

Test Material: Phenothrin

MRID: 49625701

Title: RESIDUE ANALYTICAL METHOD FOR SUMITHRIN IN SOIL

MRID: 49305301

Title: 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

EPA PC Code: 069005

OCSPP Guideline: 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

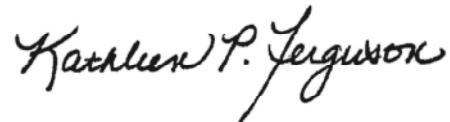
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**Date:** 4/28/16

**Secondary Reviewer:**

**Signature:**



**Date:** 4/28/16

**QC/QA Manager:** Joan Gaidos

**Signature:**



**Date:** 4/28/16

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

- Reports:** Environmental Chemistry Method (ECM): EPA MRID No. 49625701. Hirota, M. 1990. RESIDUE ANALYTICAL METHOD FOR SUMITHRIN IN SOIL. Laboratory Project ID: ER-MT-8941. Report prepared by Biochemistry and Toxicology Laboratory, Sumitomo Chemical Company, Hyogo, Japan, sponsored and submitted by Sumitomo Chemical Company, Tokyo, Japan; 15 pages. Final report issued January 18, 1990.
- Independent Laboratory Validation (ILV): EPA MRID No. 49305301. 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 PTRL Europe, Ulm, Germany, sponsored and submitted by Sumitomo Chemical Company, Tokyo, Japan; 38 pages. Final report issued November 28, 2013.
- Document No.:** MRIDs 49625701 / 49305301
- Guideline:** 850.6100
- Statements:** ECM: The study was not conducted in accordance with USEPA FIFRA standards (p. 3). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-5). Quality Assurance and Statement of Authenticity statements were not provided.
- ILV: The study was conducted in accordance with German Good Laboratory Practices (GLP; 2011), which are based on OECD GLP standards (p. 3; Appendix 3, p. 38). 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.
- Classification:** This analytical method is classified as **unacceptable**. 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. Determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The LOD was not reported in the ECM. In the ECM, no samples were prepared at the LOQ or 10×LOQ, and the number of samples was insufficient at test fortifications. In the ECM, the method could not be evaluated for specificity based on provided chromatograms. The soil matrix of the ECM was not characterized. In the ILV, linearity of the calibration curves for MS/MS analysis of trans-phenothrin were not satisfactory, and chromatograms of 10×LOQ were not included. A reagent blank was not included in the ECM and ILV.

**PC Code:** 069005

**EPA Primary Reviewer:** Kristy Crews, Chemist

**Signature:**

**EPA Secondary Reviewer:** Andrew Shelby, Physical Scientist

**Date:**  
**Signature:**

**Date:**

“ECM” written in this document refers to the submitted ECM MRID 49625701 unless otherwise noted.

### Executive Summary

This analytical method, Sumitomo Laboratory Project ID: ER-MT-8941, is designed for the quantitative determination of the total d-phenothrin (Sumithrin; sum of cis- and trans- isomers) at 0.01 mg/kg in soil using GC/MS (detection mode not reported). The LOQ is **less than** the lowest toxicological level of concern in soil. The ILV was not performed to validate the submitted ECM, but ECM (Sumitomo ID ER-31-0020, 1993). ECM (Sumitomo ID ER-31-0020), which was partially reproduced in the ILV, was validated by the ILV with the first trial using GC/MS(SIM) and GC/MS/MS with no modifications to the extraction procedure; however, only GC/MS (SIM) was used in the ECM (Sumitomo ID ER-31-0020). The ECM (Sumitomo ID ER-31-0020) extraction procedure was similar to that of the ECM MRID 49625701; however, there were multiple minor changes which could affect analyte recovery. Additionally, the GC/MS analytical parameters were incompletely reported in both ECM MRID 49625701 and ECM (Sumitomo ID ER-31-0020). Therefore, the ECM method was validated by the ILV with the first trial using substantial modifications to the extraction procedure and analytical method. The soil matrix of the ECM was not characterized; the sandy loam soil matrix of the ILV was well characterized. It could not be determined if the ILV was provided with a more difficult soil matrix than that used in the ECM. In the ILV, reagent blank and chromatograms of 10×LOQ were not included. In the ECM, no samples were prepared at the LOQ or 10×LOQ, a reagent blank was not included, the number of samples was insufficient (n = 2) at test fortifications, and the LOD for the method was not reported. Additionally, in the ECM, the method could not be evaluated for specificity based on the provided chromatograms since the axes were unlabeled and no peak integration was performed. 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.

