

Test Material: Permethrin

MRID: 49263701 & 49421001

Title: Development and Validation of a Method for the Determination of Permethrin in Soil, Water and Sediment

EPA PC Code: 109701

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: 

Date: 2/9/15

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QC/QA Manager: Joan Gaidos

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Date: 2/9/15

Analytical methods for permethrin in water, soil and sediment

Reports: **ECM:** EPA MRID No. 49263701. Shao, J. 2012. Independent Laboratory Method Validation for the Analysis of Permethrin in Water and Soil by LC-MS/MS. PASC Project No.: 058-0612C. PASC Report No.: PASC-REP-0204. FMC Study No.: 138ILV11R1. FMC Report No.: PC-0722. Report prepared by Primera Analytical Solutions Corporation, Princeton, New Jersey; sponsored and submitted by FMC Corporation, Agricultural Products Group, Ewing, New Jersey; 58 pages. Final report issued June 20, 2012.
ILV: EPA MRID No. 49421001. Shen, H. 2014. Development and Validation of a Method for the Determination of Permethrin in Soil, Water and Sediment. PTRL West Study/Project No.: 2468W. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California; sponsored and submitted by Consumer Specialty Products Association Inc. (CSPA), on behalf of the Permethrin Data Group II Steering Committee/ Joint Venture, Washington, DC; 64 pages. Final report issued June 27, 2014.

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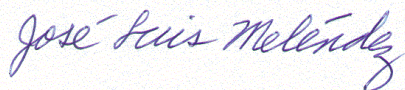
Guideline: 850.6100

Statements: **ECM:** The study was conducted in accordance with U.S. EPA GLP (CFR Title 40, Part 160; p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of the Authenticity statements were provided (pp. 2-5).
ILV: The study was conducted in accordance with U.S. EPA GLP (CFR Title 40, Part 160; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was included with the Quality Assurance statement.

Classification: This analytical method and validation are classified as supplemental. *The LOQ is greater than the lowest toxicological level of concern in water; the LOQ is less than the lowest toxicological level of concern in soil and sediment. A new water method and validation should be submitted in which the LOQ is less than the lowest toxicological level of concern for permethrin.*

Other comments: The number of samples was insufficient (n = 4) for soil samples fortified at the LOQ in the ILV. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. Extraction methods were comparable between the ILV and the ECM, but not identical, and could not always be directly compared due to lack of detail in the ECM study report. A sediment matrix was not used in the submitted ECM. Chromatographic conditions used in this ILV were similar to HPLC Method B used in the ECM.

PC Code: 109701

**Reviewed by:** José Meléndez, EPA**Date:** September 17, 2015

Executive Summary

ECM: This environmental chemistry method (ECM) analytical method, FMC Study No.: 138ILV11R1, is designed for the quantitative determination of a mixture of *trans*- and *cis*-permethrin (CAS No.s 61949-76-6 and 61949-77-7) in river water and sandy loam soil using LC/MS/MS. The method is quantitative for permethrin at the stated LOQ of 5.00 ppt (ng/L) for water and 1.00 ppb ($\mu\text{g}/\text{kg}$) for soil.

ILV: The ILV, conducted by PTRL West Study/Project No. 2468W, is designed for the quantitative determination of a mixture of *trans*- and *cis*-permethrin in surface water, sandy loam soil and clay loam sediment using LC/MS/MS. The method is quantitative for total permethrin at the stated LOQs of 5.0 ppt (ng/L) for water and 1.0 ppb ($\mu\text{g}/\text{kg}$) for soil and sediment. The recoveries of *cis*-permethrin and *trans*-permethrin were independently determined for each sample then combined for the recovery of total permethrin. The number of samples was sufficient for all analyses, except for soil samples fortified at the LOQ, where $n = 4$.

The EPA Regional Screening Level reports the lowest toxicological levels of concern for permethrin (CAS No. 52645-53-1) in water and soil as 100 $\mu\text{g}/\text{L}$ in residential tap water and 310 mg/kg in residential soil. The risk-based soil saturation limit (SSL) for the protection of ground water is 24 mg/kg (see reviewer comment 4).

The lowest available water column toxicity values for freshwater invertebrates and estuarine/marine invertebrates, expressed as LC_{50} or EC_{50} values, are 0.0066 $\mu\text{g a.i.}/\text{L}$ (amphipod *Hyaella azteca*, MRID 49513901, data in review) and 0.018 $\mu\text{g a.i.}/\text{L}$ (stone crab, MRID 40228401), respectively. The organic carbon normalized sediment NOAEC for *H. azteca* for permethrin is 322 $\mu\text{g a.i.}/\text{kgOC}$ (MRID 48593614, data in review).

*The LOQ (5.0 ng/L) is **greater than** the lowest toxicological level of concern in water* [considering an LOC of 0.5 for acute non-listed species or 0.05 for acute listed species, the lowest toxicological levels of concern are 3.3 ng/L ($= 6.6 \text{ ng}/\text{L} \times 0.5$) for acute non-listed species, and 0.33 ng/L ($= 6.6 \text{ ng}/\text{L} \times 0.05$) for acute listed species, respectively].

*The LOQ (1.0 $\mu\text{g}/\text{kg}$) is **less than** the lowest toxicological level of concern in soil and sediment.*

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Permethrin	49263701	49421001		Water	06/20/2012	FMC Corporation & CSPA ¹	LC/MS/MS	5.0 ppt (ng/L) ²
				Soil/Sediment				1.0 ppb (µg/kg) ^{2,3}

1 Consumer Specialty Products Association Inc., (CSPA), on behalf of the Permethrin Data Group II Steering Committee/Joint Venture.

