

Analytical method for total d-phenothrin (sum of cis- and trans- isomers) in soil

Reports: ECM: EPA MRID No. 50600160. Class, T. 2013. Independent Laboratory Validation (ILV) of an Analytical Method for the Determination of the cis- and trans Isomers of d-Phenothrin in Soil by GC/MS. PRTL Europe ID: P 3047 G. Report prepared by PRTL Europe, Ulm, Germany, sponsored and submitted by Sumitomo Chemical Company, Tokyo, Japan, and submitted by Sumitomo Chemical America, Inc., New York, New York; 38 pages. Final report issued November 28, 2013.

ILV: EPA MRID No. 50914201. Shen, H. 2019. Independent Laboratory Validation of an Analytical Method for the Determination of the cis- and trans Isomers of d-Phenothrin in Soil by GC/MS. Project No.: 2950W. Report prepared by EAG Laboratories-Hercules (now Eurofins EAG Agrosience, LLC), Hercules, California, sponsored and submitted by Sumitomo Chemical Company, Tokyo, Japan, and submitted by Sumitomo Chemical America, Inc., New York, New York; 106 pages. Final report issued July 3, 2019.

Document No.: MRIDs 50600160 & 50914201

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with German Good Laboratory Practices (GLP; 2011), which are based on OECD GLP standards which are accepted by Regulatory Authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI; pp. 3, 5; Appendix 3, p. 38 of MRID 50600160). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-5). A statement of the authenticity of the study report was included with the Quality Assurance statement.

ILV: The study was conducted in accordance with USEPA GLP standards (40 CFR Part 160; p. 3 of MRID 50914201). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement.

Classification: This analytical method is classified as **unacceptable**. The specificity of the method using GC/MS (SIM) analysis was not supported by ILV representative chromatograms. It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation (TFD) studies. In the ECM, chromatograms of 10×LOQ were not included. The ECM should be updated to include these

details of Florisil column packing. A reagent blank was not included in the ECM.

PC Code: 069005

Reviewer: Kristy Crews, Ph.D., Chemist

Signature:



Digitally signed
by KRISTY CREWS
Date: 2020.05.13
18:05:29 -04'00'

Date:

**CDM/CSS-
Dynamac JV**
Lisa Muto, M.S.,
Environmental Scientist

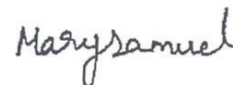
Signature:



Date: 04/30/2020

Reviewers: Mary Samuel, M.S.,
Environmental Scientist

Signature:



Date: 04/30/2020

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

This analytical method, PRTL Europe ID P 3047 G and Sumitomo ID: ER-31-0020, is designed for the quantitative determination of the total d-phenothrin (sum of cis- and trans- isomers) at 0.01 mg/kg in soil using GC/MS(SIM) and GC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in soil¹. The ECM and ILV validated the method using different characterized sandy loam soil matrices. It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the TFD studies since only one soil matrix was used. The ILV validated the method with the first trial as written with insignificant analytical instrument and equipment modifications; however, the study author noted that results obtained using GC/MS/MS demonstrated better overall selectivity and reproducibility than results obtained using GC/MS (SIM). The ECM should be updated to include missing details of Florisil column packing. All ILV and ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for d-phenothrin, as well as cis- and trans-phenothrin, except for the LOQ analysis of cis-phenothrin monitoring the *m/z* 123 ion using GC/MS (SIM). All ILV data regarding specificity was satisfactory using GC/MS/MS, but the specificity of the method using GC/MS (SIM) analysis was not supported by ILV representative chromatograms since analyte peaks for cis- and trans-phenothrin were not resolved from each other. All ECM data regarding specificity was satisfactory for cis- and trans-phenothrin using GC/MS(SIM) and GC/MS/MS; however, chromatograms of 10×LOQ fortifications were not included. Note: ECM MRID 50600160 was previously submitted as ILV MRID 49305301; no changes were made to the study report

¹ MRID 47431603

between submissions.

