Test Material:	Prallethrin
MRID:	49430401
Title:	Method Validation of an Analytical Method for the Determination of Prallethrin in Surface Water
MRID:	49472701
Title:	INDEPENDENT LABORATORY VALIDATION OF A METHOD FOR THE ANALYSIS OF PRALLETHRIN IN SURFACE WATER
EPA PC Code:	128722
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 3/13/15

Secondary Reviewer: Lynne Binari **Signature:** 

Date: 3/13/15

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

Date: 3/13/15

Analytical method for prallethrin in surface water

Reports: ECM: EPA MRID No. 49430401. Arndt, T., and L. Mannella. 2014. Method Validation of an Analytical Method for the Determination of Prallethrin in Surface Water. PRTL Study No.: 2451W. Report prepared by PTRL West (a division of EAG, LLC), Hercules, California, sponsored and submitted by Sumitomo Chemical Company, Tokyo, Japan; 89 pages. Final report issued July 3, 2014.
ILV: EPA MRID No. 49472701. Zhang, L., K.H. Martin, and E.S. Bodle. 2014. INDEPENDENT LABORATORY VALIDATION OF A METHOD FOR THE ANALYSIS OF PRALLETHRIN IN SURFACE WATER. Study No.: SQA-0010. Wildlife International Project No.: 166C-122. Report prepared by Wildlife International, Evans Analytical Group, Easton, Maryland, sponsored by Sumitomo Chemical Company, Ltd., Tokyo, Japan, and submitted by Sumitomo Chemical Company, New York, New York; 48 pages. Final report issued September 12, 2014.

Document No.: MRIDs 49430401/49472701

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA standards (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 not included; a signature page was included (p. 5).
ILV: The study was conducted in accordance with USEPA FIFRA standards and OECD Principles of Good Laboratory Practices (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 not included; a signature page was included (p. 5).

Classification: This analytical method is classified as **SUPPLEMENTAL**. In the ILV, the number of samples was inadequate (n = 4) for prallethrin at 10×LOQ. In the ECM, the means and RSDs did not meet requirements for prallethrin at the LOQ. Representative ILV chromatograms did not support the method. Determinations of the LOQ and LOD were not based on scientifically acceptable procedures. Linearity of the ILV calibration curve was not satisfactory.

PC Code: 128722

Reviewer: He Zhong, Ph.D.
Biologist, EPA/OPP/EFED

Signature: HE ZHONG
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DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=HE ZHONG, dnQualifier=0000051219
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Date: 3-23-2015

Executive Summary

This analytical method, PRTL Study No. 2451W, is designed for the quantitative determination of prallethrin at 0.01 µg/L in surface water using GC/MS/NCI. The LOQ (0.01 µg/L) is greater than the lowest toxicological level of concern (LOC = 0.0079 µg/L) in water, but the LOD (0.002 µg/L) is less than the LOC. In the ECM, acceptable reproducibility was not provided for the LOQ sample set. The method was validated at the LOQ by the ILV with the first trial. In the ILV, an inadequate number of samples was reported for the 10×LOQ sample set. Additionally, the specificity of the method could not be validated based on the provided ILV representative chromatograms. No modifications of the ECM method were reported by the ILV; however, it was noted that only one ion transition was monitored for prallethrin in the ILV, as opposed to three ion transitions in the ECM.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)	Limit of Detection (LOD)
	Environmental Chemistry Method	Independent Laboratory Validation						
Prallethrin	49430401	49472701	Surface water	03/07/2014	Sumitomo Chemical Company	GC/MS/NCI	0.01 µg/L	0.002 µg/L

