Appendix IV: DFG-WPCL SOPs

DFG-WPCL EPA Modifications and Laboratory Procedures			
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Appendix IV A: Procedure for the Management of Samples Received for Chemical Analysis

CDFG FISH AND WILDLIFE WATER POLLUTION CONTROL LABORATORY STANDARD OPERATING PROCEDURE FOR THE MANAGEMENT OF SAMPLES RECEIVED FOR CHEMICAL ANALYSIS

1.0 Scope and Application

This method describes the procedures to be followed for the receipt, handling, scheduling, storage, and disposal of samples received by the laboratory.

2.0 Summary of Method

The WPCL sample receiving area is located in the sample log-in room at the back of the main laboratory. All samples are immediately unpacked, checked for temperature, logged-in using the sample receipt log book, entered in Labworks (LIMS), labeled, checked for required preservation and preserved as necessary, checked for appropriate holding time limitations and properly stored (refrigerated or frozen). If samples are delivered frozen, they should be immediately transferred to the freezer after they are logged-in. DFG request for analysis and chain-of-custody records (Form FG 1000 Rev. 9/01) or chains of custody submitted with samples are completed and then given to the appropriate section leader for scheduling. After the analyses are completed, samples are stored until data review and reporting have been completed. Enforcement samples are held

Samples are then disposed using the evaporation pond (non-hazardous samples only) or logged into the hazardous waste storage area for scheduled pickup by a licensed hazardous waste contractor.

3.0 Sample Receipt

3.1 Samples are delivered to the Laboratory by DFG personnel, United Parcel Service (UPS), U.S. Postal Service, Federal Express, and by other commercial courier companies. Samples are shipped in Pollution Action Kit (PAK) boxes (non-hazardous samples only), hazardous materials shipping containers, and various sizes of ice chests.

3.2 Samples received by the Laboratory should be **immediately** taken by qualified Laboratory personnel to the sample log-in station located in the sample storage room at the back of the main laboratory building for unpacking. Samples should **not** be left in the sample receiving area or removed to office areas or laboratories. Samples addressed to individuals should not be left unattended if the addressee is not available.

3.3 All samples received by the Laboratory should be considered to be potentially hazardous and **caution** should be used when opening packages containing samples. Even non-hazardous samples can be a safety hazard to the person

unpacking the samples (ie. broken glass sample containers). All samples shipped in hazardous material shippers should be considered hazardous and should always be unpacked in a well ventilated area (under a fume extractor) or in a fume hood. Personnel unpacking hazardous samples should wear appropriate protective clothing, which at a minimum would include:

safety glasses
nitrile gloves
laboratory coat or apron
closed toe shoes

3.4 Cut packaging tape with a knife or scalpel. Open package or ice chest and remove paperwork which should be on top. Open laboratory log book and record the letter "L" followed by the next consecutive number followed by the last two numbers of the current year. Below the laboratory "L" number, write the date and below the date write your initials. The "L" number will be used for the entire set of samples. Record this number on the line labeled laboratory number in the upper right hand corner of the Form FG 1000 Rev. 9/01, on the chain-of-custody form (if applicable), and any other paperwork that accompanied the samples. All writing must be in ink (preferably ball point pen).

3.5 Remove samples from the shipping container and line them up on the counter. Check each sample container for cracks or breakage. Make sure that each sample is labeled. Labworks LIMS can be used to print labels for sample containers. See 3.14 for instructions. Labels may be handwritten by writing the laboratory number on each container with waterproof marker followed by consecutive numbers. For example:

sample 1 = L-345-07-01
sample 2 = L-345-07-02

3.6 Record the following information about the samples in the log book next to the laboratory number:

number of containers
type of sample (eg. sediment, water, oil, etc.)
condition of sample (broken, leaking, etc.) if necessary
where samples were collected
person requesting analyses and DFG region or other agency name
type of analysis
Index-PCA code, if given by collector

3.7 Check the Form FG 1000 Rev. 9/01 for sample descriptions (Identification/Location), if this is not filled out, do so using the information on the sample labels. Check the Form FG 1000 Rev. 9/01 for analysis requested. If instructions are unclear, contact the person who collected the samples.

3.8 If any problems are found with the samples when they are received (eg. broken containers, missing samples, samples have been shipped that are not recorded on the chain-of-custody documents), notify the individual who shipped the samples immediately and inform them of the problem.

3.9 Sign, print name and date the Form FG 1000 Rev. 9/01 and chain of custody card (if used) next to "received by". Give the submitter the goldenrod copy. Put the pink copy in the binder at the log-in area. The pink copy is used to enter all sample information into the laboratory information management system (LIMS)..

3.10 Check the holding time/sample preservation table for the analysis requested and record the holding time expiration on the Form FG 1000 Rev. 9/01 if applicable. If preservation is required, do so immediately and record the type of preservative, date preserved, and initial the Form FG 1000 Rev. 9/01. Preservation of inorganics samples is also entered in a separate log book located in the inorganic lab (this is done by inorganic lab staff). Each container should receive a label indicating that the sample was preserved and the type of preservative.

3.11 Determine where the samples will be stored and record refrigerator or freezer number in the space provided on the Form FG 1000 Rev 09/01. Keep samples together as a set. Samples should be stored as follows:

VOA samples only – Elmo (front lab, left side)
petroleum samples – Grover (front lab, left side)
inorganic samples – WPCL R5, R7, WPCL R2 (machine room), TSM F2 (sample storage room), walk in R2
ambient monitoring samples – TSM R3 (left side), TSM F2, F4, WPCL F1 (sample storage room), walk in F1
pesticides (F&W Loss) – WPCL F3 (sample storage room), WPCL R1, R2, R3, R4 (back lab), walk in R3, walk in F1

F = freezer; R = refrigerator

Enforcement and regulatory samples must be stored in a locked refrigerator/freezer.

3.12 Give all paperwork to the appropriate lead analyst:

Petroleum/semi-VOA/VOA - Bob Todd
Inorganic/hatchery monitoring - Patty Bucknell
Pesticide (F&W Loss) investigations - Abdou Mekebri
Pesticides (pesticides contracts) - Abdou Mekebri
Tissue/sediment monitoring-(PCBs, PBDEs, OC pesticides)-Kathleen Regalado

3.13 If samples were received in a PAK, photocopy the Form 1000 and write "PAK" in large letters on the front of the photocopy or if a Form FG 1000 Rev 09/01 was used, check the box labeled "PAK Requested" if not already done. Give the photocopy to Bob Todd so that a new PAK will be sent out to replace the one received.

3.14. Using Labworks for log-in samples

1. Go into Multilog
2. Load the project pre-log in group and enter site location, collection date, collection time and checking analytes, matrix, report address against COC
3. Labels can be printed from Labworks with L#, site location, analyses, collection date and time
   - 3a. Go to Labworks explore and pick L #'s for the labels
   - 3b. Highlight the accession for labels. Choose LABEL 30251.CEF and click OK
   - 3c. The program is automatically connected to access. Pick report feature and click LABEL-30251-inorg
4. Place the labels on samples

4.0 Scheduling

4.1 Laboratory analyses are scheduled by priority. Priorities are ranked as follows:

1-Enforcement samples with regulatory holding time
2-Spill and/or wildlife loss in progress
3-Routine samples with regulatory holding time
4-Enforcement samples with completion date requested
5-Routine samples with completion date requested
6-Enforcement samples with no holding time
7-Routine samples with no holding time

4.2 Samples should be completed as soon as possible after receipt. If a delay is anticipated for the completion of an analysis, the person requesting the analysis should be advised of the delay.