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						
d- Phenothrin ¹	50600160 ^{2,3}	50914201 ⁴		Soil	28/11/2013 (ECM) 17/08/1993 (Original ECM) ³	Sumitomo Chemical Company	GC/MS	0.01 mg/kg (10 ng/g)

1 Sum of cis- and trans- isomers; Sumithrin.

2 In the ECM, the soil matrix was sandy loam (Speyer 5M; pH 7.2 ± 0.1 (in CaCl₂); 61.5% ± 3.3%, silt 27.7 ± 2.5%, clay 10.9% ± 1.2%; 1.27% ± 0.50% organic carbon; 15.0 ± 3.0 meq/100 g cation exchange capacity; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34 of MRID 50600160).

3 The ECM was performed to validate another ECM (Jacobson, B, *et. al.* 1993. Dissipation of Sumithrin Applied to Bare Ground, California Location. ABC Laboratories Report ID #40310. Sumitomo ID: ER-31-0020. Pages 25-28 (total pages not reported); p. 9 of MRID 50600160).

4 In the ILV, the soil matrix was sandy loam (Specimen ID: 2705W-069; pH 7.4 (in water); pH 7.2 (in CaCl₂); sand 61%, silt 34%, clay 5%; 1.5% organic matter – Walkley Black; 19.8 meq/100 g cation exchange capacity; USDA soil texture classification); it was well characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, p. 100 of MRID 50914201). The soil was collected from Othello, Washington.

I. Principle of the Method

The ECM test material was d-phenothrin (TG, technical grade), which contained 97.0% (1R)-isomers (p. 11 of MRID 50600160). The ratio of trans/cis- isomer was 80.31/19.69 (reported by Sponsor). The ILV test material was d-phenothrin (Technical; 96.2% purity; provided by the Sponsor), which contained 96.1% (1R)-isomers (p. 19; Appendix B, p. 97 of MRID 50914201). The ratio of trans/cis- isomer was 80.4/19.6%.

Samples (20 g, dry weight) of sieved (5 mm) soil were fortified with 100 µL of 2.0 µg/mL or 20 µg/mL fortification solution, as necessary, then extracted twice using 40 mL of methanol for 10 minutes then filtered using a Büchner funnel packed with glass-fiber filter paper under vacuum (pp. 9, 14-15 of MRID 50600160). The residue was rinsed with *ca.* 30 mL of methanol. The combined organic extracts and filtrate were transferred to a separatory funnel and extracted with 80 mL of 10% aqueous sodium chloride and 40 mL of dichloromethane. The lower dichloromethane layer was passed through a bed of anhydrous sodium sulphate contained in a filter funnel. The remaining aqueous layer was extracted with 40 mL of dichloromethane. The lower dichloromethane layer was drained and filtered through the same filter funnel as before. The combined dried dichloromethane extracts were reduced to dryness by rotary evaporation at *ca.* 30°C. The residue was applied to an activated Florisil (activated overnight 130°C) in a glass column. Four 3-mL portions of hexane:ethyl acetate (20:1, v:v) were used to transfer the residue to the column, draining between each portion. The analyte was eluted using 45 mL of hexane:ethyl acetate (20:1, v:v). The first 5 mL of eluate was discarded. The following 40 mL

was collected and reduced to 1-2 mL by rotary evaporation at <40°C then to dryness under nitrogen. The residue was reconstituted in 1.0 mL of toluene prior to GC/MS analysis.

Samples were analyzed for d-phenothrin (Sumithrin) using gas chromatography with mass spectrometry (GC/MS) analysis (p. 12 of MRID 50600160). A Thermo Trace 1310 Gas Chromatograph was equipped with an Optima 5-MS Accent (Macherey-Nagel) column (30 m x 0.25 mm i.d., 0.25 µm thickness; injection temperature 225°C) and an TSQ 8000 triple-quadrupole Mass Spectrometer with positive EI. Two types of detection were used: SIM and MS/MS. Injection volume was 2 µL; carrier gas was helium (1.5 mL/minute). The oven temperature program was as follows: 95°C for 0.75 min., then with 15°C/min. to 250°C, finally with 10°C/min. to 275°C, 7 min. hold. Ions transitions monitored with SIM for d-phenothrin were m/z 183 (quantitation), m/z 123 (confirmation 1) and m/z 184 (confirmation 2). Ions transitions monitored with MS/MS for d-phenothrin were m/z 183 → 168 (quantitation), m/z 183 → 165 (confirmation 1) and m/z 183 → 153 (confirmation 2). Observed approximate retention times were 13.59 minutes for cis-phenothrin and 13.66 minutes for trans-phenothrin (Figure 8, p. 30).

