

## Appendix IV: DFG-WPCL SOPs

<b>DFG-WPCL EPA Modifications and Laboratory Procedures</b>			
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## Appendix IV A: Sample Custody, Receiving and Storage

DFG-OSPR/WPCL

SOP: WPCL-AB-001  
 Sample Receipt  
 Revision:6  
 Author: SH/GCC  
 Revision Date: 06/10/11  
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**STANDARD OPERATING PROCEDURE**  
**TITLE: Sample Custody, Receiving and Storage**

REVISION HISTORY		
Revision #	Summary of Changes	Date
6	Updated format. Added sample receiving checklist. Move sections referring to LabWorks. Added holding time tables and maps. Added sample disposal periods.	06/10/2011
5	Unknown.	08/06/2007
4	Unknown.	
3	Unknown.	
2	Unknown.	
1	Unknown.	
0	Initial release.	

Author:	<i>Scot Harris</i>	Date:
Approved:	Section Lead <i>Patricia Bucknell</i>	Date:
Approved:	Laboratory Director <i>David B. Crane</i>	Date:
Approved:	Quality Assurance <i>Gail Cho</i>	Date:
Approved:	Health and Safety <i>Thomas Lew</i>	Date:

**STANDARD OPERATING PROCEDURE**  
**TITLE: SAMPLE RECEIPT AND STORAGE**

**1.0 Scope and Application**

- 1.1 This procedure describes chain-of-custody and the procedures for receiving, handling, scheduling, storing, and disposing of samples received by the DFG-OSPR/WPCL laboratory located at 2005 Nimbus Road, Rancho Cordova, California.
- 1.2 Samples submitted to WPCL fulfill data needs for routine monitoring, research, compliance, investigations, or enforcement actions. The procedures in this SOP help ensure sample traceability, chain-of-custody, integrity, timeliness, completeness, and proper sample disposal.
- 1.3 If an individual receives samples for testing at WPCL, this procedure must be followed.

**2.0 Summary**

- 2.1 The WPCL sample receiving area is located in the sample storage room at the back of the main laboratory. All samples are immediately unpacked, logged, checked for temperature, inventoried, preserved, reviewed for holding time, volume limitations, and clarity of instructions. Chain-of-custody (COC) records (Form FG1000 Rev. 9/01) or chains of custody submitted with samples are completed. The Sample Receipt Checklist is also completed. After the COC information is verified, samples are labeled, then stored (refrigerated or frozen) in designated units. If samples are delivered frozen, they are immediately transferred to the freezer after they are logged-in.
- 2.2 Clients are notified of discrepancies or anomalies as soon as possible after discovery. If samples are delivered in-person, ask the deliverer to remain until after sample inventory is complete so that any changes can be made real-time.
- 2.3 Copies of the chain-of-custody are provided to all departments and quality assurance for scheduling and secondary review. Copies must be circulated on the same day of sample receipt.
- 2.4 Samples are stored until data review and reporting have been completed.
- 2.5 Samples are assigned a unique laboratory identifier known as the "L-number" during the sample logging process. The identifier is labeled on each sample, tracked in a manual log as well as in the laboratory information management system (LIMS).

See WPCL-AB-002 (TBD), Sample Logging for LIMS entry instructions. The L-number and sub-numbers are used to track samples through the laboratory.

- 2.6 Samples will not be stored in offices, desks, or other non-designated sample storage units. Samples will not be transferred from the Sample Custodian to laboratory departments until samples have been inspected, inventoried, assigned an L-number, and labeled. Any exceptions (i.e. holding time) must be approved by the Sample Custodian or his designee.
- 2.7 Enforcement samples are stored in designated areas or units.
- 2.8 Highly contaminated samples or pure product will be stored separately from other samples in areas designated by the Sample Custodian.
- 2.9 Hours and location.
  - 2.9.1 Routine sample receiving hours are Monday through Friday, 8:00 AM to 4:30 PM. Shipment receipt for extended hours, holidays and weekends may be prearranged with the project manager, or sample custodian or his designee.
  - 2.9.2 Samples received after hours will be stored in WPCL R2. Leave a note on the Sample Custodian's desk or write a note on the dry erase board of samples stored in WPCL R2.
  - 2.9.3 All other samples will be delivered to the WPCL sample receiving area located at the back of the main laboratory building at 2005 Nimbus Road, Rancho Cordova, California.
  - 2.9.4 Samples will be logged as soon as possible and copies of the COC distributed on the date of receipt.
- 2.10 Designated Personnel.
  - 2.10.1 Primary Sample Custodian: Scot Harris.
  - 2.10.2 Secondary contact: Patty Bucknell.

### 3.0 Responsibilities

- 3.1 The Sample Custodian is the primary individual responsible for the receipt and inspection of sample delivery groups, storage of unprocessed samples, chain-of-custody distribution, notification to the laboratory, sample disposal, and LIMS log in.
- 3.2 The Project Manager or designee has the responsibility to contact clients of any discrepancies or anomalies. All discrepancies and communications will be documented on the sample receiving documents or in Labworks.



- 3.3 All personnel receiving samples are responsible for following this procedure and for reviewing distributed COC copies for scheduled analyses.
- 3.4 All entries will be written in blue or black ink. Error correction protocols defined in WPCL-QA-002 Documentation Practices will be followed by all personnel.
- 3.5 Any corrections to original COCs or sample labels should be made by the sample deliverer.
- 3.6 Any changes will be made on the original COC located in the QA office.
- 3.7 Copies of corrections to COCs after receipt must be distributed to all departments.

#### **4.0 Definitions**

- 4.1 Traceability: Ability to recreate the sample progression through the laboratory from receipt to disposal.
- 4.2 Chain-of-custody: Sample possession from collection to disposal. A sample is under custody if:
  - 4.2.1 It is in your physical possession.
  - 4.2.2 It is in your view.
  - 4.2.3 It is in a secure area.
  - 4.2.4 It was in your possession, but the sample was stored while processing.
- 4.3 Sample Integrity: The character of a sample/analyte of interest is unaltered by collection, shipping, preservation, storage and handling activities.
- 4.4 Timeliness: Samples are preserved, processed, and reported within regulatory or contractual requirements.
- 4.5 Completeness: All requested analyses are performed, reported, and traceable.

#### **5.0 Safety**

- 5.1 Assume that samples are potentially hazardous and exercise caution when opening packages containing samples. Wear nitrile gloves, lab coats, closed-toe shoes, and safety glasses when handling all incoming samples and shipping containers.
- 5.2 Unpack samples in a well-ventilated area or in a fume hood. If a shipping container is leaking, use spill pillows to absorb spills.
- 5.3 If containers are broken upon receipt, notify the Project Manager who will contact the client to determine the next course of action. Document discussions on the chain-of-custody or on the Sample Receipt Checklist. Dispose of container contents, broken containers, and shipping container rinsates as hazardous wastes. Use caution when handling and disposing of broken glass containers- do not use your bare hands. Dispose of broken glass in broken glass containers.

5.3.1 Use spill pillows to mop up any liquids. Dispose of used pillows as hazardous waste.

5.4 Follow disposal requirements specified in WPCL-EH-049 Disposal of Hazardous Wastes.

## 6.0 Equipment and Supplies

6.1 Chain of Custody (COC) form FG 1000 (Rev. 09/01).

6.2 Sample Receipt Checklist FM 006.

6.3 Calibrated infrared temperature gun.

6.4 Calibrated thermometers.

6.5 Spill kits as needed.

6.6 Sample Receiving Log Book.

## 7.0 Procedure

7.1 Receive and unpack sample shipping containers.

7.1.1 Shipping containers may be delivered by commercial courier, mail, or in person. Sign delivery manifests (either paper or electronic). If delivered in person, ask the deliverer to remain until sample inventory is complete.