2 The LOQ (5.0 ng/L) is greater than the lowest toxicological level of concern in water; meanwhile, the LOQ (1.0 µg/kg) is less than the lowest toxicological level of concern in soil and sediment.

3 Assuming a typical range of organic carbon content for majority of sediments, of 2–10% TOC, the normalized LOQ would range from 10–50 µg/kg_{OC} for permethrin.

I. Principle of the Method

There were slight differences between the ECM and the ILV. The principle of the method is reported separately for the ECM and for the ILV.

ECM (MRID 49263701):

Water extraction:

Water samples (500 mL) were mixed with saturated sodium chloride (10 g) then extracted with hexane (100 mL; extraction details unreported; pp. 16-17; Table 3A, p. 17). The hexane extract was removed and dried with anhydrous sodium sulphate. The volume was reduced to dryness then the residue was reconstituted in hexane (evaporation details not reported; volume of reconstitution not reported). The hexane extract was purified via solid phase extraction (SPE) cartridge (SPE details not reported, including elution solvent). The eluate was reduced to dryness then the residue was reconstituted in methanol (0.5 mL) prior to analysis via LC/MS/MS.

Soil extraction:

Soil samples (10 g) were mixed with methanol:water (1:1, v:v; volume unreported) then extracted with hexane (10.0 mL; extraction details unreported; pp. 16-17; Table 3B, p. 18). The hexane extract was reduced to dryness then the residue was reconstituted in hexane (evaporation details not reported; volume of reconstitution not reported). The hexane extract was purified via solid phase extraction (SPE) cartridge (SPE details not reported, including elution solvent). The eluate was reduced to dryness then the residue was reconstituted in methanol (2.00 mL) prior to analysis via LC/MS/MS.

Samples were analyzed for permethrin by liquid chromatography with mass spectrometry (LC/MS/MS) using Method A (producing a single peak for *cis*-/*trans*-isomers) and Method B (producing separate peaks for *cis*-/*trans*-isomers; pp. 13-14). Method A employed a Sunfire C8 (20 mm x 2.1 mm i.d., 3.5 μ m) column with a gradient mobile phase of (A) 0.5% formic acid in DI water and (B) methanol (time A:B; 0 min. 80:20, 2-4 min. 10:90, 4.5-7 min. 80:20). Method B employed a Supelco Express C18 (50 mm x 2.1 mm i.d., 2.7 μ m) column with a gradient mobile phase of (A) 0.5% formic acid in DI water and (B) methanol (time A:B; 0 min. 70:30, 3-5 min. 10:90, 5.5-8 min. 70:30). Retention times for the *cis*- and *trans*-isomers of permethrin were *ca.* 3.1 min. using Method A and 4.6 min. for *trans*-permethrin and 4.9 min. for *cis*-permethrin using Method B (p. 18). Permethrin ions were monitored using MS in positive ESI mode (Q1, 391.07 m/z; Q3, 183.20 m/z; MRM scan type; p. 14). Injection volume was 20 μ L.

ILV (MRID 49421001):

The water, soil and sediment samples were fortified with a mixed stock solution containing a 1:1 racemic ratio of *cis*-permethrin and *trans*-permethrin (pp. 16-18).

Water extraction:

Water samples (500 mL) were fortified and mixed saturated sodium chloride (10 g), and methanol (50 mL; pp. 18-20; Figure 1, p. 33). The mixture was extracted twice with hexane (2 x 50 mL) with vigorous shaking for 1 minute. Each hexane extract was removed while passing through a glass funnel with glass wool plug and anhydrous sodium sulphate (20 g). After the second extraction, the sodium sulphate was rinsed with 10 mL of hexane. The volume was reduced to *ca.* 0.2 mL via turboevaporation at 40°C, then further reduced to dryness via manual evaporation with nitrogen. The residue was reconstituted in hexane (2 mL). The hexane extract was purified via solid phase extraction (SPE; Bond Elut Silica SPE 500 mg, 3 mL). The SPE cartridge was pre-conditioned with 3 mL of hexane then the residue was applied under gravity or low vacuum. Analytes were eluted with 6 mL of hexane:diethyl ether (9:1, v:v) under gravity or low vacuum. The eluate was reduced to dryness via turboevaporation under a stream of nitrogen at 40°C; the residue was reconstituted in methanol (1.0 mL) prior to analysis via LC/MS/MS.

Soil/sediment extraction:

Soil/sediment samples (50 g) were fortified in new plastic disposable centrifuge bottles and mixed with 75 mL of methanol:water (1:1, v:v) (p. 20; Figure 2, p. 34). The mixture was extracted with hexane (50.0 mL) on a shaker for 60 minutes. After centrifugation (4000 rpm for 5 minutes), the upper hexane layer (*ca.* 50 mL) was removed. A 10 mL aliquot of the hexane extract was reduced to dryness via turboevaporation at 40°C. The residue was reconstituted in hexane (2 mL). The hexane extract was purified via solid phase extraction (SPE; Bond Elut Silica SPE 500 mg, 3 mL). The SPE cartridge was pre-conditioned with 3 mL of hexane then the residue was applied under gravity or low vacuum. Analytes were eluted with 6 mL of hexane:diethyl ether (9:1, v:v) under gravity or low vacuum. The eluate was reduced to dryness via turboevaporation under a stream of nitrogen at 40°C; the residue was reconstituted in methanol (1.0 mL) prior to analysis via LC/MS/MS.