I. Principle of the Method

Samples (250 mL) of fortified sieved (200-µ) surface water were extracted twice using 25 mL of hexane with manual shaking for 40 seconds, then once using 15 mL of hexane with manual shaking for 40 seconds (pp. 14, 20; Figure 1, p. 33 of MRID 49430401). After the third extraction, the emulsion was broken with a glass rod. The combined organic extracts were passed through 10 g ± 0.1 g of anhydrous sodium sulphate contained in a glass funnel plugged with silanized glass wool. The separatory funnel used for extraction was rinsed twice with 10 mL of hexane. After drying the hexane rinses with anhydrous sodium sulphate, the rinses were combined with the extracts. The combined organic extracts and rinses were reduced to a volume of *ca*. 2 mL by rotary evaporation at *ca*. 40°C. The residue was transferred to a 15 mL conical glass tube along with hexane rinses of the original flask (3 x 3 mL). The combined residue and rinses were reduced to dryness using a Turbovap® LV evaporator with nitrogen at 40°C. The residue was reconstituted in 0.5 mL of toluene. An aliquot of the sample (0.09 mL) was combined with 0.01 mL of lindane (the internal standard, 10 µg/mL).

Samples were analyzed for prallethrin using gas chromatography with mass spectrometry (GC/MS) analysis (pp. 14, 21-22 of MRID 49430401). An Agilent Gas Chromatograph 7890A was equipped with a DB-5 ms (J&W Scientific) column (30 m x 250 µm i.d., 0.25 µm thickness; injection temperature 250°C) and an Agilent 7000 Series Triple Quad Mass Spectrometer with negative chemical ionization (NCI) and selected ion monitoring (SIM). Injection volume was 1 µL. Ions monitored for prallethrin were *m/z* 167 (quantitation ion), *m/z* 168 (confirmation ion 1)

and *m/z* 132 (confirmation ion 2; p. 22; Table 1, p. 30). For the internal standard, lindane, *m/z* 255 (most abundant ion) was monitored. Retention times for the analytes were reported as 9.5 minutes for prallethrin and 7.2 minutes for lindane.

The ILV performed the method as described, however, only *m/z* 167 (quantitation ion) was monitored for prallethrin (pp. 9-12; Table 1, p. 17; Figure 1, pp. 19-21; Appendix 1, pp. 33-42 of MRID 49472701).

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for prallethrin were reported as 0.01 µg/L and 0.002 µg/L (20% of the LOQ), respectively, in the ECM and ILV (pp. 24-25 of MRID 49430401; p. 13; Appendix 1, p. 36 of MRID 49472701).

II. Recovery Findings

ECM (MRID 49430401): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of prallethrin in surface water at 10×LOQ (0.1 µg/L; pp. 10-11, 28; Table I, p. 30; DER Attachment 2). Mean recoveries (131-137%) and RSDs (61-62%) for fortifications of prallethrin at the LOQ (0.01 µg/L) did not meet requirements based on reviewer calculations when including the "outlier" values of 287% (*m/z* 167), 284% (*m/z* 167) and 275% (*m/z* 167); those values were deemed outliers based on the Dixon test and not included in the statistical analysis of the ECM study authors. The surface water was well characterized; the source was Wildcat Creek, Richmond, California (p. 15; Appendix C, pp. 82-86).

ILV (MRID 49472701): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of prallethrin in surface water (only the *m/z* 167.00 was monitored; pp. 14-15; Table 2, p. 18). The method was validated with the first trial. Fortifications of prallethrin were performed at the LOQ (0.01 µg/L; n = 5) and 10×LOQ (0.1 µg/L; n = 4). The number of samples was inadequate for samples dosed at 10×LOQ due to a sample preparation error with one sample, which was subsequently discarded prior to analysis (Appendix 1, Appendix 1, p. 44). During processing, that sample extract was contaminated with wet sodium sulphate and would not dry completely due to excess water. The surface water was well characterized; the source was Tuckahoe Lake in Queen Anne, Maryland (p. 10; Appendix 4, p. 47).

