Test Material:	Phenothrin
MRID:	49564001
Title:	Development and Validation of an Analytical Method for the Determination of the cis- and trans Isomers of d-Phenothrin in Surface Water by GC/MS
MRID:	49564002
Title:	Independent Laboratory Validation of an Analytical Method for the Determination of the cis- and trans Isomers of d-Phenothrin in Surface Water by GC/MS
EPA PC Code:	069005
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 4/28/16

Secondary Reviewer: **Signature:** Kathleen P. Ferguson

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 surface water

Reports: Environmental Chemistry Method (ECM): EPA MRID No. 49564001. Class, T. 2013. Development and Validation of an Analytical Method for the Determination of the cis- and trans Isomers of d-Phenothrin in Surface Water by GC/MS. PRTL Europe ID: P 3046 G. Report prepared by PTRL Europe, Ulm, Germany, sponsored and submitted by Sumitomo Chemical Company, Tokyo, Japan; 31 pages. Final report issued November 28, 2013.

Independent Laboratory Validation (ILV): EPA MRID No. 49564002. Arndt, T., and L. Mannella. 2014. Independent Laboratory Validation of an Analytical Method for the Determination of the cis- and trans Isomers of d-Phenothrin in Surface Water by GC/MS. PTRL Study No.: 2578W. Report prepared by PTRL West (a division of EAG, LLC), Hercules, California, sponsored/submitted by Sumitomo Chemical Company, Ltd., Tokyo, Japan, and submitted by Sumitomo Chemical Company, New York, New York; 113 pages. Final report issued July 14, 2014.

Document No.: MRIDs 49564001 / 49564002

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 (p. 3; Appendix 3, p. 31). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Statement of Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA standards, with the exception that the certification of the isomer ratio of the test substance was not specified as GLP (p. 3). 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**. Determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The LOQ is higher than the lowest toxic endpoint. An updated ECM should be submitted to provide precautions and optional steps to prevent loss of analyte during the extraction procedure. In the ECM, a reagent blank and chromatograms of 10×LOQ were not included.

PC Code: 069005

EPA Primary Reviewer: Kristy Crews, Chemist **Signature:** Kristy Crews 2016.09.19 09:22:18 -04'00'

Date:

EPA Secondary Reviewer: Andrew Shelby, Physical Scientist **Signature:** Digitally signed by JOHN SHELBY Date: 2016.09.19 09:24:40 -04'00'

Date:

Executive Summary

This analytical method, PRTL Europe ID P 3046 G, is designed for the quantitative determination of the total d-phenothrin (sum of cis- and trans- isomers) at 0.1 µg/L in surface water using GC/MS. The LOQ is **greater than** the lowest toxicological level of concern in water. The surface water matrices of the ECM and ILV were well characterized. The method was validated by the ILV with the second trial after ILV modifications to and sponsor communication regarding the extraction procedure. Since the ILV trials and modifications showed that the loss of analyte can easily occur during the extraction procedure, the ECM method should be updated with precautions during the liquid-liquid partition step and reducing step, as well as optional steps to ensure proper recovery of analyte in case problems occur. Additionally, a reagent blank and chromatograms of 10×LOQ were not included in the ECM.

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]	49564001	49564002		Surface Water[2,3]	28/11/2013	Sumitomo Chemical Company	GC/MS	0.1 µg/L

1 Sum of cis- and trans- isomers.

2 In the ECM, the surface water matrix (pH 8.16, hardness 17.9°d, diluted organic carbon 1.7 mg/L) was obtained from the river Danube in Ulm, Germany (p. 10; Appendix 1, p. 27 of MRID 49564001).

3 In the ILV, the surface water matrix (pH 8.1, hardness 196 mg equiv. $CaCO_3$/L, dissolved organic carbon 8.7 ppm) was obtained from Wildcat Creek (flowing creek), Alvarado Park, Wildcat Canyon Regional Park in Richmond, California (37.95609°N, 122.31294°W; pp. 16-17; Appendix C, pp. 106-110 of MRID 49564002).

I. Principle of the Method

The ECM test material was d-phenothrin (TG, technical grade), which contained 97.0% (1R)-isomers (pp. 10-11 of MRID 49564001). The ratio of trans/cis- isomer was 80.31/19.69 (reported by Sponsor). The ILV test material was d-phenothrin, 97.0% purity (pp. 14-15 of MRID 49564002). The ratio of trans/cis- isomer was 8/2.

Samples (200 mL) of fortified surface water in a 0.25-L separatory funnel were extracted twice using 20 mL of hexane with manual shaking for 30 seconds, then once using 10 mL of hexane with manual shaking for 30 seconds (p. 13 of MRID 49564001). The combined organic extracts were

passed through *ca*. 10 g of anhydrous sodium sulphate contained in a glass funnel plugged with silanized glass wool. The anhydrous sodium sulphate was washed with 10 mL of hexane. The combined organic extracts and rinse were reduced to near dryness by rotary evaporation at *ca*. 40°C. The residue was further reduced to dryness under nitrogen. The residue was reconstituted in 0.5 mL of toluene prior to GC/MS analysis.