Samples (water/soil/sediment matrices) were analyzed for permethrin using an AB Sciex API 4000 Series Triple Quad Mass Spectrometer with Agilent 1100 Series Liquid Chromatograph (LC/MS/MS) employing a Supelco Express Ascentis® C18 (50 mm x 2.1 mm i.d., 2.7 µm) column with a gradient mobile phase of (A) 0.5% formic acid in HPLC grade water and (B) methanol (time A:B; 0 min. 70:30, 3.0-6.0 min. 10:90, 6.5-9.0 min. 70:30; pp. 16, 21-22). Permethrin ions were monitored using MS in positive ESI mode (391→183 m/z, primary quantitation transition; 393→183 m/z, alternative quantitation transition; MRM scan type). Injection volume was 20 µL. The *cis*- and *trans*-isomers of permethrin were separated by LC/MS/MS for quantification; however, the retention times for the isomers were not reported (pp. 22-23).

In the ILV, the Limit of Quantification (LOQ) values for permethrin were 5.0 ppt in water and 1.0 ppb in soil/sediment (corresponding to 2.5 ng/mL total permethrin; p. 23). The Limit of Detection (LOD) values were estimated at 1 ppt in water and 0.2 ppb in soil/sediment (corresponding to 0.5 ng/mL total permethrin).

II. Recovery Findings

ECM (MRID 49263701): Mean recoveries and RSDs were within guideline requirements for analysis of permethrin water and soil (p. 9). The method was validated with the first trial (p. 34). The water was obtained from the local Delaware River in Yardley, Pennsylvania and not further characterized (p. 12). The soil was a non-GLP sandy loam soil (58% sand, 27% silt, 15% clay) with 5.01% organic matter, 2.91% organic carbon and pH 7.21 (Attachment II, p. 48). Permethrin was identified by LC/MS/MS using two methods (A and B).

ILV (MRID 49421001): Mean recoveries and RSDs were within guideline requirements for analysis of permethrin in surface water, sandy loam soil and clay loam sediment at the LOQ and 10×LOQ (pp. 12, 27; Table I, p. 31; Appendix C, pp. 59-61). The fortification levels for water were 5.0 and 50 ppt. The fortification levels for soil and sediment were 1.0 and 10 ppb. The recoveries of *cis*-permethrin and *trans*-permethrin were independently determined for each sample then combined for the recovery of total permethrin. For statistics, the number of samples was five for all analyses, except for soil samples fortified at the LOQ where n = 4. No permethrin was found in the final extract of one of the five soil samples at the LOQ (Appendix D, p. 63). This sample was treated as an outlier and excluded from the calculations and results; no supporting statistical tests, such as Grubbs' and Dixon tests, were presented to justify exclusion of the recovery results as outliers. All matrices were fully characterized by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendix C, pp. 59-61). The surface water was obtained from Denton, Maryland. The soil was sandy loam soil (USDA textural class) obtained from Vacaville, California. The sediment was clay loam (USDA textural class) obtained from Goose River, Grand Forks County, North Dakota. Permethrin was identified by LC/MS/MS using MRM (pp. 16, 21-22).

Table 2. Initial Validation Method Recoveries for Permethrin in Water and Soil¹

Analyte	Fortification Level (ppt) or (ppb) ¹	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ²	Relative Standard Deviation (%)
River water						
Method A						
Permethrin ³	5.00 (LOQ)	5	84.8-111	91.9	10.8	11.9
	50.0	5	75.0-105	91.4	11.8	13.1
Method B						
Permethrin ³	5.00 (LOQ)	5	84.8-94.9	88.7	5.1	5.80
	50.0	5	72.7-107	91.2	13.9	15.1
Sandy loam soil						
Method A						
Permethrin ³	1.00 (LOQ)	5	80.3-117	96.8	13.9	14.4
	10.0	5	94.5-106	98.0	4.7	4.96
Method B						
Permethrin ³	1.00 (LOQ)	5	80.3-120	106	15.4	14.5
	10.0	5	85.6-106	94.5	9.7	10.1

Data were obtained from Table 8A, p. 28, Tables 8C-9A, pp. 30-31, Table 9C, p. 33 of the study report.

1 Fortification units were ppt for water and ppb for soil.

2 Reviewer-calculated using data from the study report since only mean and RSD values were reported in the study report (see DER Attachment 2).

3 Combined *cis*- and *trans*-permethrin.

Table 3. Independent Validation Method Recoveries for Permethrin in Water, Soil and Sediment

Analyte	Fortification Level (ppt) or (ppb) ²	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
<i>Cis</i> -Permethrin	5.0 (LOQ)	5	101-120	111	9	8
	50	5	86-106	96	7	7
<i>Trans</i> -Permethrin	5.0 (LOQ)	5	98-120	11	9	8
	50	5	95-127	107	12	11
Total Permethrin ³	5.0 (LOQ)	5	100-120	111	9	8
	50	5	91-117	102	10	10
Sandy Loam Soil						
<i>Cis</i> -Permethrin	1.0 (LOQ)	4 ⁴	72-92	84	9	11
	10	5	61-103	84	15	18
<i>Trans</i> -Permethrin	1.0 (LOQ)	4 ⁴	68-90	76	10	13
	10	5	54-88	73	12	16
Total Permethrin ³	1.0 (LOQ)	4 ⁴	72-91	80	8	10
	10	5	58-96	79	14	18

Analyte	Fortification Level (ppt) or (ppb) ²	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Sediment						
<i>Cis</i> -Permethrin	1.0 (LOQ)	5	90-130	118	17	14
	10	5	109-125	118	6	5
<i>Trans</i> -Permethrin	1.0 (LOQ)	5	102-132	113	12	11
	10	5	107-123	116	8	7
Total Permethrin ³	1.0 (LOQ)	5	97-128	116	12	10
	10	5	109-123	117	6	5

Data were obtained from p. 12; Table I, p. 31 of the study report.