Table 2. Initial Validation Method Recoveries for Prallethrin in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion (*m/z* 167)						
Prallethrin	0.01 (LOQ)	5[2]	90-287	**137**	**84**	**61**
	0.01 (LOQ)	**4**[3]	90-111	100	9	9.0
	0.1	5	97-117	110	8	7.3
Confirmation ion 1 (*m/z* 168)						
Prallethrin	0.01 (LOQ)	5[2]	90-109	**135**	**83**	**62**
	0.01 (LOQ)	**4**[3]	90-109	98	9	9.2
	0.1	5	99-116	110	7	6.4
Confirmation ion 2 (*m/z* 132)						
Prallethrin	0.01 (LOQ)	5[2]	83-105	**131**	**81**	**62**
	0.01 (LOQ)	**4**[3]	83-105	95	10	10.5
	0.1	5	97-115	108	7	6.5

Data (results) were obtained from Table I, p. 30 of MRID 49430401 and DER Attachment 2 (recalculation of means, s.d.s and RSDs for LOQ sample sets).

1 The water matrix was well characterized by Agvise Laboratories (source, Wildcat Creek, California; p. 15; Appendix C, pp. 82-86).

2 The omitted recovery value by the study authors was added back to the calculation to generate higher mean recovery (%) and standard divisions (%)

3 In the study authors' statistical analysis, one value from each set of recovery data at the LOQ was omitted based on the Dixon outlier test (recoveries of 275-287%). The mean, s.d. and RSDs reported in the ECM were 100 ± 9%, RSD 9.0% for the quantitation ion, 98 ± 9%, RSD 9.2% for the confirmation ion 1 and 95 ± 10%, RSD 10.5% for the confirmation ion 2.

Table 3. Independent Validation Method Recoveries for Prallethrin in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion (*m/z* 167.00)[2]						
Prallethrin	0.01 (LOQ)	5	78.8-99.3	90.8	7.81	8.60
	0.1	**4**[3]	81.3-91.9	86.3	4.49	5.20

Data (results) were obtained from Table 2, p. 18 of MRID 49472701.

1 The surface water matrix was obtained from Tuckahoe Lake in Queen Anne, Maryland; it was well characterized by Agvise Laboratories, Northwood, North Dakota (p. 10; Appendix 4, p. 47).

2 Only *m/z* 167.00 was monitored in the ILV.

3 Five samples were prepared but one sample was discarded prior to analysis due to sample preparation error (Appendix 1, Appendix 1, p. 44). The sample extract was contaminated with wet sodium sulphate during processing and would not dry completely due to excess water.

III. Method Characteristics

The LOQ and LOD for prallethrin were reported as 0.01 µg/L and 0.002 µg/L, respectively, in the ECM (pp. 24-25 of MRID 49430401). No calculations or comparisons to noise level were reported. The LOQ was defined as the lowest fortification level of prallethrin which was validated by the analytical method. The LOD was defined as 20% of the LOQ, as well as by the lowest calibrant (0.9 ng/mL, equivalent to 0.002 µg/L in water matrix). In the ILV, the LOQ and

LOD were reported from the ECM with no further justification (p. 13; Appendix 1, p. 36 of MRID 49472701).

Table 4. Method Characteristics

		Prallethrin
Limit of Quantitation (LOQ)		0.01 µg/L
Limit of Detection (LOD)		0.002 µg/L
Linearity (calibration curve r^2 and concentration range)	ECM[1]	r^2 = 0.9953 (*m/z* 167) r^2 = 0.9952 (*m/z* 168) r^2 = 0.9954 (*m/z* 132)
	ILV[2]	r^2 = **0.989** (*m/z* 167.00)
	Concentration range	(0.9-180 ng/mL)
Repeatable		**No** for LOQ[3] Yes for 10×LOQ
Reproducible		Yes for LOQ **No** for 10×LOQ[4]
Specific		Yes for ECM[5] **No** for ILV[6]

Data were obtained from Tables I-II, pp. 30-31; Figures 4-5, pp. 37-40; Figures 10-13, pp. 59-70 of MRID 49430401; pp. 13-15; Table 2, p. 18; Figures 2-9, pp. 22-29; Appendix 1, p. 36 of MRID 49472701.

