SUMMARY

An independent laboratory method validation study was conducted to determine the validity of a previously reported method for quantifying *cis*-d-Phenothrin and *trans*-d-Phenothrin in surface water. The test substance containing a racemic ratio of *cis*-d-Phenothrin and *trans*-d-Phenothrin (2:8) was analyzed using external standardization by Gas Chromatography with Tandem Mass Spectrometry Detection (GC-MS/MS) from water samples fortified at 0.1 µg/L (LOQ) and 1 µg/L (10X LOQ) of total d-Phenothrin (sum of cis and trans isomers). The limit of quantitation in the test system was 0.1 µg/L. The limit of detection was defined as approximately 20% LOQ (0.02 µg/L) which represented 8 ng/mL of total d-Phenothrin (sum of cis and trans isomers) in solution using the current methodology.

The experiment was conducted with two untreated controls and five control samples spiked for each fortification level; one at 0.1 µg/L (LOQ) and another at 1 µg/L (10X LOQ). The samples were extracted/partitioned in triplicate with hexane. The combined organic solvent phase was dried through sodium sulfate anhydrous, then concentrated by rotary film evaporation followed by a stream of nitrogen gas. The final concentrated extracts were reconstituted with toluene and analyzed by GC-MS/MS.

Cis-d-Phenothrin and *trans*-d-Phenothrin content was quantitated against separate 1/x weighted linear curves of the reference substances *cis*-d-Phenothrin and *trans*-d-Phenothrin whose concentration ranged from 1 ng/mL to 150 ng/mL (*cis*-d-Phenothrin) and from 3.9 ng/mL to 978 ng/mL (*trans*-d-Phenothrin). The quantitation of d-Phenothrin was based on the peak area response and concentration of the calibrants. The amount of d-Phenothrin of each isomer was determined for the quantitation ion at m/z 168 and for the confirmation ions at m/z 165 and m/z 153. Recoveries from fortified samples were determined by summing the found concentration of both isomers and dividing by the relevant fortification level.

Negligible interferences (< LOD) or no residues were detected in control matrices at the three transition ions monitored.

Matrix effects were assessed by comparing the GC-MS/MS response of cis and trans standard solutions prepared in solvent with standard solutions prepared in matrix (untreated water samples spiked with *cis* and *trans* d-Phenothrin solvent based standard solutions) at the same concentration: 10 ng/mL for *cis*-dPhenothrin and 39.1 for *trans*-d-

Phenothrin. No significant matrix suppression or enhancement was observed in the spiked controls; therefore, quantitation was conducted relative to solvent based calibrants.

MATERIAL AND METHODS

Test Substance

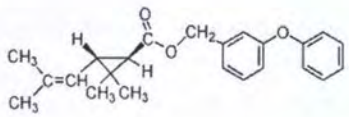
Name:	d-Phenothrin
Active Ingredient:	3-phenoxybenzyl (1*R*)-*cis*-*trans*-chrysanthemate
Isomer Ratio:	8:2 (trans:cis)
Purity:	97.0%
Supplier:	Sumitomo Chemical Company
Lot no.:	130301

Reference Substances

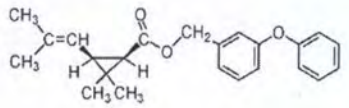
Name:	*cis*-d-Phenothrin
Supplier:	Sumitomo Chemical Company
Lot no.:	C130516
Chemical name:	3-phenoxybenzyl (1*RS*)-*cis*-*trans*-chrysanthemate
CAS no.:	51186-88-0
Molecular formula:	$C_{23}H_{26}O_3$
Molecular weight:	350.45 g/mole
Purity:	100%

Name:	*trans*-d-Phenothrin
Supplier::	Sumitomo Chemical Company
Lot no.:	120201
IUPAC name:	3-phenoxybenzyl (1*R*)-*trans*-chrysanthemate
CAS no.:	26046-85-5
Molecular formula:	$C_{23}H_{26}O_3$
Molecular weight:	350.45 g/mole
Certified content:	97.1%
Trans isomer ratio:	97.8%
Purity:	97.1 x 0.978 = 95%

Structures:



(1*R*)-*trans*-



(1*R*)-*cis*-

Origin of Samples

The test substance identified as d-Phenothrin (lot no. 130301) was provided by Sumitomo Chemical Company and received at PTRL West on February 10, 2014. Upon receipt at PTRL West, the test substance was given the PTRL inventory no. 2578W-001. The test substance was maintained refrigerated when not in use.