The ILV performed the ECM method for d-phenothrin as written with insignificant analytical instrument and equipment modifications (pp. 10, 20-29 of MRID 50914201). The ILV noted that 1) the Florisil was combined with hexane:ethyl acetate (20:1, v:v) for slurry preparation; 2) the Florisil column was topped with 1 g of anhydrous sodium sulfate; and 3) the final concentration flask was rinsed with 2 x 5 mL of hexane and those rinses were transferred to a 15 mL centrifuge tube. For GS/MS analysis, an Agilent 7000 Series Triple Quadrupole Mass Spectrometer (GC-QQQ) coupled with an Agilent 7890A Series gas chromatograph was used. GC/MS parameters were the same as the ECM with the following exceptions: DB-5MS column (30 m x 0.25 mm i.d., 0.25 µm thickness). Ions transitions monitored with SIM for d-phenothrin were m/z 184 (quantitation), m/z 183 (confirmation 1) and m/z 123 (confirmation 2). These differed from those of the ECM. Ions transitions monitored with MS/MS for d-phenothrin were m/z 183 → 168 (quantitation), m/z 183 → 165 (confirmation 1) and m/z 183 → 153 (confirmation 2). These were the same as those of the ECM. Reported approximate retention time was 14.0 minutes for d-phenothrin.

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for d-phenothrin in soil were reported as 0.01 mg/kg and 0.002 mg/kg, respectively, in the ECM and ILV (pp. 10, 16 of MRID 50600160; pp. 10, 33 of MRID 50914201).

II. Recovery Findings

ECM (MRID 50600160): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of d-phenothrin (sum of cis- and trans-isomers) in one soil at the LOQ (0.01 mg/kg) and 10×LOQ (0.1 mg/kg) using both GC/MS (SIM) and GC/MS/MS analysis (Tables 1-2, pp. 19-20). Performance data was also acceptable for the separate quantification of the cis- and trans-isomers of phenothrin. Three ions or ion transitions were monitored in either GC/MS (SIM) or GC/MS/MS analysis. Recovery results of

the quantitative and confirmatory ions or ion transitions were comparable; recovery results between GC/MS (SIM) and GC/MS/MS analysis were also comparable. The soil matrix was sandy loam (pH 7.2 ± 0.1 ; sand 61.5 ± 3.3 , silt 27.7 ± 2.5 , clay 10.9 ± 1.2 ; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34).

ILV (MRID 50914201): Mean recoveries and RSDs met requirements for analysis of d-phenothrin (sum of cis- and trans-isomers) in one soil at the LOQ (0.01 mg/kg) and 10×LOQ (0.1 mg/kg) using both GC/MS (SIM) and GC/MS/MS analysis (Table I, pp. 40-45). Performance data was also acceptable for the separate quantification of the cis- and trans-isomers of phenothrin, except for the LOQ analysis of cis-phenothrin monitoring the *m/z* 123 ion using GC/MS (SIM; RSD 65%). Three ions or ion transitions were monitored in either GC/MS (SIM) or GC/MS/MS analysis. Recovery results of the quantitative and confirmatory ions or ion transitions were comparable, except for recoveries of *m/z* 123 in GC/MS (SIM); recovery results between GC/MS (SIM) and GC/MS/MS analysis were fairly comparable, except for recoveries of *m/z* 123 in GC/MS (SIM). Results were reportedly corrected for residues quantified in the controls (pp. 30-31). The soil matrix was sandy loam (Specimen ID: 2705W-069; pH 7.4 (in water); pH 7.2 (in CaCl₂); sand 61%, silt 34%, clay 5%; 1.5% organic matter – Walkley Black; 19.8 meq/100 g cation exchange capacity; USDA soil texture classification); it was well characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, p. 100). The soil was collected from Othello, Washington. The method was validated by the ILV with the first trial as written with insignificant analytical instrument and equipment modifications; however, the study author noted that results obtained using GC/MS/MS demonstrated better overall selectivity and reproducibility than results obtained using GC/MS (SIM; pp. 10-11, 20-29, 39).