Samples were analyzed for d-phenothrin using gas chromatography with mass spectrometry (GC/MS) analysis (pp. 11-12 of MRID 49564001). 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 an TSQ 8000 triple-quadrupole Mass Spectrometer with positive EI and multiple reaction monitoring (MRM). 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 for d-phenothrin were *m/z* 183 → 168 (quantitation), *m/z* 183 → 165 (confirmation 1) and *m/z* 183 → 153 (confirmation 2; p. 12). Retention times were 13.58 minutes for cis-phenothrin and 13.67 minutes for trans-phenothrin (Figure 5, p. 25).

In the ILV, a few modifications to the ECM extraction method were performed (pp. 22-24; Figure 1, p. 40 of MRID 49564002). After the first two extractions with 20 mL of hexane, the sodium sulfate was rinsed with 10 mL hexane and collected into the flask with the combined hexane extracts. Then the sodium sulfate was replaced with new sodium sulfate prior to the third extraction with 10 mL of hexane. After this extract was dried with the new sodium sulfate and combined with the combined hexane extracts, the separatory flask and sodium sulfate were rinsed with 5 mL x 2 of hexane. These rinses were combined with the extracts. Additionally, during concentration via rotary evaporation, the extract was only concentrated to *ca*. 2 mL, instead of to near dryness, prior to further evaporation under nitrogen. The purpose of both modifications was to enhance recoveries. ECM analytical conditions were modified for the equipment available to the ILV: Agilent 7000 Series Triple Quad Mass Spectrometer (GC-QQQ) equipped with a J&W DB-5 ms column (30 m x 250 µm x 0.25 µm) connected to an Agilent 7890A Series Gas Chromatograph (pp. 16, 24). All other analytical parameters were the same as those in the ECM, including the monitored ion transitions; retention times were 12.06 min. for cis d-phenothrin and 12.14 min. for trans d-phenothrin. In the ILV, it was also specified that the lowest calibrant for the trans-phenothrin calibration was prepared at 3.9 ng/mL, instead of 10 ng/mL which was stated in the ECM, in order to cover the LOD level (p. 23).

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for d-phenothrin in water were reported as 0.10 µg/L and *ca*. 0.02 µg/L (20% of the LOQ), respectively, in the ECM and ILV (p. 17 of MRID 49564001; pp. 10, 28-29 of MRID 49564002).

II. Recovery Findings

ECM (MRID 49564001): 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 surface water at the LOQ (0.10 µg/L) and 10×LOQ (1.0 µg/L; Table 1, p. 18). Recovery results of the quantitative and confirmatory ion transitions were comparable. The surface water

matrix (pH 8.16, hardness 17.9°d, diluted organic carbon 1.7 mg/L) was obtained from the river Danube in Ulm, Germany; it was well characterized by Institute Alpha (Ulm, Germany; p. 10).

ILV (MRID 49564002): Mean recoveries and RSDs met requirements for analysis of d-phenothrin (sum of cis- and trans-isomers) in surface water at the LOQ (0.10 µg/L) and 10×LOQ (1.0 µg/L; Table I, p. 37). Recovery results of the quantitative and confirmatory ion transitions were comparable. The surface water matrix (pH 8.1, hardness 196 mg equiv. $CaCO_3$/L, dissolved organic carbon 8.7 ppm) was obtained from Wildcat Creek (flowing creek), Alvarado Park, Wildcat Canyon Regional Park in Richmond, California (37.95609°N, 122.31294°W); it was well characterized by Agvise Laboratories, Northwood, North Dakota (pp. 16-17; Appendix C, pp. 106-110). The method was validated with the second trial (p. 35). During the first trial, low recoveries were observed at the 10×LOQ fortification; the low recoveries were attributed to incomplete partitioning during the liquid-liquid partitioning steps in the extraction.

Table 2. Initial Validation Method Recoveries for d-Phenothrin in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion transition (*m/z* 183 → 168)						
d-Phenothrin[2]	0.10 (LOQ)	5	92-115	105	9	9
	1.0	5	90-117	106	10	10
Confirmation ion transition 1 (*m/z* 183 → 165)						
d-Phenothrin[2]	0.10 (LOQ)	5	92-117	106	11	10
	1.0	5	91-120	106	11	10
Confirmation ion transition 2 (*m/z* 183 → 153)						
d-Phenothrin[2]	0.10 (LOQ)	5	94-116	106	9	9
	1.0	5	88-113	102	9	9

Data (uncorrected results, pp. 13-14) were obtained from Table 1, p. 18 of MRID 49564001 and DER Attachment 2 (calculation of s.d.).