5.0 Sample Storage

5.1 Samples remain refrigerated or frozen until they are needed for analysis. Samples are removed from storage for analysis and then are returned to refrigerated storage. Tissue samples are always returned to a freezer after they are analyzed. Samples remain refrigerated/frozen until results are reported.

5.2 Samples that will not spoil may be moved to locked non-refrigerated storage after the report has been completed. Retain chain-of-custody cards or the original copy of the Form FG 1000 Rev. 09/01 for all stored samples.

5.3 Samples retained by the laboratory for six months that are not required as physical evidence should be disposed using a hazardous materials disposal contractor. The person submitting the samples should be contacted and told the samples will be disposed unless a request is made to store the samples at the laboratory for a longer period of time. When samples are disposed, the word "Disposed" and the disposal date are recorded next to the logbook entry (lab accession number) for that sample with the person's initials that authorized the disposal. Inorganics samples are also entered in separate disposal log book located in the inorganic lab (this is done by inorganic lab staff).

QA Officer Approval:_____ Date: _____

SOP Final Approval:_____ Date: _____

Appendix IV B: SO-TISS Determination of OC and PCB in Sediment and Tissue (Modifications to EPA 8081B and 8082)

ANALYSIS OF EXTRACTABLE SYNTHETIC ORGANIC COMPOUNDS IN TISSUE AND SEDIMENT
(Organochlorine Pesticides, Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers)

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 organochlorine (OCs) pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and shellfish tissues and sediments. Dual column gas chromatography with dual electron capture detectors (GC-ECD) and/or gas chromatography with triple quadrupole mass spectrometry (GC-MSMS) are used to analyze OC pesticides, PCBs and PBDEs. Table 1 lists the target OC pesticide compounds currently analyzed with their method detection limits and reporting limits. Table 2 lists the PCB congeners and Aroclor mixtures analyzed with their reporting limits. Table 3 lists the PBDE congeners analyzed with their method detection limits and reporting limits.

1.2 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.

Table 1. Organochlorine Compounds Analyzed and their Minimum Detection Limits (MDL) and Reporting Limits (RL) in Tissue (ng/g, wet wt.) and Sediment (ng/g dry weight), based on 50 % moisture.

	Tissue		Sediment	
	MDL, ng/g wet wt.	RL, ng/g wet wt.	MDL, ng/g dry wt.	RL, ng/g dry wt.
aldrin	0.414	1.00	0.800	2.00
chlordane, cis	0.400	1.00	0.800	2.00
chlordane, trans	0.450	1.00	0.900	2.00
chlorpyrifos	0.204	1.00	0.400	2.00
dacthal	0.096	1.00	0.200	2.00
DDD, o,p'	0.096	1.00	0.200	2.00
DDD, p,p'	0.124	1.00	0.250	2.00
DDE, o,p'	0.178	2.00	0.400	4.00
DDE, p,p'	0.480	2.00	1.00	4.00
DDMU, p,p'	0.108	3.00	0.200	6.00
DDT, o,p'	0.216	3.00	0.400	6.00
DDT, p,p'	0.156	5.00	0.300	10.0
diazinon	4.80	20.0	10.0	40.0
dieldrin	0.432	0.500	1.00	1.00
endosulfan I	0.560	2.00	1.00	4.00
endosulfan II	0.682	5.00	1.40	10.0
endosulfan sulfate	0.546	5.00	1.00	10.0
endrin	0.180	2.00	0.400	4.00
HCH, alpha	0.262	0.500	0.500	1.00
HCH, beta	0.210	1.00	0.400	2.00
HCH, gamma	0.144	0.500	0.300	1.00
heptachlor	0.356	1.00	0.700	2.00
heptachlor epoxide	0.246	1.00	0.500	2.00
hexachlorobenzene	0.346	0.692	0.700	1.40
methoxychlor	0.146	3.00	0.300	6.00
mirex	0.300	1.50	0.600	3.00
nonachlor, cis	0.308	1.00	0.600	2.00
nonachlor, trans	0.194	1.00	0.400	2.00
oxadiazon	0.544	1.00	1.00	2.00
oxychlordane	0.474	1.00	1.00	2.00
parathion, ethyl	0.524	2.00	1.00	4.00
parathion, methyl	0.756	4.00	1.50	8.00
tedion	1.07	2.00	2.00	4.00
DBOB(surrogate)	NA	NA	NA	NA
DBCE(surrogate)	NA	NA	NA	NA
DDD*deuterated (surrogate)	NA	NA	NA	NA

Table 2. PCB Congeners and Aroclor mixtures Analyzed and their Reporting Limits (RL) in Tissue (ng/g, wet weight) and Sediment (ng/g, dry weight).

NIST PCB Congeners:	
PCB Congener 8	PCB Congener 128
PCB Congener 18	PCB Congener 138
PCB Congener 28	PCB Congener 153
PCB Congener 44	PCB Congener 170
PCB Congener 52	PCB Congener 180
PCB Congener 66	PCB Congener 187
PCB Congener 87	PCB Congener 195
PCB Congener 101	PCB Congener 206
PCB Congener 105	PCB Congener 209
PCB Congener 118	PCB Congener 209 C^{13}(surrogate)

Additional PCB Congeners:	
PCB Congener 27	PCB Congener 141
PCB Congener 29	PCB Congener 146
PCB Congener 31	PCB Congener 149
PCB Congener 33	PCB Congener 151
PCB Congener 49	PCB Congener 156
PCB Congener 56	PCB Congener 157
PCB Congener 60	PCB Congener 158
PCB Congener 64	PCB Congener 169
PCB Congener 70	PCB Congener 174
PCB Congener 74	PCB Congener 177
PCB Congener 77	PCB Congener 183
PCB Congener 95	PCB Congener 189
PCB Congener 97	PCB Congener 194
PCB Congener 99	PCB Congener 198_199
PCB Congener 110	PCB Congener 200
PCB Congener 114	PCB Congener 201
PCB Congener 126	PCB Congener 203
	PCB Congener 137

All individual PCB Congener reporting limits (RL) are 0.2 ng/g (wet weight) or 0.4 ng/g (dry weight, based on 50 % moisture). Estimated Aroclor concentrations calculated from the congener concentrations have the following RLs:

Aroclors:	RL ng/g (wet wt.)	RL ng/g (dry wt.)
Aroclor 1248	25	50
Aroclor 1254	10	20
Aroclor 1260	10	20

Table 3. Polybrominated Diphenyl Ethers (PBDEs) and their Minimum Detection Limits (MDL) and Reporting Limits (RL) in Tissue (ng/g, wet wt.) and Sediment (ng/g, dry wt., based on 50 % moisture.)

	Tissue		Sediment	
	MDL, ng/g wet wt.	RL, ng/g wet wt.	MDL, ng/g dry wt.	RL, ng/g dry wt.
BDE 17	0.139	0.600	0.278	1.20
BDE 28	0.148	0.600	0.296	1.20
BDE 47	0.196	0.800	0.391	1.60
BDE 66	0.135	0.600	0.269	1.20
BDE 100	0.157	0.600	0.314	1.20
BDE 99	0.197	0.800	0.394	1.60
BDE 85	0.177	0.800	0.354	1.60
BDE 154	0.165	0.600	0.329	1.20
BDE 153	0.185	0.800	0.370	1.60
BDE 138	0.200	0.800	0.400	1.60
BDE 183	0.297	1.20	0.594	2.40
BDE 190	0.437	1.80	0.874	3.60
BDE 209	1.00	10.0	2.00	20.0

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 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.

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, sample matrix type and analysis type.

2.2 A 1-5 g (tissue or sediment homogenate) sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48 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® 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 pesticide, PCB and

PBDE 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.

