Analytical method for lambda-cyhalothrin in soil

Reports:	ECM: EPA MRID No.: 49520001. Mayer, L.C. 2014. Lambda-cyhalothrin: Lambda-cyhalothrin –Residue Method (GRM043.05B) for the Determination of Lambda-cyhalothrin in Soil – Analytical Method. Report No.: GRM043.05B. Task No.: TK0251818. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 40 pages. Final report issued September 12, 2014. ILV: EPA MRID No. 49721701. Guo, D. 2015. Lambda-cyhalothrin: Lambda-cyhalothrin – Independent Laboratory Validation of Residue Method (GRM043.05B) for the Determination of Lambda-cyhalothrin in Soil – Final ILV Report. Report No: PASC-REP-0646. PASC Project No.: 141-1172. Task No.: TK0261528. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey; sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 90 pages. Final report issued July 13, 2015.
Document No.:	MRIDs 49520001 & 49721701
Guideline:	850.6100
Statements:	ECM: The study was conducted with no claim of compliance with USEPA or OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 49520001). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not provided. A signed and dated Summary of Revisions to the original method (GRM043.05A) was provided (p. 4). ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49721701). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity was not provided.
Classification:	This analytical method is classified as **Acceptable**. However, the determinations of the LOQ and LOD were not based on scientifically acceptable procedures. In the ILV, soil characterization data was insufficient to establish that the most difficult matrix was used to validate the method. In the ECM, the number of samples (n = 3) was insufficient at the LOQ; no samples were prepared at 10×LOQ; significant residues (*ca*. 25% of the LOQ) were observed in controls; recoveries were corrected for residues in the controls; and representative chromatograms were only provided for one of the two soil matrices.
PC Code:	128897
Reviewer:	Lewis Ross Brown, III Environmental Biologist

Signature: Brown, Lewis 2016.03.07 07:28:34 -05'00'

Date: Mar. 2, 2016

Page numbers for MRID 49721701 refer to those listed in the bottom-most right-handed corner of the pages.

Executive Summary

This analytical method, Syngenta Residue Method (GRM043.05B), is designed for the quantitative determination of lambda-cyhalothrin in soil at the stated LOQ of 0.001 mg/kg (1 ppb) using GC/MS. Lambda-cyhalothrin was identified and quantified using one ion transition (*m/z* 241). Two confirmatory ions (*m/z* 243 and *m/z* 205) were monitored, but the recoveries were not quantified. The LOQ is less than the lowest toxicological level of concern in soil. The ECM was performed using sand and loam soils; however, the number of samples (n = 3) was insufficient at the LOQ, and no samples were prepared at 10×LOQ. The independent laboratory validated the method after one trial with no modifications using clay loam and sandy loam soils; however, due to the insufficient soil characterization data in the ILV, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. Additionally, in the ECM, recoveries were corrected for significant residues in the controls (*ca*. 25% of the LOQ) and representative chromatograms were only provided for one of the two 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						
Lambda-cyhalothrin	49520001	49721701		Soil[1,2]	12/09/2014	Syngenta	GC/MS	0.001 mg/kg

1 In the ECM, sand soil (pH 7.8; 73% sand, 16% silt, 11% clay; 0.9% organic carbon) and loam soil (pH 7.3; 55% sand, 28% silt, 17% clay; 4.9% organic carbon) were used (USDA soil texture classification; Table 1, p. 23 of MRID 49721701).

2 In the ILV, clay loam soil (CAPY081512; pH 6.9) and sandy loam soil (EXCEL FARM; pH 7.8) were used (USDA soil texture classification; p. 10; Table 1, p. 16 of MRID 49721701). Organic matter or carbon percentages and sand/silt/clay percentages were not reported.