7.1.2 As soon as possible after receipt, inspect the shipping container for leaks or damage.

7.1.2.1 If leaking, refer to WPCL-EH-049 and the Safety section of this SOP.

7.1.2.2 If damaged, open the container cautiously. Be prepared to move the damaged container to a hood if odors are detected.

7.1.2.3 Note any leakage or damage on the COC or Sample Receiving Checklist.

7.1.3 Remove any paperwork, shipping manifests that are attached to the outside of the shipping container. Cut packaging tape to open the package or ice chest.

7.1.4 Inspect the contents for broken, leaking, or damaged containers. If intact, continue.

7.1.5 Remove any COCs or other paperwork.

7.1.5.1 If no COC is provided, initiate a COC (FG 1000). Fill out the header information as completely as possible.

7.2 Assign a unique sample delivery group tracking number.

- 7.2.1 Locate the hardbound Sample Receipt Log. Locate the most recent last entry. The assigned internal tracking number will be L-number-YY where "number" is a sequential number and YY indicates the current year. Record this number in the far left margin of the logbook page. Date and initial the entry. Example: L-345-11 indicates the 345<sup>th</sup> delivery group received in 2011. "Number" resets to -001- at the beginning of each calendar year. Record the following in the logbook next to the L-number:
- 7.2.1.1 Client name.
  - 7.2.1.2 Number of samples.
  - 7.2.1.3 Matrix of sample.
  - 7.2.1.4 Project location. Include Index-PCA code if provided.
  - 7.2.1.5 General description of analyses.
- 7.2.2 Pull a FG 1000 Chain of Custody Record and FM 006 Sample Receiving Checklist for completion. Write the L-number on client-provided documentation and in the Lab Number space indicated on the forms.
- 7.3 Measure and record sample temperature on the COC.
- 7.3.1 Turn on the infrared temperature gun. Allow to equilibrate.
    - 7.3.1.1 If the IR gun is unavailable, place a thermometer in the cooler, close, allow to equilibrate for 15 minutes prior to reading.
  - 7.3.2 Aim the IR gun on sample containers. Do not aim the gun at ice or packing material or at other people.
  - 7.3.3 Record the temperature on the COC under "Water Temp:" and on the Sample Receipt Checklist, "cooler temperature upon arrival." Read in Fahrenheit.
  - 7.3.4 Acceptable temperature ranges:
    - 7.3.4.1  $\leq 43^{\circ}\text{F}$
    - 7.3.4.2  $\leq 6^{\circ}\text{C}$ .
  - 7.3.5 Note any exceedances on the Sample Receipt Checklist and notify the client.
- 7.4 Inventory and inspect samples.
- 7.4.1 Note the presence or absence of custody seals on shipping containers on the Sample Receipt Checklist.
  - 7.4.2 Remove samples from the shipping container and arrange them on the counter in client identifier order (if possible). If multiple containers are

- provided, group containers according to client identifiers. Count sample containers and compare to the COC.
- 7.4.3 On the COC, record the number of containers for each bottle type under "# of Containers."
  - 7.4.4 Check each sample for cracks, leaks, or breakage. Note any problems on the COC.
  - 7.4.5 Compare client ID labels to the provided COC. Note any discrepancies on the COC for immediate client notification by the Project Manager or designee.
    - 7.4.5.1 COC information and sample labels must match exactly.
    - 7.4.5.2 If provided, the date and time of Collection indicated on the COC must match sample labels.
  - 7.5 Check holding times and preservation.
    - 7.5.1 Using the table in Attachment 1, calculate the remaining holding times. Compare the remaining days and/or hours against the table.
    - 7.5.2 If 50% or more of the holding time has elapsed, contact the Project Manager and the affected department immediately and make a note on the COC.
    - 7.5.3 If the COC does not indicate that the sample is preserved and the table indicates that the sample should be preserved, notify the affected department immediately. Note any preservation problems on the COC.
  - 7.6 Check volumes and containers.
    - 7.6.1 Confirm with affected departments if you are unsure that there is sufficient volume to perform the requested analyses. Contact the Project Manager or the Sample Custodian if there is a shortage of volume.
  - 7.7 Verify that requested analyses are present and that instructions are clear.
    - 7.7.1 Assign and write sub-numbers on the COC.
      - 7.7.1.1 For organics analyses: One line = one sample = sub-number.  
Example: L-number = L-100-11.  
Sample A is listed on line 1 of the COC.  
All containers of Sample A will be labeled as L-100-11-001.
      - 7.7.1.2 For inorganics and biologicals tests: Each sample container = unique number.
      - 7.7.1.3 For combined organics and inorganics:
        - 7.7.1.3.1 Label organics as in 7.7.1.1.

- 7.7.1.3.2 After all lines are assigned, the next sequential sub-numbers are assigned to each bottle received for inorganics testing.
- 7.8 Complete the COC and Sample Receipt Checklist. Fill out all spaces.
  - 7.8.1 Indicate the storage location of samples on the COC in the upper right hand box under "Lab Storage."
  - 7.8.2 Sign and print your name in the box Received By.
  - 7.8.3 Record the date and time of receipt.
- 7.9 Distribute copies of the COC.
  - 7.9.1 Yellow or make a copy = Client.
  - 7.9.2 Pink copy, FM 006, and original paperwork = Sample Receiving Binder.
  - 7.9.3 Xerox copies for all departments: Back Lab, Petroleum, TSM, Inorganics, Dissection.
  - 7.9.4 The original = Quality Assurance to be included in the client report.
- 7.10 Samples are ready for Labworks log in by the Sample Custodian or designee.

**8.0 Designated Storage Locations for unprocessed samples. See Figure 1.**

- 8.1 All temperatures will be monitored daily by the Sample Custodian. Exception: Saturday and Sunday.
- 8.2 Refrigerators will be monitored and maintained at  $<6^{\circ}\text{C}$ .
- 8.3 Freezers will be monitored and maintained at  $\leq -20^{\circ}\text{C}$ .
- 8.4 Sample storage locations for unprocessed samples.
  - 8.4.1 Volatiles/VOAs/Method 8260 : ELMO.
  - 8.4.2 Petroleum samples: GROVER.
  - 8.4.3 Inorganics samples.
    - 8.4.3.1 Walk-in R2 (primary)
    - 8.4.3.2 WPCL R7 (Enforcement; must be locked).
    - 8.4.3.3 CMAP (frozen AFDM, chlorophyll a)
    - 8.4.3.4 Environmental Walk-In (9 month archive pre-disposal).
  - 8.4.4 Tissue Samples.
    - 8.4.4.1 TSM F1.
    - 8.4.4.2 Walk-In F1
  - 8.4.5 Pesticides.
    - 8.4.5.1 WPCL R1, R2, R3, R4 (extracts, back lab).
    - 8.4.5.2 Walk-in R3 (waters).

