

Determination of Pyrethroid Pesticides in Sediment

1.0 Scope and Application

1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of pyrethroid pesticides in sediment. Dual column gas chromatography with dual electron capture detectors (GC-ECD) and/or gas chromatography with triple quadrupole mass spectrometry (GC-MSMS) are used for analysis. Table 1 lists the target pyrethroid compounds currently analyzed with their method detection limits (MDL) and reporting limits (RL), based on dry weight. Detection limits were calculated using USEPA procedures found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11). Sediment results can be found in Appendix A. The average percent recoveries, amount recovered and standard deviations are listed in Table 5.

These procedures are applicable when low parts per billion analyses are required to monitor differences between burdens in soils and sediment from relatively uncontaminated reference areas and contaminated areas.

Table 1. Pyrethroid Compounds Analyzed, CAS Numbers, Minimum Detection Limits (MDL) and Reporting Limits (RL) in Sediment, Based on 50 % Moisture.

Target Analytes	CAS#	GC-ECD		GC-MSMS	
		MDL, ng/g dry wt.	RL, ng/g dry wt.	MDL, ng/g dry wt.	RL, ng/g dry wt.
Allethrin	584-79-2	1.0	2.0	1.5	3.0
Bifenthrin	82657-04-3	0.50	1.0	0.25	0.50
Cyfluthrin	68359-37-5	2.0	4.0	0.20	0.40
Cypermethrin	52315-07-8	2.0	4.0	0.30	0.60
Deltamethrin/ Tralomethrin	52918-63-5/ 66841-25-6	2.0	4.0	0.20	0.40
Esfenvalerate/ Fenvalerate	66230-04-4/ 51630-58-1	1.0	2.0	0.20	0.40
Fenpropathrin	64257-84-7	2.0	4.0	0.80	1.6
Flucythrinate	70124-77-5	5.0	10	NA	NA
Lambda- cyhalothrin	91465-08-6	1.0	2.0	0.15	0.30
Permethrin, cis	54774-45-7	4.0	8.0	0.70	1.40
Permethrin, trans	51877-74-8	4.0	8.0	1.20	2.40
Phenothrin	26002-80-2	5.0	10	0.11	0.22
Prallethrin	23031-36-9	1.0	2.0	0.40	0.80
Resmethrin	10453-86-8	NA	NA	0.40	0.80
Tetramethrin	7696-12-0	5.0	10	0.30	0.60

2.0 Summary of Method

2.1 Sets of 12-18 homogenized sediment samples are scheduled for extraction by the project lead chemist. Extraction method employed was developed and validated by the Water Pollution Control Laboratory (WPCL) and is a modification of EPA Method 3545A Pressurized Fluid Extraction (PFE). Extract cleanup and partitioning methods are modifications of EPA Methods 3640A Gel Permeation Cleanup and 3620C Florisil Cleanup and the multi-residue methods for fatty and non-fatty foods described in the U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. 1, 3rd Edition 1994, Chapter 3, Multi-residue Methods, Section 303-C1.

Sediment samples are removed from the freezer and allowed to thaw.

2.2 A 1-5 g sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 24 hours to determine moisture content. A 10 g sample is mixed using a clean glass stirring rod with approximately 7 g of pre-extracted Hydromatrix[®] (Varian Corp) in a 250 mL Trace Clean Wide Mouth Jar until the mixture is free flowing. The mixture is then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) extractor cell and packed by tamping the mixture. A solution containing cis-permethrin (C¹³) surrogate is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted twice with a 50/50 mixture of acetone/dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in 60 mL VOA vials.

2.3 The combined extracts are dried using sodium sulfate, evaporated to approximately 0.5-1.0 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder columns and micro-Snyder apparatus and diluted to 10 mL using DCM. The extracts are then filtered through a 0.45 µm syringe filter into 12 mL culture tubes fitted with Teflon-septa open style caps.

2.4 The culture tubes are then placed on the GPC autosampler for initial sample cleanup.

2.5 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroleum ether. The extracts are then further cleaned using Florisil[®] in a 22 mm x 300 mm column. The Florisil[®] columns are eluted with 70% diethyl ether/petroleum ether. The extracts are concentrated to an appropriate volume using K-D/micro K-D apparatus prior to analysis by GC-ECD and/or GC-MSMS.