I. Principle of the Method

Samples (10 g) of soil were fortified, as necessary, in a round bottom flask and extracted with 40 mL of acetonitrile via refluxing for 30 minutes using a reflux condenser and a heating block (pp. 12-13; Appendix 1, p. 38; Appendix 3, p. 40 of MRID 49520001). After cooling, an aliquot (2.5 mL) was transferred to a polypropylene centrifuge tube and centrifuged for 5 minutes at 3000 rpm. An aliquot (1.25 mL) of the supernatant (equivalent to 0.25 g) was transferred to a screw-cap, polypropylene centrifuge tube and diluted with ultra-pure water (4 mL). The mixture was extracted twice with 4 mL of n-hexane via shaking for 30 minutes. The upper layer was transferred to a clean polypropylene tube. The combined extracts were reduced to *ca*. 1 mL under a stream of nitrogen in a sample concentrator with heating block (40°C). A Florisil cartridge (Bond Elut Florisil SPE cartridges; 1 g, 6 mL) was pre-conditioned with methanol and dichloromethane: hexane (5:95, v:v; 5 mL each). The reduced extract was applied to the Florisil cartridge under gravity. The analyte was eluted using dichloromethane:hexane (40:60, v:v; 1 x 5 mL) into 15-mL test tubes. The eluate was evaporated to dryness under a stream of nitrogen and heating block set to 40°C. Toluene (1 mL) was added, and the sample was mixed via ultrasonication for 5 minutes in a bath of cold water. After vortexing, the sample was transferred to an auto-sampler vial and analyzed by GC/MS.

The ECM study author noted the following precautions for performing the extraction procedure: 1) for low level residue analysis, the use of sample container rinses and disposable labware was recommended to increase procedural recoveries and avoid cross-contamination; 2) if different SPE cartridges are used, the elution profile should be checked before analysis; 3) if the Florisil cartridge is larger than 1 g, 6 mL, the wash should be increased from 5 mL to 8 mL; and 4) a pH of 6 should be maintained since epimerization of lambda-cyhalothrin was observed at high pH (pp. 13-14 of MRID 49520001).

Samples were analyzed for lambda-cyhalothrin using a Hewlett Packard 6890 GC using a Hewlett Packard 5973 detector in negative ion chemical ionization mode (NICI; pp. 13-15; Appendix 1, p. 38 of MRID 49520001). The GC/MS conditions were as follows: HP-5MS column (30.0 m x 0.25 mm, 0.25 µm); helium carrier gas (1.0 mL/min.); injector temperature 275°C; oven temperature gradient, 1 min. 150°C to 1.5 min. 300°C at 20°C/min.; injection volume 4 µL; and mass spectrometer in chemical (SIM) mode with negative polarity. Lambda-cyhalothrin was identified and quantified using three ions (primary, confirmatory 1 and confirmatory 2): *m/z* 241, *m/z* 243 and *m/z* 205, respectively. The retention time for lambda-cyhalothrin was *ca*. 9.1 minutes. No further confirmation technique was employed.

The ILV was performed exactly as above using the same analytical instruments (p. 11; Appendix 1, pp. 34-83 of MRID 49721701). Lambda-cyhalothrin was identified and quantified using same three ions (primary, confirmatory 1 and confirmatory 2) as the ECM. The retention time for lambda-cyhalothrin was *ca*. 8.9 minutes (Figures 9-12, pp. 28-31). No modifications to the ECM were required.

In the ECM and ILV, the LOQ and LOD for lambda-cyhalothrin were 0.001 mg/kg (1 ppb) and 0.0002 µg/mL (equivalent to 0.8 pg injected on the column), respectively (pp. 18 of MRID 49520001; p. 8, 11-12 of MRID 49721701).

II. Recovery Findings

ECM (MRID 49520001): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of lambda-cyhalothrin in soil at the fortification level of 0.001 mg/kg (1 ppb; LOQ; Table 2, p. 23; DER Attachment 2). The number of samples (n = 3) was insufficient at the LOQ. No samples were fortified at 10×LOQ. Lambda-cyhalothrin was identified and quantified using one ion transition (*m/z* 241). Two confirmatory ions (*m/z* 243 and *m/z* 205) were monitored, but the recoveries were not quantified (p. 15; Figure 10, p. 35). The recovery results were corrected for residues quantified in the controls (pp. 15-16; Figures 9-10, pp. 34-35). The soil matrices were characterized (USDA soil texture classification; Table 1, p. 23). Sand soil (pH 7.8; 73% sand, 16% silt, 11% clay; 0.9% organic carbon) and loam soil (pH 7.3; 55% sand, 28% silt, 17% clay; 4.9% organic carbon) were used.