2.3 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.

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

2.5 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 tissue samples are eluted with 6% diethyl ether/PE (Fraction 1), 15% diethyl ether/PE (Fraction 2), and 50% diethyl ether/PE (Fraction 3). Florisil® columns prepared for sediment samples are eluted with 6% diethyl ether/PE (Fraction 1) and 50% diethyl ether/PE (Fraction 2).The fractions are concentrated to an appropriate volume using K-D/micro K-D apparatus prior to analysis by dual column high resolution gas chromatography and/or GC-MSMS. The distribution of synthetic organic compounds in the fractions is listed in Table 4.

Table 4. Distribution of Synthetic Organic Compounds Among the Three Fractions of a Standard Florisil® Column.

6% Fraction 1/	**15% Fraction** 2/	**50% Fraction** 3/
aldrin	dacthal	endosulfan II 5/
chlordane (cis-)	DBCE*	endosulfan sulfate
chlordane (trans-)	dieldrin	
DBOB*	endosulfan I 4/	
DDE, o,p'	endosulfan II 5/	
DDE, p,p'	endrin	
DDD, o,p'	oxadiazon	
DDD, p,p'/DDD-d10*,p,p'	tetradifon	
DDMU, p,p'		
DDT, o,p'		
DDT, p,p'		
endosulfan I 4/		
heptachlor		
heptachlor epoxide		
hexachlorobenzene		
HCH-alpha		
HCH-beta		
HCH-gamma		
methoxychlor		
nonachlor (cis-)		
nonachlor (trans-)		
oxychlordane		
polybrominated diphenyl ethers (PBDEs)		
polychlorinated biphenyls (PCBs)/PCB 209*(C^{13})		
toxaphene		

* surrogate
1/ 6% ethyl ether in petroleum ether (analysis by GC-MSMS)
2/ 15% ethyl ether in petroleum ether (analysis by GC-ECD)
3/ 50% ethyl ether in petroleum ether (analysis by GC-ECD).
4/ In both 6% and 15% fractions.
5/ In both 15% and 50% fractions.

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 250 mL reservoir and 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 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.

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. Water bath is equipped with solvent recovery system.

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, 2.5 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 and lipid determination.

4.13 Desiccator with indicating desiccant.

4.14 Glass funnel, 75 mm.

4.15 Graduated cylinder, 250 mL and 100 mL.

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

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

4.19 Gas chromatographs (GC) (3): Hewlett-Packard HP 6890 plus, equipped with dual micro-ECD. All are equipped with split-splitless injector with EPC and autosampler.

4.20 GC Capillary columns, 60 meter DB5 and 60 meter DB17MS (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) connected to a single injection port using a "Y" press fit connector.

4.21 GC Data System, Hewlett-Packard, 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.

4.22 Gas chromatograph-mass spectrometer (triple quadrupole), Varian Model 1200L with Varian Model 3800 gas chromatograph, split-splitless injector with EPC and Combi-Pal autosampler.

4.23 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).

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

4.25 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).

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.

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

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

5.13 OC/PCB/PBDE Surrogate Mix containing: 40 ppb of deuterated p,p'-DDD-d10, PCB 209(C^{13}), and dibutylchlorendate (DBCE).

5.14 Standard Reference Material (SRM), National Institute of Standards and Technology (NIST): SRM 1588b (Organics in Cod Liver Oil) and SRM 1944 (New York/New Jersey Waterway sediment).

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. 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.

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 Resection should be carried out by or under the supervision of a competent biologist. 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.

6.4 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.

6.5 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 $\leq$-20° C and analyzed as soon as possible, but within 12 months of sample receipt.

6.6 Sediment samples may be refrigerated at 4°C for up to 14-days maximum or must be stored frozen at minus (-) 20°C for up to 12 months maximum.

7.0 Sample Extraction

7.1 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.

7.2 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.

7.3 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.

7.4 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).

7.5 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. 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.

7.6 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 48 hours and re-weigh dry weight.

7.7 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®)*.

7.8 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®.

7.9 All of the extraction cells are spiked with the OC/PCB/PBDE pesticide surrogate standard. Spike each cell with exactly 0.5 mL of the appropriate surrogate solution. Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.10 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCS) and its duplicate (LCSD) (if used) are spiked with exactly 0.5 mL of the OC/PCB/PBDE matrix spike solution (40 ng/mL). A separate MS/MSD and LCS/LCSD (if used) is required for each class of compounds being analyzed. Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.11 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.

7.12 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%

7.13 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.

7.14 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-pipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 80-82°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.

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 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 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)

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 '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 SOPAH which is used for both pesticides (SO) and petroleum (PAH) clean-up. 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 SOPAH 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 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.

8.9.4 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.

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. 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. After it has finished cooling, remove the concentrator tube and bring to a final volume of 10 mL in DCM. Split the sample using a 5 mL volumetric pipette. One aliquot is transferred to a labeled 13 x 100 mm test tube. Add a new micro boiling chip to the remaining aliquot 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. Exactly one-half of the extract is removed and placed in a GC autosampler vial for PAH silica/alumina column cleanup or for archive if PAHS are not requested.

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.

8.12 SEDIMENT SAMPLES ONLY: Check the GPC chromatogram for a sulfur peak. If a sulfur peak is present, add acid rinsed copper to the 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 tissue and sediment samples analyzed for pesticides and PCBs.

9.2 **PCB ONLY:** When the samples are to be analyzed for only PCBs prepare only the 6% 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 40 mL per sample for the 6% eluant.

9.3 **TISSUE:** Prepare the reagents to be used for Florisil® cleanup <u>for tissue</u>: 6% ethyl ether in petroleum ether, 15% ethyl ether in PE, and 50% ethyl ether in

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 40 mL per sample for the 6%, 50 ml per sample for the 15% (F2), and 40 ml per sample for the 50% (F3) fractions.

9.4 **SEDIMENT:** Prepare the reagents to be used for Florisil® cleanup for sediment: 6% ethyl ether in petroleum ether and 50% ethyl ether in 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 40 mL per sample for the 6% and 40 ml per sample for the 50% fraction.

9.5 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.

9.6 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.***

9.7 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.

9.8 When the combined sample and rinses reach the sodium sulfate layer, add 40 mL of 6% 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. Place a 50 mL clean, dry, petroleum ether rinsed beaker over the top of the reservoir to prevent evaporation during the elution process. If only **PCB** analyses are requested, allow the column to completely drain and stop here.

TISSUE SAMPLES

9.9 Just as the last of the 6% diethyl ether/PE solvent reaches the top of the sodium sulfate layer, add 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D containing the 6% elution, add 50 mL of the 15% diethyl ether/PE mixture to the column reservoir, replace the 50mL beaker, and elute as before. Add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask containing the 6% diethyl ether/PE fraction 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.

9.10 Repeat the above adding 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D flask containing the 15% eluant. Add 40 mL of 50% diethyl ether/PE mixture to the solvent reservoir. Allow all of the eluant to drain into the K-D flask.

SEDIMENT SAMPLES

9.11 Just as the last of the 6% diethyl ether/PE solvent reaches the top of the sodium sulfate layer, add 0.5 mL of iso-octane to a new K-D flask and exchange it for the K-D flask containing the 6% eluant, add 40 mL of the 50% diethyl ether/PE mixture to the column reservoir, replace the 50mL beaker, and elute as before. Add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask containing the 6% diethyl ether/PE fraction 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.

9.12 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.

9.13 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 rinsate 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. A Vortex

Genie mixer may be used for this step. Transfer the extract to a clean labeled culture tube and cap.