**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						

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 <sup>1</sup>	49625701	49305301 <sup>2</sup>		Soil <sup>3,4</sup>	18/01/1990	Sumitomo Chemical Company	GC/MS	0.01 mg/kg (10 ng/g)

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

2 The ILV was not performed to validate ECM MRID 49625701. The ILV 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 49305301). ECM (Sumitomo ID ER-31-0020) was partially reproduced in the ILV. The extraction procedure from ECM (Sumitomo ID ER-31-0020) was inserted into the ILV report (pp. 14-15). The ECM analytical parameters of ECM (Sumitomo ID ER-31-0020) were briefly reported in the ILV. No recovery results or chromatograms from ECM (Sumitomo ID ER-31-0020) were included in the ILV.

3 In the ECM, the soil matrix was not characterized or described.

4 In the ILV, the soil matrix was sandy loam (pH  $7.2 \pm 0.1$ ; sand  $61.5 \pm 3.3$ , silt  $27.7 \pm 2.5$ , clay  $10.9 \pm 1.2$ ; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34 of MRID 49305301).

## I. Principle of the Method

The ECM test material was d-phenothrin (Sumithrin; 97.0% purity; p. 6 of MRID 49625701). The ratio of trans/cis- isomer was not reported. The ILV test material was d-phenothrin (TG, technical grade), which contained 97.0% (1R)-isomers (p. 11 of MRID 49305301). The ratio of trans/cis- isomer was 80.31/19.69 (reported by Sponsor).

Samples (20 g, dry weight) of fortified, sieved (5 mm) soil were extracted twice using 30 mL of methyl alcohol for 10 minutes then filtered using a Kiriya funnel (equivalent of Buchner funnel) packed with Hyflo Super-cel (*ca.* 1 cm thickness) under vacuum (pp. 6-7 of MRID 49625701). The residue was rinsed with 10 mL of methyl alcohol "by portions" (p. 7). The combined organic filtrates were transferred to a separatory funnel and extracted with 80 mL of 10% aqueous sodium chloride and 40 mL of dichloromethane for 10 minutes. The lower dichloromethane layer was passed through *ca.* 50 g of anhydrous sodium sulphate contained in a filter funnel. The remaining aqueous layer was extracted with 30 mL of dichloromethane for 5 minutes. The lower dichloromethane layer was drained and filtered through the same filter funnel as before. The anhydrous sodium sulphate was washed with 15 mL of dichloromethane. The combined dried dichloromethane extracts and rinse were reduced to dryness by rotary evaporation at  $<40^{\circ}\text{C}$ . The study author noted that concentration must be stopped soon after the solvent is evaporated in order to prevent loss of solvent (p. 10). The residue was applied to a activated florisil PR column (activated overnight  $130^{\circ}\text{C}$ ; hexane:ethyl acetate, 20:1, v:v; 18-mm diameter column) topped with 1 g of anhydrous sodium sulfate (p. 7). 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 70 mL of hexane:ethyl acetate (20:1, v:v). The first 20 mL of eluate was discarded. The following 50 mL was collected and reduced to dryness by rotary evaporation at  $<40^{\circ}\text{C}$ . The residue was reconstituted in acetone prior to GC/MS analysis. The study author noted that the eluting fraction of d-phenothrin should be checked using the standard prior to method validation; recovered standard should be  $\geq 90\%$  for confirmation of eluting fraction (p. 10).

Samples were analyzed for d-phenothrin (Sumithrin) using gas chromatography with mass spectrometry (GC/MS) analysis (p. 8 of MRID 49625701). A Finnigan 4000 Gas Chromatograph - Mass Spectrometer with PROMIN was equipped with a 5% Silicone SE-30 on Chromosorb W AW DMCS column (60-80 mesh, 2 mm i.d., 1.1 m length; column temperature 245°C). Mass spectrometer detection mode and ion polarity were not reported. Ions monitored for d-phenothrin were not reported and could not be determined from the spectra provided (Figures 2-4, pp. 13-15). Only one peak was detected in the spectra; the retention time could not be determined from the spectra provided.