ILV (MRID 49721701): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements for analysis of lambda-cyhalothrin in soil at fortification levels of 0.001 mg/kg (1 ppb; LOQ) and 0.01 mg/kg (10 ppb; 10×LOQ; pp. 12-13; Table 3, p. 18). Lambda-cyhalothrin was identified and quantified using one ion transition (*m/z* 241). Two confirmatory ions (*m/z* 243 and *m/z* 205) were monitored, but the recoveries were not quantified (Figures 9-10, pp. 28-29). The results were not corrected since no residues were found in the controls (Figures 7-8, pp.

26-27). The method was validated for lambda-cyhalothrin at both fortification levels in both matrices after one trial with no modifications (p. 8). The soil matrices were not adequately characterized by Agvise Laboratories, Inc., Northwood, North Dakota (USDA soil texture classification; p. 10; Table 1, p. 16). Clay loam soil (CAPY081512; pH 6.9) and sandy loam soil (EXCEL FARM; pH 7.8) were used (organic matter or carbon percentages not reported).

Table 2. Initial Validation Method Recoveries for Lambda-cyhalothrin in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sand Soil						
Primary ion (*m/z* 241)						
Lambda-cyhalothrin	0.001 (LOQ)	**3**	75-92	86	10	11.4
	0.01		Not performed			
Loam Soil						
Primary ion (*m/z* 241)						
Lambda-cyhalothrin	0.001 (LOQ)	**3**	89-114	101	13	12.5
	0.01		Not performed			

Data (corrected recovery results; pp. 15-16; Figures 9-10, pp. 34-35) were obtained from Table 2, p. 23 of MRID 49520001. Standard deviations were reviewer-calculated from the data in the study report since the study author only reported means and RSDs (see DER Attachment 2).

1 The soil matrices were characterized (USDA soil texture classification; Table 1, p. 23). Sand soil (pH 7.8; 73% sand, 16% silt, 11% clay; 0.9% organic carbon) and loam soil (pH 7.3; 55% sand, 28% silt, 17% clay; 4.9% organic carbon) were used.

2 Recoveries for the confirmatory ions (*m/z* 243 and *m/z* 205) were not quantified.

Table 3. Independent Validation Method Recoveries for Lambda-cyhalothrin in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Soil						
Primary ion (*m/z* 241)						
Lambda-cyhalothrin	0.001 (LOQ)	5	98-115	103	7	7
	0.01	5	91-105	97	6	6
Sandy Loam Soil						
Primary ion (*m/z* 241)						
Lambda-cyhalothrin	0.001 (LOQ)	5	89-131	110	15	14
	0.01	5	101-109	104	4	3

Data (uncorrected recovery results; Figures 7-8, pp. 26-27) were obtained from pp. 12-13; Table 3, p. 18 of MRID 49721701.

1 The soil matrices were insufficiently characterized by Agvise Laboratories, Inc., Northwood, North Dakota (USDA soil texture classification; p. 10; Table 1, p. 16 of MRID 49721701). Clay loam soil (CAPY081512; pH 6.9) and sandy loam soil (EXCEL FARM; pH 7.8) were used. Organic matter or carbon percentages and sand/silt/clay percentages were not reported.

2 Recoveries for the confirmatory ions (*m/z* 243 and *m/z* 205) were not quantified.

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for lambda-cyhalothrin were 0.001 mg/kg (1 ppb) and 0.0002 µg/mL (equivalent to 0.8 pg injected on the column), respectively (pp. 9, 18 of MRID 49520001; pp. 8, 11-12 of MRID 49721701). In the ECM and ILV, the LOQ was defined as the lowest analyte concentration in a sample at which the method has been validated (mean recovery 70-110%, RSD ≤20%). The ECM study author also advised that the response for the analyte peak should be no less than four times the mean amplitude of the background noise at the analyte retention time in the control sample. In the ECM and ILV, the LOD was defined as the lowest analyte concentration which can be detected above the mean amplitude of the background noise at the analyte retention time in the control sample. The ECM and ILV study authors also noted that the LOD can be estimated as three times the background noise and will vary between instruments and analytical runs.