3.0 Interferences

- 3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

- 3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4 . Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination.
- 3.3 Interferences co-extracted from tissue and sediment samples limit the method detection and quantitation limits. For this reason, sample extract cleanup is necessary to yield reproducible and reliable analyses of low level contaminants.

4.0 Apparatus and Materials

- 4.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.
- 4.2 Chromatographic Column - (300 mm x 11 mm) borosilicate glass chromatography column with a Teflon stopcock.
- 4.3 Glass wool, Pyrex - solvent washed prior to use.
- 4.4 Kuderna-Danish (K-D) Apparatus
- 4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.
- 4.4.2 Evaporation flask - 500 mL (Kontes K-570050-0500, or equivalent), attached

to concentrator tube with blue clamp (Kontes K-662750-0012).

4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).

4.4.5 Boiling chips, Chemware® Ultra-pure PTFE, extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.5 Water bath, Organomation Assoc. Inc. (OA-SYS/S-EVAP-KD), 115 V, thermostatically controlled with stainless steel cover to fit 5 K-D apparatus, installed in a fume hood.

4.6 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.

4.6.1 Extraction Cells, 33 mL, Dionex P/N 049562

4.6.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.

4.6.3 VOA Vials, 60 mL, pre-cleaned and certified.

4.7 Sample vials - glass, 2 mL with PTFE-lined screw cap.

4.8 Analytical balance - capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Balance - capable of 100 g to the nearest 0.01 g.

4.11 Disposable Pasteur Pipettes - (rinsed with solvents before use).

4.12 Aluminum dishes for moisture determination.

4.13 Desiccator with indicating desiccant.

4.14 Glass funnel, 75 mm.

4.15 Graduated cylinder, 250 mL and 100 mL.

4.16 Culture tubes, 13 x 100 mm and 16 x 100 mm, with PTFE lined cap.

- 4.17 Gas chromatographs: 1) Agilent 6890*plus*, equipped with two micro ECD detectors with EPC, split-splitless injector with EPC, and autosampler. Capillary columns, 60 meter DB5 and 60 meter DB17MS (J&W Scientific) (0.25 mm I.D. and 25 μ m film thickness) connected to a single injection port using a "Y" press fit connector. 2) Gas chromatograph-mass spectrometer (triple quadrupole), Varian Model 1200L with Varian Model 3800 gas chromatograph, split-splitless injector with EPC and Combi-Pal autosampler. The capillary column is a XLB (Waters) 60 m x 0.25 mm I.D. x 0.25 μ m film thickness.
- 4.18 Data Systems (Agilent and Varian Workstations) to collect and record GC data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.
- 4.19 Homogenizer, Buchi Model B-400 (Brinkman P/N 16-07-200-1) or equivalent equipped with titanium knife assembly (Brinkman P/N 16-07-222-2) and glass sample vessel (Brinkman P/N 16-07-245-1).
- 4.20 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).
- 4.21 Gel Permeation (size exclusion) Chromatograph, automated, J₂ Scientific AccuPrep 170, equipped with 70 g S-X3 BioBeads J₂ Scientific P/N C0070G (100% DCM).

5.0 Reagents

- 5.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.
- 5.2 Acetone. (Same as above).
- 5.3 Iso-Octane. (Same as above).
- 5.4 Diethyl ether preserved with 2% ethanol. (Same as above).
- 5.5 Dichloromethane (DCM). (Same as above).
- 5.6 Chem Elut-Hydromatrix®, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.
- 5.7 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

- 5.8 Florisil[®], 60/100 mesh, PR grade, U. S. Silica.
- 5.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE and GPC.)
- 5.10 Nitrogen, ultra-pure (99.99999%) for ECD makeup and blowdowns.
- 5.11 Helium, ultra-pure (99.99999%) for GC carrier gas.
- 5.12 Air, compressed, breathing quality, for ASE pneumatics.
- 5.13 Pyrethroid Surrogate Solution containing 20 ppb of cis-permethrin (C¹³) obtained from Cambridge Isotope Laboratories (Andover, MA). P/N CLM-7322-1.2.
- 5.14 Pyrethroid Spiking Solution Mix A (for GC/ECD): Individual compounds obtained from Chem Service, Inc., West Chester, PA. Solution made in iso-octane.