9.14 Repeat for 15% (tissue only) and 50% extracts. The extracts are ready for analysis by GC-ECD and GC-MSMS.

10.0 Analytical Procedure

10.1 Before the sample extracts can be analyzed, 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 (GC) or Varian (GC-MSMS) Software.

10.2 Each sequence includes a minimum of seven calibration standards. The calibration curve concentration for chlorinated hydrocarbons differs for different analytes, but in general the range is 0.5 ppb to 500 ppb. The calibration curve concentration range for polychlorinated biphenyl congeners (PCBs) is 0.5 ppb to 100 ppb. Higher concentrations of PCB standards (50 ppb to 1000 ppb) are analyzed with samples containing higher concentrations of PCBs.

10.3 To verify the calibration standards, second source pesticide check standards (Radian Corp., Pesticide Check Standard Mix A, ERP-009L; Pesticide Check Standard Mix B, ERP-011L) and PCB congener check standard (Ultra Scientific, RPC-EPA) are analyzed. The second source analytes and their concentrations are listed in Table 5 (pesticides) and Table 6 (PCB congeners).

Table 5. Radian Pesticide Calibration Check Standards (Mix A and B)

Mix A	Certified Concentration (ng/µL)
Aldrin	10.0
Gamma-HCH	5.00
DDT, p,p'	20.0
Dieldrin	10.0
Endosulfan I	10.0
Endosulfan II	20.0
Heptachlor	10.0
Heptachlor epoxide	10.0
Methoxychlor	80.0
Mix B	
Alpha-HCH	5.00
Beta-HCH	20.0
Delta-HCH	10.0
Cis-chlordane	10.0

Trans-chlordane		10.0
DDD, p,p'		20.0
DDE, p,p'		10.0
Endosulfan sulfate	20.0	
Endrin		20.0

Table 6. Ultra Scientific PCB Congener Check Standard

RPC-EPA	Certified Concentration (ng/µL)*
PCB 8	4.0
PCB 18	4.0
PCB 28	4.0
PCB 52	4.0
PCB 44	4.0
PCB 66	4.0
PCB 101	4.0
PCB 118	4.0
PCB 153	4.0
PCB 105	4.0
PCB 138	4.0
PCB 187	4.0
PCB 128	4.0
PCB 180	4.0
PCB 170	4.0
PCB 195	4.0
PCB 206	4.0
PCB 209	4.0

* Initial concentration of RPC-EPA is 0.2 µg/mL in iso-octane. This solution is diluted 2:100 in iso-octane

10.4 An initial calibration blank and initial calibration verification standard is analyzed after the calibration standards and prior to the first sample extract. For the 6% Fraction and 15% Fraction runs, continuing calibration blanks (CCBs) and calibration verification standards (CCVs) are analyzed after ten sample extracts have been analyzed. The 50% Fraction extracts contain more lipid material and can cause the CCVs to fail to meet the % recovery criteria, therefore the CCBs and CCVs are analyzed after every five sample extracts. 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 (5 – 10 ppb).

10.6 As the run proceeds, sample extracts are monitored for analyte concentrations that are greater than the calibration curve and need dilution.

10.7 Instrumentation

Gas Chromatographs with Electron Capture Detectors:

10.7.1 Agilent 6890*plus* gas chromatograph equipped with two ^{63}Ni micro-electron capture detectors with EPC and autosampler. Two 60 meter, 0.25 mm ID, 0.25 µm (film thickness) fused silica columns (J&W) are used. A 5 meter length of DB-5 column is connected to a press fit "Y" union which splits the column effluent into two 60 m columns, a DB-5 and a DB-17MS. The injector is a split-splitless injector with EPC.

10.7.2 Chromatograph conditions:
The injector is operated isothermal at 240°C. The oven has an initial temperature of 80°C which is held for 1 minute and then temperature programmed to 210°C at a rate of 15°C/min and held for 10 min. It is then programmed to 280°C at a rate of 2°C/min and is held for 51 min (for PBDE analysis the oven is held at 280°C for 110 min). Helium is used as the carrier gas at a linear velocity of 35 cm/sec. Nitrogen is used for the detector makeup at 30 mL/min.

10.7.3 Sample volume:
Three microliters of samples and standards are injected and split approximately 50/50 onto the 60 m DB-5 and the 60 m DB-17MS.

10.7.3 Instrument calibration:
External standard calibration is used.

10.7.4 Data acquisition and processing:
Detector signals are acquired and processed with a Agilent 3365 Series II Chemstation. Data processing may also be done using Enviroquant Software.

Gas Chromatograph-Triple Quadrupole Mass Spectrometer:

10.7.5 Varian Model 3800/1200L gas chromatograph/triple quadrupole mass spectrometer equipped with a Model 1177 split-splitless injector with EPC and CombiPal autosampler. A J&W 60 meter, 0.25 mm ID, 0.25 µm (film thickness) XLB fused silica columns (J&W) is used. The injector is a split-splitless injector with EPC.

10.7.6 Chromatograph Conditions:
The injector is operated isothermal at 280°C in splitless mode with pressure pulse (45 psi for 1.05 min). The oven has an initial temperature of 80°C which is held for 1 minute and then temperature programmed to 210°C at a rate of 15°C/min and held for 10 min. It is then programmed to 280°C at a rate of 2°C/min and is held for 8 min.

Helium is used as the carrier gas at a constant column flow of 1 mL/min.

10.7.7 Mass Spectrometer Conditions:
The mass spectrometer is operated in electron impact (EI) ionization and MSMS mode using argon as the CID gas. A collision energy of 10 to 30 volts is used depending on the analyte. Q1 and Q3 mass fragments were selected to optimize selectivity and sensitivity. See Table 7.

Table 7. Varian 1200 MS collision energies and mass fragments (Q1 and Q3) for targeted analytes.

	Segment	Q1	Q3	Collision Energy	Internal Standard
DBOB	1	296	246	-20	HCH, alphaC^{13}
HCH, alpha	2	219	183	-10	HCH, alphaC^{13}
HCH, alphaC^{13}	2	223	187	-10	Internal Std
HCB	3	284	214	-30	HCB C^{13}
HCBC^{13}	3	290	220	-30	Internal Std
HCH, gamma	4	219	183	-15	HCH, alphaC^{13}
HCH, beta	4	219	183	-15	HCH, alphaC^{13}
Heptachlor	5	272	237	-15	HeptachlorC^{13}
HeptachlorC^{13}	5	277	242	-15	Internal Std
Chlorpyrifos	6	314	258	-10	ChlorpyrifosC^{13}
ChlorpyrifosC^{13}	6	325	260	-15	Internal Std
Aldrin	6	293	258	-10	ChlorpyrifosC^{13}
Oxychlordane	7	387	263	-10	Nonachlor, transC^{13}
Heptachlor epoxide	7	387	353	-10	HeptachlorC^{13}
DDE, o,p'	8	318	246	-10	DDE, p,p'C^{13}
DDMU, p,p'	9	284	212	-15	DDE, p,p'C^{13}
Chlordane, trans	9	373	266	-15	Nonachlor, transC^{13}
Chlordane, cis	9	373	266	-15	Nonachlor, transC^{13}
Nonachlor, trans	10	409	310	-15	Nonachlor, transC^{13}
Nonachlor, transC^{13}	10	418	310	-15	Internal Std
DDE, p,p'	12	318	246	-15	DDE, p,p'C^{13}

