INTRODUCTION

An independent laboratory validation (ILV) of the analytical method for the determination of Prallethrin in surface water was performed by Wildlife International. The analytical method, "Method Validation of an Analytical Method for the Determination of Prallethrin in Surface Water" is referenced in Appendix I. The study was performed to satisfy regulatory requirements for independent laboratory validation of methods as set forth by the U.S. Environmental Protection Agency Series 860 – Residue Chemistry Test Guidelines, OPPTS 860.1340 (1) and OCSPP 850.6100 (2), EU Guidelines SANCO/825/00 REV. 8.1 16/11/2010 (3), ENV/JM/MONO (2007) (4), TNsG (Part A, Chapter 2, Point 4 and Part B, Chapter 2, Point 4) (5) and PR Notice 96-1, Notice to Manufacturers, Formulators, Producers and Registrants of Pesticides Products, Tolerance Enforcement Methods – Independent Laboratory Validation by Petitioner (6). The study was performed at the Wildlife International analytical chemistry facility in Easton, Maryland and was identified as Project Number 166C-122. The method provided by the Sponsor (7) was validated by fortifying natural surface water with Prallethrin and quantitation of the recoveries of fortification samples. The analysis of samples was performed using gas chromatography with mass selective detection (GC/MS). The samples were prepared and analyzed between July 10 and 11, 2014. All raw data generated by Wildlife International and the final report are filed under Project Number 166C-122 in archives located on the Wildlife International site.

OBJECTIVE

The objective of this study was to fulfill EPA requirements set forth in guideline OPPTS 860.1340 (1) and OCSPP 850.6100 (2), EU Guidelines SANCO/825/00 REV. 8.1 16/11/2010 (3) , ENV/JM/MONO (2007) (4) , TNsG (Part A, Chapter 2, Point 4 and Part B, Chapter 2, Point 4) (5) and PR Notice 96-1 (6). This study was validated to demonstrate that an independent researcher can reproduce the results of the analytical method with minimal contact with the method developers.

EXPERIMENTAL DESIGN

Surface water was fortified with Prallethrin at two different concentrations and analyzed according to a validated method. One reagent and two matrix blank samples were analyzed with the verification analysis to evaluate potential analytical interferences. A calibration curve was prepared from external standards of Prallethrin to determine the test substance concentrations in samples.

MATERIALS AND METHODS

Test Substance

The test substance was received from PTRL West on May 29, 2014. It was assigned Wildlife International identification number 11735 upon receipt and was stored under refrigerated conditions. The test substance, a liquid, was identified as Prallethrin; Lot number: 13SC8359513. The test substance had a reported purity of 100% and an expiration date of September 20, 2016. The Certificate of Analysis is presented in Appendix 2.

Internal Standard

The internal standard was received from Sigma Aldrich on June 9, 2014. It was assigned Wildlife International identification number 11752 upon receipt and was stored under ambient conditions. The internal standard, a solid, was identified as Lindane; Lot number: SZBB321XV; CAS No.: 58-89-9. The internal standard had a reported purity of 99.8% and an expiration date of November 17, 2016. The Certificate of Analysis is presented in Appendix 3.

Reagents and Solvents

All solvents were of HPLC/GC grade. All reagents were of ACS grade.

Test System

Surface Water

The test system, defined as subsamples of surface water, a number of which were fortified with Prallethrin was used for purposes of determining the quantitative recoveries. The surface water was obtained from Tuckahoe Lake in Queen Anne, Maryland on July 2, 2014.

The characterization of the surface water was performed by Agvise Laboratories, Inc. (604 Highway 15 West, Northwood, North Dakota). The water was characterized for pH, calcium, magnesium, sodium, hardness, sodium adsorption ratio, dissolved organic carbon content and conductivity and the result is presented in Appendix 4.

Analytical Methods

The method used for the analysis of Prallethrin in surface water was provided by the Sponsor and was followed by Wildlife International. Triplicate hexane extractions of Prallethrin from matrix fortification samples were performed. The combined extracts were filtered through anhydrous

sodium sulphate prior to drying with rotary film evaporation followed by a stream of nitrogen gas. The dried residues were reconstituted in toluene and combined with an aliquot of the internal standard (lindane) prior to analysis by gas chromatography with mass selective detection (GC/MS). A method flowchart is provided in Figure 1.