Table 2. Initial Validation Method Recoveries for d-Phenothrin in Soil¹

Analyte	Fortification Level (mg/kg)	Number of Tests ²	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
GC/MS (SIM)						
<i>m/z</i> 183						
cis-Phenothrin	LOQ ⁴	5	95-105	100	4	3.5
	10×LOQ ⁴	5	81-88	85	3	3.8
trans-Phenothrin	LOQ ⁴	5	104-109	106	2	2.1
	10×LOQ ⁴	5	83-94	87	4	5.0
d-Phenothrin ⁵	0.01 (LOQ)	5	103-108	105	2	2.1
	0.1	5	83-93	87	4	4.7
<i>m/z</i> 123						
cis-Phenothrin	LOQ ⁴	5	79-96	89	7	7.5
	10×LOQ ⁴	5	76-85	80	3	3.9
trans-Phenothrin	LOQ ⁴	5	105-110	106	2	1.8

Analyte	Fortification Level (mg/kg)	Number of Tests ²	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
	10×LOQ ⁴	5	75-89	81	6	6.7
d-Phenothrin ⁵	0.01 (LOQ)	5	102-104	103	1	0.9
	0.1	5	75-88	81	5	6.1
<i>m/z</i> 184						
cis-Phenothrin	LOQ ⁴	5	99-103	102	2	1.5
	10×LOQ ⁴	5	84-90	87	3	2.9
trans-Phenothrin	LOQ ⁴	5	102-107	104	2	2.1
	10×LOQ ⁴	5	82-93	86	4	5.0
d-Phenothrin ⁵	0.01 (LOQ)	5	102-106	103	2	1.9
	0.1	5	82-92	87	4	4.6
GC/MS/MS						
Quantitation ion transition (<i>m/z</i> 183 → 168)						
cis-Phenothrin	LOQ ⁴	5	92-98	94	2	2.3
	10×LOQ ⁴	5	81-94	87	6	6.7
trans-Phenothrin	LOQ ⁴	5	100-105	102	2	1.9
	10×LOQ ⁴	5	84-96	88	5	6.0
d-Phenothrin ⁵	0.01 (LOQ)	5	99-103	101	2	2.0
	0.1	5	83-95	88	5	6.1
Confirmation ion transition 1 (<i>m/z</i> 183 → 165)						
cis-Phenothrin	LOQ ⁴	5	96-101	98	2	2.2
	10×LOQ ⁴	5	82-96	88	6	6.8
trans-Phenothrin	LOQ ⁴	5	100-105	102	2	2.3
	10×LOQ ⁴	5	84-96	88	5	6.2
d-Phenothrin ⁵	0.01 (LOQ)	5	99-105	102	2	2.3
	0.1	5	83-96	88	5	6.3
Confirmation ion transition 2 (<i>m/z</i> 183 → 153)						
cis-Phenothrin	LOQ ⁴	5	92-99	95	3	2.8
	10×LOQ ⁴	5	82-94	87	5	6.2
trans-Phenothrin	LOQ ⁴	5	99-103	101	2	1.9
	10×LOQ ⁴	5	82-95	87	6	6.2
d-Phenothrin ⁵	0.01 (LOQ)	5	98-103	100	2	2.0
	0.1	5	83-95	87	5	6.1

Data (uncorrected results, p. 15) were obtained from Tables 1-2, pp. 19-20 of MRID 50600160 and DER Attachment 2 (calculation of s.d.).

1 The soil matrix was sandy loam (Speyer 5M; pH 7.2 ± 0.1 (in CaCl₂); 61.5% ± 3.3%, silt 27.7 ± 2.5%, clay 10.9% ± 1.2%; 1.27% ± 0.50% organic carbon; 15.0 ± 3.0 meq/100 g cation exchange capacity; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34). The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 The recovery value for the first sample of each set of five was the mean of two injections.

3 Standard deviations were reviewer-calculated using the reported values in the study report since these values were not reported in the study report. Rules of significant figures were followed.

4 Recoveries from d-phenothrin LOQ and 10×LOQ samples.

5 Sum of cis- and trans- isomers. The recoveries of the cis and trans isomers were independently calculated then summed to determine total d-phenothrin recovery (p. 15; Tables 1-2, pp. 19-20).