1 All matrices were fully characterized with USDA textural classifications by Agvise Laboratories, Northwood, North Dakota (p. 16; Appendix C, pp. 59-61). The surface water was obtained from Denton, Maryland. The soil was obtained from Vacaville, California. The sediment was obtained from Goose River, Grand Forks County, North Dakota.

2 Fortification units were ppt for water and ppb for soil and sediment.

3 Combined *cis*- and *trans*-permethrin.

4 No permethrin was found in the final extract of one of the five soil samples at the LOQ. This sample was treated as an outlier and excluded from the calculations and results (pp. 12, 27; Table 1, p. 31; Appendix D, p. 63).

III. Method Characteristics

In the **ECM**, the LOQ values for permethrin were 5.00 ppt in water and 1.00 ppb in soil (pp. 22-23). At this LOQ, signal-to-noise ratios (S/N) in water samples were >120 for HPLC Method A and >128 for HPLC Method B; S/N in soil samples were >144 for HPLC Method A and >45.7 for HPLC Method B. The LODs were estimated by multiplying the standard deviation of replicate samples fortified at LOQ level with the t-distribution value at 99% confidence level ($t_{0.99} = 3.747$ for 5 replicates). The LOD values for permethrin in water were 2 ppt for Method A (S/N *ca.* 60) and 0.5 ppt (*trans*-permethrin; S/N *ca.* 27) and 0.6 ppt (*cis*-permethrin; S/N *ca.* 56) for Method B; LOD values in soil were 0.5 ppb for Method A (S/N *ca.* 102) and 0.3 ppb (*trans*-permethrin, S/N *ca.* 50 and *cis*-permethrin, S/N *ca.* 71) for Method B. The study author reported the lowest toxicity values for freshwater invertebrates and estuarine/marine invertebrates as EC₅₀ values of 0.039 µg a.i./L (waterflea) and 0.018 µg a.i./L (stone crab), respectively (based on the EPA EFED Registration Review for Permethrin, 06/20/11). The study author also reported that “permethrin has no adverse effect on various soil microorganisms or their soil activity” (p. 23). Based on this data, the study author concluded that the LOQ levels validated by the study report are below levels of ecological concern in soil and water (p. 24).

In the **ILV**, the Limit of Quantification (LOQ) values for permethrin were 5.0 ppt in water and 1.0 ppb in soil/sediment (corresponding to 2.5 ng/mL total permethrin; pp. 23, 27-28). The LOQ was validated by the demonstration of acceptable linearity and recovery at the LOQ in each matrix. The Limit of Detection (LOD) values were estimated at 1 ppt in water and 0.2 ppb in soil/sediment (corresponding to 0.5 ng/mL total permethrin; *ca.* 20% of the LOQ). The LOD was validated by the demonstration of acceptable linearity and sensitivity at the LOD in each matrix (Figure 13, p. 46). No calculations or other justifications were provided.

Table 4a. ECM: Method Characteristics*

	Permethrin			
	River water		Sandy loam soil	
	Method A	Method B	Method A	Method B
Limit of Quantitation (LOQ)	5.00 ppt	5.00 ppt	1.00 ppb	1.00 ppb
Limit of Detection (LOD)	2 ppt	0.5-0.6 ppt	0.5 ppb	0.3 ppb
Linearity (calibration curve r^2 and concentration range) ¹	$r^2 = 0.991$ (2.5-200 ng/mL)	$r^2 = 0.994$ (1.25-100 ng/mL; <i>trans</i>) $r^2 = 0.994$ (1.25-100 ng/mL; <i>cis</i>)	$r^2 = 0.997$ (2.5-200 ng/mL)	$r^2 = 0.996$ (1.25-100 ng/mL; <i>trans</i>) $r^2 = 0.992$ (1.25-100 ng/mL; <i>cis</i>)
Repeatable	Yes			
Reproducible	No ²		No ³	
Specific	Yes			

* Data were obtained from pp. 9, 22-24 and Tables 5A-6C, pp. 20-22 of the ILV study report unless noted otherwise.

1 Reviewer-calculated calibration curves yielded similar linearity, r^2 values of 0.9939-0.9994 (DER Attachment 2).

2 The study utilized LC/MS/MS analysis while the ECM stated in this study utilized GC/MS analysis. The previous ECM's (MRID 48638501) reviewer calculated LOQs ranged 1.6-45 ng/L (ppt) for influent and effluent wastewater (GC/MS analysis; data from previous DER for MRID 48638501).

3 The study utilized LC/MS/MS analysis while the ECM stated in this study utilized GC/MS analysis. The previous ECM's (MRID 47053002) reported LOQ was 1.0 µg/kg (ppb) for sediment (GC/MS analysis; data from previous DER for MRID 47053002).

Table 4b. Final Method Characteristics (Method B of the ECM)*

	Permethrin		
	River water	Sandy Loam Soil	Clay Loam Sediment
Limit of Quantitation (LOQ)	5.0 ppt	1.0 ppb	
Limit of Detection (LOD)	1 ppt	0.2 ppb	
Linearity ¹	calibration curve r^2	$r^2 = 0.9971$ (<i>cis</i> -permethrin) $r^2 = 0.9983$ (<i>trans</i> -permethrin)	
	concentration range	(0.25-50 ng/mL <i>cis</i> - or <i>trans</i> -permethrin) (0.5-100 ng/mL total permethrin)	(0.5-50 ng/mL <i>cis</i> - or <i>trans</i> -permethrin) (1-100 ng/mL total permethrin)
Repeatable	Yes		
Reproducible	Yes ²		
Specific	Yes		

* Data were obtained from pp. 12, 23, 26-28; Table I, p. 31 of the study report.