Table 4. Method Characteristics for Lambda-cyhalothrin in Soil

		Lambda-cyhalothrin
Limit of Quantitation (LOQ)		0.001 mg/kg (1 ppb)
Limit of Detection (LOD)		0.0002 µg/mL (equivalent to 0.8 pg injected on the column)
Linearity (calibration curve r^2 and concentration range)[1]	ECM	r^2 = 0.997 (*m/z* 241) (0.08-40 pg or 0.0002–0.01 µg/mL)
	ILV	r^2 = 1.00 (*m/z* 241) (0.08-200 pg or 0.00002–0.05 µg/mL)
Repeatable	ECM[2]	Yes at LOQ, but n= **3** **No** at 10×LOQ (no samples prepared)
	ILV[3]	Yes at LOQ and 10×LOQ
Reproducible		Yes
Specific	ECM	Representative chromatograms were only provided for one of the two soils (sand soil matrix). Residues in the controls were *ca*. 25% of the LOQ (*ca*. 30% of the LOQ) at the analyte retention time.[4]
	ILV	Yes; no matrix interferences were observed.

Data were obtained from pp. 9, 18; Table 2, p. 23; Figures 9-11, pp. 34-36 of MRID 49520001; p. 8, 11-13; Table 3, p. 18; Figures 7-13, pp. 26-32 of MRID 49721701.

1 Recoveries were only quantified for the primary ion transition (*m/z* 241). Two confirmatory ions (*m/z* 243 and *m/z* 205) were monitored, but the recoveries were not quantified. A confirmatory method is not required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.

2 In the ECM, sand soil (pH 7.8; 73% sand, 16% silt, 11% clay; 0.9% organic carbon) and loam soil (pH 7.3; 55% sand, 28% silt, 17% clay; 4.9% organic carbon) were used (USDA soil texture classification; Table 1, p. 23 of MRID 49721701).

3 In the ILV, clay loam soil (CAPY081512; pH 6.9) and sandy loam soil (EXCEL FARM; pH 7.8) were used (USDA soil texture classification; p. 10; Table 1, p. 16 of MRID 49721701). Organic matter or carbon percentages and sand/silt/clay percentages were not reported.

4 Based on Figure 4, p. 29 and Figures 9-10, pp. 34-35 of MRID 49520001.

IV. Method Deficiencies and Reviewer's Comments

1. The estimations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 9, 18 of MRID 49520001; pp. 8, 11-12 of MRID 49721701). In the ECM and ILV, the LOQ was defined as the lowest analyte concentration in a sample at which the method has been validated (mean recovery 70-110%, RSD ≤20%). The ECM study author also advised that the response for the analyte peak should be no less than four times the mean amplitude of the background noise at the analyte retention time in the control sample. In the ECM and ILV, the LOD was defined as the lowest analyte concentration which can be detected above the mean amplitude of the background noise at the analyte retention time in the control sample. The ECM and ILV study authors also noted that the LOD can be estimated as three times the background noise and will vary between instruments and analytical runs.

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

2. Due to the insufficient soil characterization data in the ILV, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. ILV soil matrices were reported with USDA soil texture classification; however, organic matter or carbon percentages and sand/silt/clay percentages were not provided (p. 10; Table 1, p. 16 of MRID 49721701).

3. The ECM report MRID 49520001 contained several deficiencies for satisfying OCSPP Guideline 850.6100 criteria.

   The number of samples (n = 3) was insufficient at the LOQ (Table 2, p. 23 of MRID 49520001). OCSPP Guidelines require that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10×LOQ) for each analyte.

   No samples were prepared at 10×LOQ. OCSPP Guidelines require that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10×LOQ) for each analyte.