8.4.5.3 Walk-in F1.

8.4.5.4 TSM F2 (tissues).

8.4.5.5 TSM F1 (tissues).

8.4.6 Sediments.

8.4.6.1 WPCL F1

#### **9.0 Sample Archive and Disposal (minimum periods)**

- 9.1 All samples and extracts will be disposed according to WPCL-EH-049 Disposal of Hazardous Wastes.
- 9.2 Non-enforcement water samples, SWAMP water and algae samples may be disposed 9 months after reported to the client or 20 months after receipt.
- 9.3 Tissues and sediments will be stored at  $\leq -20^{\circ}\text{C}$  until directed to dispose.
- 9.4 Enforcement samples, California Department of Fish and Game samples, samples from the Pesticides Investigation Unit will be held in proper storage until directed to dispose.
- 9.5 Solvent extracts may be disposed 18 months after results are reported to the client. Extracts are stored in the dark at  $\leq -20^{\circ}\text{C}$ .

#### **10.0 References**

- 10.1 WPCL-EH-049, "Disposal of Hazardous Wastes"
- 10.2 WPCL-QA-002, "Documentation Practices"
- 10.3 USEPA, Table II, "Required Containers, Preservation Techniques, and Holding Times," Federal Register 40 CFR Part 136, March 26, 2007.
- 10.4 USEPA, Office of Solid Waste, Chapter 3 and Chapter 4, SW-846.

#### **11.0 Attachments**

- 11.1 Attachment 1: Analyses and Holding Times-Metals
- 11.2 Attachment 2: Analysis and Holding Times-Inorganics
- 11.3 Attachment 3: Analysis and Holding Times-Organics
- 11.4 Figure 1: Storage Locations Map.
- 11.5 Figure 2: FM006 Sample Receipt Checklist

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METALS CONTAINERS, PRESERVATION, HOLD TIMES												
Updated 2/17/10	EPA	Standard	LabWorks Code	Container	Preservation		Holding Time		Minimum Volume		Same Container	
Analyses	Method	Methods			Water (includes dissolved)	Solids, Tissues	Water	Solids	Water	Solids		
Aluminum by GFAAS		SM 3113B	AL_GF_*	P,FP,G	HNO3 to pH<= 2	<= -20°C	6 months	6 months	250 mL		100 g	1
Aluminum, Total monomeric		SM 3500 Al-E	ALIM_FIA				6 months	6 months	250 mL			
Aluminum, Organic monomeric		SM 3500 Al-E	ALOM_FIA				6 months	6 months	250 mL			
Arsenic by GFAAS	EPA 204.2		AS*				6 months	6 months	250 mL			
Arsenic by Hydride			AS_HYDR*				6 months	6 months	250 mL			
Cadmium by GFAAS		SM 3113B	CD_GF*				6 months	6 months	250 mL			
Cadmium by FLAAS		SM 3111B	CD*				6 months	6 months	250 mL			
Calcium by FLAAS		SM 3111B	CA*				6 months	6 months	250 mL			
Copper by FLAAS		SM 3111B	CU*				6 months	6 months	250 mL			
Total Copper by GFAAS		SM 3113B	CU_GF				6 months	6 months	250 mL			
Dissolved Copper by GFAAS		SM 3113B	CU_GF_DIS				6 months	6 months	250 mL			
Iron by FLAAS		SM 3111B	FE				6 months	6 months	250 mL			
Iron by GFAAS		SM 3113B	FE_GF*				6 months	6 months	250 mL			
Total and Dissolved Lead by FLAAS		SM 3111B	PB*				6 months	6 months	250 mL			
Total and Dissolved Lead by GFAAS		SM 3113B	PB_GF*				6 months	6 months	250 mL			
Magnesium by FLAAS (0.05-2.0ppm)		SM 3111B	MG*				6 months	6 months	250 mL			
Magnesium by FAAS (1.00-20.0ppm)		SM 3113B	MG*				6 months	6 months	250 mL			
Manganese by GFAAS		SM 3113B	MN_GF*				6 months	6 months	250 mL			
Mercury in Water	EPA 245.5		HG_COLD*				28 days	6 months	500 mL			
Molybdenum by GFAAS		SM 3113B	MO_GF				6 months	6 months	250 mL			
Nickel by FLAAS		SM 3111B	NI*				6 months	6 months	250 mL			
Nickel by GFAAS		SM 3113B	NI_GF*				6 months	6 months	250 mL			
Potassium Permanganate (KMnO4)	Calculation from Mn						6 months	6 months	250 mL			
Potassium by FLAAS (0.50-20.0ppm)		SM 3111B	K*				6 months	6 months	250 mL			
Se in Tissue by Hydride on PE300, in soln	EPA 7742M		SE_HY*				6 months	6 months	250 mL			
Se in Tissue by Hydride on PE300, wet wt.	EPA 7742M						6 months	6 months	250 mL			
Se in Tissue by Hydride on PE300, dry wt.	EPA 7742M						6 months	6 months	250 mL			
Se in Sediment by Hydride on PE300, in soln	EPA 7742M						6 months	6 months	250 mL			
Se in Sediment by Hydride on PE300, wet wt.	EPA 7742M	% Moisture					6 months	6 months	250 mL			
Se in Sediment by Hydride on PE300, dry wt.	EPA 7742M		6 months				6 months	250 mL				
Sodium by FLAAS		SM 3111B	NA*	6 months	6 months	250 mL						
Silver		SM 3111B	AG*	6 months	6 months	250 mL						
Zinc by GFAAS		SM 3113B	ZN_GF*	6 months	6 months	250 mL						
Zinc by FLAAS		SM 3111B	ZN	6 months	6 months	250 mL						

NA = not applicable

Selenium Digestion = JAOAC65

Methods for Chemical Analysis of Water and Wastewater, EPA-600/4-79-020, March 1983.

Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992, American Public Health Association, American Water Works Association, WPCF.

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Organics: TBD

Analyses	ORGANICS CONTAINERS, PRESERVATION, HOLD TIMES (INCOMPLETE)													Same Container	Additional for QC
	EPA Method	Other Method	LabWorks Code	Container		Preservation		Holding Time		Minimum Volume		Water	Solid		
				Water	Solid	Water	Solid	Water	Solid	Water	Solid				
Anatoxin a				G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g			2	
Anticoagulants	HPLC			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Carbamates	8321	LCMSMS		G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g	1			
Glyphosate	547	HPLC		G amber	G jar	<=8°C	<= -20°C	14/18 months	NA	30 mL	NA				
Herbicides (flpronil*, metalochlor, trifluralin)	8270M	GCMSMS		G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Microcystins				G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g	2		2	
Organochlorine Pesticides (OCH)	8081			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Organophosphorus (organophosphate) Pesticides (OP, OPP)	8141	8270		G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Organotin	8323	GC-FPD		G amber	G jar	<=8°C	<= -20°C	7/40	6 months	1000 mL	50 g				
PCBs	8081			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Pharmaceuticals and Personal Care Products				G amber	G jar	<=8°C	<= -20°C	48 hours/40	7/40	1000 mL	50 g				
Phenols (PCP, TCP)	8321			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Piperonyl Butoxide (PBO)				G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g	1			
Polybrominated Diphenyl Ethers (PBDE)	8270M	GCMSMS		G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Polyuclear Aromatic Hydrocarbons (PAH)	8270 SIM			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Pyrethrins	LCMSMS			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Pyrethroids	8081M	GCMSMS		G amber	G jar	<=8°C	<= -20°C	72 hours, 7/40	1 yr, 14/40	1000 mL	50 g				
Semi-Volatiles by GCMS	8270	PAH, PAH-SIM		G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Surfactants (Nonylphenol, nonylphenol ethoxylates)	HPLC			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Triazines	GC-TSD			G amber	G jar	<=8°C	<= -20°C	7/40	1 yr, 14/40	1000 mL	50 g				
Volatiles	8260	BTEX, MTBE		G vial	G	<=8°C	<=8°C	14 days	14 days	40 mL	50 g				