<u>Compound</u>	<u>P/N</u>	<u>Concentration (ppb)</u>
Bifenthrin	PS2003	50
Cyfluthrin	F2460	200
Cypermethrin	PS1068	200
Deltamethrin	PS2071	200
Es-fenvalerate	PS2004	100
Fenpropathrin	PS2002	200
Lambda-cyhalothrin	PS2018	100
Permethrin, cis	PS758-1	250
Permethrin, trans	PS758-2	250

Pyrethroid Spiking Solution Mix B (for GC/MSMS): Individual compounds obtained from Chem Service, Inc., West Chester, PA except for tetramethrin, which was purchased from AccuStandard, New Haven, CT. Solution made in iso-octane.

<u>Compound</u>	<u>P/N</u>	<u>Concentration (ppb)</u>
Allethrin	PS791	25
Bifenthrin	PS2003	5
Cyfluthrin	F2460	25
Cypermethrin	PS1068	25
Deltamethrin	PS2071	25
Es-fenvalerate	PS2004	10
Fenpropathrin	PS2002	50
Lambda-cyhalothrin	PS2018	10

Permethrin, cis	PS758-1	5
Permethrin, trans	PS758-2	5
Phenothrin	PS1030	5
Prallethrin	PS2109	25
Resmethrin	F2293	25
Tetramethrin	P-406	25

5.15 Pyrethroid Instrument Calibration Standards: Individual compounds obtained from Chem Service (see 5.14) are mixed in iso-octane with concentrations ranging from 0.50 ppb to 500 ppb (based on bifenthrin).

5.16 Second Source Standards: Pyrethroid analytes were obtained from AccuStandard, New Haven, CT (P/N AE-00023, Pesticide Mix 14 - components include cyfluthrin, cypermethrin, deltamethrin, fenvalerate, L-cyhalothrin, and permethrin and P-5252 (esfenvalerate)) and Ultra Scientific, North Kingstown, RI (P/N PST-1915 (bifenthrin) for verification of calibration standards.

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 Sample Collection, Preparation, and Storage

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination.

6.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, rinsed with a high-purity acetone, and finally rinsed with Type II water.

- 6.3 Sediment samples are collected in wide-mouth glass jars and must be frozen if not extracted within 14 days.

7.0 Sample Extraction

- 7.1 Frozen sediment samples are removed from the freezer and allowed to thaw. Prior to extraction, sediment samples are thoroughly mixed by hand using a clean glass rod or may be homogenized using a Polytron homogenizer equipped with stainless steel generator equipped with Teflon bearings. Sample sets of 12-18 should be extracted when possible. The ASE-200 extractor will extract 24 cells. Be sure to reserve enough cells for method blanks, matrix spikes and duplicates, laboratory control spikes and sample duplicates.
- 7.2 Prepare a glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle.
- 7.3 Label 60 mL VOA vials for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap; if they are placed outside of this area, they will interfere with the ASE optical sensor. Use two VOA vials for each sample. Label the first VOA vial with the ASE position number, bench sheet number and the sample name. Label the second VOA vial the same but add "RE" to distinguish between the two vials. Label and weigh aluminum planchets for moisture determinations (samples ID can be made on the bottom of planchets using a ball point pen).
- 7.4 Tare a 250 mL glass jar. Using a clean (solvent rinsed) glass rod, stir the sediment so that the mixture is homogeneous. Weigh 10 g of sample into the jar, record the weight on the bench sheet, and add the twice-extracted Hydromatrix[®] from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free flowing.
- 7.5 Weigh 1-5 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for 24 hours and re-weigh dry weight.
- 7.6 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter (*the filter is the one that was used to pre-extract the Hydromatrix[®]*).
- 7.7 Pour the sediment/Hydromatrix[®] mixture through the powder funnel back into the extraction cell that the Hydromatrix[®] was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be

necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the method blank and laboratory control spike and its duplicate (if used) will contain only Hydromatrix®.