Table 3. Independent Validation Method Recoveries for d-Phenothrin (Sumithrin) in Soil¹

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
GC/MS (SIM)						
<i>m/z</i> 184						
cis-Phenothrin	LOQ ²	5	97-120	104	9	9
	10×LOQ ²	5	103-117	108	6	6
trans-Phenothrin	LOQ ²	5	106-127	113	9	8
	10×LOQ ²	5	88-98	93	4	4
d-Phenothrin ³	0.01 (LOQ)	5	105-126	111	9	8
	0.1	5	90-101	96	5	5
<i>m/z</i> 183						
cis-Phenothrin	LOQ ²	5	93-114	101	8	8
	10×LOQ ²	5	96-112	103	7	7
trans-Phenothrin	LOQ ²	5	101-123	110	8	7
	10×LOQ ²	5	93-100	96	3	3
d-Phenothrin ³	0.01 (LOQ)	5	100-122	108	8	7
	0.1	5	94-102	97	4	4
<i>m/z</i> 123						
cis-Phenothrin	LOQ ²	5	53-244	119	77	65
	10×LOQ ²	5	89-120	102	13	13
trans-Phenothrin	LOQ ²	5	95-116	102	8	8
	10×LOQ ²	5	90-104	95	6	6
d-Phenothrin ³	0.01 (LOQ)	5	87-141	105	21	20
	0.1	5	90-107	96	7	7
GC/MS/MS						
Quantitation ion transition (<i>m/z</i> 183 → 168)						
cis-Phenothrin	LOQ ²	5	104-116	110	4	4
	10×LOQ ²	5	99-107	105	3	3
trans-Phenothrin	LOQ ²	5	113-122	118	3	3
	10×LOQ ²	5	105-111	109	2	2
d-Phenothrin ³	0.01 (LOQ)	5	112-120	116	3	3
	0.1	5	104-110	108	2	2
Confirmation ion transition 1 (<i>m/z</i> 183 → 165)						
cis-Phenothrin	LOQ ²	5	101-112	108	4	4
	10×LOQ ²	5	98-108	105	4	4
trans-Phenothrin	LOQ ²	5	112-122	117	4	3
	10×LOQ ²	5	105-110	108	2	2
d-Phenothrin ³	0.01 (LOQ)	5	110-119	115	3	3
	0.1	5	104-110	108	2	2
Confirmation ion transition 2 (<i>m/z</i> 183 → 153)						
cis-Phenothrin	LOQ ²	5	104-113	109	3	3
	10×LOQ ²	5	99-108	105	4	4
trans-Phenothrin	LOQ ²	5	113-122	117	3	3
	10×LOQ ²	5	105-110	108	2	2
d-Phenothrin ³	0.01 (LOQ)	5	111-119	115	3	3
	0.1	5	104-110	108	2	2

Data (results were reportedly corrected for residues quantified in the controls, pp. 30-31) were obtained from Table I, pp. 40-45 of MRID 50914201.

- 1 The soil matrix was sandy loam (Specimen ID: 2705W-069; pH 7.4 (in water); pH 7.2 (in CaCl₂); sand 61%, silt 34%, clay 5%; 1.5% organic matter – Walkley Black; 19.8 meq/100 g cation exchange capacity; USDA soil texture classification); it was well characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, p. 100). The soil was collected from Othello, Washington. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.
- 2 Recoveries from d-phenothrin LOQ and 10×LOQ samples.
- 3 Sum of cis- and trans- isomers. The recoveries of the cis and trans isomers were independently calculated then summed to determine total d-phenothrin recovery.

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for d-phenothrin (sum of cis and trans isomers) in soil were reported as 0.01 mg/kg and 0.002 mg/kg, respectively (pp. 10, 16 of MRID 50600160; pp. 10, 32-33 of MRID 50914201). In the ILV, the LOQ and LOD were calculated for each isomer of phenothrin based on the ratios; the LOQ and LOD were calculated as 160.8 ng/mL and 32.2 ng/mL, respectively, for trans-phenothrin and 39.2 ng/mL and 7.8 ng/mL, respectively, for cis-phenothrin. In the ECM, the LOQ was supported by the successful validation of the analytical method at that fortification level, and the LOD was defined as 20% of the LOQ. No justifications for the LOQ and LOD were reported in the ILV. No calculations for the LOQ and LOD were reported in the ILV or ECM.