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Document1 - Microsoft Word

File Edit View Insert Format Tools Table Window Help

Normal + Cent Arial 12 B I U Final Showing Markup Show

TYPE HERE TO SEARCH FOR HELP

CALIFORNIA Department of Pesticide Regulation  
 WATER PESTICIDES CONTROL LABORATORY  
 2825 S. HIGHWAY 99  
 RANCHO COSTA, CA 95070

**SAMPLE RECEIPT CHECKLIST**

Lab #: \_\_\_\_\_ Receipt Date: \_\_\_\_\_  
 Client: \_\_\_\_\_ Received By: \_\_\_\_\_

	YES	FedEx / UPS / Other	COMMENTS
Were samples shipped?	NO	Courier/Hand Delivery	
Cooler temperature upon arrival:	_____ °C or _____ °F	NA	
Chain-of-Custody Present	YES	NO	
Chain-of-Custody Complete	YES	NO	
Custody seals present on shipping container?	YES	NO	NA
Custody seals present on samples?	YES	NO	NA
Were sample containers intact?	YES	NO	
Were samples preserved?	YES	NO	NA
Appropriate sample volumes for requested analyses?	YES	NO	
Samples have multiple phases?	YES	NO	
Samples and COC match?	YES	NO	
Date/Time of collection on COC?	YES	NO	
Appropriate sample container?	YES	NO	OTHER
Samples within holding times?	YES	NO	
All analyses specified on the COC?	YES	NO	
Fish and/or Wildlife loss?	YES	NO	If yes, notify Stella McMillan or Pesticides Investigation Unit.
If any problems, client notified? By whom?	YES	NO	

F M D D RECEIPT

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Start Novell GroupWise - Mailbox N:\QA\Form Templates Microsoft Excel - (0) L-72... WPCL\_AB\_001 Receipt.d... Document1 - Microso...

4:10 PM

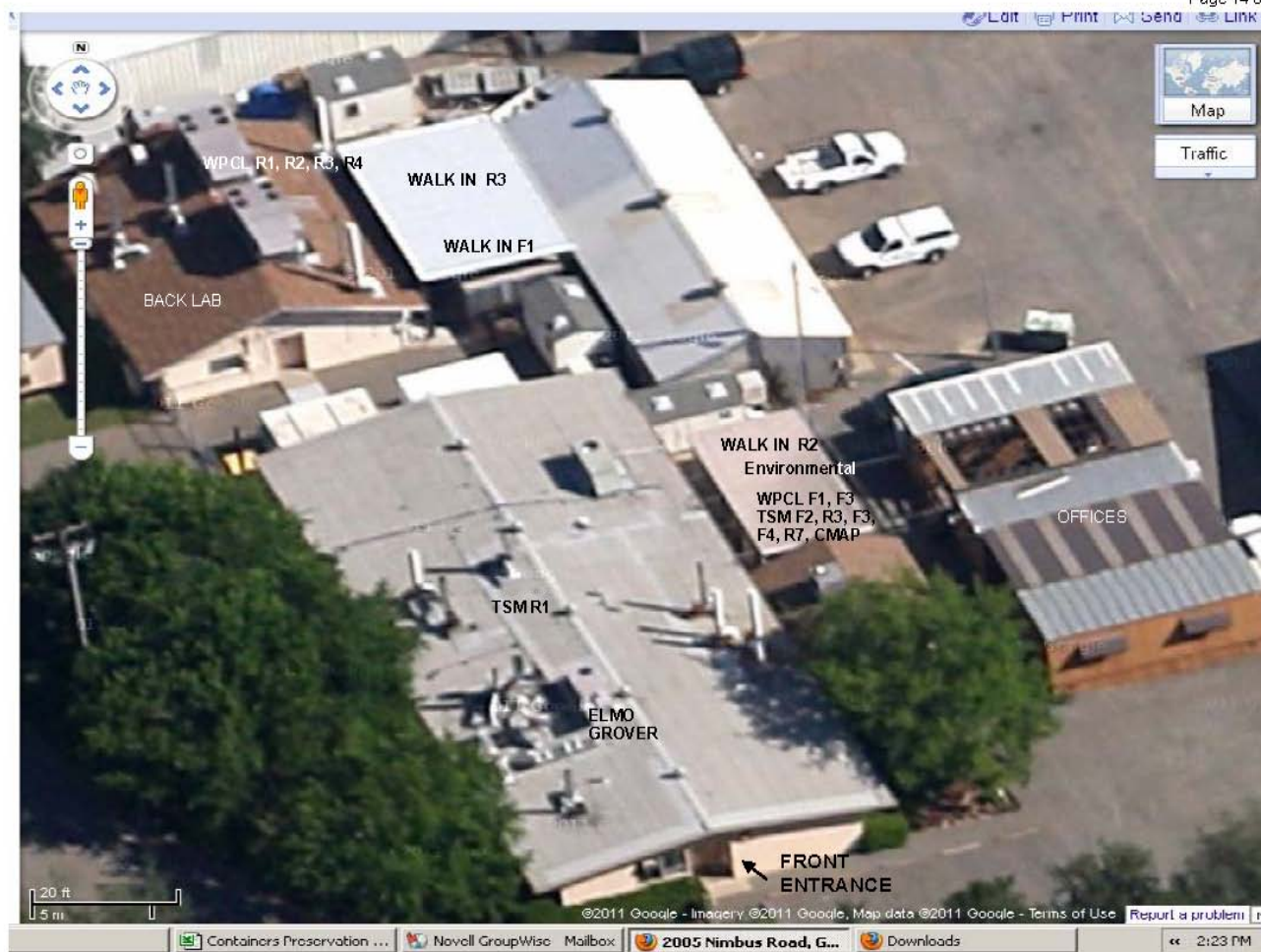
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## **Appendix IV B: Determination of OC and PCB in Sediment and Tissue (Modifications to EPA 8081B and 8082)**

WPCL Method: OCH, PCBs  
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### **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.

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**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	RL, ng/g	MDL, ng/g	RL, ng/g
	<u>wet wt.</u>	<u>wet wt.</u>	<u>dry wt.</u>	<u>dry wt.</u>
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

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**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 <sup>13</sup> (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:

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

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**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	RL, ng/g	MDL, ng/g	RL, ng/g
	<u>wet wt.</u>	<u>wet wt.</u>	<u>dry wt.</u>	<u>dry wt.</u>
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, 3<sup>rd</sup> 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 3-4 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<sup>®</sup> 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

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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  $\mu$ m syringe filter into J<sub>2</sub> 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<sup>®</sup> in a 11 mm x 300 mm column with a 250 mL reservoir. The Florisil<sup>®</sup> columns prepared for tissue samples are eluted with 6% diethyl ether/PE (Fraction 1), 15% diethyl ether/PE (Fraction 2), and Florisil<sup>®</sup> 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.



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

<b>6% Fraction <u>1</u>/</b>	<b>15% Fraction <u>2</u>/</b>
aldrin	dacthal
chlordane (cis-)	DBCE*
chlordane (trans-)	dieldrin
DBOB*	endosulfan I <u>4</u> /
DDE, o,p'	endosulfan II <u>5</u> /
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 <u>4</u> /	
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 <sup>13</sup> )	
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.



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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  $\text{Na}_2\text{SO}_4$ . 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<sup>®</sup> Ultra-Pure PTFE, extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

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- 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  $\mu$ m film thickness) connected to a single injection port using a "Y" press fit connector.