   Residues in the controls were observed at *ca*. 25% of the LOQ (*ca*. 30% of the LOQ) at the retention time of lambda-cyhalothrin (Figure 4, p. 29; Figures 9-10, pp. 34-35 of MRID 49520001). OCSPP Guidelines require that the matrix blank was free of interference.

   Method recoveries were corrected for residues quantified in the controls (pp. 15-16; Figures 9-10, pp. 34-35 of MRID 49520001). OCSPP Guidelines require that recoveries were not corrected for reagent blanks, matrix blanks, or other recoveries.

   Representative chromatograms were only provided for one of the two soil matrices (sand soil; Figures 9-10, pp. 34-35 of MRID 49520001). OCSPP Guidelines require that chromatograms were provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix.

4. One significant typographical error was noted in the ILV: the LOQ was reported as "0.10 µg/L (ppb) for lambda-cyhalothrin in soil", instead of 0.001 mg/kg (1 ppb; p. 14 of MRID 49721701).

   Three typographical errors were noted in the ECM: the appendix for the analytical procedure flow-chart was reported as "**Appendix 4**", instead of "**Appendix 3**" (pp. 12, 40 of MRID 49520001); the GC column dimensions were reported as "30.0 m x **0.25 m**, 0.25 µm", instead of "30.0 m x **0.25 mm**, 0.25 µm" in Appendix 1 (p. 38); and the calibration range was incorrectly reported as "0.00002 to **0.5** µg/mL injected on column (equivalent to 0.08 to **2000** pg on column", instead of 0.00002 to **0.05** µg/mL injected on column (equivalent to 0.08 to **200** pg on column" (p. 19; Figure 11, p. 36).

5. In the ECM, lambda-cyhalothrin was stable in the final extracts in n-hexane when stored at *ca*. 0-9°C for up to 12-13 days (p. 19; Table 4, p. 24 of MRID 49520001).

6. In the ECM, matrix effects were assessed. Although no matrix effects were observed in the soil matrices tested, the study author recommended that matrix-matched standards be used to "compensate for any matrix effects observed" (p. 19; Table 3, p. 23 of MRID 49520001).

7. It was reported in the ECM that one analyst could complete a batch of 12 soil samples in 8 hours (one working day; p. 20 of MRID 49520001). No timeframe for analysis was reported in the ILV.

8. The summary of communications between the independent laboratory and Syngenta study monitors and study director was provided (p. 13; Appendix 4, p. 90 of MRID 49721701). The only communication was the clarification of the fortification standard solvent.

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

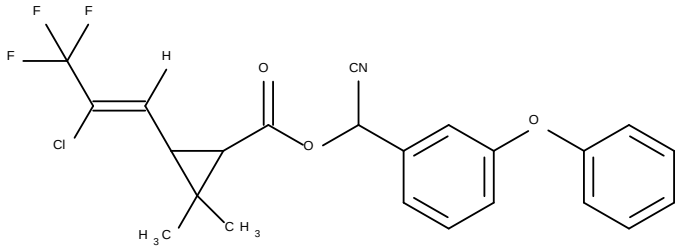
Lambda-cyhalothrin

IUPAC Name: Reaction product comprising equal quantities of (R)-α-cyano-3-phenoxybenzyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and (S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate

CAS Name: (R)-cyano(3-phenoxyphenyl)methyl (1S,3S)-rel-3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate

CAS Number: 91465-08-6

SMILES String: FC(F)(F)C(Cl)=CC1C(C)(C)C1C(=O)OC(C#N)c2cc(Oc3ccccc3)ccc2



Test Material:	Lambda-cyhalothrin
MRID:	49520001
Title:	Lambda-cyhalothrin: Lambda-cyhalothrin –Residue Method (GRM043.05B) for the Determination of Lambda-cyhalothrin in Soil – Analytical Method
MRID:	49721701
Title:	Lambda-cyhalothrin: Lambda-cyhalothrin – Independent Laboratory Validation of Residue Method (GRM043.05B) for the Determination of Lambda-cyhalothrin in Soil – Final ILV Report
EPA PC Code:	128897
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 1/12/16

Secondary Reviewer: Kathleen Ferguson **Signature:**

Date: 1/12/16

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 1/12/16