Table 4. Method Characteristics

		cis-Phenothrin	trans-Phenothrin	d-Phenothrin ¹	
Limit of Quantitation (LOQ)	ECM	Not reported		0.01 mg/kg	
	ILV	39.2 ng/mL (calc)	160.8 ng/mL (calc)		
Limit of Detection (LOD)	ECM	Not reported		0.002 mg/kg	
	ILV	7.8 ng/mL (calc)	32.2 ng/mL (calc)		
Linearity (calibration curve <i>r</i> and concentration range) ²	ECM	SIM	<i>r</i> = 0.9990 (<i>m/z</i> 183) <i>r</i> = 0.9986 (<i>m/z</i> 123) <i>r</i> = 0.9991 (<i>m/z</i> 184)	<i>r</i> = 0.9975 (<i>m/z</i> 183) <i>r</i> = 0.9989 (<i>m/z</i> 123) <i>r</i> = 0.9976 (<i>m/z</i> 184)	N/A ³
		MS/MS	<i>r</i> = 0.9989 (Q & C1) <i>r</i> = 0.9986 (C2)	<i>r</i> = 0.9969 (Q) <i>r</i> = 0.9967 (C1) <i>r</i> = 0.9970 (C2)	N/A ³
	ILV	SIM	<i>r</i> = 0.9994 (<i>m/z</i> 184) <i>r</i> = 0.9973 (<i>m/z</i> 183) <i>r</i> = 0.9991 (<i>m/z</i> 123)	<i>r</i> = 0.9990 (<i>m/z</i> 184) <i>r</i> = 0.9993 (<i>m/z</i> 183) <i>r</i> = 0.9986 (<i>m/z</i> 123)	N/A ³
		MS/MS	<i>r</i> = 0.9999 (Q) <i>r</i> = 0.9999 (C1) <i>r</i> = 1.0000 (C2)	<i>r</i> = 0.9994 (Q, C1 & C2)	N/A ³
	Range		4.0-1000 ng/mL	40-2500 ng/mL	N/A
Repeatable	ECM ^{4,5}	Yes for LOQ and 10×LOQ			
	ILV ^{6,7}	Yes for LOQ and 10×LOQ, except for <i>m/z</i> 123 (SIM; RSD 65%).	Yes for LOQ and 10×LOQ		
Reproducible	Yes for LOQ and 10×LOQ				
Specific	ECM		Yes, matrix interferences were <10% of the LOQ (based on peak area) for all three monitored ions.		N/A ³
		SIM	Multiple nearby contaminants (peak height <i>ca.</i> 40-70% of cis-Phenothrin LOQ peak) were noted. Significant interference was noted in <i>m/z</i> 123 SIM for cis-Phenothrin at LOQ.		
		MS/MS	Yes, matrix interferences were <1% of the LOQ (based on peak area) for all three monitored ion transitions.		
		No 10×LOQ representative chromatograms were provided.			
ILV	SIM	No , matrix interferences were <5% of the LOQ (based on peak area) for all three monitored ions; however, analyte peaks for cis- and trans-phenothrin were not resolved from each other. ⁸ Multiple nearby contaminants (peak height <i>ca.</i> 50-100% of cis-Phenothrin LOQ peak) were noted.		N/A ³	
	MS/MS	Yes, no matrix interferences were observed for all three monitored ion transitions			

Data were obtained from pp. 10, 16 (LOQ/LOD); Tables 1-2, pp. 19-20 (recovery data); Figures 2-3, pp. 24-25 (calibration curves); Figures 7-11, pp. 29-33 (chromatograms) of MRID 50600160; pp. 10, 32-33 (LOQ/LOD); Table I, pp. 40-45 (recovery data); Figure 2, pp. 54-65 (calibration curves); Figures 4-7, pp. 74-89 (chromatograms)

of MRID 50914201; DER Attachment 2. Q = quantitative ion transition (m/z 183 \rightarrow m/z 168); C1 = confirmatory 1 ion transition (m/z 183 \rightarrow m/z 165); C2 = confirmatory 2 ion transition (m/z 183 \rightarrow m/z 153).

1 Sum of cis- and trans- isomers. 3-Phenoxybenzyl (1R)-*cis-trans*-chrysanthemate; 3-Phenoxybenzyl (1RS)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate.

2 Reported r values were reviewer-calculated from r^2 values reported in the study reports (Figures 2-3, pp. 24-25 of MRID 50600160; Figure 2, pp. 54-65 of MRID 50914201). Reported r values were limited to 4 significant figures.

3 The recoveries of the cis and trans isomers were independently calculated then summed to determine total d-phenothrin recovery.