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- 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, Buechi 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<sub>2</sub> 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<sup>®</sup>, 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<sup>®</sup>, 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.

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- 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<sup>13</sup>), and dibutylchlorodate (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

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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  $\leq -20^{\circ}\text{C}$  and analyzed as soon as possible, but within 12 months of sample receipt.
- 6.6 Sediment samples may be refrigerated at  $4^{\circ}\text{C}$  for up to 14-days maximum or must be stored frozen at minus ( $-$ )  $20^{\circ}\text{C}$  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 Buchi B-400 mixer equipped with a titanium knife assembly or for small samples a Brinkman Polytron<sup>®</sup> 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.

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- 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<sup>®</sup> 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 3-4 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<sup>®</sup>*).
- 7.8 Pour the tissue or sediment/Hydromatrix<sup>®</sup> mixture through the powder funnel back into the extraction cell that the Hydromatrix<sup>®</sup> 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<sup>®</sup>.
- 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

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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 carousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carousel with the position numbers corresponding to the position numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.

7.12 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 carousel. Check each of the extraction cells to make sure that the caps are (*firmly tightened*) as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.

7.14 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.

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- 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 dichloromethane 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



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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 Whatman filter (0.45  $\mu\text{m}$ ) 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<sub>2</sub> 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

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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 has had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero Signal' button to set the baseline.

8.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the 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

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and add that to the K-D flask. Add 0.5 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. Attach the inverted Hopkins condenser to the top of the Snyder column and turn to water on to the solvent recovery system (~1500-2000 cc/min). When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, remove the inverted Hopkins condenser and secure out of the way. Lift the K-D apparatus up enough to be able to angle it slightly and add 40-50 mL Petroleum Ether through the top of the Snyder column. By holding the K-D apparatus at an angle, it allows the solvent to more easily drain back into the flask. Return to the K-D apparatus back into the hot water bath. Repeat this step 2 more times to successfully solvent exchange the sample from DCM to Petroleum Ether. When the apparent volume in the concentrator tube is 5-10 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus and allow to completely drain into the concentrator tube. Add a new micro boiling chip to the aliquot and place it in a 400 mL beaker containing water heated to approximately 75°C on a hot plate (4-5 tubes can be evaporated at one time). Evaporate the solvent down to 1-2 mL. Remove it from the water bath and allow it to cool.

- 8.11 Transfer the solution to a 13 x 100 culture tube with a Pasteur pipette, rinse the concentrator tube with 0.5 ml of Petroleum Ether, vortex, and transfer the rinse to the culture tube. Repeat the rinse step two more times, and add each rinse to the culture tube. Cap the culture tube with a Teflon faced cap. Mark the volume on the tube with a permanent marker.
- 8.12 SEDIMENT SAMPLES ONLY: Add acid rinsed copper to the culture tubes to remove any residual sulfur from the extract. Allow copper to stay in contact with extract overnight.

## 9.0 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 50 mL per sample for the 6% eluant.

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- 9.3 **TISSUE:** Prepare the reagents to be used for Florisil® cleanup for tissue: 6% ethyl ether in petroleum ether, 15% 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 50 mL per sample for the 6%, 50 ml per sample for the 15% (F2).
- 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 50 mL per sample for the 6% and 50 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 50 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

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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 chip and attach a Snyder column with a blue 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. 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 blue 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,

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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 (sediments only). 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)

<u>Mix A</u>	<u>Certified Concentration (ng/<math>\mu</math>L)</u>
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
<u>Mix B</u>	
Alpha-HCH	5.00
Beta-HCH	20.0

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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

<u>RPC-EPA</u>	<u>Certified Concentration (ng/μL)*</u>
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).



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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 <sup>63</sup>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



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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.

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

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Nonachlor, transC <sup>13</sup>	10	418	310	-15	Internal Std
DDE, p,p'	12	318	246	-15	DDE, p,p'C <sup>13</sup>
DDE, p,p'C <sup>13</sup>	12	329	258	-15	Internal Std
DDD, o,p'	13	235	165	-20	DDE, p,p'C <sup>13</sup>
DDT, o,p'	15	235	165	-15	DDT, p,p'C <sup>13</sup>
Nonachlor, cis	16	409	275	-15	Nonachlor, transC <sup>13</sup>
DDD, p,p-deuterated	16	243	173	-20	DDT, p,p'C <sup>13</sup>
DDD, p,p'	16	235	165	-15	DDT, p,p'C <sup>13</sup>
DDT, p,p'	17	235	165	-25	DDT, p,p'C <sup>13</sup>
DDT, p,p'C <sup>13</sup>	17	248	177	-20	Internal Std
Methoxychlor	18	227	169	-20	DDT, p,p'C <sup>13</sup>
Mirex	20	272	237	-15	DDE, p,p'C <sup>13</sup>

#### 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<sup>13</sup>) – 4Cl congeners  
PCB 97 (C<sup>13</sup>) – 5Cl congeners  
PCB 128 (C<sup>13</sup>) – 6Cl and 7Cl congeners  
PCB 194 (C<sup>13</sup>) – 8Cl congeners  
PCB 206 (C<sup>13</sup>) – 9Cl congeners  
PCB 209 (C<sup>13</sup>) – 10Cl congeners

##### OC Internal Standards

HCH, alpha (C<sup>13</sup>)  
HCB, (C<sup>13</sup>)  
Heptachlor, (C<sup>13</sup>)  
Chlorpyrifos, (C<sup>13</sup>)  
Nonachlor, trans (C<sup>13</sup>)  
DDE, p,p' (C<sup>13</sup>)  
DDT, p,p' (C<sup>13</sup>)

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:

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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**

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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  
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### 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 60°C (140°F);
- 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.

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**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.**

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## 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.

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- 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)

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- 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.



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- 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

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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, section 25200.3.1

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\_\_\_\_\_  
Safety Officer

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Laboratory Director

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

## Appendix IV D: Protocol for Corrective Action Procedures

DFG-OSPR/WPCL

SOP: WPCL-QA-050  
 Nonconformance, Corrective Action  
 Revision: 1  
 Author: DBC  
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**STANDARD OPERATING PROCEDURE**  
**TITLE: Nonconformance, Corrective Action and Preventative Action**

REVISION HISTORY		
Revision #	Summary of Changes	Date
1	Reformatted.	02/01/10
0	Initial release.	09/18/06

Author:	<i>David Crane</i>	Date:
Approved:	Laboratory Director <i>David B. Crane</i>	Date:
Approved:	Quality Assurance <i>Gail Cho</i>	Date:
Approved:	Health and Safety <i>Thomas Lew</i>	Date:

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## STANDARD OPERATING PROCEDURE

### TITLE: Nonconformance, Corrective Action and Preventative 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 QA 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.

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- 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 manager 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 QA 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, QA 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 QA 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).

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- 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 Procedure

- 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.
  - 4.1.3 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.
  - 4.1.4 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.
  - 4.1.5 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.
  - 4.1.6 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.
  - 4.1.7 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.
  - 4.1.8 The Section Lead Chemist and QA Officer review the NCR for assignment of cause analysis investigation and potential corrective actions. If following the

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determination of cause, a corrective action is deemed appropriate; procedures are followed as described in Section 4.3.

#### 4.2 Root Cause Analysis

- 4.2.1 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.
- 4.2.1.1 The root cause of the nonconformance may not always be the obvious source of the problem.
- 4.2.1.2 Root causes that result in nonconformances can include: staff skills and training, client requirements, sample composition, methods requested, equipment, calibration, supplies, etc.
- 4.2.1.3 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.
- 4.2.1.4 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.
- 4.2.2 Root cause analysis studies must be appropriate to the scope and severity of the nonconformance identified.
- 4.2.3 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.
- 4.2.4 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.

