STANDARD OPERATING PROCEDURE
TITLE: Extraction of Tissues and Sediments for Pyrethroid Pesticides (Pressurized Fluid Extraction)

REVISION HISTORY		
Revision #	**Summary of Changes**	**Date**
1	Reformatted SOP. Separated the extraction from the instrument analysis. Added extraction benchsheet documentation requirements. Added spike witness responsibilities. Changed Florisil column specifications. Added GPC calibration requirements. Added muffle furnace preparation of sodium sulfate. Expanded safety section.	07/19/2013
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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 selected pyrethroids in fish and shellfish tissues and sediments.

1.2 The extracts may be analyzed by GC/MS/MS. Target analytes and routine spiking concentrations are listed in Table 1.

1.3 These procedures are applicable when low parts per billion analyses are required to monitor differences between burdens in organisms and sediment concentrations from relatively uncontaminated reference areas and contaminated areas. In addition, the procedures are applicable when low detection limits are required for the estimation of potential health effects of bioaccumulated substances.

1.4 Only analysts who have been trained according to WPCL-QA-005 Training may be allowed to generate client data using this procedure.

2.0 Summary of Method.

2.1 Sets of 10-18 homogenized tissue or 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 SW3545A Pressurized Fluid Extraction (PFE). Extract cleanup and partitioning methods are modifications of EPA Methods SW3640A Gel Permeation Cleanup, SW 3620C Florisil Cleanup, and 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.

2.2 Homogenized tissue or sediment samples are removed from the freezer and allowed to thaw. A separate extraction bench sheet is initiated for each set of samples which are distinguished by project and sample matrix type.

2.3 To determine moisture/solids content a 3-4 g tissue sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48. Moisture/solid content is determined using a 3-4 g sediment sample weighed into a pre-weighted aluminum planchet and placed in a 100°C oven for 48 hours.

2.4 The extraction is performed on a 10 g sample mixed using a clean glass stirring rod with approximately 7 g of pre-extracted Hydromatrix® in a 250 mL TraceClean® 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 pyrethroid surrogate compounds 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 two 60 mL VOA vials.

2.5 The combined extracts (~100 mL) are dried using sodium sulfate, evaporated to approximately 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 J_2 Scientific AccuPrep 170 (GPC) autosampler tubes. If the lipid content needs to be determined, two milliliters each of the filtered extracts are removed and placed in a pre-weighed aluminum planchet.

2.6 The GPC autosampler tubes are then placed on the GPC autosampler for initial sample cleanup by gel permeation (size exclusion) chromatography.

2.7 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroleum ether. The extracts are then fractionated using 5 grams of Florisil® in a 11 mm x 300 mm column with a 250 mL reservoir. The Florisil® columns prepared for pyrethroids are eluted with 15% diethyl either/PE. The fractions are concentrated to an appropriate volume using K-D/micro K-D apparatus. Internal standards are add just prior to analysis by dual column high resolution gas chromatography and/or GC-MSMS.

3.0 Interferences and Comments.

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.

3.2 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.

3.3 Before use, Na_2SO_4 will be baked in a muffle furnace at 450°C for 4 hours.

3.4 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.

3.5 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.

3.6 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. This will usually require that resection (i.e., surgical removal) of tissue be performed in a controlled environment (e.g., a laboratory). The samples should be double wrapped in aluminum foil and immediately frozen with dry ice in a covered ice chest. Ice should be in water tight plastic bags for transporting live shellfish.

3.7 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.

3.8 Resection should be carried out by or under the supervision of a competent biologist. See WPCL-PR-070 “Preparation of Tissues and Bivalves.” Each organism should be handled with clean high carbon steel, titanium, quartz, or Teflon instruments (except for external surfaces). The specimens should come into contact with pre-cleaned glass surfaces only. Polypropylene and polyethylene surfaces are a potential source of contamination and should not be used. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for resecting tissue for analysis. For fish samples, special care must be taken to avoid contaminating target tissue (especially muscle) with slime and/or adhering sediment from the fish interior (skin) during resection. The incision "troughs" are subject to such contamination; thus, they should not be included in the sample. In case of muscle, a "core" of tissue is taken from within the area bordered

by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that constitutes the rest of the muscle tissue mass.