4 In the ECM, the soil matrix was sandy loam (Speyer 5M; pH 7.2 ± 0.1 (in CaCl_2); $61.5\% \pm 3.3\%$, silt $27.7 \pm 2.5\%$, clay $10.9\% \pm 1.2\%$; $1.27\% \pm 0.50\%$ organic carbon; 15.0 ± 3.0 meq/100 g cation exchange capacity; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34 of MRID 50600160).

5 Although the title of ECM MRID 50600160 identified the study report as an ILV; the MRID was submitted as an 5ECM. ILV MRID 50914201 identified MRID 50600160 as its ECM (p. 10 of MRID 50914201).

6 In the ILV, the soil matrix was sandy loam (Specimen ID: 2705W-069; pH 7.4 (in water); pH 7.2 (in CaCl_2); sand 61%, silt 34%, clay 5%; 1.5% organic matter – Walkley Black; 19.8 meq/100 g cation exchange capacity; USDA soil texture classification); it was well characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, p. 100 of MRID 50914201). The soil was collected from Othello, Washington.

7 The ILV validated the method with the first trial as written with insignificant analytical instrument and equipment modifications; however, the study author noted that results obtained using GC/MS/MS demonstrated better overall selectivity and reproducibility than results obtained using GC/MS (SIM; pp. 10-11, 20-29, 39 of MRID 50914201).

8 Based on Figure 6, p. 82, of MRID 50914201.

IV. Method Deficiencies and Reviewer's Comments

1. A method validation for total d-phenothrin (sum of cis- and trans- isomers) in soil was previously submitted and reviewed by CDM Smith/CSS-Dynamac JV. A DER was prepared for MRIDs 49625701 & 49305301 in 2016 by primary reviewer Lisa Muto and QC/QA manager Joan Gaidos. MRID 49305301 was submitted as the ILV for that method validation. The following deficiencies were found with that method validation submission: 1) The ILV was not performed to validate the submitted ECM; an updated ECM should be provided with the full detailed method which was validated by the ILV, as well as recovery results and chromatograms supporting the LOQ using that method; 2) Determinations of the LOQ and LOD were not based on scientifically acceptable procedures; 3) The LOD was not reported in the ECM; 4) In the ECM, no samples were prepared at the LOQ or $10\times$ LOQ, and the number of samples was insufficient at test fortifications; 5) In the ECM, the method could not be evaluated for specificity based on provided chromatograms; 6) The soil matrix of the ECM was not characterized; 7) In the ILV, linearity of the calibration curves for MS/MS analysis of trans-phenothrin were not satisfactory, and chromatograms of $10\times$ LOQ were not included; and 8) A reagent blank was not included in the ECM and ILV.

MRID 49305301 is the exact same report as MRID 50600160. For this DER, information for MRID 50600160 was validated. The recovery results of cis- and trans-phenothrin were added. Representative chromatograms were reassessed. One of the previous deficiencies was no longer valid (linearity of the calibration curves for MS/MS analysis

- of trans-phenothrin were not satisfactory) since OSCPP 850.6100 guideline linearity requirement guidance was updated in November 2019.
2. The specificity of the method using GC/MS (SIM) analysis was not supported by ILV representative chromatograms since analyte peaks for cis- and trans-phenothrin were not resolved from each other (Figure 6, p. 82, of MRID 50914201). Accurate integration and quantification were not possible using the analytical method. Additionally, multiple nearby contaminants (peak height *ca.* 50-100% of cis-Phenothrin LOQ peak) were noted.
 3. Only one soil matrix was included in the ILV: sandy loam (Specimen ID: 2705W-069; pH 7.4 (in water); pH 7.2 (in CaCl₂); sand 61%, silt 34%, clay 5%; 1.5% organic matter – Walkley Black; 19.8 meq/100 g cation exchange capacity; USDA soil texture classification; p. 21; Appendix C, p. 100 of MRID 50914201). It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation (TFD) studies. 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. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies. In the submitted d-phenothrin aerobic soil metabolism study (MRID 50600159), three soils were studied: two silt loam soils and one sandy loam soil (Table 1, p. 40 of MRID 50600159).
 4. In the ECM, chromatograms of 10×LOQ were not included. Representative chromatograms should be provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix to evaluate the specificity of the method.
 5. The communications between the ILV laboratory and study sponsor was summarized (p. 34 of MRID 50914201). Communications involved clarification of details of Florisil column packing, isomer ratio of test material, and exchange of ILV trial results. The details of Florisil column packing were termed “missing experimental details” which presumably included that 1) the Florisil was combined with hexane:ethyl acetate (20:1, v:v) for slurry preparation; and 2) the Florisil column was topped with 1 g of anhydrous sodium sulfate (pp. 25-27, 34 of MRID 50914201). These details were not included in the ECM. The reviewer suggests that the ECM should be updated to include these details of Florisil column packing.
 6. ILV performance data was not acceptable for the LOQ analysis of cis-phenothrin monitoring the *m/z* 123 ion using GC/MS (SIM; RSD 65%; Table I, pp. 40-45 of MRID 50914201). Performance data was acceptable for the summation of the cis- and trans-phenothrin into d-phenothrin for *m/z* 123 ion using GC/MS (SIM). The *m/z* 123 ion was the confirmatory 2 ion for GC/MS (SIM) analysis; the reviewer noted that a confirmatory method is not usually required when LC/MS and/or GC/MS is the primary method for