#### 4.3 Corrective Action



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- 4.3.1 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.
  - 4.3.2 Common corrective actions include: recalibration, instrument maintenance, sample reparation, analysis of spiking solutions for degradation, etc. but must be appropriate to the scope and the magnitude of the nonconformance identified.
  - 4.3.3 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.
- 4.4 Corrective Action and Follow-Up
- 4.4.1 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.
  - 4.4.2 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.
  - 4.4.3 Following this review, the NC/CAR form is then completed by the QA Officer.
  - 4.4.4 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.
  - 4.4.5 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 4.2 – 4.3.
  - 4.4.6 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.
- 4.5 Preventive Actions Procedure.
- 4.5.1 Preventive action are a pro-active process to determine the areas where potential improvements can be made to reduce the likelihood of problems or complaints.
  - 4.5.2 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.

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- 4.5.3 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.
- 4.5.4 Preventive actions generally result from the Section Lead Chemists or the Laboratory Director as a result of conversations with laboratory staff or daily activities.
- 4.5.5 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.
- 4.5.6 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.
- 4.5.7 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.
- 4.5.8 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.
- 4.5.9 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.
- 4.5.10 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.

## 5.0 Appendices

- 5.1 NC/CAR Report (2 pages)

## 6.0 References

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

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Appendix 5.1 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:** \_\_\_\_\_

DFG-OSPR/WPCL

SOP: WPCL-QA-050  
 Nonconformance, Corrective Action  
 Revision:1  
 Author: DBC  
 Revision Date: 12/31/09  
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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:** \_\_\_\_\_

## Appendix IV E: Method: Microcystins and Biotoxins by LC/MS/MS

WPCL Method: Microcystins  
Date: 06/17/08  
SOP# WPCL-LC-065  
Revision #1  
Prepared by: GJB/AM/DC  
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### Determination of Microcystins and Microcystin Metabolites in Water and Tissue by Enhanced Liquid Chromatography Tandem Mass Spectrometry

#### 1.0 Scope and Application

A liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method has been developed and thoroughly validated to identify and quantify trace levels of cyanotoxins or microcystins (MC) in water, bivalves and fish tissue with enhanced sensitivity and specificity. The method enables confirmation and quantification of six MCs (MC-LA, LF, LR, LW, RR and YR) with a single chromatographic run. The applied chromatography also allows determination of certain MC metabolites (Desmethyl-LR and -RR). By using LC-ESI-MS/MS in Multiple Reaction Monitoring (MRM) mode, the limit of detection and quantitation for the microcystins studied, were determined to be between 0.2 pg and 1 pg on column (5:1 S/N ratio).

#### 2.0 Summary of Method

An aliquot of water sample is mixed with 10% acidified methanol and extracted using sonication techniques. Each batch of samples (20 or less) contains a blank, laboratory control sample (LCS), matrix spike and duplicate (MS/MSD) and field sample duplicate, when provided (100 mL total). Identification and quantification of MCs is performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in MRM mode. All quantitation is performed using certified standards, except the demethylated (dm) congeners which are quantified as the parent non-methylated analog since no certified standard is commercially available. All extracts are analyzed using a five level calibration curve and second source standards are obtained when available. The microcystins currently analyzed are MC-RR, -dmRR, -LR, -dmLR, -YR, -LA, -LF, and -LW. Nodularin is used for internal standard. The reporting limit for all microcystins is 1 µg/L (ppb). The average method recovery range for MCs in water is 65-120%.

#### 3.0 Interferences

**3.1** Solvents, reagents, glassware, and other sample processing hardware may cause LC 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.

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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** Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. Solid phase extraction (SPE) can be used to overcome many of these interferences.

**3.3 SPE Clean Up Procedure**

Pre-filtered water samples (100 mL) were extracted with J.T.Baker™ C18, 6 mL, 500 mg solid phase cartridges (Milford, MA) mounted on a Resprep™ vacuum manifold, (Restek Corp., Bellefonte, PA). The cartridges were first pre-conditioned with 10 mL methanol followed by 10 mL water. The samples were loaded through the cartridges at a rate of 5 mL/min, not to exceed 20 psi. The cartridges were then dried for 5 minutes with vacuum and finally, eluted with 2 mL methanol, vortexed and filtered through 0.45 µm filters.

**4.0 Material and methods**

**4.1 Chemicals and reagents**

Certified MC standards (LR, RR, LF, LW and NOD-R) were purchased from Calbiochem (EMD Chemicals, La Jolla, CA) and LR, RR, YR, LA were purchased from Sigma-Aldrich (Allentown, PA). Burdick and Jackson HPLC grade solvents (acetonitrile, methanol, water), glass fiber filters (Type A/E, 90mm, 1 µm) and Gelman Acrodisc® CR PTFE syringe filters (13 mm, 0.45 µm) were obtained from Pall Corp., Ann Arbor MI, USA. Mobile phase additives, ACS grade formic acid (98%) and trifluoroacetic acid (99%) were purchased from Sigma Aldrich, Milwaukee, WI, USA). For method validation purposes, Sacramento River water and Rainbow Trout tissue and livers were obtained from the Nimbus Fish hatchery, Rancho Cordova, CA. Mussels were purchased from a local fish market. A combined intermediate working solution of MCs was made in methanol from the purchased standards.

**4.2 Sample storage**

Tissue samples are kept frozen until time of extraction. Water samples for cyanotoxin analysis should be refrigerated in the dark to prevent toxin degradation but it is essential that storage be kept to a minimum (preferably less than 72 hours). Where prolonged storage is required, samples can be frozen, although this will release toxins from the cells and only the total amount of toxin in the sample can then be determined.

### 4.3 Sample preparation

#### 4.3.1. Liquid sample extraction

MCs in water bodies at the time of a bloom will be present in both the water (free, dissolved or extra cellular toxins) and the cyanobacterial cells (intracellular toxins). In order to determine total MC in the water the cell wall must be ruptured or lysed by repeated freeze-thawing and sonication. An aliquot of sample (100 mL) was filtered under vacuum through a glass fiber filter (1  $\mu$ m). The water and filters were extracted separately, as follows: 1) Pre-filtered water samples were acidified with 0.1 % FA and 0.05 % TFA to obtain pH~2 and extracted by SPE using JT BakerBond C18, 6 cc, 500 mg solid phase cartridges (Mallinckrodt Baker, Phillipsburg, NJ) mounted on a Resprep™ vacuum manifold (Restek Corp., Bellefonte, PA). The cartridges were first pre-conditioned with 10 mL methanol followed by 10 mL acidified water. The samples were loaded through the cartridges at a rate of 5 mL/min, not to exceed 20 psi. The cartridges were then dried for 5 minutes with vacuum and finally, eluted with 2 x 1 mL mixture methanol:water (90:10) acidified with 0.1% TFA, vortexed and filtered through 0.45  $\mu$ m filters. Extracts are now ready for analysis. 2) Filters with planktonic material or lyophilized biomass shellfish were extracted twice with 15 mL of methanol:acidified water (90:10, v/v) by homogenizing for 1-2 minutes using a Polytron, followed by 10 minute sonication in ultrasonic bath. The extracts were centrifuged and the supernatant was evaporated at 35°C to 5 mL with rotary-evaporator. The concentrated extract was diluted to 100 mL in order to decrease the methanol concentration, acidified and followed by the SPE procedure.