DDE, p,p'C^{13}	12	329	258	-15	Internal Std
DDD, o,p'	13	235	165	-20	DDE, p,p'C^{13}
DDT, o,p'	15	235	165	-15	DDT, p,p'C^{13}
Nonachlor, cis	16	409	275	-15	Nonachlor, transC^{13}
DDD, p,p-deuterated	16	243	173	-20	DDT, p,p'C^{13}
DDD, p,p'	16	235	165	-15	DDT, p,p'C^{13}
DDT, p,p'	17	235	165	-25	DDT, p,p'C^{13}
DDT, p,p'C^{13}	17	248	177	-20	Internal Std
Methoxychlor	18	227	169	-20	DDT, p,p'C^{13}
Mirex	20	272	237	-15	DDE, p,p'C^{13}

10.7.8 Instrument Calibration:

Internal standard calibration is used. Internal standards are added to the standards and sample extracts just prior to analysis. The following internal standards are used at 1.0 ng/µL:

PCB Internal Standards
PCB 52 (C^{13}) – 4Cl congeners
PCB 97 (C^{13}) – 5Cl congeners
PCB 128 (C^{13}) – 6Cl and 7Cl congeners
PCB 194 (C^{13}) – 8Cl congeners
PCB 206 (C^{13}) – 9Cl congeners
PCB 209 (C^{13}) – 10Cl congeners

OC Internal Standards
HCH, alpha (C^{13})
HCB, (C^{13})
Heptachlor, (C^{13})
Chlorpyrifos, (C^{13})
Nonachlor, trans (C^{13})
DDE, p,p' (C^{13})
DDT, p,p' (C^{13})

Nine target analyte calibration levels are used (0.25, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100 ng/µL).

10.7.9 Sample volume:

Two microliters of samples and standards are injected.

10.7.10 Data processing:
Mass spectrometer signals are acquired and processed using Varian 1200L software .

11.0 References

Tetra Tech, Inc. 1986. Bio Accumulation monitoring Guidance: 4. Analytical Methods for U.S. Priority Pollutants and 301 (h) Pesticides in tissues from Estuarine and Marine Organisms. TC-3953-03. U.S. EPA Washington, DC.

U.S. Environmental Protection Agency. 1993. Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories, Volume I, Fish Sampling and Analysis. EPA 823-R-93-002. U.S. EPA, Office of Water, Washington D.C.

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.

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]
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 IV C: Procedure for the Handling, Storage and Disposal of Hazardous and General Laboratory Waste

File: HAZMAT_Rev4_SOP
Date: 3/23/09
Revision #: 4
Prepared By: DBC
Page 1 of 8

CDFG Fish and Wildlife Water Pollution Control Laboratory (WPCL) and Petroleum Chemistry Laboratory (PCL) Standard Operating Procedure for the Handling, Storage, and Disposal of Hazardous and General Laboratory Waste

1. Scope and Application

1.1 Federal and state regulatory control over hazardous waste has become extraordinarily stringent in recent years. These changes have dramatically increased the complexity of handling the hazardous waste produced by laboratories.

1.2 These procedures are provided to ensure safe, efficient, and legally compliant handling and disposal of hazardous waste.

2. Summary of Hazardous Waste Disposal

2.1 **NEVER DISPOSE OF LIQUIDS, SOLID CHEMICALS, LABORATORY SAMPLES, HAZARDOUS WASTE OR HAZARDOUS MATERIALS IN THE LABORATORY TRASH RECEPTICALS OR DUMPSTERS. NEVER DISCHARGE LIQUID HAZARDOUS WASTE TO THE SANITARY SEWER (bathroom drains) OR EVAPORATION POND (laboratory sinks, fume hood drains, floor drains).** Non-hazardous aqueous laboratory waste can be disposed of by discharging to the evaporation pond.

2.2 Characteristics of Hazardous Waste (these definitions apply to waste potentially generated by WPCL, for complete definitions see Title 22 Article 2 section 66261.10)

Ignitability – Hazardous Waste Number **D001**

- is liquid, other than an aqueous solution containing less than 24 percent alcohol by volume, with flash point less than 60ºC (140ºF);
- is not a liquid and is capable of causing fire through friction, absorption of moisture or spontaneous chemical changes and, when ignited burns so vigorously and persistently that it creates a hazard;
- is an ignitable compressed gas;
- is an oxidizer defined in 49 CFR section 173.151.

Corrosivity – Hazardous Waste Number **D002**

- is aqueous and has a pH less than or equal to 2 or greater than or equal to 12.5;
- is not aqueous and, when mixed with an equivalent weight of water, produces a solution having a pH less than or equal to 2 or greater than or equal to 12.5.

Reactivity – Hazardous Waste Number **D003**

- is normally unstable and readily undergoes violent change without detonating;
- reacts violently with water;
- forms potentially explosive mixtures with water;
- when mixed with water, generates toxic gases, vapors or fumes in a quantity sufficient to present a danger to human health or the environment;
- is a cyanide or sulfide bearing waste which, when exposed to pH conditions between 2 and 12.5, can generate toxic gases, vapors or fumes in a quantity sufficient to present a danger to human health or the environment;
- is capable of detonation or explosive reaction if it is subjected to a strong initiating source or if heated under confinement;
- is readily capable of detonation or explosive decomposition at STP;
- is a Class A explosive.

Toxicity – Hazardous Waste Number **D004-D043**

See attached definitions of toxicity.

2.3 All chemical hazardous waste must be properly identified, labeled, segregated, and stored prior to removal by a qualified and licensed hazardous waste contractor.

2.4 Maximum Storage Times

- The maximum length of time that hazardous waste may be stored by the laboratory is 270 days from the initial date of accumulation.
- On the date that 55 gallons of waste have accumulated, the laboratory has 90 days to have the waste removed.
- Hazardous waste should be transferred from the laboratory to the hazardous material storage building within 6 months of the initial date of accumulation. The date that the waste is transferred to the hazardous material storage building, that date must be entered on the hazardous waste label under "Accumulation Start Date". Waste must be removed within 90 days of the Accumulation Start Date.
- Any hazardous waste container stored over 270 days is a violation.

2.5 Labeling Hazardous Waste Containers

- All hazardous waste containers must be labeled properly.
- Hazardous waste labels must be completely filled out.
- The first date of accumulation (WPCL waste log-in code) must always be entered on the upper right corner of the label with the individual's initials. **The date that the waste is transferred from the lab to the hazardous waste storage building must be entered on the label in the "Accumulation Start Date" section and that starts the 90 day removal requirement.**

2.6 Neutralization of Aqueous Acids and Bases

Small volumes of aqueous corrosive hazardous waste may be neutralized and discharged to the evaporation pond if the following procedures are followed. A hazardous waste facilities permit or other grant of authorization is not required for treatment of laboratory hazardous waste (neutralization of aqueous acid and base waste) generated onsite, if all of the following requirements are met:

- The hazardous waste is treated in containers using recommended procedures and quantities for treatment of laboratory wastes published by the National Research Council (NRC) or procedures for treatment of laboratory wastes published in peer-reviewed scientific journals.
- The waste is treated at a location that is as close as practical to the location where the laboratory hazardous waste is generated, **and the treatment is conducted within 10 calendar days after the date that the waste is generated.**
- The amount of laboratory hazardous waste treated in a single batch does not exceed the quantity limitation specified in subparagraph (A) or (B), whichever is the smaller quantity:
  - (A) **Five gallons** or 18 kilograms, whichever is greater.
  - (B) (i) Except as otherwise provided in clause (ii), the quantity limit recommended in the procedures published by the NRC or in other peer reviewed scientific journals for the treatment procedure being used. (ii) a qualified chemist has demonstrated that a larger quantity can be safely treated and documentation to that effect is maintained onsite.
- The laboratory hazardous waste treated is from a single procedure, or set of procedures that are part of the same laboratory process.
- The person performing the treatment has knowledge of the laboratory hazardous waste being treated, including knowledge of the procedure that generated the laboratory waste, and has received hazardous waste training, including how to conduct the treatment, manage treatment residuals, and respond effectively to emergency situations.
- Training records for all persons performing treatment of laboratory hazardous wastes pursuant to this subdivision are maintained for a minimum of three years.
- All records maintained by the laboratory pertaining to treatment conducted pursuant to this subdivision are made available for inspection upon request by a representative of the department or the CUPA or other authorized agency.