The reference substances identified as *cis*-d-Phenothrin (lot no. C130516) and *trans*-d-Phenothrin (lot no. 120201) were provided provided by Sumitomo Chemical Company. Upon receipt at PTRL West, the reference substances were given PTRL inventory no. shown below:

Reference Substance	PTRL Inventory no.	Receipt Date
cis-d-Phenothrin	2578W-003	February 10,2014
trans-d-Phenothrin	2578W-002	February 10, 2014

The reference substances were stored refrigerated when not in use. Certificates of Analysis for the reference and test substances are provided in Appendix B.

Other Chemicals

HPLC acetone, toluene, and hexane were obtained from Burdick & Jackson; sodium sulfate was obtained from Fisher Scientific.

Equipment List

Laboratory Balances
0.2 mm sieve
Thermometers
Silanized glass wool
Pasteur pipettes
Beakers
Glass funnels (6 cm diameter)
Graduated glass cylinders
Hamilton glass precision syringes
Volumetric flasks
Pipetmen with plastic disposable tips
Separatory funnels (500 mL capacity)
Pear shaped flasks (100 mL capacity)
Glass conical tubes (15 mL capacity)
Amber bottles and vials with Teflon® lined caps
Vortex mixer
Büchi rotavapor with water bath
Turbovap® LV nitrogen evaporator

Agilent 7000 Series Triple Quad Mass Spectrometer (GC-QQQ) with Agilent 7890A Series gas chromatograph
Mass Hunter Data System Software

Test System

Source of Test System

Creek water collected in Richmond, California (37.95609°N 122.31294°W) was collected by PTRL West personnel. The test system was collected in a plastic bucket on March 3, 2014 and upon arrival at PTRL West, the inventory no. 2578W-004 was given. The water sample was stored refrigerated (typically < 4°C) in the dark when not in use.

Characterization of the Test System

The natural water used in the study was characterized 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. Aliquots of the test system (1.8L for 10X LOQ subsample set and 1.4L for LOQ subsample set) were sieved through a 200 micron sieve prior to fortification and analysis. The water characterization report, methods of characterization as well as collection documentation are presented in Appendix C.

Test Method

The analytical method for the analysis of d-Phenothrin was validated at PTRL West by Gas Chromatography with Tandem Mass Spectrometry Detection (GC-MS/MS) based on the analytical method (Reference 1), provided by the sponsor.

The water samples were spiked with known concentrations of d-Phenothrin (racemic ratio of *cis*-d-Phenothrin and *trans*-d-Phenothrin (2:8)). The samples were partitioned with hexane. The organic solvent phase was dried through sodium sulfate, then concentrated by rotary film evaporation over a water bath at approximately 40°C, and dried with a stream of nitrogen gas (approximately 40°C). The final concentrated extract was reconstituted with toluene and analyzed by GC-MS/MS. The percent recovery was determined using external standardization where separate linear curves for each isomer were analyzed along with the samples.

Preparation of cis-d-Phenothrin Stock Solution

A stock solution of the *cis*-d-Phenothrin reference substance was prepared by weighing an aliquot of the reference substance (10.25 mg) directly in a 10 mL volumetric flask. The stock solution was dissolved and diluted to the mark with toluene. An additional volume of toluene (0.250 mL) was added into the flask to yield a nominal concentration of 1,000 µg/mL. The concentration of stock solution was corrected for the purity of the reference substance (100%). The stock solution was transferred into an amber bottle and stored refrigerated (typically < 10°C) when not in use.