3.9 The resected tissue sample should be placed in a clean glass or PTFE container which has been washed with detergent, rinsed twice with tap water, rinsed once with distilled water, rinsed with acetone, and, finally, rinsed with high-purity petroleum ether.

4.0 Preservation and Holding Times

4.1 The U.S. EPA has published a guidance document containing specific recommendations regarding holding times and temperatures for tissue samples to be analyzed for semi-volatile organic compounds. The following holding conditions should be observed. Tissue samples should be maintained at <-20^{o} C and analyzed as soon as possible, but within 12 months of sample receipt.

4.2 Sediment samples may be refrigerated at <6°C for up to 14-days maximum or must be stored frozen at minus (-) 20°C for up to 12 months maximum.

4.3 Extracts must be analyzed within 40 days after extraction (concentration). Coordinate workloads to allow instrument analysis to be completed within the 40 days.

5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound and sample 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 file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets should be made available to all personnel involved in this procedure. It is the responsibility of the analyst to read the MSDS as part of the training process.

5.2 Wear gloves, lab coats, safety glasses,or face shields while processing samples. All processes must be performed in an operating hood.

5.3 Wear a face shield while performing any operations involving vacuum.

5.4 Wash your hands at the completion of sample processing.

5.5 Dispose of waste solvents and spiking solutions according to WPCL-EH-049 “Disposal of Hazardous Wastes.”

5.6 The following chemicals have the potential to be highly toxic or hazardous. For details, read the MSDS associated with each chemical.

5.6.1 Methylene chloride AKA dichloromethane (DCM).

5.6.2 Iso-octane (ISO).

5.6.3 Petroleum ether (PE).

5.6.4 Acetone.

5.6.5 Diethyl ether.

6.0 Equipment and Supplies

6.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.

6.2 Chromatographic Column - (300 mm x 11 mm) borosilicate glass chromatography column with 250 mL reservoir and Teflon stopcock.

6.3 Glass wool, Pyrex - solvent washed prior to use.

6.4 Kuderna-Danish (K-D) Apparatus.

6.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or quivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

6.4.2 Evaporation flask - 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with blue clamp (Kontes K-662750-0012).

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

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

6.4.5 Boiling stones, 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.

6.5 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. Water bath is equipped with solvent recovery system.

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

6.6.1 Extraction Cells, 33 mL, Dionex P/N 049562.

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

6.6.3 Amber VOA Vials, 60 mL, pre-cleaned and certified.

6.7 Amber Sample vials - glass, 2.5 mL with PTFE-lined screw cap.

6.8 Analytical balance - capable of weighing 0.1 mg.

6.9 Drying oven.

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

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

6.12 Aluminum dishes for moisture and lipid determination.

6.13 Glass funnel, 75 mm.

6.14 Graduated cylinder, 250 mL and 100 mL.

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

6.16 Centrifuge tubes, 15 mL, graduated to 0.1 mL and calibrated to 1.0 mL and 2.0 mL.

6.17 Homogenizer, Bucchi 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).

6.18 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).

6.19 Gel Permeation (size exclusion) Chromatograph, automated, J2 Scientific AccuPrep 170, equipped with 70 g S-X3 BioBeads J_2 Scientific P/N C0070G (100% DCM).

6.20 GPC Calibration Solution, Ultra Scientific Analytical Solutions, Product #CLP-340 containing corn oil, bis(2-ethylhexyl)phthalate, methoxychlor, perylene, and elemental sulfur.

7.0 Reagents and Standards

7.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

7.2 Acetone. (Same as above).

7.3 Iso-Octane. (Same as above).

7.4 Diethyl ether preserved with 2% ethanol.(Same as above).

7.5 Dichloromethane (DCM). (Same as above).

7.6 Chem Elut-Hydromatrix®, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use. Muffle at 450º C for about 4 hours prior to use. After muffling, can be stored in a cleaned, glass jar.

7.7 Sodium sulfate. Anhydrous granular reagent grade, muffle at 450º C for about 4 hours. After muffling, can be stored in a cleaned glass jar. Rinse with DCM just prior to use.

7.8 Florisil®, 60/100 mesh, PR grade, U.S. Silica. Muffle prior to use.

7.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE and GPC).