- 7.8 **All** of the extraction cells are spiked with the pyrethroid surrogate standard. Spike each cell with exactly 1.0 mL of the pyrethroid surrogate solution (20 ng/mL). Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.
- 7.9 The extraction cells used for the matrix spike (MS) and matrix spike duplicate (MSD) and laboratory control spike (LCS) and its duplicate (LCSD) (if used) are spiked with exactly 1.0 mL of the pyrethroid matrix spike solution (20 ng/mL). Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.
- 7.10 The extraction cells are capped (*Firmly tightened but do not over tighten*) and placed on the ASE 200 carousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carousel with the position numbers corresponding to the position numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.
- 7.11 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

Pre-heat	0 min.
Heat	5 min.
Static	5 min.
Flush	60%
Purge	300 sec.
Cycles	1
Pressure	1500 psi
Temp	100 °C
Sol A Other	100%

- 7.12 After the initial extraction is complete, remove full VOA vials and place in a Wheaton rack. Place the second set of collection VOA vials labeled "RE" on the ASE carousel. Check each of the extraction cells to make sure that the caps are (*firmly tightened*) as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.
- 7.13 When extraction is completed, place VOA vials in a Wheaton rack with the "RE" vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they

are removed for the GPC cleanup procedure.

8.0 Gel Permeation Chromatography

IMPORTANT: *All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.*

- 8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps. Allow them to sit out until they are at room temperature.
- 8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the funnel to prevent any possible splashing out.
- 8.3 Pour sample extracts from the VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial, cap and shake and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~50 mL) by pouring from a clean and rinsed 400 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~50 mL) from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).
- 8.4 Add 0.5 mL Iso-Octane using a macro-pipette and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask and place the flask on the hot water bath set at 80-82°C. Evaporate the solvent until the apparent volume is 2-5 mL. Remove the inverted Hopkins condenser and secure using the set clamps so that it is out of the way. At this point there should be between 2-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and 10 mL or less of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-D flask to prevent any of it from entering the concentrator tube upon removal.
- 8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed aluminum foil. Rinse the Snyder column with petroleum ether and place back in the column rack for storage. After all of the flasks have

been removed from the hot water bath, repeat steps 2-5 for the remaining samples extracted with this set.

- 8.6 Add a new micro-boiling stone and place a clean micro-Snyder column on the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath. Evaporate the solvent until only 0.5-1.0 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.
- 8.7 When the solvent has been evaporated to 1.0 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add DCM to the concentrator tube to reach a final volume of 10.0 mL.
- 8.8 Gelman filter (0.45 µm) the sample into a 12 mL culture tube. Using a volumetric pipette remove 2.0 mL of the filtered sample and place it in a pre-weighed aluminum planchet if lipid determination is needed. Cap the culture tube with the Teflon-insert style caps. Mark the bottom of the meniscus with a pen in case of evaporation before clean-up on GPC.
- 8.9 All samples are cleaned using a J₂ Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1" ID glass column with 70g Bio-Beads SX-3 in 100% DCM)
 - 8.9.1 From the desktop double click on the AccuPrep.exe shortcut to open the program. Click on the Use Injector button and allow the instrument time to initialize. Activate the pump by using the top left hand button. A solvent Control Pump window will open up. Click on the Apply Defaults button and then OK on the Selected Pressure Limit 30 psi. The pump should audibly be heard coming on and the green light should show that the system is on line and status flowing. Make sure that the bottle of clean DCM is full and the waste bottle is empty. Allow the system to pump for about 5 minutes before switching the column in-line (gray button next to Column that has 'Put in line' on it). The pressure will be observed to normally go up to the 12-16 psi range. Turn the power on to the detector to allow it at least 30 minutes of time to warm up before use. Because the scale is auto-adjusted in the software now it is no longer necessary to manually adjust the range on the unit itself.
 - 8.9.2 While the system is equilibrating, the sequence can be entered. Click on the Seq button next to the Pump button. An 'Editing new sequence' window will pop up. This gives a view of the instrument which clearly shows the sample tray locations and the corresponding sample collection locations. By clicking on the sample tray position, a new window 'Adding sample at tray position #' will pop up.