The ILV was performed to validate another ECM (Sumitomo ID ER-31-0020. Jacobson, B, et. al. 1993; see Reviewer's Comment #1; p. 9 of MRID 49305301). The ECM extraction procedure of Sumitomo ID ER-31-0020 was similar to that of the ECM 49625701, except for the following: 1) 40 mL of methanol, instead of 30 mL, were used per initial extraction; 2) glass-fiber filter paper was used instead of Hyflo Super-cel for filtration; 3) 30 mL, instead of 10 mL of methanol was used to rinse the filter paper; 4) for the second dichloromethane extraction 40 mL, instead of 30 mL, was used; 5) after the dichloromethane partitioning, no rinsing of the sodium sulfate was performed; 6) only 45 mL, instead of 70 mL, of hexane:ethyl acetate (20:1, v:v) was used to elute d-phenothrin from the florisil column; 7) the reduction of the analyte solution was performed to 1-2 mL on the rotary evaporator, then to dryness under nitrogen; and 8) the residue was reconstituted in 1.0 mL of toluene. In the ILV, samples were analyzed for d-phenothrin using gas chromatography with mass spectrometry (GC/MS) analysis (p. 12). The ECM analytical parameters of ECM (Sumitomo ID ER-31-0020) were briefly reported (see Reviewer's Comment #6). In the ILV, a Thermo Trace 1310 Gas Chromatograph was equipped with an Optima 5-MS Accent (Macherey-Nagel) column (30 m x 250 µm i.d., 0.25 µm thickness; injection temperature 225°C) and a TSQ 8000 triple-quadrupole Mass Spectrometer with positive EI. Two types of detection were used: SIM and MS/MS. Injection volume was 2 µL. 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). Retention times were 13.59 minutes for cis-phenothrin and 13.66 minutes for trans-phenothrin (Figure 8, p. 30).

The Limit of Quantification (LOQ) for d-phenothrin in soil was reported as 0.01 mg/kg in the ECM and ILV; the Limit of Detection (LOD) was reported as 0.002 mg/kg (20% of the LOQ) in the ILV (p. 9 of MRID 49625701; pp. 10, 16 of MRID 49305301). The LOD was not reported in the ECM.

## II. Recovery Findings

ECM (MRID 49625701): Individual recoveries met requirements (70-120%) for analysis of d-phenothrin (Sumithrin) in one soil at 6×LOQ (0.06 mg/kg) and 60×LOQ (0.6 mg/kg; p. 9). No samples were prepared at the LOQ or 10×LOQ. Only two samples were prepared at each test fortification; therefore, statistical analysis could not be performed on the results. Only one ion or ion transition was monitored by GC/MS; however, this ion or ion transition was not reported and could not be determined by the reviewer. Only one peak was detected in the spectra; cis- and trans-

isomers were not quantified separately (Figures 2-4, pp. 13-15). The soil matrix was not characterized or described.

ILV (MRID 49305301): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of d-phenothrin (sum of cis- and trans-isomers) in one soil at the LOQ (0.01 mg/kg) and 10 $\times$ LOQ (0.1 mg/kg) using either GC/MS (SIM) or GC/MS/MS analysis (Tables 1-2, pp. 19-20). 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 fairly comparable. The soil matrix was sandy loam (pH  $7.2 \pm 0.1$ ; sand  $61.5 \pm 3.3$ , silt  $27.7 \pm 2.5$ , clay  $10.9 \pm 1.2$ ; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34). The ILV was not performed to validate ECM MRID 49625701. The method from a similar ECM (Sumitomo ID ER-31-0020. Jacobson, B, et al. 1993) was validated by the ILV with the first trial with no modifications to the extraction procedure, but an augmented analytical method [only GC/MS (SIM) was used in the ECM (Sumitomo ID ER-31-0020); p. 9; Tables 1-2, pp. 19-20; see Reviewer's Comments #1 and 6]. The ECM (Sumitomo ID ER-31-0020) extraction procedure was similar to that of the ECM MRID 49625701; however, there were changes to volumes of solvents for extraction and elution, changes to filter components, no rinsing of sodium sulfate and changes to the concentration procedure and reconstitution solvent for GC/MS (pp. 9, 14-15). Additionally, the GC/MS analytical parameters were incompletely reported in both ECM MRID 49625701 and ECM (Sumitomo ID ER-31-0020; as reported in the ILV). Therefore, the ECM MRID 49625701 method was validated by the ILV with the first trial using substantial modifications to the extraction procedure and analytical method.