7.10 Nitrogen, ultra-pure (99.99999%) for ECD makeup.

7.11 Helium, ultra-pure (99.99999%) for GC carrier gas.

7.12 Air, compressed, breathing quality, for ASE pneumatics.

7.13 Universal Surrogate mix containing 40ppb DBOB, 40ppb of DBCE and 400ppb of TPP in acetone.

7.14 Pyrethroid 9 compound Spiking Solution: Add 1 mL of a 50 ng/mL solution.

8.0 Calibration and Standardization.

8.1 GPC Calibration Standard Solution

8.1.1 In a clean and DCM rinsed 100 mL volumetric flask, quantitatively transfer one vial of the CLP-340 calibration standard to the volumetric flask.

8.1.2 Fill with DCM to approximately 80% of the final volume, cap, and mix well.

8.1.3 Bring up to final volume with DCM.

8.1.4 Transfer to a clean 125 mL amber glass bottle and label as GPC Calibration Standard. Include on the label the lot number, preparation date, and preparer's initials.

8.2 GPC Calibration

8.2.1 Cut times are required for the GPC. Run a standard to obtain a chromatogram of the standard.

8.2.1.1 Between 15-25 minutes, two peaks representing the fats/lipids will appear.

8.2.1.2 At about 27-30 minutes, bis(2-ethyhexyl)phthalate appears as the third peak.

8.2.1.3 At about 33-36 minutes, methoxychlor appears as the fourth peak.

8.2.1.4 At about 44-47 minutes, perylene appears as the fifth peak.

8.2.1.5 At about 51-55 minutes, sulfur will appear as the last peak.

8.2.2 Create cut times that collect everything except the fats/lipids and sulfur. Cut times are generally 25 minutes and 50 minutes but as the column gets older, the cut times drift and need to be readjusted. Print the chromatogram and with a ruler, draw cut times as shown on the attachment. Display for reference purposes near the GPC.

8.3 Refrigerators and freezers. Record temperatures daily using calibrated thermometers.

8.4 Balances: calibrate prior to use with certified weights. Record the checks.

9.0 Procedure

9.1 Remove homogenized tissue or sediment samples from freezer and allow to thaw. Prior to extraction, the tissue samples are homogenized using a Bucchi B-400 mixer equipped with a titanium knife assembly or for small samples a Brinkman Polytron®

equipped with a titanium and Teflon generator. Decant any excess water from the sediment samples prior to thoroughly mixing 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 10-18 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 laboratory control spikes.

9.2 A separate extraction bench sheet is initiated for each project, sample matrix type, and analysis type. Several bench sheets may be used for an extraction set.

9.2.1 The extraction benchsheet must include:

9.2.1.1 Customer/client name.

9.2.1.2 Lab number (L-number).

9.2.1.3 Sample number, sample ID.

9.2.1.4 Test name.

9.2.1.5 Extraction date.

9.2.1.6 Analyst name.

9.2.1.7 Spike witness.

9.2.1.8 Initial sample volume.

9.2.1.9 Final extract volume.

9.2.1.10 Lot number and brand of any filters used.

9.2.1.11 Solvent lot numbers used.

9.2.1.12 Final solvent.

9.2.1.13 Quality control lot/tracking number.

9.2.1.14 Spike solution ID number and concentration.

9.2.1.15 Spike solution volume added.

9.2.1.16 Surrogate solution ID number and concentration.

9.2.1.17 Surrogate solution volume added.

9.2.1.18 Clean-ups used.

9.2.1.19 If added. Internal standard volume, ID, initials, date added.

9.3 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.

9.4 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 lipid and moisture determinations (write sample ID on the bottom of planchets using a ball point pen).

9.5 Tare a 250 mL glass jar. Using a clean (solvent rinsed) glass rod, stir the tissue or 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.

9.6 Weigh 3-4 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for tissues and 100°C oven for sediments for 48 hours and re-weigh until a constant dry weight.

9.7 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®)*.

9.8 Pour the tissue or 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®.

9.9 All of the extraction cells are spiked with the pyrethroid surrogate standard. Spike each cell with exactly 1.0 mL of the appropriate surrogate solution (Table 2). Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

9.10 A witness is required to confirm that samples have been spiked properly.

9.10.1 The spike witness must verify that the spike solutions are not expired, are the proper pesticide group, are the proper concentration and are documented in the standards log.