Concentrations of Prallethrin in the matrix fortification samples were determined using an Agilent Model 7890A gas chromatograph, equipped with an Agilent Model 5975C mass selective detector operated in selected ion monitoring (SIM) mode. Representative full mass spectral scans of Prallethrin and Lindane are presented in Figure 10 and Figure 11 respectively. Ion 167 m/z was chosen to quantitate Prallethrin and ion 255 m/z to quantitate Lindane. Chromatographic separations were achieved using a DB-5MS UI column (30 m x 250 µm, 0.25 µm film thickness). The instrument parameters are summarized in Table 1.

Internal Standard Preparation

A stock solution of Lindane was prepared by accurately weighing 0.01002 g (weight corrected for purity of 99.8%) of the internal standard on an analytical balance. The internal standard was transferred to a 10-mL volumetric flask and the contents were brought to volume with toluene. The primary stock solution (1.00 mg a.i./mL) was diluted in toluene to prepare a 10.0 µg a.i./mL working solution.

Prallethrin Stock Preparation

A stock solution of Prallethrin was prepared by accurately weighing 10.01 mg of the test substance on an analytical balance. The weight was not adjusted for purity because the purity is 100% according to the Certificate of Analysis. The test substance was transferred to a 10.0-mL volumetric flask and the contents were brought to volume with toluene. The primary stock solution (1.00 mg/mL) was diluted in toluene to prepare a 10.0 µg/mL intermediate solution. The 10.0 µg/mL intermediate solution was diluted in toluene to prepare a 1.00 µg/mL intermediate solution. The 1.00 µg/mL intermediate solution was diluted in toluene to prepare 0.100 and 0.0200 µg/mL intermediate solutions.

Fortification Preparation

The 10.0 µg/mL Prallethrtin intermediate solution was diluted in acetone to prepare a 250 µg/L fortification solution. The 250 µg/L fortification solution was diluted in acetone to prepare a

25.0 µg/L fortification solution. One hundred microliters of the 250 µg/L acetone fortification solution was used to fortify 250 mL of surface water to prepare quality control samples at a concentration of 0.10 µg/L. One hundred microliters of the 25.0 µg/L acetone fortification solution was used to fortify 250 mL of surface water to prepare quality control samples at a concentration of 0.01 µg/L.

Standards Preparation

The 10.0, 1.00, 0.100 and 0.0200 µg/mL Prallethrtin intermediate solutions were used to prepare Prallethrin solvent calibrants in toluene. The following shows the dilution scheme for the set of solvent calibrants:

Stock Concentration (µg/mL)	Aliquot (mL)	Final Volume (mL)	Solvent Calibrant Concentration (µg/L)
0.100	0.100	10.0	1.00
0.0200	1.00	10.0	2.00
1.00	0.0500	10.0	5.00
1.00	0.100	10.0	10.0
1.00	0.200	10.0	20.0
10.0	0.0500	10.0	50.0
10.0	0.100	10.0	100
10.0	0.200	10.0	200

An aliquot of 0.0900 mL of each above Prellethrin solvent calibrants in toluene was combined with 0.0100 mL of the internal standard working solution (10.0 µg a.i./mL) in an autosampler vial with glass insert. The concentration of Prellethrin calibration standards are shown below:

Solvent Calibrant Concentration (µg/L)	Standard Concentration (µg/L)
1.00	0.900
2.00	1.80
5.00	4.50
10.0	9.00
20.0	18.0
50.0	45.0
100	90.0
200	180

Table 1

Typical Gas Chromatograph (GC) Operational Parameters

INSTRUMENT:	Agilent Model 7890A gas chromatograph (GC)
DETECTOR:	Agilent Model 5975C mass selective detector (MSD) NCI mode
ANALYTICAL COLUMN:	DB-5MS UI (30 m x 250 µm, 0.25 µm film thickness)
INJECTOR TEMPERATURE:	250°C
RUN TIME:	14 minutes
OVEN:	Initial temperature: 120°C Initial hold time: 2.00 minute Ramp: 15.0°C/minute Final temperature: 255°C Final hold time: 3.00 minute
INJECTION VOLUME:	1.00 µL (splitless)
CARRIER GAS:	Helium
INITIAL FLOW:	1 mL/min
INITIAL PRESSURE:	11.654 psi
ACQUISITION MODE:	Selected ion monitoring (SIM)
MSD TRANSFER LINE TEMP:	260 °C
MS SOURCE TEMPERATURE:	150°C
MS QUAD TEMPERATURE:	150°C
IONS MONITORED:	167.00 m/z (Prallethrin) and 255.00 m/z (Lindane)
APPROXIMATE RETENTION TIMES:	Prallethrin 11.2 min. Lindane: 8.9 min.