- generating study data. OSCPP 850.6100 guidelines state that mean recoveries are 70-120% and RSDs are $\leq 20\%$. The ILV study author noted that results obtained using GC/MS/MS demonstrated better overall selectivity and reproducibility than results obtained using GC/MS (SIM; p. 39 of MRID 50914201).
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, 16 of MRID 50600160; pp. 10, 32-33 of MRID 50914201). In the ECM, the LOQ was supported by the successful validation of the analytical method at that fortification level, and the LOD was defined as 20% of the LOQ. No justifications for the LOQ and LOD were reported in the ILV. No calculations for the LOQ and LOD were reported in the ILV or ECM. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
 8. In the ECM, no reagent blank was included. OCSPP guidelines recommend that a minimally complete sample set includes a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at $10\times$ LOQ for each matrix.
 9. In the ILV, it was reported that linear regression of both *cis* and *trans* d-Phenothrin was conducted for GC-MS/MS analysis results in MRM mode (p. 36 of MRID 50914201). Quadratic regression of both *cis* and *trans* d-Phenothrin was conducted for GC-MS analysis results in SIM mode, although the ECM used linear regression. The different GC/MS instrument from different manufacturers used in the ILV caused a difference in the method sensitivity and linearity range.
 10. In the ECM and ILV, no significant matrix effects were observed using either GC/MS (SIM) or GC/MS/MS analysis ($<20\%$; p. 14; Table 3, p. 21 of MRID 50600160; pp. 35-36; Table II, pp. 46-49 of MRID 50914201).
 11. It was reported in the ILV that 13 samples required *ca.* 16 person-hours with *ca.* 6 hours for preparation of standard solutions, *ca.* 8 hours to complete the sample processing, and *ca.* 2 hours for GC/MS (SIM) and GC/MS/MS analysis (pp. 33-34 of MRID 50914201).

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.

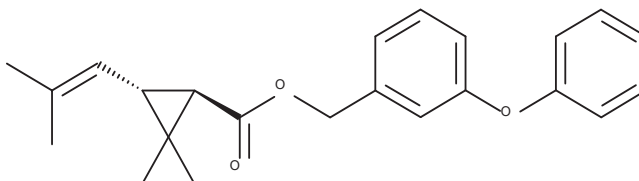
Attachment 1: Chemical Names and Structures**d-Phenothrin (1R trans/cis ratio = ca. 80/20) [Sumithrin]**

IUPAC Name: 3-Phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate.
3-Phenoxybenzyl (1*RS*)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate.
3-Phenoxybenzyl (±)-*cis-trans*-chrysanthemate.
3-Phenoxybenzyl (1*R*)-*cis-trans*-chrysanthemate.

CAS Name: (3-Phenoxyphenyl)methyl 2,2-dimethyl-3-(2-methyl-1-propen-1-yl)cyclopropanecarboxylate.

CAS Number: 26002-80-2
51186-88-0 (cis)
26046-85-5 (trans)

SMILES String: CC(C)=CC3C(C(=O)OCc2cccc(Oc1cccc1)c2)C3(C)C (EpiSuite version 4.0).

(1*R*)-*trans*-Phenothrin(1*R*)-*cis*-Phenothrin