1 The surface water matrix (pH 8.16, hardness 17.9°d, diluted organic carbon 1.7 mg/L) was obtained from the river Danube in Ulm, Germany; it was well characterized by Institute Alpha (Ulm, Germany; p. 10).

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. 14).

Table 3. Independent Validation Method Recoveries for d-Phenothrin in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion transition (*m/z* 183 → 168)						
d-Phenothrin[2]	0.10 (LOQ)	5	69-96	84	11	13.1
	1.0	5	74-81	78	3	3.8
Confirmation ion transition 1 (*m/z* 183 → 165)						
d-Phenothrin[2]	0.10 (LOQ)	5	69-89	81	8	9.9
	1.0	5	74-80	76	3	3.9
Confirmation ion transition 2 (*m/z* 183 → 153)						
d-Phenothrin[2]	0.10 (LOQ)	5	67-90	81	9	11.1
	1.0	5	72-79	76	3	3.9

Data (uncorrected results, pp. 24-26; Appendix D, pp. 111-113) were obtained from Table I, p. 37 of MRID 49564002.

1 The surface water matrix (pH 8.1, hardness 196 mg equiv. $CaCO_3$/L, dissolved organic carbon 8.7 ppm) was obtained from Wildcat Creek (flowing creek), Alvarado Park, Wildcat Canyon Regional Park in Richmond, California (37.95609°N, 122.31294°W); it was well characterized by Agvise Laboratories, Northwood, North Dakota (pp. 16-17; Appendix C, pp. 106-110).

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 (pp. 26-27).

III. Method Characteristics

The LOQ and LOD for d-phenothrin (sum of cis and trans isomers) in water were reported as 0.10 µg/L and *ca*. 0.02 µg/L (20% of the LOQ), respectively, in the ECM and ILV (pp. 9, 15, 17 of MRID 49564001; pp. 10, 28-29 of MRID 49564002). No calculations or comparisons to noise level were reported. No justification of the LOQ and LOD was provided in the ECM. In the ILV, the LOQ was defined as the lowest fortification level of d-phenothrin which was validated by the analytical method. No justification of the LOD was provided, but it was reported that the LOD represented 8 ng/mL of total d-phenothrin (sum of cis and trans isomers) in solution.

Table 4. Method Characteristics

		d-Phenothrin[1]	
		cis-Phenothrin	**trans-Phenothrin**
Limit of Quantitation (LOQ)		0.10 µg/L	
Limit of Detection (LOD)		*ca*. 0.02 µg/L	
Linearity (calibration curve r^2 and concentration range)	ECM	r^2 = 1.0000 (*m/z* 168) r^2 = 0.9999 (*m/z* 165) r^2 = 1.0000 (*m/z* 153) (1-150 ng/mL)	r^2 = 0.9978 (*m/z* 168) r^2 = 0.9977 (*m/z* 165) r^2 = 0.9973 (*m/z* 153) (10-1000 ng/mL)
	ILV	r^2 = 0.9984 (*m/z* 168) r^2 = 0.9976 (*m/z* 165) r^2 = 0.9989 (*m/z* 153) (1-150 ng/mL)	r^2 = 0.9993 (*m/z* 168) r^2 = 0.9990 (*m/z* 165) r^2 = 0.9991 (*m/z* 153) (3.9-978 ng/mL)
Repeatable	ECM[2]	Yes for LOQ (n = 5) and 10×LOQ (n = 5)	
	ILV[3]		
Reproducible		Yes for LOQ and 10×LOQ	
Specific	ECM	Yes, matrix interferences were <5% of the LOQ for all three monitored ion transitions.	
	ILV	Yes, matrix interferences were <6% of the LOQ for all three monitored ion transitions based on the sum of the two isomers.[4]	

Data were obtained from p.12; Table 1, p. 18; Figure 2, p. 22; Figures 5-6, pp. 25-26 of MRID 49564001; Tables I-II, pp. 37-38; Figures 2-3, pp. 41-46; Figures 5-8, pp. 48-59; Figures 13-20, pp. 72-95 of MRID 49564002. Q = quantitative ion transition; C1 = confirmatory 1 ion transition; C2 = confirmatory 2 ion transition.

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

2 In the ECM, the surface water matrix (pH 8.16, hardness 17.9°d, diluted organic carbon 1.7 mg/L) was obtained from the river Danube in Ulm, Germany; it was well characterized by Institute Alpha (Ulm, Germany; p. 10; Appendix 1, p. 27 of MRID 49564001).