9.10.2 The witness must verify that the correct volume was added to all samples.

9.10.3 The witness must sign and date the extraction records.

9.11 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCS) and its duplicate (LCSD) are spiked with exactly 1.0 mL of pyrethroid spike solution (Table 1). A separate MS/MSD and LCS/LCSD is required for each class of compounds being analyzed. Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

9.12 The extraction cells are capped *(Firmly tightened but do not overtighten)* and placed on the ASE 200 carrousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carrousel 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.

9.13 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 ^{o}C
Sol A Other	100%.

9.14 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 carrousel. 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.

9.15 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.

9.16 Gel Permeation Chromatography

IMPORTANT: ***All glassware should be triple rinsed with methylene chloride. Glass wool should be triple rinsed with petroleum ether. Sodium sulfate should be muffled at 450°C and rinsed with methylene chloride before use.***

9.16.1 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.

9.16.2 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.

9.16.3 Pour sample extracts from the two VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to both VOA vials, 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).

9.16.4 Add 0.5 mL Iso-Octane using a macro-pipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask, clamp with a blue clamp and place the flask on the hot water bath set at 80-82^{o}C. Drop down the inverted Hopkins condenser from the solvent recovery system and attach it to the top of the Snyder column. Turn the water supply on to the solvent recovery system until the water flow is between 1500-2000 cc/min. 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.

9.16.5 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 place a pre-rinsed glass stopper on top. Rinse the Snyder column with DCM 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.

9.16.6 Add a new micro-boiling chip 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^{o}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 1.0 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

9.16.7 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.

9.16.8 Whatman 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.

9.16.9 All samples are cleaned using a J_2 Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1" ID glass column with 70g Bio-Beads SX-3 in 100% DCM).

9.16.9.1 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.

9.16.9.2 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 'ZGPC Calib' 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. By default the Method File will be on the program OP_PYR. 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), laboratory control spike (Spiked Blank), and the SRM (Lab Control Std) are encountered. After all the samples have been added to the sequence, save it as the bench sheet number (BS###). 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 ZGPC method is being used for the calibration standard and the OP_PYR 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.

9.16.9.3 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) and cover with pre-cut pieces of aluminum. 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 bench sheet and laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump as 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.

9.16.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the sample 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. Empty the waste container into a 4L waste bottle labeled with a hazardous waste label.

9.16.10 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. Attach the inverted Hopkins condenser to the top of the Snyder column and turn to water on to the solvent recovery system (~1500-2000 cc/min). When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, remove

the inverted Hopkins condenser and secure out of the way. 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. Add a new micro boiling chip to the aliquot and place it in a 400 mL beaker containing water heated to approximately 75^{o}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.

9.16.11 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. Mark the volume on the tube with a permanent marker.

9.16.12 SEDIMENT SAMPLES ONLY: Add acid rinsed copper to the culture tubes to remove any residual sulfur from the extract. Allow copper to stay in contact with extract overnight.

9.17 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^{o}C for at least 24 hours prior to use.***

9.17.1 This procedure is performed after the GPC cleanup procedure for all tissue and sediment samples.

9.17.2 When the samples are to be analyzed for Pyrethroids prepare the 15% ethyl ether in petroleum ether Florisil® column eluant. 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 75 mL per sample for the 15% eluent.

9.17.3 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the 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 5 grams of Florisil® that has been measured using a dedicated pre-calibrated culture tube. 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.

9.17.4 Place a 600 mL beaker under the column and pre-wet the column with about 25 mL of petroleum ether.

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.***

9.17.5 When approximately 1 inch of PE remains above the surface of the column, add 0.5 mL of iso-octane to a K-D flask and place it under the 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 PE rinse reaches the column bed surface, decant the sample onto the column. Immediately add approximately 0.5 mL of PE to the tube, vortex, and add the rinse to the sample extract on the column. Add another 0.5 ml of 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.

9.17.6 When the combined sample and rinses reach the sodium sulfate layer, add 75 mL of 15% diethyl ether/petroleum ether that has been carefully measured out using a graduated cylinder to the column reservoir. Make sure that the stopcock is fully open in order to achieve the desired flow rate of 2 to 3 mL per minute. Allow the column to completely drain into the KD Flask.