This allows information to be included about each specific sample. Sample position 1 will always be a calibration standard (CLP-340) which is run prior to any sequence of runs to verify instrument integrity. In the Sample ID field just type in 'CLP-340'. In the Descrip (optional), information pertaining to the project, laboratory control number, bench sheet number and date are typically added. The Method File needs to be changed to 'GPC-Sed' for only this sample and in the Sample Type field the 'Calibration' type can be chosen. After this information is completed click on the OK to continue. This returns you back to the main sequence window but now the first position will be highlighted in green. Continue by adding the next sample information to tray position 2, again following the same steps as before. The Method File name to be used is 'Sed-Pest'. Also by default, the Sample Type field will already be set at 'Sample'. This will not need to be changed until a duplicate sample (Duplicate), matrix spike (Matrix Spike), matrix spike duplicate (Spike Duplicate) and laboratory control spike (Spiked Blank) are encountered. After all the samples have been added to the sequence, save it as the WPCL log-in number (L-###-##). From the Editing sequence window print out the sample list. Compare the information to your original bench sheet to insure there are no mistakes. Make sure the GPC-Sed method is being used for the calibration standard and 'Sed-Pest' method is being used for the samples. Next verify that the samples are still at the marked line on the culture tubes (add DCM to the marked line if they are not). Place a tube with the GPC Calibration Standard Solution (CLP-340) in sample tray position 1 and then follow as the sequence was made in the remaining positions.

8.9.3 Get two boxes of the 125 mL Trace Clean amber bottles for sample collection. A bottle does not need to be placed in collection position #1 because that is the GPC Calibration Std (all goes to waste). Remove the white caps from the bottles and place them on top of the detector (so that Teflon side is not exposed to possible contamination). Label the boxes with laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump has had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero Signal' button to set the baseline.

8.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the samples positions and collection positions have been loaded, then click on the large button with the stop watch to begin the program. A window will pop up asking if the correct column method is loaded (100%DCM). Click on 'yes' to engage the syringe pump to begin priming. The sample probe will move over to sample position #1 and aspirate the sample. After the samples have all been processed (~1 hour per sample), remove the label from the sample position and place it on the bottle in corresponding collection position. Cap the bottle and place it back in the box that was retained for their storage. At the end of the sequence there will be a window that pops up saying that the 'Sequence has been

successfully completed'. The column will switch offline and the pump will automatically shut down. The only thing that has to manually be turned off is the power to the detector.

- 8.10 Pour the GPC eluate into a rinsed K-D flask. Rinse the bottle with some DCM and add that to the K-D flask. Add 0.5 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, lift the K-D apparatus up enough to be able to angle it slightly and add 40-50 mL Petroleum Ether through the top of the Snyder column. By holding the K-D apparatus at an angle, it allows the solvent to more easily drain back into the flask. Return to the K-D apparatus back into the hot water bath. Repeat this step 2 more times to successfully solvent exchange the sample from DCM to Petroleum Ether. When the apparent volume in the concentrator tube is 5-10 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus and allow to completely drain into the concentrator tube. After it has finished cooling, remove the concentrator tube and place a micro-Snyder column on it. Add a new micro boiling chip and place it in a 400 mL beaker containing water heated to approximately 75°C on a hot plate (4-5 tubes can be evaporated at one time). Evaporate the solvent down to 1-2 mL. Remove it from the water bath and allow it to cool.
- 8.11 Transfer the solution to a 13 x 100 culture tube with a Pasteur pipette; rinse the concentrator tube with 0.5 mL of Petroleum Ether, vortex, and transfer the rinse to the culture tube. Repeat the rinse step two more times and add each rinse to the culture tube. Cap the culture tube with a Teflon faced cap. Place extracts in a refrigerator for storage until the final Florisil® column cleanup is done.
- 8.12 Add acid rinsed copper to all culture tubes to remove any residual sulfur from the extract.