1 ECM r^2 values are reviewer-generated from reported r values of *ca.* 0.9986-0.9992 (Figures 8-9, pp. 41-42; DER Attachment 2). Linearity of the ECM calibration curves could not be verified by the reviewer since only raw data for two calibration standards was included in the study report (Figure 10, p. 43).

2 Method MRID 49421001 is reproducible when compared to study MRID 49263701 (Method B).

IV. Method Deficiencies and Reviewer's Comments

Comparison of MRID 49263701 and ECM MRIDs 48638501 & 47053002

1. Note: The report 49263701 was submitted in support of the ECM MRIDs 47053002 & 48638501 which were reviewed in 2011. Overall, the submitted study was not suitable

for verifying the procedures reported by the 2011 ECM since the quantification method was changed from GC/MS to LC/MS/MS. Additionally, the matrices for these reports differed slightly, in that the ECMs considered wastewater and sediment (not characterized) and the current study considered river water and sandy loam soil. For the water procedure, the current study made at least one modification, indicating that ECM procedure was not validated for all types of water sources.

2. The reviewer could not determine the full extent of method deviation in this study from the ECM stated in the study, since the method details of ECM were not provided in MRID 49263701 (p. 17). The study author reported the following method deviations/modifications: the quantification method was changed from GC/MS to LC/MS/MS; the final reconstitution solvent was changed from acetone to methanol due to the LC/MS/MS analysis; and sodium chloride was added to the water sample prior to hexane extraction (p. 18). In addition to these modifications, the reviewer also noted the possibility of more modifications based on the summary of the ECM methods reported in the Environmental Chemistry Method Review Report of MRID 47053002, including the elimination of the addition of methanol to the water sample prior to hexane extraction. The study protocol (Attachment III, pp. 49-58) was included in the study report; however, it did not contain method details, only references to the original ECM.
3. The LOQs of this study were the same or within range of the LOQs of the previous ECM; however, since the analytical method was changed, these LOQ values cannot be compared.
4. The lowest toxicological levels of concern for permethrin (CAS No. 52645-53-1) in water and soil are found in the EPA Regional Screening Level (<http://www.epa.gov/region9/superfund/prg/> archived). Screening levels for permethrin are 100 µg/L in residential tap water and 310 mg/kg in residential soil. The risk-based soil saturation limit (SSL) for the protection of ground water is 24 mg/kg. The lowest available water column toxicity values for freshwater invertebrates and estuarine/marine invertebrates, expressed as LC₅₀ or EC₅₀ values, are 0.0066 µg a.i./L (amphipod *Hyaella azteca*, MRID 49513901, data in review) and 0.018 µg a.i./L (stone crab, MRID 40228401), respectively. The organic carbon normalized sediment NOAEC for *H. azteca* for permethrin is 322 µg a.i./kg_{OC} (MRID 48593614, data in review). An LOQ above toxicological level of concern could result in an unacceptable method classification.
5. Communication between the ECM personnel and the current study author was reportedly recorded, but not discussed or included in the ILV study report (p. 34).
6. The time requirement for the sample sets of this experiment was not reported.

Comparison of ECM (MRID 49263701) and ILV (MRID 49421001)

7. Note: The ILV was developed based on methods reported in the EPA MRID 49263701 (pp. 13, 30 of MRID 49421001). In this DER, MRID 49263701 was considered as the ECM and MRID 49421001 was considered as the ILV.
8. Extraction methods were comparable between the ILV and the ECM, but not identical, and could not always be directly compared due to lack of detail in the ECM study report. A sediment matrix was not analysed in the submitted ECM. Chromatographic conditions used in this ILV were similar to HPLC Method B used in the ECM. LOQ values for permethrin were 5.00 ppt in water and 1.00 ppb in soil in the ECM using HPLC Method B, with LOD values of 0.5-0.6 ppt and 0.3 ppb, respectively.
9. The number of samples was insufficient ($n = 4$) for soil samples fortified at the LOQ (pp. 12, 27; Table I, p. 31; Appendix D, p. 63). No permethrin was found in the final extract of one of the five soil samples at the LOQ. This sample was treated as an outlier and excluded from the calculations and results. No supporting statistical tests, such as Grubbs' and Dixon tests, were presented to justify exclusion of the recovery results as outliers.
10. The estimations of the LOQ and LOD in the ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ was validated by the demonstration of acceptable linearity and recovery at the LOQ in each matrix (pp. 27-28; Figure 13, p. 46). The LOD was validated by the demonstration of acceptable linearity and sensitivity at the LOD in each matrix. No calculations or comparison to background levels was provided for the LOQ or LOD. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
11. Representative chromatograms of reagent blanks, matrix blanks, and fortifications at the LOD, LOQ and 10×LOQ were included for all three matrices (Figures 3-4, pp. 35-37; Figures 11-13, pp. 44-46). The reviewer noted that the two separate peaks of the enantiomers of permethrin were unequal in the chromatograms. The baseline noise was close to 50% of the height of the smaller peak in the water sample fortified at the LOD (Figure 13, p. 46). There was an interfering peak at the retention time of *trans*-permethrin in the sediment matrix blank with the MS/MS transition 391→183; therefore, the alternative MS/MS transition 393→183 was used to eliminate the interference (p. 25; Figure 4c-d, p. 37).
12. Matrix effects were assessed in the ILV (pp. 25-26; Table II, p. 32; Figures 5-7, pp. 38-40). Solvent-based calibrants were used for the quantitation of permethrin since the percent recoveries of matrix-based calibrants were within acceptable limits for all matrices.
13. The reviewer noted a typographical error on p. 25 of the study report where the MS/MS transition for primary quantitation was reported as 391→**193** instead of 391→**183** (pp. 10, 22).

