 (LOQ/LOD); Tables 1-4, pp. 32-35 (recovery results); p. 26; Figures 21-28, pp. 58-65 (calibration coefficients); Figures 1-20, pp. 38-57 (chromatograms) of MRID 50564602; pp. 20, 23 (LOQ/LOD); Tables 1-4, pp. 27-30 (recovery results); pp. 15, 23; Figure 1, p. 34; Figure 10, p. 38; Figure 19, p. 43; Figure 28, p. 47 (calibration curves); Figures 2-36, pp. 34-51 (chromatograms) of MRID 50564601; DER Attachment 2. Q = quantitation ion transition; C = confirmation ion transition.

1 Ipconazole was a mixture of cis/cis ipconazole (ipconazole cc) and cis/trans ipconazole (ipconazole ct). Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l- (1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol; and ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol.

2 Correlation coefficients (r) were reviewer-calculated from r^2 values provided in the study report (p. 26 of MRID

50564602; DER Attachment 2).

3 In the ECM, loamy sand soil (78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black; 1.06 g/cm^3 bulk density; 9.7 meq/100 g cation exchange capacity) obtained from Rochester, Massachusetts, and freshwater sediment (96% sand, 4% silt, 0% clay; pH 5.2 in 1:1 soil:water ratio; 2.0% organic matter – Walkley Black) obtained from Glen Charlie Pond, Wareham, Massachusetts, were characterized by Agvise Laboratories, Northwood, North Dakota, and used in the study (USDA Soil Texture Classification not reported; pp. 13-14 of MRID 50564602).

4 In the ILV, sandy loam soil (Speyer 5M, CS 31/17; 59% sand, 30% silt, 11% clay; pH 7.3 in 0.01M $CaCl_2$; pH 8.3 in water; 1.0% organic carbon; 15.7 meq/100 g cation exchange capacity) and silt loam sediment (Calwich Abbey, CS 55/17; 29% sand, 57% silt, 14% clay; pH 7.3 in 0.01M $CaCl_2$; pH 7.7 in water; 4.7% organic carbon; 17.4 meq/100 g cation exchange capacity) were used in the study (USDA Soil Texture Classification; pp. 13-14; Appendix 2, pp. 56-57 of MRID 50564601). The soils were characterized by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom, and Lufa Speyer.

5 The ILV validated the method for ipconazole in soil and sediment with the first trial as written, except for insignificant analytical instrument and equipment modifications (pp. 11, 14-18, 21; Appendix 4, p. 59 of MRID 50564601). For the sediment validation, samples were re-injected after dilution errors were corrected. No updated ECM is required.

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV soil/sediment matrices covered the range of soils/sediments used in the aerobic and anaerobic aquatic metabolism and terrestrial field dissipation (TFD) studies since only one soil matrix and one sediment matrix was included in the ILV: sandy loam soil (Speyer 5M, CS 31/17; 59% sand, 30% silt, 11% clay; 1.0% organic carbon) and silt loam sediment (Calwich Abbey, CS 55/17; 29% sand, 57% silt, 14% clay; 4.7% organic carbon; USDA Soil Texture Classification; pp. 13-14; Appendix 2, pp. 56-57 of MRID 50564601). More than one soil and sediment should be included to encompass the range of soils/sediments used in the aquatic metabolism and TFD studies. The submitted ipconazole aerobic aquatic metabolism study (MRID 49910306) contained two UK sediment/water systems: Bury Pond (sandy clay loam) and Emperor Lake (loamy sand; Table 2, p. 64 of MRID 50920946; Table III, p. 41 of MRID 49910306). No ipconazole TFD studies were submitted; however, six soils were included in the four submitted aerobic metabolism studies (MRIDs 45542224, 46008402, 49910304, & 49910305).

2. No communication between the method developers and ILV occurred. The communication log reported that the Study Sponsor (Study Monitor was Keiichi Sudo of Kureha Corporation) and ILV communicated via email to exchange the signed study protocol and final validation results (p. 22; Appendix 5, p. 60 of MRID 50564601).

3. The determinations of LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 23-25, 28-29 of MRID 50564602; pp. 20, 23 of MRID 50564601). In the ECM, the LOQ was specified for each isomer of the ipconazole as 46.4 µg/kg for ipconazole cc and 3.6 µg/kg for ipconazole ct in loamy sand soil and 46.5 µg/kg for ipconazole cc and 3.7 µg/kg for ipconazole ct in freshwater sediment. In the ECM, the LOQ was determined as the lowest fortification level and as the fortification level at which the blank values did not exceed 30%. In the ECM, the LOD was defined as three times the signal-to-noise ratio of the control samples. In the ILV, the LOD was defined as three times the baseline noise of the control and sediment for ipconazole cc and ipconazole ct (primary and confirmatory). In the ECM, the LOD values for ipconazole in artificial and marine sediment were calculated based on the following equation: $LOD = (3x(N_{ctl})/(Resp_{LS}) \times Conc_{LS} \times DF_{CTRL}$,

where, LOD is the limit of detection of the analysis, N_{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. 10). In the ILV, the LOD values for ipconazole were calculated, based on the following equation: LOD = 3 x height of control baseline noise x control dilution factor x calibration standard concentration (µg/L) / height of calibration standard peak. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV.

MDLs were also calculated in the ECM and ILV (pp. 23-25, 28-29 of MRID 50920983; pp. 20, 23 of MRID 50564601).

4. The extraction procedure was not sufficiently exhaustive. The soil samples were extracted twice using 20 mL of acetonitrile:purified reagent water:formic acid (90:10:0.1, v:v:v) with shaking (150 rpm) for 30 minutes.

5. Matrix effects of the test soil/sediment matrices were studied in the ECM and ILV and found to be insignificant (<20%; pp. 26, 28-29; Tables 5-6, pp. 36-37 of MRID 50564602; p. 23; Tables 5-6, pp. 31-32 of MRID 50564601). Solvent-based calibration standards were used for quantification of the residues (Appendix B, p. 74 of MRID 50564602).

6. The timeframe required to complete the validation 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.

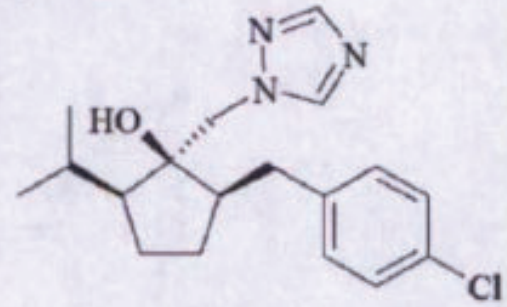
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

Ipconazole

IUPAC Name:	Ipconazole cc: (1*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol Ipconazole ct: (1*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol
CAS Name:	Not reported
CAS Number:	Ipconazole (CAS [125225-28-7]) Ipconazole cc (CAS [115850-69-6]) Ipconazole ct (CAS [115937-89-8])
SMILES String:	c1cc(Cl)ccc1CC2CCC(C(C)C)C2(O)Cn3ncnc3

Ipconazole cc



Ipconazole ct