3 In the ILV, the surface water matrix (pH 8.1, hardness 196 mg equiv. $CaCO_3$/L, dissolved organic carbon 8.7 ppm) was obtained from Wildcat Creek (flowing creek), Alvarado Park, Wildcat Canyon Regional Park in Richmond, California (37.95609°N, 122.31294°W); it was well characterized by Agvise Laboratories, Northwood, North Dakota (pp. 16-17; Appendix C, pp. 106-110 of MRID 49564002).

4 Residues of the cis and trans isomers were present in the majority of the control chromatograms, and the cis isomer was present in greater magnitude, relative to the LOQ response, than the trans isomer [Figures 13-14, pp. 72-77 (cis); Figures 17-18, pp. 84-89 (trans); Appendix D, pp. 111-113 of MRID 49564002].

IV. Method Deficiencies and Reviewer's Comments

1. 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, 15, 17 of MRID 49564001; pp. 10, 28-29 of MRID 49564002). In the ILV, the LOQ was defined as the lowest fortification level of d-phenothrin which was validated by the analytical method, and it was reported that the LOD represented 8 ng/mL of total d-phenothrin (sum of cis and trans isomers) in solution.

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not

reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

2. The ILV modifications of the ECM extraction method were performed in the first and second trial (pp. 22-24; Figure 1, p. 40 of MRID 49564002). The hexane three-fold extraction sequence was interrupted after the first two extractions for rinses and replacement of the sodium sulfate. Then, the third hexane extraction was performed followed by rinsing. Additionally, during concentration via rotary evaporation, the extract was only concentrated to *ca*. 2 mL, instead of to near dryness, prior to further evaporation under nitrogen. The purpose of both modifications was to enhance recoveries. During the first trial, low recoveries in the 10×LOQ sample set were attributed to "different degrees of emulsion during liquid-liquid partitioning steps" (p. 30). The sponsor recommended "a more consistent and robust shaking during the partition step for the second attempt" (pp. 30-31). Since the ILV trials and modifications highlighted the fact that the loss of analyte can easily occur during the extraction procedure, the ECM method should be updated with precautions during the liquid-liquid partition step (Step 3) and the reducing step (Step 4), as well as optional steps to ensure proper recovery of analyte in case problems occur (p. 13 of MRID 49564001).

3. No chromatograms of 10×LOQ were included in the ECM. 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.

4. No reagent blank was included in the ECM. 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. In the ILV, the procedural recoveries were not corrected for residues found in the controls even though the calculations included corrections for control residues and residues were found in many of the controls (pp. 24-26; Appendix D, pp. 111-113 of MRID 49564002). The residues found in the controls were quantified and determined to be "<LOD"; therefore, no correction to the procedural recovery was made. The peak areas of the residues in the controls were 0-30% of the LOQ response due to slightly low LOQ recovery [Figures 13-14, pp. 72-77 (cis); Figures 17-18, pp. 84-89 (trans)].

6. Communications between the method developing laboratory and study sponsor was reported (pp. 29-30 of MRID 49564002). Sponsor suggestions for the second trial and reports of the success of the second trial comprised the communications.

7. In the ECM, no significant matrix effects were observed (<20%; p. 13; Table 2, p. 19 of MRID 49564001).

   In the ILV, matrix effects were assessed by comparing the response ratio of a solvent-based calibrant to a matrix-based calibrant (10 ng/mL for cis; 39.1 ng/mL for trans; p. 33; Table III, p. 39 of MRID 49564002). Accuracy for the matrix-based calibrant ranged 89-106% at all three ions, so solvent-based calibrants were used.

8. It was reported in the ILV that one subset of samples (one reagent blank, two matrix blanks, five samples dosed at the LOQ or 10×LOQ) required *ca*. six hours to complete the sample processing (p. 29 of MRID 49564002). Subsequent GC/MS analysis and evaluation required an additional *ca*. six. The overall time for a sample subset was one-and-a half calendar days. For a complete trial of two subsets, the overall time requirement was 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]

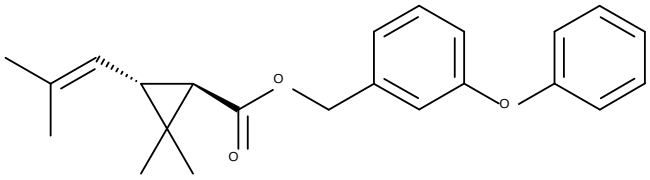
IUPAC Name: 3-Phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate.
3-Phenoxybenzyl (1*RS*)-*cis*-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate.
3-Phenoxybenzyl (±)-*cis*-*trans*-chrysanthemate.
3-Phenoxybenzyl (1R)-*cis*-*trans*-chrysanthemate.

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(Oc1ccccc1)c2)C3(C)C (EpiSuite version 4.0).

(1R)-*trans*-Phenothrin



(1R)-*cis*-Phenothrin