14. The time requirement for the sample sets of this experiment was reported as *ca.* 6 hours for sample preparation and for LC/MS/MS analysis; the total amount of time required to complete a subsample set was *ca.* 12 hours or 1.5 calendar days (p. 24).

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.

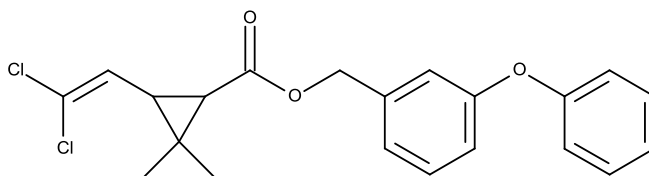
Attachment 1: Chemical Names and Structures**Permethrin**

IUPAC name 3-Phenoxybenzyl(1RS)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Name (3-Phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

CAS # 52645-53-1

SMILES CC1(C)C(C=C(Cl)Cl)C1C(=O)OCc3cccc(Oc2ccccc2)c3

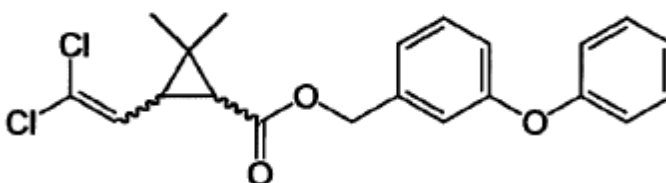
***Cis*-Permethrin**

IUPAC name 3-Phenoxybenzyl (1RS)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Name Not reported

CAS # 61949-76-6

SMILES Not found

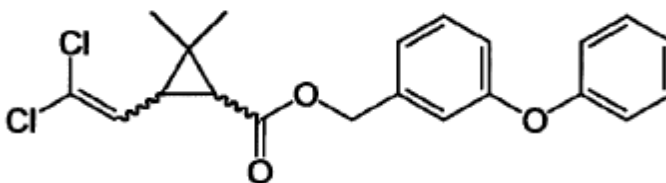
***Trans*-Permethrin**

IUPAC name 3-Phenoxybenzyl (1RS)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Name Not reported

CAS # 61949-77-7

SMILES Not found



DER Attachment 2: Spreadsheets

Chemical: Permethrin
 PC: 109701
 MRID: 49263701
 Guideline: 850.6100

Determination of Permethrin in River Water and Sandy Loam Soil

Fortified (ppt)	Permethrin in River Water						
	Recovery (%)	Mean (%)	SD ¹ (%)	RSD ² (%)	Max	Min	n =
Method A (Single peak)							
5.00 LOQ	87.0 86.8 111.0 84.8 89.7	91.9	10.8	11.8	111.0	84.8	5
50.00	75.0 89.8 86.3 105.0 100.0	91.2	11.8	12.9	105.0	75.0	5
Method B (Combined data of separate peaks)							
5.00 LOQ	84.8 84.9 85.1 94.9 93.7	88.7	5.1	5.8	94.9	84.8	5
50.00	107.0 103.0 87.4 86.4 72.7	91.3	13.9	15.2	107.0	72.7	5
Fortified (ppb)	Permethrin in Sandy Loam Soil						
	Recovery (%)	Mean (%)	SD ¹ (%)	RSD ² (%)	Max	Min	n =
Method A (Single peak)							
1.00 LOQ	91.0 80.3 117.0 92.6 103.0	96.8	13.9	14.3	117.0	80.3	5
10.00	94.5 106.0 98.1 94.9 96.1	97.9	4.7	4.8	106.0	94.5	5
Method B (Combined data of separate peaks)							
1.00 LOQ	111.0 80.3 120.0 105.0 114.0	106.1	15.4	14.5	120.0	80.3	5
10.00	86.8 104.0 90.7 106.0 85.6	94.6	9.7	10.2	106.0	85.6	5

Results from Tables 8A-9C, pp. 28-33 of MRID 49263701.

Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).

Any discrepancies between reviewer calculated values and reported results most likely due to rounding.