9.17.6.1.1 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 0.5-1 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

9.17.6.1.2 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and transfer the contents to a calibrated centrifuge tube rinsing the concentrator tube with a small amount of PE and adding the rinse to the centrifuge tube. If the volume in the centrifuge tube is greater than 1 mL, evaporate to 1 mL using nitrogen. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. Transfer the extract to a clean labeled culture tube.

10.0 Quality Control

10.1 A batch of samples is composed of field samples of similar matrix processed concurrently using the same reagents, clean-ups, and procedures. Because the size of the batch is limited by the number of extraction cells available on the ASE (24 cells), up to 18 field samples plus the quality control samples make up an extraction batch. Quality control samples are method blanks, lab control samples, matrix spike, matrix spike duplicate, certified reference material, and duplicates.

10.2 A method blank (MBLK) is processed with every batch of samples. A blank will include Hydromatrix (as appropriate), sodium sulfate, surrogate spike, and reagents used in the extraction. The MBLK will undergo all procedures applied to samples including clean-ups.

10.3 A Laboratory Control Sample (LCS) is processed with every batch of samples. The LCS will include Hydromatrix (as appropriate), sodium sulfate, surrogate spike, matrix spike, and reagents used in the extraction. The LCS will undergo all procedures applied to samples including clean-ups. If insufficient field sample is available for performing a matrix spike, a duplicate LCS (LCSD) will be included in the batch.

10.4 A certified reference material (CRM or SRM) will be processed as a sample with every batch. CRMs are purchased from third party vendors accompanied by certificates of analysis.

10.5 Matrix spike/ matrix spike duplicate (MS/MSD) will be processed with every batch. A matrix spike is a well-mixed sub-sample of a selected field sample where a known concentration of known analytes are added ("spiked") prior to processing. A MSD is a third sub-sample where a spike is added prior to processing the samples within the batch.

10.6 A duplicate will be processed with every batch as required by project plans or client request. A duplicate is a well-mixed sub-sample of a selected field sample processed with the batch.

10.7 If quality control samples indicate that contamination or poor recoveries are due to errors during the extraction, all samples associated with failed controls will be re-extracted in a new batch if there is sufficient sample volume available. The new batch must include a new set of quality control samples.

11.0 References

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11.3 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.

11.4 U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line, Method 3545A, *Pressurized Fluid Extraction*, Revision 1, February 2007, http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3545a.pdf [11/10/08]

11.5 U.S. Environmental Protection Agency, Office of Solid Waste, Method 3620C, *Florisil Cleanup*, Revision 3, February 2007, http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/3620c.pdf [11/10/08]

11.6 U.S. Environmental Protection Agency, Office of Solid Waste, Method 3640A, *Gel Permeation Cleanup*, Revision 1, September 1994, http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3640a.pdf [03/29/07].

11.7 WPCL-EH-049, "Disposal of Hazardous Wastes."

11.8 WPCL-QA-005, “Training”

11.9 WPCL-PR-070. “Preparation of Tissues and Bivalves.”

12.0 Attachments

12.1 Table 1: Pyrethroids 9 Compound Spiking Solution.

12.2 Table 2: Universal Surrogate Spike Solution.

12.3 Figure 1: GPC Calibration and Cut Times

Table 1: "50 ppb Pyrethroids 9 Compound Spiking Solution"

CAS Number	ANALYTE NAME	Working Spike Concentration ng/mL in Acetone
82657-04-3	Bifenthrin	10
68359-37-5	Cyfluthrin, total	50
52315-07-8	Cypermethrin, total	50
52918-63-5/66841-25-6	Deltamethrin/Tralomethrin	50
66230-04-4/51630-58-1	Esfenvalerate/Fenvalerate, total	20
64257-84-7	Fenpropathrin	10
54774-45-7	Permethrin, cis-	50
51877-74-8	Permethrin, trans-	100
91465-08-6	Cyhalothrin, lambda, total	20

Table 2: "Universal Surrogate Spike for Pesticides in Sediment"

CAS Number	ANALYTE NAME	Working Spike Concentration ng/mL in Acetone
1770-80-5	Dibutychlorendate (DBCE)	40
10386-84-2	Dibromooctafluorobiphenyl (DBOB)	40
115-86-6	Triphenylphosphate (TPP)	400

Figure 1 : GPC Calibration and Cut Times