3. Source Reduction and Waste Minimization

3.1 Whenever possible, experiment protocols should include provisions to both reduce the volume of the source, and minimize the generation of hazardous waste.

3.2 Waste such as motor oil, paper, corrugated boxes, toner cartridges etc. that is eligible for recycling should be recycled.

4. Storage of Waste in the Laboratory

4.1 Each laboratory should have a designated location in which to store hazardous materials to be discarded. This location should be out of the way of the normal lab activities, but should be easily accessible and recognizable. This space should be properly labeled. Fume hoods may be used temporarily to store small quantities of materials being generated but hazardous materials should not be allowed to accumulate in hoods because it could block air flow. Cabinets under fume hoods are appropriate storage locations for small quantities of hazardous waste.

4.2 All waste materials must be kept in secondary containers and segregated by hazard class (i.e., oxidizing agents such as potassium permanganate or hydrogen peroxide should be separated from organics or corrosives, acids should be separated from bases, etc.). Secondary containers can be lab trays, or any such device that will contain 110% of the largest container.

5. Labeling

5.1 Containers must be labeled prior to being used as receptacles for hazardous waste. Printed Hazardous Waste Labels must be used and filled out completely with all mandatory information including (see attached examples and summary of hazardous waste labeling codes commonly used at WPCL):

- the words "Hazardous Waste"
- starting date of accumulation in upper right corner and initials of person labeling waste container (waste identification number)
- CDFG Fish and Wildlife Water Pollution Control Lab
  2005 Nimbus Road (916) 358-2858
  Rancho Cordova, CA 95670
- WPCL's EPA ID Number (**CAD980815401**)
- the "Accumulation Start Date" or the date the waste is transferred to the hazardous materials storage building which starts the 90 day storage period (satellite storage rule)
- the composition (name of the waste) and physical state (gas, liquid, solid, sludge)
- a description of the hazardous properties of the waste (i.e. flammable, reactive, toxic, corrosive)
- EPA waste code and California waste code
- Approved D.O.T. Shipping Name and "UN" number (proper shipping names must be written exactly as listed in the D.O.T. regulations)

5.2 Handwritten labels made from tape or unprinted labels are **not** acceptable .

5.3 Chemical names must be specific. Nonspecific labels such as "organic waste", "waste solvents", "acid waste", etc., are not sufficient.

5.4 Chemical formulas or abbreviated chemical names are **not** acceptable.

5.5 Specific waste identification labels may be used **in addition to** the Hazardous Waste Label, such as PCB waste labels.

5.6 Hazardous Waste Labels are available in the cabinet in the photocopier room in the main laboratory.

6. Documentation (Hazardous Waste Inventory Logs)

6.1 When a waste container is started in the laboratory or moved to the hazardous materials storage building, it must be logged-in on laboratory's (lab room or hazmat storage building) Hazardous Waste Inventory Log. Each laboratory room where hazardous waste is generated and the hazardous materials storage building must have a logbook for recording the information described in 6.2.

6.2 The log must include the following information:
Container Log # (Identification No. – month/day/year, initials)
- -Date In (date that waste accumulation started)
- -Date Out (date that waste is transferred to the hazardous material storage building)
- -Waste Description
- -Waste Amount (size of container)
- -Hazard (Toxic, Flammable, etc)
- -First initial and surname of the person starting the waste

6.3 When the hazardous waste container is moved to the hazardous materials storage building, the waste must be logged out of the laboratory and logged into the hazardous materials storage building. The date that the waste is transferred must be entered on the Hazardous Waste label in the "Accumulation Start Date" section.

7. Containers

7.1 Containers must be properly labeled and in good condition (i.e., structurally sound and leak-proof) and **kept closed** unless you are adding or removing wastes. Liquids must be in a screw-capped container that will not leak if tipped over. Corks, parafilm, lab beakers, or other open containers are **not** acceptable. If waste is not in a proper container, transfer the waste.

7.2 The size of the container should correspond with the quantity of materials discarded. For example, it is not cost effective to ship 50 mL of material in a 4 L container, etc.

7.3 Contaminated lab ware such as glassware, gloves, paper towels, etc., must not have liquid in them. They must be placed in clear, double plastic bags and properly labeled with a Hazardous Waste Label. For disposal of broken glassware and sharps see Section 11.

7.4 Glass, paper, or plastic must not be placed in liquid waste containers.

7.5 The material must be compatible with the container - acids or bases cannot be stored in metal containers or solvents in plastic.

7.6 Containers must be inspected weekly for leaks and deterioration (**this must be documented with any deficiencies and corrective action**).

7.7 Hazardous waste storage building must be inspected weekly by the WPCL safety officer or his designate. **This inspection must be documented with any deficiencies and corrective action.**

8. Waste Segregation

8.1 Proper segregation of waste chemicals in the laboratory helps facilitate waste disposition options such as recycling. This can also result in cost savings for disposal. Any questions about waste segregation should be directed to the WPCL safety officer.

8.2 Examples of responsible and cost-effective segregation include:

- Separating halogenated from non-halogenated solvents
- Isolating metals from other wastes
- Keeping waste acetone separate from other solvents

9. Empty Containers

9.1 Empty (nothing can be poured out if the container is inverted) chemical containers of **five gallons or less** that have had the caps removed and labels defaced or removed may be disposed of as regular refuse. **Full or partially full containers should never be thrown in the regular trash.**

9.2 Empty containers that held extremely hazardous materials (waste) must be triple rinsed prior to disposal. **All rinsate** must be handled as hazardous waste.

10. Unknowns

10.1 Unknowns must be characterized prior to disposal. If the Laboratory can

not characterize the material it will have to be characterized by the hazardous waste contractor or another lab specializing in the characterization of hazardous waste prior to disposal.

11. Sharps

11.1 Broken glass, pipets, and any other sharp material that is not contaminated with hazardous material must be disposed of in a rigid sharps or broken glassware container.

11.2 Sharps that have been contaminated with hazardous materials should either be triple rinsed and discarded with non-contaminated sharps or should be discarded in a separate rigid sharps container designated and labeled as hazardous waste.

12. Tissue and Sediment Waste

12.1 Tissue waste resulting from dissection of fish should remain frozen until it is to be discarded. The frozen fish tissue should be bagged and transported to the sanitary landfill where it is discarded in a designated area used for that purpose. The county landfill should be contacted ahead of time to find out dates that these types of refuse are accepted.

12.2 Waste sediment and soils that meet the definition of hazardous waste must be labeled and treated accordingly. Waste sediment and soils that are not hazardous waste must be disposed of as non-hazardous laboratory waste and removed by the laboratory's hazardous waste contractor.

13. Hazardous Materials Storage Building Weekly Inspections

13.1 The hazardous materials storage building must be inspected weekly. A permanent record of the inspections, inspection log and deficiency and corrective action reports must be kept. Examples of the checklist, inspection log and deficiency report are attached.

14. References

California Environmental Protection Agency, Department of Toxic Substances Control (DTSC) Fact Sheets January 2002 and December 2006.