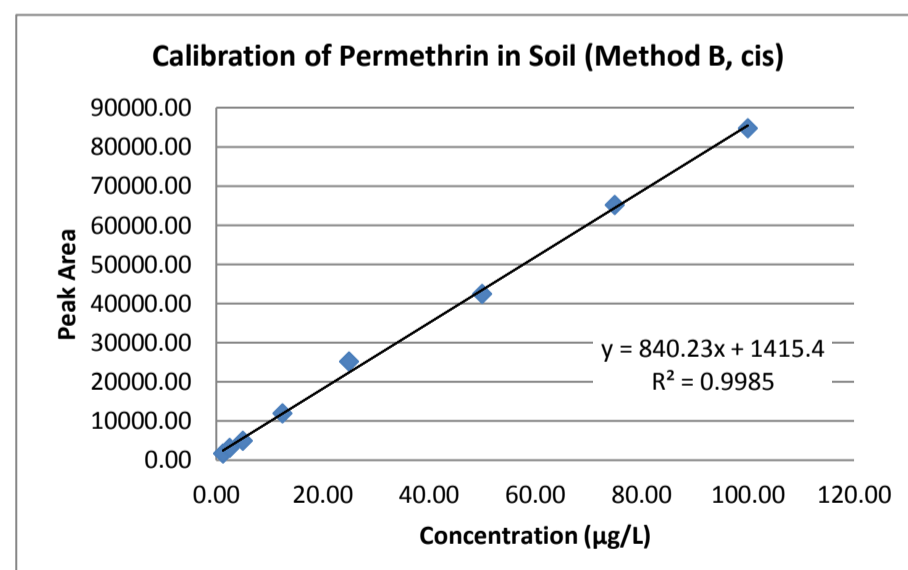
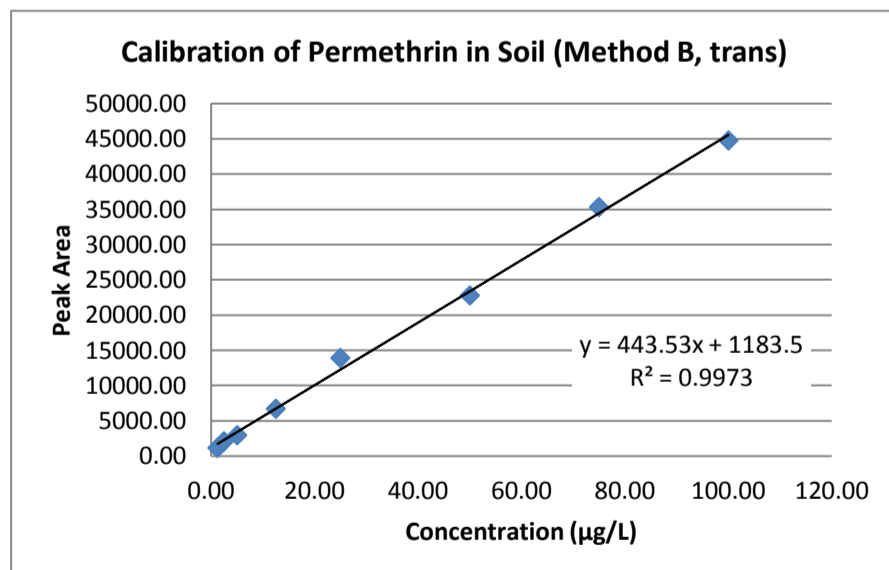
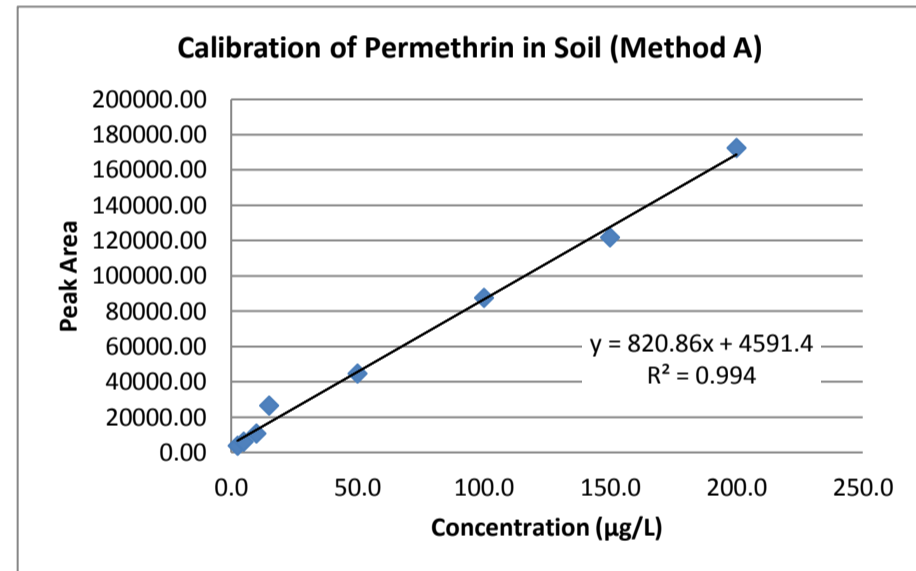
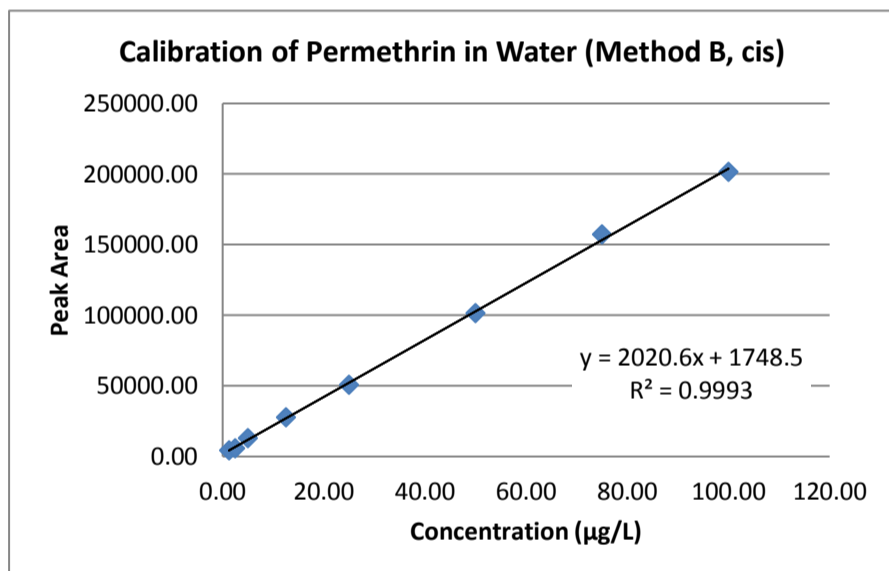
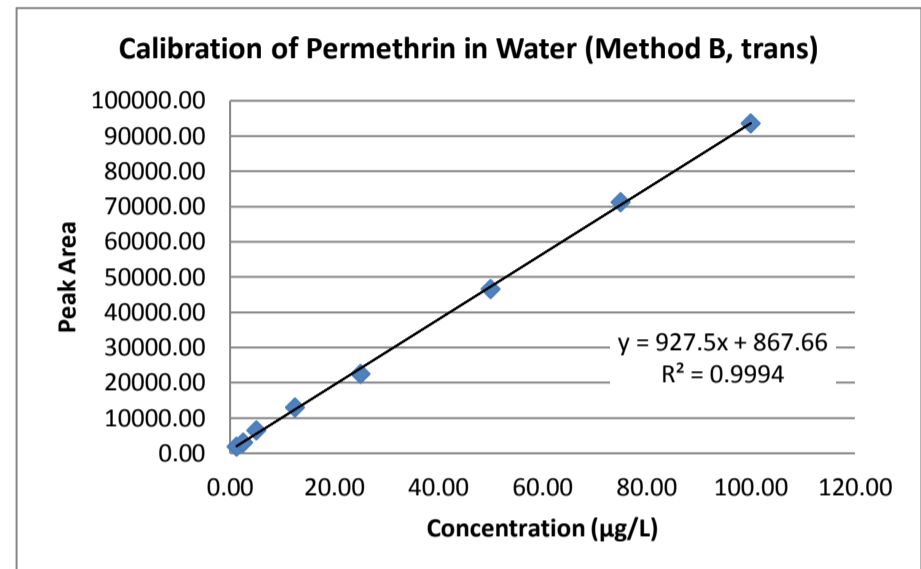
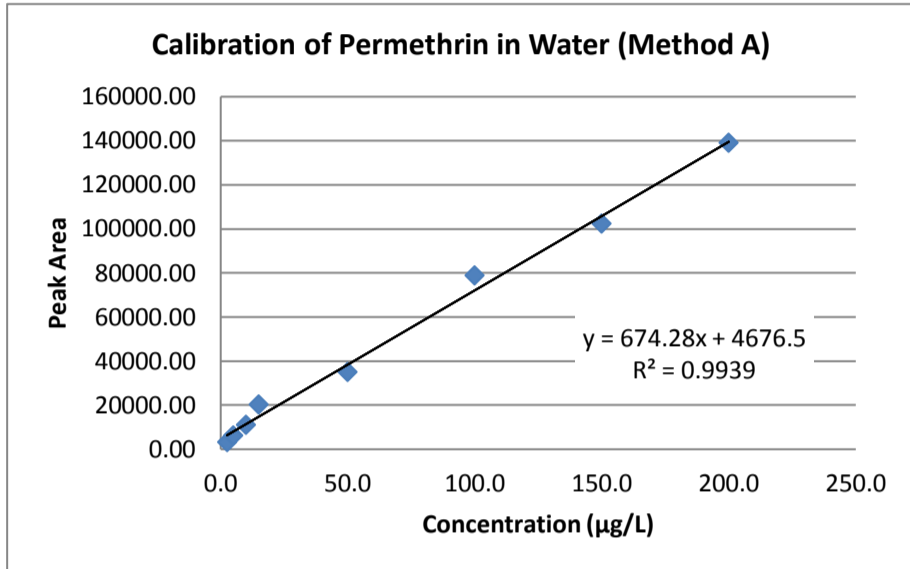
1 SD = Standard Deviation; determined using the "unbiased" or "n-1" method.