9.0 Florisil® Column Fractionation

IMPORTANT: All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether (PE) before they are used for this procedure. Florisil® must be activated in an oven at 130°C for at least 24 hours prior to use.

- 9.1 This procedure is performed after the GPC cleanup procedure for all sediment samples analyzed for pyrethroid pesticides.
- 9.2 Prepare the reagent to be used for Florisil® cleanup: 70% ethyl ether in petroleum ether (EE/PE). Make an amount slightly in excess of what is actually needed to

allow for any loss which may occur during solvent transfer. The required volume is 280 mL per sample.

- 9.3 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the 22 x 300 mm column and tap into place with a PE rinsed glass rod. Cover with a small portion (0.5 inch) of sodium sulfate. Fill the column with four inches of Florisil[®]. Tap column with rubber "mallet" to firmly settle the Florisil[®]. Top the column with 3/4-1 inch of sodium sulfate. This will prevent the column from being disrupted when solvent is added and will remove any residual water.
- 9.4 Place a 600 mL beaker under the column and pre-wet the column with about 25 mL of 70% EE/PE.

IMPORTANT: From this point and through the elution process, the solvent level should never be allowed to go below the top of the sodium sulfate layer.

- 9.5 When approximately 1 inch of solvent remains above the surface of the column, place a K-D flask under column making sure that the stopcock is in the full open position. This will allow for a flow rate of about 2 to 3 mL/min. When the meniscus of the solvent rinse reaches the column bed surface, decant the sample onto the column. Immediately add approximately 0.5 mL of 70% EE/PE to the tube, vortex, and add the rinse to the sample extract on the column. Add another 0.5 ml of 70% EE/PE to the tube, vortex, and add this final rinse to the sample extract on the column. Start the columns in a sequential fashion, and the lag time will be adequate to perform the necessary tasks for up to six columns.
- 9.6 When the combined sample and rinses reach the sodium sulfate layer, add 280 mL of 70% EE/PE that has been carefully measured out using a graduated cylinder to the column. Make sure that the stopcock is fully open in order to achieve the desired flow rate of 5 mL per minute. Collect directly into a clean PE rinsed 500 mL KD flask.
- 9.7 After all of the solvent drains through the column, add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask and place vessel in the hot water bath with the temperature set at 80-82°C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.
- 9.8 When the vessels are cool, remove the concentrator tube from the K-D flask add a new micro boiling stone and attach a clean micro-Snyder column to the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. Evaporate the solvent until

only 0.5 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

9.9 Final preparation procedures:

9.9.1 PYR Mix A by GC/ECD: When the solvent has been evaporated to 1 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 0.5 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step. Transfer the extract to a labeled amber GC vial. The extracts are ready for analysis.

9.9.2 PYR Mix B by GC/MSMS: When the solvent has been evaporated to 1 mL remove the tube from the bath. Remove the micro-Snyder column and reduce the volume to 0.500 mL using nitrogen (Ultra-high purity). Transfer the extract to a labeled amber GC vial. The extracts are ready for analysis.

10.0 Analytical Procedure

10.1 Before the sample extracts can be analyzed by the GC-ECD or GC-MSMS, a sequence listing the order of calibration standards, second source check standards, initial and continuing calibration blanks, initial and continuing calibration verification standards and sample extracts is written using Agilent Chemstation Software or Varian Workstation (GC/MS/MS) software.

10.2 Each GC sequence includes a minimum of seven calibration standards. The calibration curve concentration for pyrethroids differs for different analytes, but in general the range is 0.1 ppb to 500 ppb.

10.3 To verify the calibration standards, second source check standards (see 5.16) are analyzed. No second source existed. We have purchased individual analytes and a mix from AccuStd and UltraSci to be used in the future.

10.4 An initial calibration blank and initial calibration verification standard is analyzed after the calibration standards and prior to the first sample extract. Continuing calibration blanks (CCBs) and calibration verification standards (CCVs) are analyzed after ten sample extracts have been analyzed. If a CCV fails, the five samples prior to the failed CCV and the five samples after the failed CCV are re-analyzed after a new calibration curve is analyzed.