Preparation of cis-d-Phenothrin Working Solutions

Working solutions were prepared by measuring aliquots of the *cis*-d-Phenothrin stock solution prepared above (1,000 µg/mL) via pipetman with disposable plastic tips and/or of the previously prepared diluted solutions and transferring into separate volumetric flasks containing some toluene. Final solutions were diluted to the mark with same solvent. Actual concentrations are shown below:

Solution used	Aliquot soln (mL)	Final volume (mL)	Theoretical conc. (µg/mL) [1]
1,000 µg/mL	1.0	10	100.0
100 µg/mL	1.0	10	10.0
10 µg/mL	1.0	10	1.0
1 µg/mL	1.0	10	0.1

[1] Theoretical conc. (µg/mL) = [theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)

Working solutions were vortexed to mix, transferred into amber bottles and stored refrigerated (typically < 10°C) when not in use.

Preparation of cis-d-Phenothrin Solvent Based Calibrants

Six calibrants were prepared by transferring an appropriate volume of the *cis*-d-Phenothrin working solutions via pipetman with disposable plastic tips into separate 10 mL volumetric flasks and diluted to the mark with toluene. Final calibrants were vortexed to mix, transferred into amber bottles and stored refrigerated (typically < 10°C) when not in use. The concentration of *cis*-d-Phenothrin calibrants ranged from 1 ng/mL to 150 ng/mL as shown below:

Aliquot soln (mL)	Solution used	Final volume (mL)	Theoretical conc. (ng/mL)[2] cis-d-phenothrin
0.100	0.1 µg/mL	10	1.0
0.200	0.1 µg/mL	10	2.0
0.400	0.1 µg/mL	10	4.0
0.100	1 µg/mL	10	10.0
0.500	1 µg/mL	10	50.0
0.150	10 µg/mL	10	150.0

[2] Theoretical conc stds (ng/mL) = [theoretical conc soln used (µg/mL) x 1,000 ng/µg x aliquot (mL)] ÷ final volume (mL)

Preparation of trans-d-Phenothrin Stock Solution

A stock solution of the *trans*-d-Phenothrin reference substance was prepared by weighing an aliquot of the reference substance (10.85 mg) in a 10 mL volumetric flask containing some toluene. The stock solution was dissolved and diluted to the mark with toluene. An additional volume of toluene (0.540 mL) was added into the flask to yield a nominal concentration of 978 µg/mL. The concentration of stock solution was corrected for the purity of the reference substance (95%). The stock solution was transferred into an amber bottle and stored refrigerated (typically < 10°C) when not in use.

Preparation of trans-d-Phenothrin Working Solutions

Working solutions were prepared by measuring aliquots of the *trans*-d-Phenothrin stock solution prepared above (978 µg/mL) via pipetman with disposable plastic tips and/or of the previously prepared diluted solutions and transferring into separate volumetric flasks containing some toluene. Final solutions were diluted to the mark with same solvent. Actual concentrations are shown below:

Solution used	Aliquot soln (mL)	Final volume (mL)	Theoretical conc. (µg/mL)[4]
978 µg/mL	1.0	10	97.8
97.8 µg/mL	1.0	10	9.8

[4] Theoretical conc. (µg/mL) = [theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)

Working solutions were vortexed to mix, transferred into amber bottles and stored refrigerated (typically < 10°C) when not in use.

Preparation of trans-d-Phenothrin Solvent Based Calibrants

Six calibrants were prepared by transferring an appropriate volume of the *trans*-d-Phenothrin working solutions via pipetman with disposable plastic tips into separate volumetric flasks and diluted to the mark with toluene. Final calibrants were vortexed to mix, transferred into amber bottles and stored refrigerated (typically < 10°C) when not in use. The concentration of *trans*-d-Phenothrin calibrants ranged from 3.9ng/mL to 978 ng/mL as shown below:

Aliquot soln (mL)	Solution used	Final volume (mL)	Theoretical conc. (ng/mL)[5] trans-d-phenothrin
1.000	39.1 ng/mL	10	3.9
0.100	978 ng/mL	10	9.8
0.200	978 ng/mL	10	19.6
0.400	978 ng/mL	10	39.1
0.500	9.8 µg/mL	10	490
0.100	97.8 µg/mL	10	978

[5] Theoretical conc stds (ng/mL) = [theoretical conc (µg/mL) x 1,000 ng/µg x aliquot (mL)] ÷ final volume (mL)
[5] Theoretical conc stds (ng/mL) = [theoretical conc (ng/mL) x aliquot (mL)] ÷ final volume (mL)

Preparation of Matrix Based Calibrants

Matrix based calibrants were prepared at one concentration level for each isomer: 10 ng/mL for *cis*- d-Phenothrin and 39.1 ng/mL for *trans*-d-Phenothrin as follows:

Two control samples (untreated water, control no. 3 and 4) were combined and vortexed to mix.

A 97.8 ng/mL trans-d-Phenothrin solvent based standard was prepared by measuring 1 mL of the 978 ng/mL calibrant from the trans-d-Phenothrin curve and transferring into a 10 mL volumentric flask containing some toluene. The final solution was diluted to the mark with same solvent. Standard solution was vortexed to mix and transferred into an amber bottle.

An aliquot (0.2 mL) of the combined control was spiked with 0.05 mL of 50 ng/mL *cis*-d-Phenothrin solvent based calibrant in a GC vial containing a 0.4 mL glass insert with flat bottom.

An aliquot (0.15 mL) of the combined control was spiked with 0.1 mL of 97.8 ng/mL *trans*-d-Phenothrin solvent based standard in a GC vial containing a 0.4 mL glass insert with flat bottom.

Pipetmen with plastic disposable tips were used for measuring all aliquots. Resultant spiked solutions (matrix based calibrants) were mixed with Pasteur pipettes, capped, and vortexed to mix. Matrix based calibrants were stored refrigerated (typically < 10°C) after analysis.

Preparation of Test Substance Solutions

Preparation of d-Phenothrin Stock Solution

A stock solution of the d-Phenothrin test substance was prepared by weighing an aliquot of the test substance (10.37 mg) in a 4 mL amber vial fitted with septum cap containing 2 mL of toluene. The amber vial was previously tared prior to the addition of the test substance. Test substance was added into the vial using a Hamilton glass precision syringe. The contents of the vial were transferred into a 10 mL volumetric flask rinsing the walls of the vial several times with toluene. The solution was diluted to the mark with toluene. An additional volume of toluene (0.059 mL) was added into the flask to yield a nominal concentration of 1,000 µg/mL. The concentration of the stock solution was corrected for the purity of the test substance (97.0%). The stock solution was transferred into an amber bottle and stored refrigerated (typically < 10°C) when not in use.

Preparation of d-Phenothrin Working and Fortification Solutions

Test substance solutions were prepared by volumetrically measuring aliquots of the d-Phenothrin stock solution (1,000 µg/mL) and/or of the previously prepared diluted solution and transferring into separate volumetric flasks containing some acetone. Final solutions were diluted to the mark with same solvent. Actual concentrations are shown below:

Solution used	Aliquot soln (mL)	Final volume (mL)	Theoretical conc. (µg/mL) [7]	Fort Level
1,000 µg/mL	0.1	10	10	-
10 µg/mL	0.2	10	0.2	LOQ (0.1 µg/L)
1,000 µg/mL	1.0	10	100	-
100 µg/mL	0.2	10	2.0	10X LOQ (1 µg/L)

[7] Theoretical conc. (µg/mL) = [theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)

Working and fortification solutions were vortexed to mix, transferred into amber bottles and stored refrigerated (typically < 10°C) when not in use.

Fortification Procedure

Fortification of untreated water samples was conducted at two fortification levels as shown below:

Fortification Level (µg/L)	d-Phenothrin
0.1	0.1 mL of 0.2 µg/mL in 200 mL water
1	0.1 mL of 2 µg/mL in 200 mL water

Fortification was conducted to determine the percent recovery within the method validation. This procedure was performed in quintuplicate during method validation at each fortification level.