CCR Title 22, sections 66261.10, 66262.20-24, 66262.34

California Health and Safety Code, section25200.3.1

Safety Officer	Signature	Date
Laboratory Director	Signature	Date

Appendix IV D: Protocol for Corrective Action Procedures

California Department of Fish and Game
Fish and Wildlife Water Pollution Control Laboratory
2005 Nimbus Road
Rancho Cordova, CA 95670

STANDARD OPERATING PROCEDURE

NONCONFORMANCE, CORRECTIVE ACTION AND PREVENTIVE ACTION

1.0 SCOPE AND APPLICATION

1.1 The purpose of this document is to describe the procedure used by the DFG Water Pollution Control Laboratory (WPCL) for the identification and documentation of nonconforming events, items, or procedures and the assessment of their impact on the quality of data generated by the laboratory.

1.2 This procedure also describes the laboratory's corrective action and preventive action procedures and monitoring.

1.3 This SOP is applicable to all laboratory systems involved in the quality system and analytical processes in the laboratory, including but not limited to, sample receiving and logging, storage, preparation, analysis, reporting, auditing, and proficiency testing.

1.4 This SOP also addresses instances of nonconformance for which no corrective action is possible or appropriate, but documentation of the nonconformance is still required.

1.5 Documentation of all nonconformances is required by WPCL and is maintained on file by the QC Officer.

1.6 This procedure also requires documentation of nonconformances resulting from errors made by persons submitting samples to the laboratory. The documentation required is maintained and archived with the appropriate data set.

1.6.1 Errors made by persons submitting samples to the laboratory (i.e. errors on chain of custody documents, sample labeling, etc.) are documented and resolved by immediately contacting the sampler or person responsible for the samples.

1.6.2 Errors that are identified following receipt of analytical results that do not result from any laboratory mistake (i.e. incorrect sample identifier, test method requested, etc.) are resolved using directions taken from consultation with the data user.

2.0 DEFINITIONS

2.1 **Nonconformance** – An item, event, or procedure which does not comply or agree with the governing documents, procedures, policies or requirements (e.g. QAPP, QAMP, WPCL QA Manual, etc.).

2.2 **Corrective action** – A twofold measure that is taken to correct a nonconforming event and to eliminate or severely restrict the reoccurrence of the same type of nonconformance.

2.3 **Preventive action** – A measure taken to address needed improvements and potential sources of nonconformances.

3.0 RESPONSIBILITIES

3.1 Any individual within the laboratory can stop an analysis when nonconformance occurs (i.e. nonconformance not caused by sample matrix or similar unpreventable condition, etc.). Problems will be identified, documented and resolved prior to continuing the analysis.

3.1.1 The quality assurance officer also possesses the authority and responsibility to stop any work that does not meet quality standards of the laboratory and to take all necessary steps to return the system in question to a state of control.

3.1.2 The Laboratory Director is responsible for the determination of "official" work stoppages and for notifying all parties of concern regarding work stoppages, redistribution, subcontracting, if necessary, and subsequent work resumption.

3.1.2.1 The Laboratory Director, in conjunction with the Section Lead Chemist and QC Officer is responsible for redistributing workload during work stoppages to ensure that requirements are met with respect to hold and turnaround times.

3.1.2.2 The Section Lead Chemist, QC Officer, or Laboratory Director are responsible for notifying the data user of significant problems requiring work stoppages.

3.2 Each employee who detects a deficiency is responsible for initiating documentation of the nonconformance and forwarding the documentation to the appropriate Section Lead Chemist and /or QA Officer for review and assessment.

3.3 The Section Lead Chemist, in conjunction with the QA Officer and Laboratory Director, is responsible for analyzing the source of the nonconforming item, determining the impact of the nonconformance on the quality of the data and /or operations and implementing corrective actions to correct and/or restrict the noted deficiency according to the requirements detailed in the project QAPP or laboratory QA manual.

3.4 The Quality Assurance Officer is responsible for maintaining nonconformance/corrective action records and aiding personnel in the identification of nonconforming items, determining the extent of the nonconformance, and planning corrective action.

3.5 Laboratory personnel are responsible for participating in cause analysis and implementing corrective actions in response to nonconformances and for timely written response(s).

3.5.1 Generally, corrective actions, taken in response to nonconformances and cause analysis, are to be initiated immediately upon the identification of the event.

3.5.2 Some nonconformances, cause analyses and appropriate corrective actions may require additional time due to external factors, including purchasing new materials, obtaining additional training etc.

3.5.3 The QA Officer is responsible for participating in and monitoring nonconformance identification, cause analysis and corrective actions to ensure that each nonconformance is addressed quickly and effectively.

3.6 The Laboratory Director is ultimately responsible for assuring that laboratory procedures are performed in accordance to written instructions.

4.0 NONCONFORMANCE PROCEDURE AND DISCUSSION

4.1 Each performance event is documented by the individual who identifies the event. Documentation is effected using the Nonconformance/Corrective Action Record (NC/CAR). The NC/CAR is completed following the instructions on the form and is forwarded to the Section Lead Chemist followed by the QA Officer for review.

4.1.1 All laboratory staff have access to electronic NC/CAR forms.

4.1.2 The person(s) identifying the nonconformance will complete the Set ID, Sample Matrix, Analysis, Date Documented, and Date of Occurrence along with a brief description of the nonconformance.

4.1.2.1 If a specific data set is not appropriate to identify the nonconformance, complete a descriptive title in the space marked Set ID to allow for clear and concise identification of the nonconformance addressed.

4.1.3 The identifier will also sign the initiated form and forward it to the Section Lead Chemist who will pass it on to the QA Officer.

4.1.4 The Section Lead Chemist and/or QA Officer will assess the impact of the nonconformance on the data generated and will formulate a cause analysis study, if necessary.

4.2 Nonconformances may also be generated by the QA Officer in response to specific Measurement Quality Objectives and Method Quality Objectives. These reports do not require the Section Lead Chemist's review.

4.3 One NCR is completed for each nonconformance identified; however multiple sample sets may be documented on one NCR form if the deficiency is the same for each set listed.

4.4 The Section Lead Chemist and QA Officer review the NCR for assignment of cause analysis investigation and potential corrective actions. If following the determination of cause, a corrective action is deemed appropriate; procedures are followed as described in Section 6.

5.0 ROOT CAUSE ANALYSIS PROCEDURE AND DISCUSSION

5.1 Root cause analysis may involve any number of people, from 1 or 2 to the entire laboratory staff, and may involve informal conversations to lengthy reports to various individuals but must include a sufficient number of people to effectively and efficiently identify what happened and more importantly the cause of the nonconforming event (why it happened) or item and all related factors that contribute to the nonconformance.

5.1.1 The root cause of the nonconformance may not always be the obvious source of the problem.

5.1.2 Root causes that result in nonconformances can include: staff skills and training, client requirements, sample composition, methods requested, equipment, calibration, supplies, etc.

5.1.3 Investigations include historical sample performance (i.e. samples from the same site submitted by the same client), method performance, analyst training, and any other factors relating to system performance that could make an impact on the nonconformance identified.

5.1.4 Additionally upon the identification of cause of the nonconformances, internal audits may be performed where appropriate areas of activity are audited as soon as possible if the identification of the nonconformance casts doubts on the laboratory's compliance with its own policies and procedures or project QAMP or QAPP.

5.2 Root cause analysis studies must be appropriate to the scope and severity of the nonconformance identified.

5.3 Root cause analysis is generally undertaken by the QA Officer, in conjunction with the specific Section Lead Chemist and staff, and is monitored by the QA Officer for effectiveness in addressing the original nonconformance identified.