**Table 2. Initial Validation Method Recoveries for d-Phenothrin (Sumithrin) in Soil<sup>1</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
d-Phenothrin <sup>2</sup>	0.06	2	87, 96	--	--	--
	0.6	2	88, 93	--	--	--

Data (uncorrected results, p. 10) were obtained from p. 9 of MRID 49625701. Statistical analysis for means, s.d.s and RSDs could not be determined due to the insufficient number of samples (n =2).

1 The soil matrix was not characterized.

2 Only one peak was detected in the spectra; cis- and trans- isomers were not quantified separately (Figures 2-4, pp. 13-15).

**Table 3. Independent Validation Method Recoveries for d-Phenothrin (Sumithrin) in Soil<sup>1</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>GC/MS (SIM)</b>						
<i>m/z</i> 183						
d-Phenothrin <sup>2</sup>	0.01 (LOQ)	5	103-108	105	2	2
	0.1	5	83-93	87	4	5
<i>m/z</i> 123						
d-Phenothrin <sup>2</sup>	0.01 (LOQ)	5	102-104	103	1	1
	0.1	5	75-88	81	5	6
<i>m/z</i> 184						
d-Phenothrin <sup>2</sup>	0.01 (LOQ)	5	102-106	103	2	2

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.1	5	82-92	87	4	5
<b>GC/MS/MS</b>						
Quantitation ion transition ( $m/z$ 183 $\rightarrow$ 168)						
d-Phenothrin <sup>2</sup>	0.01 (LOQ)	5	99-103	101	2	2
	0.1	5	83-95	88	5	6
Confirmation ion transition 1 ( $m/z$ 183 $\rightarrow$ 165)						
d-Phenothrin <sup>2</sup>	0.01 (LOQ)	5	99-105	102	2	2
	0.1	5	83-96	88	5	6
Confirmation ion transition 2 ( $m/z$ 183 $\rightarrow$ 153)						
d-Phenothrin <sup>2</sup>	0.01 (LOQ)	5	98-103	100	2	2
	0.1	5	83-95	87	5	6

Data (uncorrected results, p. 15) were obtained from Tables 1-2, pp. 19-20 of MRID 49305301 and DER Attachment 2 (calculation of s.d.).

1 The soil matrix was sandy loam (pH  $7.2 \pm 0.1$ ; sand  $61.5 \pm 3.3$ , silt  $27.7 \pm 2.5$ , clay  $10.9 \pm 1.2$ ; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34).

2 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).

### III. Method Characteristics

The LOQ and LOD for d-phenothrin (sum of cis and trans isomers) in soil were reported 0.01 mg/kg and 0.002 mg/kg (20% of the LOQ), respectively (pp. 9-10 of MRID 49625701; pp. 10, 16 of MRID 49305301). In the ECM, the LOQ was supported by the fact that the signal-to-noise ratio was more than 10 at that fortification level; no calculations were provided, but the LOQ was reportedly equal to 0.4 ng by GC/MS. The LOD was not reported in the ECM. In the ILV, the LOQ was supported by the successful validation of the analytical method at that fortification level. No justification of the LOD was provided.

**Table 4. Method Characteristics**

		<b>d-Phenothrin<sup>1</sup></b>	
		<b>cis-Phenothrin</b>	<b>trans-Phenothrin</b>
Limit of Quantitation (LOQ)		0.01 mg/kg	
Limit of Detection (LOD)		0.002 mg/kg	
Linearity (calibration curve $r^2$ and concentration range)	ECM		Not reported <sup>2</sup> (0.4-6 ng/mL)
	ILV	SIM	$r^2 = 0.9981$ ( $m/z$ 183) $r^2 = 0.9973$ ( $m/z$ 123) $r^2 = 0.9983$ ( $m/z$ 184) (4.0-1000 ng/mL)
			$r^2 = 0.9950$ ( $m/z$ 183) $r^2 = 0.9979$ ( $m/z$ 123) $r^2 = 0.9953$ ( $m/z$ 184) (40-2500 ng/mL)
	ILV	MS/MS	$r^2 = 0.9978$ ( $m/z$ 168) $r^2 = 0.9979$ ( $m/z$ 165) $r^2 = 0.9972$ ( $m/z$ 153) (4.0-1000 ng/mL)
$r^2 = \mathbf{0.9939}$ ( $m/z$ 168) $r^2 = \mathbf{0.9935}$ ( $m/z$ 165) $r^2 = \mathbf{0.9941}$ ( $m/z$ 153) (40-2500 ng/mL)			
Repeatable	ECM <sup>3</sup>		<b>No</b> for LOQ and 10 $\times$ LOQ; no samples were prepared. Yes for 6 $\times$ LOQ and 60 $\times$ LOQ, but (n = <b>2</b> ) for both.
	ILV <sup>4</sup>		Yes for LOQ (n = 5) and 10 $\times$ LOQ (n = 5)