2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

Chemical: Permethrin
 PC: 109701
 MRID: 49263701
 Guideline: 850.6100
 Calibration Curves

Calibration Curve Data	Permethrin											
	Water- Method A		Water- Method B (trans)		Water- Method B (cis)		Soil- Method A		Soil- Method B (trans)		Soil- Method B (cis)	
	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts
	2.5	3246.00	1.25	1961.00	1.25	4463.00	2.5	3712.00	1.25	1173.00	1.25	1690.00
	5	6201.00	2.50	3066.00	2.50	5727.00	5	6186.00	2.50	2089.00	2.50	3177.00
	10	11049.00	5	6562.00	5	12833.00	10	10746.00	5	2966.00	5	4920.00
	15	20360.00	13	13044.00	13	27746.00	15	26587.00	13	6685.00	13	11947.00
	50	35159.00	25	22465.00	25	50777.00	50	44788.00	25	13948.00	25	25141.00
	100	78810.00	50	46540.00	50	101612.00	100	87573.00	50	22807.00	50	42471.00
	150	102413.00	75	71283.00	75	157204.00	150	121816.00	75	35352.00	75	65117.00
	200	139227.00	100	93606.00	100	201713.00	200	172432.00	100	44755.00	100	84772.00

Results (Peak Area) from Tables 5A-6C, pp. 20-22 of MRID 49263701.



Chemical: Permethrin
PC: 109701
MRID: 49421001
Guideline: 850.6100

Calibration Curve Correlation Coefficients r (1/x weighting) converted to r^2

Analyte	Reported r	Calculated r^2
cis-Permethrin	0.998560103369	0.9971
trans-Permethrin	0.999169403651	0.9983

Results (r values) from Figures 8-9, pp. 41-42 of MRID 49421001.