5.4 Upon identification of the root cause, the QA Officer and/or Section Lead Chemist will complete the Cause of Nonconformance section on the NC/CAR report and will then decide if a corrective action is needed, what steps should be performed to implement that corrective action to remedy and restrict the reoccurrence of the nonconformance and will designate the laboratory personnel who will be assigned to implement the steps required.

6.0 CORRECTIVE ACTION PROCEDURE AND DISCUSSION

6.1 Upon identification of the root cause, the QA Officer and Section Lead Chemist then decide if a corrective action is needed, what steps should be performed to correct the nonconformance and to severely restrict reoccurrence and will also determine the personnel who will be assigned to implement the steps selected.

6.2 Common corrective actions include: recalibration, instrument maintenance, sample repreparation, analysis of spiking solutions for degradation, etc. but must be appropriate to the scope and the magnitude of the nonconformance identified.

6.3 The corrective action portion of the NC/CAR form is then completed and retained by the QA Officer for further review and a copy is placed with the documentation for the project affected by the nonconformance/corrective action.

7.0 PROCEDURES FOR CORRECTIVE ACTION AND FOLLOW-UP

7.1 Allowing an appropriate period of reasonable time to fully implement the corrective action, the QA Officer will then perform a review of the subsequent implementation and effectiveness of all corrective actions.

7.1.1 This follow-up is usually performed within 3-5 days, but the time frame may vary depending on the complexity of the corrective action required.

7.1.2 Following this review, the NC/CAR form is then completed by the QA Officer.

7.1.2.1 If corrective action has been successful, the NC/CAR form is copied and the copy placed in the appropriate sample set for archive and the original is retained by the QA Officer in the NC/CAR file.

7.1.2.2 If corrective actions have not been successful, the Section Lead Chemist and QA Officer will conduct another review to determine other possible courses of action and repeat procedures in Sections 5 – 6.

7.1.2.3 If no corrective action has been taken by the individual assigned to implement the corrective action, the issue will be reported to the Laboratory Director for further action.

8.0 PREVENTIVE ACTIONS DISCUSSION AND PROCEDURE

8.1 Preventive action is a pro-active process to determine the areas where potential improvements can be made to reduce the likelihood of problems or complaints.

8.2 Preventive actions may originate with any member of the laboratory, from analyst to Laboratory Director, and should be brought to the attention of the Section Lead Chemist and/or QA Officer for consideration.

8.2.1 It may be necessary for the originator to prepare a short report regarding the type of improvement needed and potential improvements to be made to provide ample information for a thorough discussion among the laboratory's lead chemists and director.

8.2.2 Preventive actions generally result from the Section Lead Chemists or the Laboratory Director as a result of conversations with laboratory staff or daily activities.

8.2.3 Preventive actions can result from needed changes as instrumentation or procedures become outdated, newer technology is created to improve the laboratory's throughput and data quality, or as a result of trends identified during control charting or data analysis/review, etc.

8.3 Once issues are identified for possible preventive actions and the QA Officer is informed, the issues are discussed with the Section Lead Chemist and analysts.

8.4 The issue will be discussed with the laboratory staff affected by the proposed preventive actions, including possible benefits and costs, for formulation of an action plan.

8.4.1 If subsequent investigations are necessary, they will be assigned to specific personnel and will be monitored by the QA Officer for resolution by the date assigned for completion of the investigation.

8.5 Following the reception of all required supporting information, the Laboratory Director is responsible for determining the need for the proposed preventive action, for assigning personnel to perform the preventive action duties, and for determining the time frame in which the duties will be completed.

8.6 If the situation becomes an actual nonconformance or the result of nonconformances prior to the resolution of the preventive action; the preventive actions taken will be assistive, but the issue is then addressed using the procedure for nonconformance/cause analysis/corrective action and that procedure will take precedence over the preventive action activities.

9.0 APPENDICIES

9.1 NC/CAR Report (2 pages)

10.0 REFERENCES

10.1"General Requirements for the Competence of Testing and Calibration Laboratories," ISO/IEC 17025:1999(E).

California Department of Fish and Game
Fish and Wildlife Water Pollution Control Laboratory
2005 Nimbus Road
Rancho Cordova, CA 95670

SOP Contract QA Officer Approval: _____ Date: _____

SOP Final Approval: _____ Date: _____

SOP QA Officer Approval: _____ Date: _____

California Department of Fish and Game
Fish and Wildlife Water Pollution Control Laboratory
2005 Nimbus Road
Rancho Cordova, CA 95670

Date: 9-18-06
SOP# CORR_ACTION
Revision #0
Prepared by: DBC
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Appendix 9.1 NC/CAR Report

CDFG WATER POLLUTION CONTROL LABORATORY
NONCONFORMANCE/CORRECTIVE ACTION REPORT

NC/CAR# _____
(assigned by QA Officer)

Directions: Fill in all information in the top box and briefly explain the nonconformance. Forward this document to the QA Officer for review/follow-up and archive in the project folder.

SUBMIT THIS REPORT TO THE QA OFFICER WITHIN ONE WORKING DAY AFTER NONCONFORMANCE HAS BEEN DOCUMENTED

Set ID#(s): _____

Sample Matrix: _____ **Analysis:** _____

Date Documented: _____ **Date of Occurrence:** _____

Briefly describe nonconformance: *(Check all that apply)*

1. _____ LCS compounds outside warning/control limits.
2. _____ Contamination in blank outside warning/control limits
3. _____ MS/MSD compounds outside warning/control limits.
4. _____ Surrogate outside warning/control limits.
5. _____ Calibration curve/check standard outside warning/control limits.
6. _____ SRM outside warning/control limits.
7. _____ Other: (describe) _____

_____

Was client contacted? _____ Yes (If yes, complete the following information) _____ No

Client contact: _____ **Organization:** _____

Date: _____ **Time:** _____

Signature: _____ **Date:** _____

Supervisor's Signature: _____ **Date:** _____

QUALITY ASSURANCE USE ONLY

Date NCR received by QA: _____

Signature: _____ **Date:** _____

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Fish and Wildlife Water Pollution Control Laboratory
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CDFG WATER POLLUTION CONTROL LABORATORY
NONCONFORMANCE/CORRECTIVE ACTION REPORT

NC/CAR# _____
(assigned by QA Officer)

Directions: Cause and corrective action are to be completed by the Section Lead Chemist with the aid of any responsible parties. A two-week (or appropriate) follow-up to the corrective action will be given to resolve the issue by the personnel assigned to address the nonconformance. If corrective action is not addressed, the report will be forwarded to the Lab Director for resolution.

Section Lead Chemist

Cause of Nonconformance:

_____ Matrix Effect.
_____ Spiking solution/Standard mix degradation.
_____ Instrument malfunction.
_____ Preparation error.
_____ Other (describe): _____
_____

Section Lead Chemist

Corrective Action: (Check all that apply)

_____ Sample was re-prepared and reanalyzed.
_____ Standards were re-prepared and reanalyzed.
_____ Instrument maintenance was performed.
_____ Spiking solution/standard solution was analyzed for degradation.
_____ Other (describe): _____
_____
_____ No action necessary/possible. Why? _____

Person assigned to correct nonconformance: _____

Date corrective action is to be initiated: _____

Assigned by: _____

QA USE ONLY (Follow Up Comments)

Was corrective action initiated? _____ Yes _____ No* _____ Not Required

Did corrective action correct nonconformance? _____ Yes _____ No*

** (if no to either, forward to Laboratory Director for further action)*

Comments:

Signature: _____ **Date:** _____

Laboratory Director (if applicable)

Comments:

Signature: _____ **Date:** _____

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