Reproducible		<b>Could not be determined<sup>5</sup></b>
Specific	ECM	<b>Could not be determined<sup>6</sup></b>
	ILV	Yes, matrix interferences were <10% of the LOQ for all three monitored ions or ion transitions.

Data were obtained from p. 9; Figures 1-4, pp. 12-15 of MRID 49625701; p. 16; Tables 1-2, pp. 19-20; Figures 2-3, pp. 24-25; Figures 7-11, pp. 29-33 of MRID 49305301. Q = quantitative ion transition; C1 = confirmatory 1 ion transition; C2 = confirmatory 2 ion transition.

- 1 Sum of cis- and trans- isomers. Sumithrin. 3-Phenoxybenzyl (1R)-*cis-trans*-chrysanthemate; 3-Phenoxybenzyl (1R*S*)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate.
- 2 The linear regression equation and coefficient was not reported for the calibration curve (Figure 1, p. 12 of MRID 49625701). The reviewer could not calculate the linear regression coefficient since the raw data was not provided. Only one peak was detected in the spectra; cis- and trans- isomers were not quantified separately (Figures 2-4, pp. 13-15).
- 3 In the ECM, the soil matrix was not characterized or described.
- 4 In the ILV, the soil matrix was sandy loam (pH  $7.2 \pm 0.1$ ; sand  $61.5 \pm 3.3$ , silt  $27.7 \pm 2.5$ , clay  $10.9 \pm 1.2$ ; USDA soil texture classification); it was well characterized by and obtained from LUFA Speyer (p. 11; Appendix 1, p. 34 of MRID 49305301).
- 5 The ILV was not performed to validate ECM MRID 49625701. The method from a similar ECM (Sumitomo ID ER-31-0020, 1993) was validated by the ILV (see Reviewer's Comment #1). Also, the LOQ of the method was not validated by ECM MRID 49625701.
- 6 The reviewer could not evaluate the provided chromatograms for specificity since the axes were unlabeled and no peak integration was performed. The reviewer observed residues in the control soil spectra at the retention time of d-phenothrin, but could not determine the proportions of the control residues to the LOQ peak (Figure 2, p. 13 of MRID 49625701). Based on the definition of the LOQ provided by the ECM study author, the reviewer assumed that the study author reported that the matrix interferences were 10% of the LOQ (p. 10).  
Linearity is satisfactory when  $r^2 \geq 0.995$ .

## V. Method Deficiencies and Reviewer's Comments

1. The reproducibility of the method could not be determined since the ILV was not performed to validate ECM MRID 49625701. The ILV 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 49305301). ECM (Sumitomo ID ER-31-0020) was partially reproduced in the ILV. The extraction procedure from ECM (Sumitomo ID ER-31-0020) was inserted into the ILV report (pp. 14-15). The ECM (Sumitomo ID ER-31-0020) extraction procedure was similar to that of the ECM MRID 49625701; however, there were changes to volumes of solvents for extraction and elution, changes to filter components, no rinsing of sodium sulfate and changes to the concentration procedure and reconstitution solvent for GC/MS (pp. 9, 14-15). These changes could affect analyte recovery. The ECM analytical parameters of ECM (Sumitomo ID ER-31-0020) were briefly reported in the ILV (see Reviewer's Comment #6). No recovery results or chromatograms from ECM (Sumitomo ID ER-31-0020) were included in the ILV.

The analytical method portion of the method in ECM MRID 49625701 lacked important mass spectrometer detailed, such as detection mode, ion polarity and ions or ion transitions monitored (p. 8 of MRID 49625701). None of these details could be determined from the provided chromatograms (Figures 2-4, pp. 13-15). Additionally, only one peak was detected in the spectra. The cis- and trans- isomers were not quantified separately in ECM MRID 49625701, as they were in the ILV.