10.5 The CCV analyte concentrations are mid-range of the calibration curve (20-60 ppb).

hold time 0.50 min

- MS-MS Operating Conditions

Ion source temperature:	200 °C	Ionization pressure:	45 mTorr
Manifold temperature:	40 °C	Ion preparation:	MS/MS
Transferline temperature:	280 °C	Collision gas:	Argon
		Filament delay:	5 min

Table 3. Retention times, parent (Q1)-daughter (Q3) ion transitions and Collision Energy for selected pyrethroids.

	Segment	Q1	Q3	Collision Energy	Internal Standard
Allethrin	1	123	81	-10	permethrin,transC ¹³
Paraethrin	1	123	81	-10	permethrin,transC ¹³
Resmethrin	1	123	81	-10	permethrin,transC ¹³
Bifenthrin	2	181	166	-15	permethrin,transC ¹³
Fenpropathrin	2	181	152	-25	permethrin,transC ¹³
Tetramethrin	2	164	107	-20	permethrin,transC ¹³
Phenothrin	3	123	81	-15	permethrin,transC ¹³
Lambda-cyhalothrin	4	181	152	-25	permethrin,transC ¹³
Permethrin, cis	5	183	153	-10	permethrin,transC ¹³
Permethrin, cisC ¹³ - (surrogate)	5	189	159	-15	permethrin,transC ¹³
Permethrin, trans	5	183	153	-10	permethrin,transC ¹³
Permethrin, trans C ¹³	5	189	159	-15	Internal std
Cyfluthrin	6	163	127	-10	permethrin,transC ¹³
Cypermethrin	6	163	127	-10	permethrin,transC ¹³
Esfenvalerate- Fenvalerate	7	167	125	-10	permethrin,transC ¹³
Deltamethrin	8	181	152	-20	permethrin,transC ¹³

Table 4. Concentrations and Signal to Noise (S/N-RMS) Results for Selected Pyrethroids Using EI-MSMS

	Pyreth 0.1 Std conc (ppb)	Conc in sediment Mult=0.2	Signal to Noise
permethrin, trans-L (int std)			416
allethrin	0.5	0.1	18
bifenthrin	0.1	0.02	109
cyfluthrin 1-4	0.5	0.1	94
cyfluthrin 1	0.5	0.1	81
cyfluthrin-2	0.5	0.1	80
cyfluthrin-3	0.5	0.1	50
cyfluthrin-4	0.5	0.1	77
cypermethrin 1-4	0.5	0.1	118
cypermethrin 1	0.5	0.1	77
cypermethrin-2	0.5	0.1	125
cypermethrin-3	0.5	0.1	197
cypermethrin-4	0.5	0.1	93
deltamethrin	0.5	0.1	22
esfenvalerate_fenvalerate-1	0.2	0.04	22
esfenvalerate_fenvalerate-2	0.2	0.04	124
fenpropathrin	1.0	0.2	73
lambda-cyhalothrin-2	0.2	0.04	37
paraethrin	0.5	0.1	54
permethrin-cis	0.1	0.02	19
permethrin-trans	0.1	0.02	24
phenothrin	0.1	0.02	18
resmethrin	0.5	0.1	55
tetramethrin	0.5	0.1	34

11.0 Method Performance

11.1 Pyrethroid analyte average percent recoveries, amount recovered and standard deviations in American River sediment are listed in Table 5.

Table 5. Summary of method validation results for pyrethroids in American River sediment; spike level at 5-20 ng/g by GC/ECD.

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	Standard Deviation (n=8)
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Bifenthrin	106	5.30	0.14
Cyfluthrin	108	10.4	0.85
Cypermethrin	108	11.1	0.81
Deltamethrin	62.0	3.10	0.69
Es/Fenvalerate	107	5.39	0.27
Lambda- cyhalothrin	104	5.17	0.38
Permethrin	99.0	16.2	1.27

12.0 References

U.S. Food and Drug Administration. 1994. Pesticide Analytical Manual. Volume 1, Chapter 3, Multiclass Multiresidue Methods. U.S. Food and Drug Administration, Rockville, MD.