Extraction Method

1. Sieve natural surface water through a 200 micron sieve.

2. Measure 200 mL aliquots of water in 500 mL glass separatory funnels. All glassware: funnels, 100 mL pear shaped flasks, 15 mL conical tubes were previously rinsed with hexane and acetone.

3. Fortify as necessary using a 100 µL glass precision syringe.

4. Extract samples with 20 mL x 2 hexane for 30 seconds by manual shaking.

5. Combine hexane extracts in 100 mL pear shaped flasks.

6. Dry combined hexane extracts through a funnel plugged with silanized glass wool, topped with approximate 10 g ± 0.1 g sodium sulphate anhydrous in the 100 mL pear shaped flasks.

7. Wash sodium sulfate with 10 mL hexane collecting it into the pear shaped flasks.

8. Discard sodium sulfate aliquots and reweigh new aliquots.

9. Extract samples with 10 mL x 1 hexane for 30 seconds and combine hexane extracts.

10. Rinse separatory funnels with 5 mL x 2 hexane and collect the rinse through the funnels with sodium sulphate into the pear shaped flasks.

11. Concentrate extracts by rotary evaporation (approximately 1-2 mL) over a water bath at approximately 40°C.

12. Transfer concentrated extracts into 15 mL conical glass tubes by Pasteur pipette.

13. Concentrate concentrated extracts using Turbovap® LV evaporator with nitrogen at 40°C to dryness.

14. Reconstitute samples in 0.5 mL toluene via a 1 mL pipetman. Vortex to mix.

15. Transfer 250 µL reconstituted samples into separate GC vials containing glass inserts (400 µL with flat bottom).

16. Cap vials and analyze by GC-MS/MS.

17. Remaining extracts are transferred in GC vials with glass inserts and stored refrigerated (typically < 10°C) when not in use.

A schematic diagram of the extraction method is presented in Figure 1.

Modification of the Method During Independent Laboratory Validation

The following minor modifications were employed during conduct of the independent laboratory study:

l. The lowest calibrant solution for *trans*- d-Phenothrin was prepared at 3.9 ng/mL to cover the required LOD (20% LOQ of total d-Phenotrhin). The lowest *trans*- d-Phenothrin calibrant stated in the original method was 10 ng/mL.

2. During the partitioning step (liquid-liquid extraction), after the first two extractions with hexane (20 mL), the sodium sulfate was washed with 10 mL hexane and collected into the pear shape flask containing the combined hexane extracts. The salt was discarded and replaced with an additional 10 g in the glass funnel for the third and last extraction (10 mL hexane). The separatory funnel and sodium sulfate were rinsed with 5 mL x 2 hexane and collected into the pear shaped flasks. In the original method, only one aliquot of sodium sulfate was used and rinsed with hexane (10 mL) collecting into the pear shaped flasks.

3. During the concentration step, hexane concentrated extracts (approximate 2 mL) were transferred to 15 mL glass conical tubes and evaporated to dryness under a stream of nitrogen and over a water bath at approximately 40°C to minimize surface area and hence achieve higher recoveries. In the original method, hexane concentrated extracts were concentrated to near dryness in the pear shaped flasks and removed the remaining solvent under a nitrogen stream.

Gas Chromatography with Tandem Mass Spectrometry Analytical Method (GC-MS/MS)

GC conditions

Column: J&W DB-5 ms, 30 m x 250 µm x 0.25 µm
Injection volume: 2 µL
Injector temp: 225°C
Splitless mode
Splitless time: 2 min
Double Goose Neck liner (Restek Sky™)
Temp program:

- Initial conditions: 95°C for 0.75 minute
- Ramp: 15°C/minute to 250°C
- Ramp 2: 10°C to 275°C
- Final temp: 275°C hold for 7 minutes

Flow rate (He): 1.5 mL/min
Pressure: 15.8 psi
Run time: 20.5 minutes
Retention times:

- *Cis* d-Phenothrin: 12.06 min
- *Trans* d-Phenothrin: 12.14 min

MS conditions

Electron Impact mode
Transfer line temp: 275°C
MS source temp: 230°C
Solvent delay: 8 minutes

Time segments

Time segment	Start Time (min)	Scan Type	gain
1	8	MRM	50

Time Events

Time segment 1: MRM

Compound name	Precursor ion	MS1 resolution	Product ion	MS2 resolution	Dwell (ms)	CE (V)
Phenothrin	183	unit	168	unit	20	10
Phenothrin	183	unit	165	unit	20	10
Phenothrin	183	unit	153	unit	20	10

Note: Based on the reference and test substance chromatograms (Figures 8 to 19), m/z ion 168 was considered as the quantitation ion (most abundant ion) and m/z 165 and 153 were considered as the confirmation (qualifier) ions.

GC-MS/MS Analysis

Samples were analyzed interspersed between the calibrants so as to assess the response of the calibrants if they had been affected by matrix samples (signal suppression or enhacement). Since separate linear curves were prepared for each isomer, samples were interspersed between calibrants of *cis*-d-Phenothrin and then reanalyzed (in different GC vials) interspersed between calibrants of *trans*-d-Phenothrin within the same sequence.

Calibrants and samples were analyzed in single injection. Toluene was analyzed as the solvent blank to determine if there was carryover of target analytes between sample injections. Toluene was analyzed at the beginning and among samples during analysis.

A standard solution of each isomer was reanalyzed at the end of each isomer analysis as check standards (quality control standards) to ensure good chromatography and good instrument performance. The stability of the signal was monitored by comparing the response of a quality control standard injection at the end of isomer analysis to the response of a quantitation standard (calibrant) within the sequence.

A representative injection of the partial sequence for the *cis*-d-Phenothrin analysis was: solvent blank, cis 1 ng/mL calibrant, cis 2 ng/mL calibrant, reagent blank (10X LOQ set), reagent blank (LOQ set), control sample 1 (10X LOQ set), control sample 2 (10X LOQ set), control sample 1 (LOQ set), control sample 2 (LOQ set), solvent blank, cis 4 ng/mL calibrant, fortified sample A1, fortified sample A2, fortified sample A3, fortified sample A4, fortified sample A5, cis 10 ng/mL calibrant, fortified sample B1, fortified sample B2, fortified sample B3, fortified sample B4, fortified sample B5, cis 50 ng/mL calibrant, cis 10 ng/mL check standard, cis 10 ng/mL matrix spike (matrix based calibrant), cis 150 ng/mL calibrant, cis 10 ng/mL QC.

A similar sequence to that above was used for the trans isomer analysis.

Methods of Calculation

Preparation of Stock Standard Solutions

$$\text{Volume of solvent (mL)} = \frac{(W) \times 1000\,\mu g/mg \times (P)}{(FC)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (µg/mL)

Quantitation

Separation of *cis*-d-Phenothrin and *trans*-d-Phenothrin was achieved by GC-MS/MS. The isomers were identified by the coincidence of their retention times with their respective reference standards and MS characteristics. The quantitation of *cis*-d-Phenothrin and *trans*-d-Phenothrin was conducted by peak area of each isomer relative to the theoretical concentration of the calibrants. The content of *cis*-d-Phenothrin and *trans*-d-Phenothrin in

samples was quantitated against separate 1/x weighted linear curves (y = mx + b) of *cis*-d-Phenothrin and *trans*-d-Phenothrin calibrants where:

y = peak area
x = ng/mL isomer injected
m = slope
b = intercept

Weighting of the calibration curve of each isomer was applied so as to provide better curve fit at the lower concentration levels of each isomer. The calculation of weighted curve equations (linear regression) and concentration (ng/mL) present in samples and calibrants was conducted using Mass Hunter software. The amount of d-Phenothrin of each isomer was determined for the quantitation ion at m/z 168 and for the confirmation ions at m/z 165 and 153.