When evaluating ECM MRID 49625701 and ILV MRID 49305301 as a method validation set, the ECM method was validated by the ILV with the first trial using substantial modifications to the extraction procedure and analytical method. Therefore, 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. 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. The LOQ and LOD were not adequately supported by calculations or comparison to background levels in the ECM and ILV (pp. 9-10 of MRID 49625701; pp. 10, 16 of MRID 49305301). In the ECM, the LOQ was supported by the fact that the signal-to-noise ratio was more than 10 at that fortification level; no calculations were provided, but the LOQ was reportedly equal to 0.4 ng by GC/MS. In the ILV, the LOQ was supported by the successful validation of the analytical method at that fortification level. No justification of the LOD was provided in the ILV.

The LOD was not reported in the ECM.

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. The ECM contained several additional significant issues with number and fortification of samples, inadequate chromatographic support and lack of a reagent blank.

No samples were prepared at the LOQ or 10×LOQ. Also, a reagent blank was not included in the ECM. The LOQ of the method was not validated by ECM since 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× LOQ for each matrix. A chromatogram of the LOQ was provided in the ECM; however, the axes were unlabeled and no peak integration was performed (p. 9; Figure 2, p. 13 of MRID 49625701).

The number of samples was insufficient ( $n = 2$ ) at both test fortifications (6×LOQ and 60×LOQ). 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× LOQ for each matrix.

The reviewer could not evaluate the provided chromatograms for specificity since the axes were unlabeled and no peak integration was performed. The reviewer observed residues in the control soil spectra at the retention time of d-phenothrin, but could not determine the proportions of the control residues to the LOQ peak (Figure 2, p. 13 of MRID 49625701). Based on the definition of the LOQ provided by the ECM study author, the reviewer assumed the study author reported that the matrix interferences were 10% of the LOQ. OCSPP guidelines recommend that representative 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.

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× LOQ for each matrix.

4. The ILV contained several issues with unsatisfactory linearity, insufficient chromatographic support and lack of a reagent blank.

The linearity of the calibration curves for MS/MS analysis of trans-phenothrin were not satisfactory ( $r^2 \geq 0.995$ ). The correlation coefficients were 0.9935-0.9941 for all three ion transitions monitored.

No chromatograms of 10×LOQ were included. OCSPP guidelines recommend that representative 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.

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× LOQ for each matrix.

5. The soil matrix of the ECM was not characterized; the sandy loam soil matrix of the ILV was well characterized (p. 11; Appendix 1, p. 34 of MRID 49305301). It could not be determined if the ILV was provided with a more difficult soil matrix than that used in the ECM.
6. In the ILV, the study author reported that the analytical method of ECM (Sumitomo ID ER-31-0020) used GC/MS(SIM; p. 9 of MRID 49305301). The cis- and trans- isomers of phenothrin were evaluated separately in ECM (Sumitomo ID ER-31-0020), monitoring only two fragment ions ( $m/z$  183 and 123). Only the ion  $m/z$  183 was quantified in recovery results. No additional instrument description or parameters for ECM (Sumitomo ID ER-31-0020) were reported in the ILV. The ILV analytical method used GC/MS(SIM) and GC/MS/MS in which three ions or ion transitions were monitored and quantified.
7. No communication between the method developing laboratory and study sponsor was reported.
8. In the ILV, no significant matrix effects were observed (<20%; p. 13; Table 3, p. 21 of MRID 49305301).
9. It was reported in the ILV that 13 samples required *ca.* sixteen person-hours to complete the sample processing (p. 17 of MRID 49305301). Subsequent GC/MS analysis and evaluation required an additional *ca.* sixteen to seventeen. The overall time for 13 samples was about two to three calendar days.

## 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 = 80.31/19.69) [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.

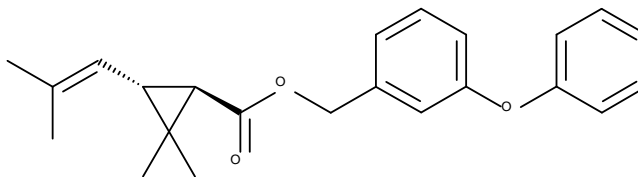
**CAS Number:** 26002-80-2

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