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Method 3620C, *Florisil Cleanup*, Revision 3, February 2007, <http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3620c.pdf> [11/10/08]
Method 3640A, *Gel Permeation Cleanup*, Revision 1, September 1994, <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3640a.pdf> [03/29/07]

SOP Section Approval: _____

Date: _____

SOP Final Approval: _____

Date: _____

SOP QA Officer Approval: _____

Date: _____

APPENDIX A

METHOD DETECTION LIMIT VALIDATION FOR PYRETHROIDS IN SEDIMENT BY GC/ECD

Pyrethroid Pesticides	Spike Amount Expected (ng/g)	Average Amount Recovered (ng/g)	Average Percent Recovery	Variance Amount Recovered (ng/g)	Standard Deviation Amount Recovered	MDL=t*Stdev (ng/g)
Bifenthrin	5.00	5.30	106	0.021	0.143	0.43
Cyfluthrin-1	10.0	10.4	104	0.723	0.850	2.55
Cyfluthrin-2	10.0	10.8	108	1.16	1.08	3.23
Cyfluthrin-3	10.0	10.6	106	0.421	0.649	1.94
Cyfluthrin-4	10.0	11.2	112	1.47	1.21	3.64
Cypermethrin-1	10.0	11.1	111	0.662	0.814	2.44
Cypermethrin-2	10.0	10.9	109	0.916	0.957	2.87
Cypermethrin-3	10.0	10.4	104	0.665	0.815	2.44
Cypermethrin-4	10.0	11.0	110	0.467	0.683	2.05
Deltamethrin	5.00	3.10	62	0.481	0.693	2.08
Esfenvalerate/Fenvalerate-1	5.00	5.39	108	0.072	0.268	0.80
Esfenvalerate/Fenvalerate-2	5.00	5.31	106	0.126	0.355	1.06
Lambda-cyhalothrin-1	5.00	5.17	103	0.147	0.384	1.15
Lambda-cyhalothrin-2	5.00	5.26	105	0.070	0.265	0.79
Permethrin-cis	15.0	16.2	108	1.60	1.27	3.80
Permethrin-trans	15.0	13.5	90	9.28	3.05	9.13

American River sediment was used for validation (29.2 % moisture)

Students' t value for 99% confidence, where n=8, is 2.998

METHOD DETECTION LIMIT VALIDATION FOR PYRETHROIDS IN SEDIMENT BY GC/MSMS					
	RSD	Average	STD DEV	Std Dev x Student's T (7 Deg of Freedom) 2.998	MDL Dry Wt mult=0.2
permethrin, trans-L					
allethrin	13	18.6	2.48	7.43	1.486
paraethrin	9	7.6	0.65	1.95	0.391
resmethrin	8	8.1	0.64	1.93	0.386
tetramethrin	10	4.3	0.44	1.31	0.262
Bifenthrin	18	2.2	0.41	1.24	0.247
Fenpropathrin	12	11.3	1.33	4.00	0.799
phenothrin	12	1.5	0.19	0.56	0.113
lambda-cyhalothrin-2		2.1	0.25	0.75	0.150
permethrin-cis	32	3.8	1.22	3.67	0.734
permethrin-trans	44	4.5	2.01	6.03	1.205
Cyfluthrin 1-4	6	5.8	0.35	1.06	0.211
Cyfluthrin 1	6	6.0	0.34	1.02	0.205
Cyfluthrin-2	5	6.0	0.32	0.97	0.195
Cyfluthrin-3	7	6.0	0.40	1.19	0.237
Cyfluthrin-4	8	5.9	0.45	1.36	0.271
cypermethrin 1-4	8	6.9	0.53	1.59	0.318
Cypermethrin 1	6	7.2	0.40	1.21	0.242
Cypermethrin-2	10	6.7	0.69	2.06	0.411
Cypermethrin-3	10	7.6	0.74	2.22	0.445
Cypermethrin-4	7	6.8	0.46	1.38	0.276
Esfenvalerate_Fenvalerate-1	12	4.6	0.56	1.68	0.335
Esfenvalerate_Fenvalerate-2	10	2.8	0.27	0.82	0.163
Deltamethrin	11	2.7	0.31	0.92	0.184