#### 4.3.2 Bivalve sample extraction

Tissue (mussel, liver, fish tissue) samples were homogenized using a Buchi B-400 mixer equipped with a titanium knife assembly. A 2-5 g sample was transferred to conical centrifuge tubes with 10 mL methanol:acidified water (90:10, v/v) and finely-ground with an Arrow 850 tissue grinder (Arrow Engineering Co., Inc., Hillside, NJ) equipped with a glass pestle for five minutes, followed by sonication with a Branson® 3510 Ultrasonic for one hour. The extracts were then centrifuged at 3500 rpm for 30 minutes using a HN-S centrifuge (Damon-IEC Division, Needham Heights, MA). The extract is reduced to minimum volume and diluted with water (not to exceed 5 % methanol), acidified and finally, cleaned-up using SPE, as described above. For extremely dirty samples, an extra step is recommended by washing the HLB cartridge with 10-20% methanol:water solution before eluting the target analytes.

### 4.4 Analysis parameters and set up

The LC-MSD single quadrupole used was an Agilent 1100 liquid chromatograph connected to Hewlett Packard MSD-SL and the LC-MS/MS used was an Agilent 1200 liquid chromatograph connected to a 6410 triplequadrupole (QqQ). Both LCs were equipped with a vacuum degasser, a binary pump, an autosampler and a thermostatted column compartment kept at 40°C. These instruments were purchased from Agilent Technologies, Santa Clara, CA. Agilent Chemstation

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software was used to collect and process data from LC-MS, while Agilent Mass Hunter software was used for LC triplequadrupole.

**4.4.1. Chromatography**

The mobile phase consisted of HPLC water (A) and acetonitrile (B) (both contained 0.1 % formic acid (v/v)). The gradient elution program started with 5 % B and held for 2 min. The first linear gradient from 5 % B to 50 % B over 8 min, a second linear gradient from 50 % B to 75 % B over 3 min and held at this gradient for 5 min before returning to initial mobile phase ratio at 19 min and held for 1 min. The run time was 20 minutes. The flow rate was set at 0.25 mL/min. The injection volume was 10 µL. After each run, the column was equilibrated for 5 min at the initial conditions before the next injection. A Guard column C18, 3.5 µm, 2.1 x 30 mm was used to protect the analytical column; dC18, 3 µm and 2.1 X 100mm (Waters Atlantis). The effluent from the LC column was directed from the waste to the mass spectrometer source after the first 5 minutes of the run.

**4.4.2. MS-SIM parameters**

Nitrogen (less than 1 ppm oxygen, Praxair, Rancho Cordova, CA) was used as the nebulizing and drying gas. The MSD was run using electrospray ionization (ESI) interface operated in positive mode as follows: 350°C drying gas temp, 13.0 L/min drying gas flow, 40 psi nebulizer pressure, 110 fragment voltage and 4.0 kV electrospray capillary voltage. MS detection was performed in Selected Ion Monitoring (SIM). The following MC ions (m/z) were monitored: 519.8 RR and 512.8 desmethyl-RR are both [M+2H]<sup>2+</sup>; 1045.6 YR, 995.7 LR, 981.7 demethyl-LR, 910.6 LA, 1026.6 LW, 987.6 LF and 825.5 NOD-R were monitored using [M+H]<sup>+</sup>. Full scan was also collected over the range 100-1100 Da. The UV-Diode Array Detector (DAD) was set at 238 nm , Agilent ChemStation was used to collect data.

First, experiments were carried out by direct injection of high concentrations of individual toxin standards into the mass spectrometer using the Flow Injection Automated (FIA) program. The obtained full scan spectra showed the exclusive presence of protonated molecular ions [M+H]<sup>+</sup> for all microcystins (MC) except MC-RR, which had a doubly charged [M+2H]<sup>2+</sup> ion. This correlates well with the presence of two arginine residues in MC-RR whose side chains are capable of retaining external protons and producing stabilized [M+2H]<sup>2+</sup>. Those ions were then chosen for SIM mode. Table 1 shows the toxin fragments monitored and their respective m/z values. Nodularin could be used as surrogate (Sur) or internal standard (IS) since it is a pentacyclicpeptide and found primarily in marine water.

MC toxin fragment	m/z [M+H] <sup>+</sup>	m/z [M+2H] <sup>2+</sup>
Adda fragment	135.1	
MC-RR		519.8



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dm-MC-RR		512.8
MC-LR	995.5	
dm-MC-LR	981.5	
MC-YR	1045.5	
MC-LA	910.6	
MC-LF	987.5	
MC-LW	1026.5	
NOD-(IS)	825.5	

The fragment voltage was optimized by re-analyzing the individual microcystin standards in SIM mode using FIA programming and changing the fragment voltage over the range from 10-130 volts. The fragment voltage corresponding to the most intense peak was chosen (110V). Instrument default settings were used for drying gas, capillary voltage and the remaining MS parameters. Finally, the LC system was attached to the MS and MCs standard mixture was analyzed. All analytes were well separated under the LC conditions listed above. It was possible to increase analyte response in the SIM mode by using multiple acquisitions and time programming modes, where five segments were used.

#### 4.4.3. MS/MS-MRM parameters

The triplequadrupole was operated using the same conditions as the single quadrupole, except the detection was in multiple reaction monitoring (MRM) mode. The parameters for running MRM are as follows: ultra-pure nitrogen gas was used for collision induced dissociation (CID). The protonated fragment ions used for SIM mode served as the precursor ions for MRM mode, therefore, only the transition from the precursor to the product ion needed to be optimized by varying the voltage of collision induced dissociation (CID) gas from 0 to 50 eV. As a result, the most intense product ions obtained by these voltage settings were selected. The collision energy (CE) was set at 50V for MC-RR/dm-RR and 70V for the remaining MCs. The MRM windows were established for MCs using the daughter ions, which are the Adda fragments of m/z 135.2 and m/z 213 produced by the transition of the protonated precursor ions (SIM). Fragment at m/z 135 corresponding to the O-methylphenylacetaldehyde [Ph-CH<sub>2</sub>-CHOMe]<sup>+</sup> structure from the Adda moiety and the fragment at m/z 213 corresponding to [Glu-Mdha+H]<sup>+</sup> resulted from the MRM transition were the predominate product ions for all MC analytes. The fragment ions, m/z 135.2 and m/z 213 were chosen as quantifier and qualifier ions, respectively. Fragment of Adda at m/z 135.2 and fragment ion at m/z 227.1 correspond to [Glu-Mdha+H]<sup>+</sup> were obtained for the internal standard, NDLN. Table 2 shows the optimum CE setting for all MCs and time segments of the MRM method.