METHOD OUTLINE FOR THE PROCESSING OF PRALLETHRIN IN SURFACE WATER

Preparation of Internal Standard (IS) Solutions

1. Prepare an IS stock solution of 1.00 mg a.i./mL by dissolving Lindane in toluene.
2. Prepare IS working solution in toluene using volumetric flasks, volumetric pipettes and gas-tight syringes or equivalent.
3. Transfer all IS solutions to amber bottles and store frozen when not in use.

Preparation of Prallethrin Standard Solutions in Toluene

1. Prepare a Prallethrin stock solution of 1.00 mg/mL by dissolving the test substance in toluene.
2. Prepare Prallethrin intermediate solutions in toluene using volumetric flasks, volumetric pipettes and gas-tight syringes or equivalent.
3. Transfer all Prallethrin solutions to amber bottles and store frozen when not in use.

Preparation of Prallethrin Solvent Calibrants

1. Prepare eight calibrants by diluting Prallethrin intermediate solutions with toluene using volumetric flasks and gas-tight syringes or equivalent.
2. Transfer all Prallethrin solvent calibrants to amber bottles and store frozen when not in use.

Preparation of Prallethrin Calibration Standards

1. Aliquot 0.0900 mL of Prallethrin solvent calibrants into GC vials containing 0.35 mL glass inserts using a 100-µL (or equivalent) auto-pipette. Add 0.0100 mL of IS working solution (10.0 µg a.i./mL) to each calibrant using an appropriate sized auto-pipette.
2. Cap and vortex to mix.

(Continued)

Figure 1. Analytical method outline for the processing of Prallethrin in surface water.

METHOD OUTLINE FOR THE PROCESSING OF PRALLETHRIN IN SURFACE WATER (Continued)

Preparation of Prallethrin Fortification Solutions in Acetone

1. Prepare Prallethrin fortification solutions in acetone starting with Prallethrin toluene intermediate solution using volumetric flasks and gas-tight syringes or equivalent.

2. Prepare all Prallethrin fortification solutions to amber bottles and store frozen when not in use.

Extraction Procedure

1. Sieve natural surface water through a 212 µm sieve.

2. Rinse all glassware with hexane and acetone.

3. Measure 250 mL aliquots of water using a graduated cylinder and pour into 500 mL glass separatory funnels.

4. Fortify as necessary using a 100-µL gas-tight syringe.

5. Extract all samples with hexane three times. For the first two extractions, add 25 mL of hexane and for the third extraction add 15 mL of hexane using a graduated cylinder to each sample. Stopper and shake each sample (with venting) for 40 seconds. Allow the organic and aqueous layers to separate. Drain the water (lower) layer into a beaker. Drain the hexane extract through a funnel plugged with silanized glass wool, topped with approximately 10 g ± 0.1 g anhydrous sodium sulphate into a 250-mL roundbottom flask.

6. Pour the water sample back to its original separatory funnel before the next extraction.

7. Rinse separatory funnels twice, each time with 10 mL of hexane and drain through the same funnels plugged with silanized glass wool, topped with approximately 10 g ± 0.1 g anhydrous sodium sulphate into the same roundbottom flask.

8. Concentrate extracts by rotary evaporation to approximately 2-mL over a waterbath at approximately 40°C.

9. Transfer concentrated extracts into 15 mL conical glass tubes by Pasteur pipettes.

10. Rinse roundbottom flask with approximately 3-mL of hexane three times and combine rinsates into the 15-mL conical tubes.

(Continued)

METHOD OUTLINE FOR THE PROCESSING OF PRALLETHRIN IN SURFACE WATER (Continued)

11. Blow dry concentrated extracts to dryness on N-Evap evaporator with nitrogen in a waterbath at approximately 40°C.

12. Reconstitute samples in 0.500 mL of toluene using a 1000-µL autopipette. Vortex to mix.

13. Aliquot 0.0900 mL of reconstituted samples into GC vials containing 0.35 mL glass inserts using a 100-µL (or equivalent) auto-pipette. Add 0.0100 mL of IS working solution (10.0 µg a.i./mL) to each sample, except for the matrix control (MAB) samples, using an appropriate sized auto-pipette. Add 0.0100 mL of toluene to the matrix control (MAB) samples using an appropriate sized auto pipette. Cap and vortex to mix.

14. Analyze samples by GC/MS. The reagent blank is toluene.