1 The reviewer verified the linearity of the calibration curves of the ECM (r^2 = 0.99 for *m/z* 167 and *m/z* 168; r^2 = 0.9906 for *m/z* 132; see DER Attachment 2).

2 The reviewer verified the linearity of the calibration curves of the ILV (r^2 = 0.9822; see DER Attachment 2).

3 Mean recoveries (131-137%) and RSDs (61-62%) for fortifications of prallethrin at the LOQ (0.01 µg/L) did not meet requirements based on reviewer calculations when including the "outlier" values of 275-287%; those values were deemed outliers by the study authors based on the Dixon test and not included in the ECM statistical analysis (Table I, p. 30 of MRID 49430401 and DER Attachment 2).

4 The number of samples was inadequate for samples dosed at 10×LOQ (n = 4). Five samples were prepared but one sample was discarded prior to analysis due to sample preparation error (Appendix 1, Appendix 1, p. 44 of MRID 49472701). The sample extract was contaminated with wet sodium sulphate during processing and would not dry completely due to excess water.

5 Figures 10-13, pp. 59-70 of MRID 49430401.

6 The analyte signal was barely visible above the baseline in the representative chromatogram of the LOQ fortification (Figure 8, p. 28 of MRID 49472701). It was not possible to assess the raw data for baseline interferences from the matrix or sample processing. A very small peak response at the retention time of prallethrin was noted in the chromatogram of the matrix blank, but the magnitude of this peak compared to the LOQ peak could not be assessed since peak areas were not reported for any validation samples (Figure 7, p. 27).

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, the number of samples was inadequate for the surface water dosed at 10×LOQ (n = 4; Table 2, p. 18). Five samples were prepared but one sample was discarded prior to analysis due to sample preparation error (Appendix 1, Appendix 1, p. 44). The sample extract was contaminated with wet sodium sulphate during processing and would not dry completely due to excess water. OCSPP guidelines recommend a minimum of five spiked replicates to be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

2. In the ECM, mean recoveries (131-137%) and RSDs (61-62%) for fortifications of prallethrin at the LOQ (0.01 µg/L) did not meet requirements based on reviewer calculations when including the "outlier" values of 287% (*m/z* 167), 284% (*m/z* 167) and 275% (*m/z* 167); those values were deemed outliers by the study authors based on the Dixon test and not included in the statistical analysis of the ECM (pp. 10-11, 28; Table I, p. 30; DER Attachment 2). The mean, s.d. and RSDs reported in the ECM were 100 ± 9%, RSD 9.0% for the quantitation ion, 98 ± 9%, RSD 9.2% for the confirmation ion 1 and 95 ± 10%, RSD 10.5% for the confirmation ion 2. All procedural recoveries should be included in the statistical analysis of method validation experiments, even those assessed as outliers, and mean recoveries and RSDs should be 70-120% and ≤20%, respectively.

3. 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. The LOQ was defined as the lowest fortification level of prallethrin which was validated by the analytical method. The LOD was defined as 20% of the LOQ, as well as by the lowest calibrant (0.9 ng/mL, equivalent to 0.002 µg/L in water matrix).

   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.

4. The specificity of the method could not be verified in the ILV because the analyte signal was barely visible above the baseline in the representative chromatogram of the LOQ fortification (Figure 8, p. 28 of MRID 49472701). It was not possible to assess the chromatographic raw data for baseline interferences from the matrix or sample processing. The reviewer noted that the problem could have been caused by providing a representative chromatogram which was not "zoomed-in" (the "zoomed-in" spectra was provided in the ECM chromatograms).

   A very small peak response at the retention time of prallethrin was noted in the ILV chromatogram of the matrix blank, but the magnitude of this peak compared to the LOQ peak could not be accessed since peak areas were not reported for any validation samples (Figure 7, p. 27 of MRID 49472701).