Recoveries from fortified samples were determined by summing the found concentration of both isomers and dividing by the relevant fortification level.

Residue in water (µg/L)

µg/L total d-Phenothrin =

$$\frac{\text{sum conc (ng/mL) of cis and trans d - Phenothrin x dilution factor x Final vol. (mL)}}{\text{1000 ng/µg x sample volume (L)}}$$

Example: Fortified sample FA1 (m/z 168)

$$\text{µg/L Total d-Phenothrin} = \frac{(5.950\,\text{ng/mL} + 21.753\,\text{ng/mL}) \text{x} 1 \text{x} 0.5\,\text{mL}}{1000\,\text{ng/µg x} 0.2\,\text{L}} = 0.0693$$

Percent recovery of total d-Phenothrin in water (%)

$$\text{\% Recovery} = \frac{\text{µg/L detected - µg/L avg Control}}{\text{Fortification Level (µg/L)}} \text{ x } 100$$

Example: Fortified sample FA1 (m/z 168)

$$\% \text{ Total d-Phenothrin} = \frac{0.0693\ \mu g/L}{0.1\ \mu g/L} \times 100 = 69\%$$

*The amount of d-Phenothrin residue detected in the control for the LOQ sample set (m/z 168 ion) was < LOD, equivalent to 8 ng/mL of total d-Phenothrin (sum of cis and trans isomers) in solution.

Expected concentration of total d-Phenothrin in fortified samples at LOQ

$$\frac{\text{Fortification level } (\mu g/L) \times \text{ sample volume (L)} \times 1000\ ng/\mu g}{\text{final volume (mL)}}$$

Example: Fortified sample A1 (m/z 168)

$$\% \text{ Expected concentration of total d-Phenothrin} = \frac{0.1\ \mu g/L \times 0.2\ L \times 1000\ ng/\mu g}{0.5 mL} = 40$$

ng/mL

d-Phenothrin isomer ratio was cis: trans (2:8) where:

cis-d-Phenothrin = 40 ng/mL x 0.2% = 8 ng/mL

trans-d-Phenothrin = 40 ng/mL x 0.8% = 32 ng/mL

Transcriptions (spreadsheets) of the raw data to support calculations for this study are presented in Appendix D.

Limit of Detection

The limit of detection (LOD) was defined as approximately 20% LOQ (0.02 µg/L) which represented 8 ng/mL of total d-Phenothrin (sum of cis and trans isomers) in solution.

Limit of Quantitation

The limit of quantitation (LOQ) was assigned as the lowest fortification level of total d-Phenothrin (sum of cis and trans isomers) validated by the analytical method. The LOQ for d-Phenothrin in water was 0.1 µg/L.

Time Required for Completion of a Sample Set

A sample set was divided into two subsample sets for efficient handling. A subsample set consisted of a reagent blank, two controls (untreated water samples) and five fortified water samples (at one level i.e. LOQ). Time required for one subsample set from initiation of extraction until the completion of instrumental analysis and data evaluation is as follows:

- Sample preparation, including sieving of natural water, liquid-liquid partition, and extract concentration take approximately 6 hours
- GC-MS/MS analysis and data processing (three MS/MS transitions for each isomer) take approximately 6 hours

TOTAL = approximately 12 hours for one analyst to complete a subsample set (approximately one a half calendar days) or 24 hours (3 calendar days) to complete two subsample sets to satisfy the ILV requirements.

Statistical Methods

Means, standard deviation, relative standard deviation, and 1/x linear regression were the only statistical methods employed in this study.

Communication Pertaining to Independent Laboratory Validation

Communication between the Study Director and the Sponsor Representative was limited to e-mail correspondence as follows:

1. April 3, 2014: Discussion of low recovery in the 10X LOQ subsample set. Different degrees of emulsions during the liquid-liquid partitioning steps in the extraction were observed which may have led to lower recoveries. A more

Figure 1. Schematic Diagram of the Analytical Method.