   The ILV study authors reported that inferences were <30% of the LOQ during sample analysis (p. 13; Table 2, p. 18 of MRID 49472701).

5. The linearity of the ILV calibration curve was not satisfactory; linearity is satisfactory when $r^2 \geq 0.995$.

6. The ILV study authors reported that no modifications of the ECM method were performed, required or suggested. The ILV study authors reported that "no particular step [was] more critical than others" and that "volumes in extraction procedure step 13 can be changed proportionally" (p. 15 of MRID 49472701). However, the reviewer noted that only one ion transition of prallethrin was monitored in the ILV, whereas three ion transitions were monitored in the ECM. This modification did not affect the validity of the ILV study results since OCSPP guidelines typically do not require a confirmation method when analytical methods such as GC/MS and LC/MS are employed; however, the ILV study should be more rigorous than the ECM study.

7. The ECM study authors reported that the samples were originally analyzed by GC/MS with electron impact mode (m/z 123 and 105) without the lindane internal standard (p. 26 of MRID 49430401). This original analytical method yielded poor specificity for prallethrin with baseline and non-related peak interferences, as well as poor sensitivity at the lower end of the calibration curve. Chemical ionization mode and the addition of lindane were employed in order to reduce interferences from the test system.

8. Lindane was added as an internal standard (p. 10 of MRID 49430401; p. 11 of MRID 49472701). The quantification of prallethrin was based on the peak area response ratio between prallethrin and lindane. The calculations also showed correction for residues detected in the controls; however, no prallethrin residues were detected in the untreated controls or reagent blank (pp. 23-24, 27; Figure 10, pp. 59-61; Figure 12, pp. 65-67; Appendix D, pp. 87-89 of MRID 49430401). In the ILV, quantification of prallethrin was also based on the peak area response ratio between prallethrin and lindane; no correction for residues found in the blanks was shown (p. 14).

9. In the ECM, the matrix was free of interferences; however, the reviewer noted that the confirmation ion *m/z* 168 chromatogram in Figure 10 (p. 60; control for the LOQ) was very light in print (Figure 10, pp. 59-61; Figure 12, pp. 65-67 of MRID 49430401). A peak at *ca*. 9.4 was noted in the confirmation ion *m/z* 132 chromatogram in Figure 10 (p. 61; control for the LOQ), but this peak did not interfere with the baseline of the prallethrin peak (*m/z* 132) at the LOQ (Figure 11, p. 64 of MRID 49430401).

10. The water matrices were well characterized in the ECM and ILV (p. 15; Appendix C, pp. 82-86 of MRID 49430401; p. 10; Appendix 4, p. 47 of MRID 49472701).

11. No communication between the independent laboratory personnel and the method developing laboratory or study sponsor occurred (p. 15 of MRID 49472701).

12. In the ECM, matrix effects were assessed by comparing the response ratio of a solvent-based calibrant (45 ng/mL) to a matrix-based calibrant (45 ng/mL; pp. 27-28; Table III, p. 32 of MRID 49430401). Accuracy for the matrix-based calibrant ranged 104-106% at all three ions.

13. It was reported in the ILV that one set of thirteen samples (one reagent blank, two matrix blanks, five samples dosed at the LOQ and five samples dosed at 10×LOQ) required ten worker-hours to complete the sample processing (pp. 9, 15; Appendix 1, p. 37 of MRID 49472701). Subsequent GC/MS analysis and evaluation required 22 hours. The overall time for a sample set 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

Prallethrin (1R trans/cis ratio = 98/2)

IUPAC Name: (S)-2-Methyl-4-oxo-3-(2-propynyl)-2-cyclopent-2-enyl (1R)-cis,trans-chrysanthemate

CAS Name: (S)-2-Methyl-4-oxo-3-(2-propynyl)-2-cyclopenten-1-yl (1R)-cis,trans-2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate

CAS Number: 23031-36-9 (mixture of 8 isomers)

SMILES String: Not found