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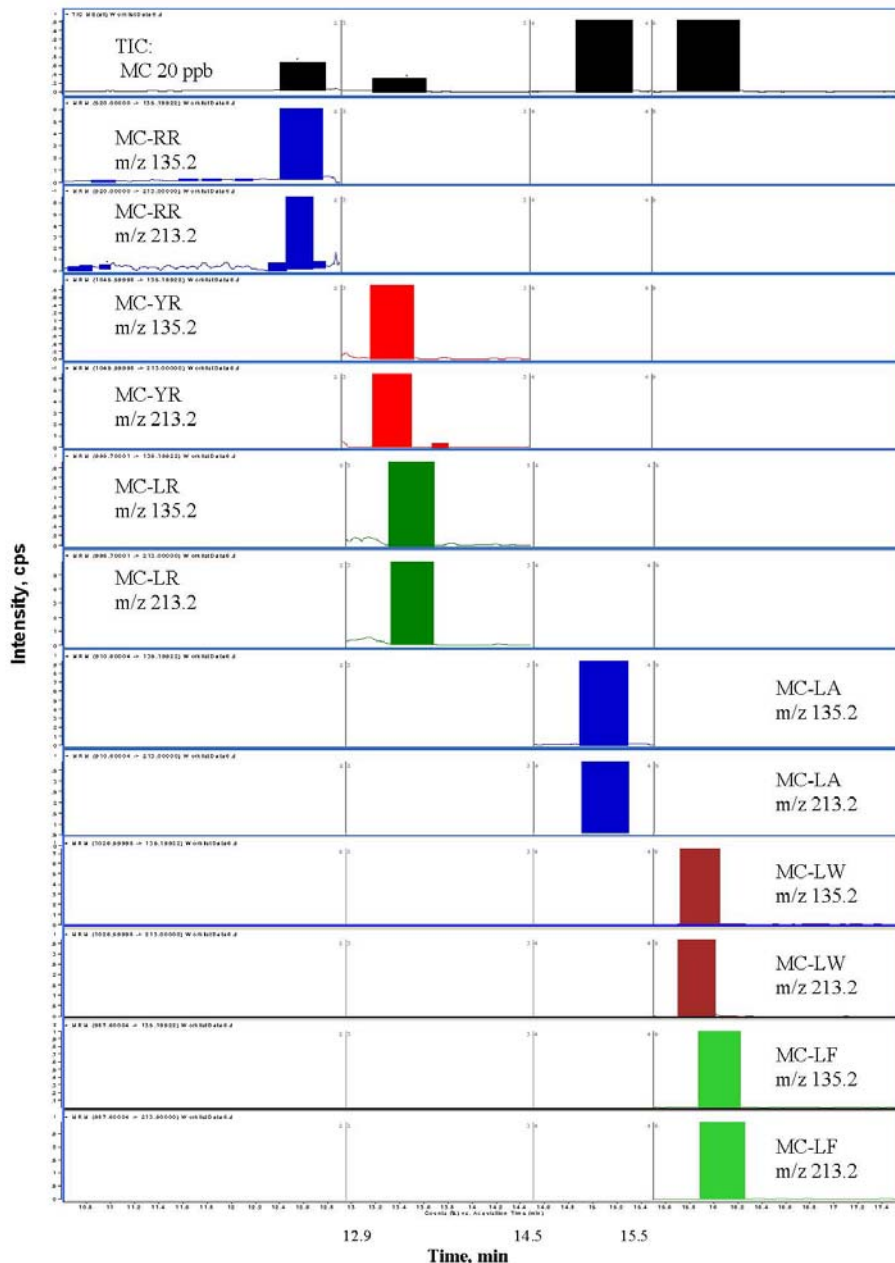
Table 2. Time segments and MRM parameters.

Time Segment #	Time (min)	Target Compound	Scan Type	Precursor Ion	Product Ions	Fragment (V)	Collision Energy (V)
1	0	na*	MS2 Scan (to waste)				
2	5	MC-RR	MRM	520	213, 135.2	110	50
		dm-MC-RR	MRM	512.8	213, 135.2	110	50
3	12.9	MC-LR	MRM	995.7	213, 135.2	110	70
		dm-MC-LR	MRM	981.7	213, 135.2	110	70
		MC-YR	MRM	1045.6	213, 135.2	110	70
4	14.5	MC-LA	MRM	910.6	213, 135.2	110	70
5	15.5	MC-LF	MRM	987.6	213, 135.2	110	70
		MC-LW	MRM	1026.6	213, 135.2	110	70
* na: not applicable							

Under these LC-MS/MS conditions a 0.2 µg/L microcystins standard mixture was analyzed and easily identified with S/N greater than 5.0 for most toxins. Typical MRM and reconstructed ion chromatograms are shown in Fig. 1.

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Fig. 1. MRM constructed ion chromatogram for microcystin standard.



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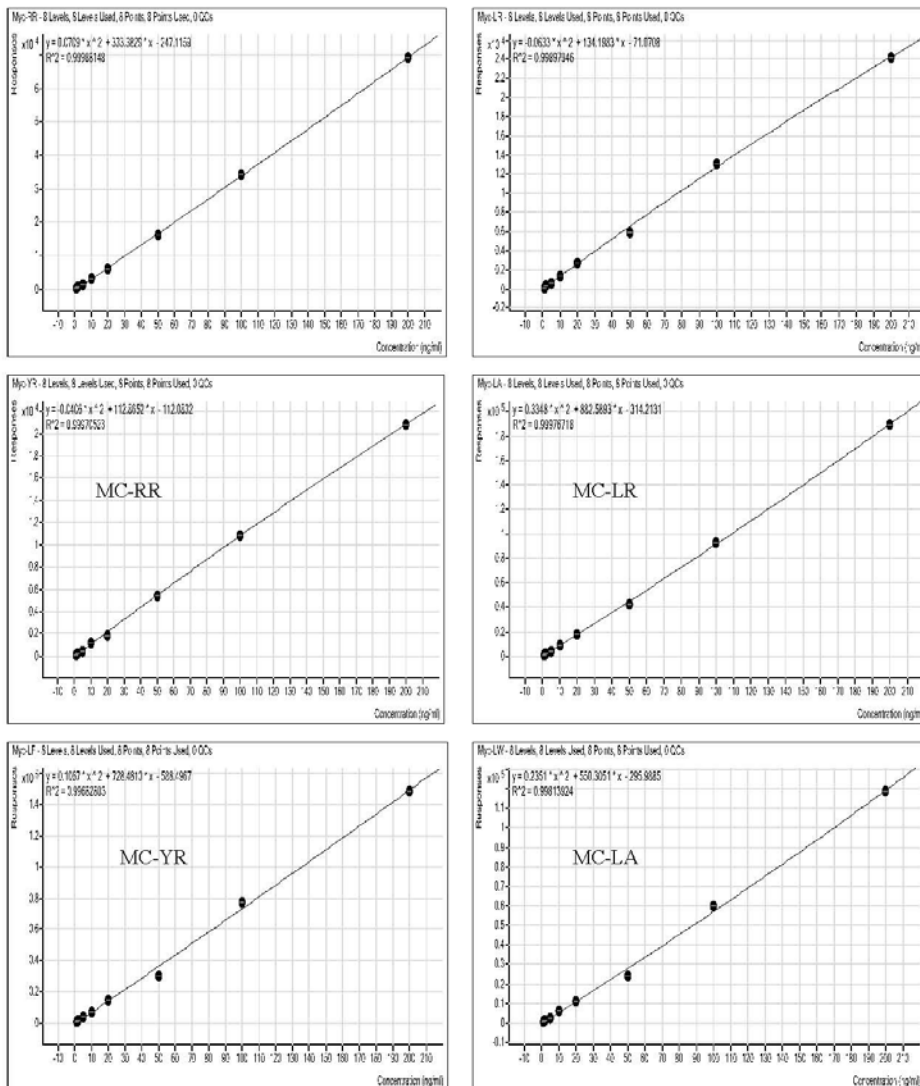
#### 4.5 Calibration curve

To evaluate the linearity of the system various concentrations of certified MC mixture standard solution were prepared in methanol-water (90:10) (v/v) to obtain a seven level calibration curve ranging from 0.2 µg/L to 200 µg/L. An individual calibration curve was drawn for all the toxins except the demethylated variants. A linear response was found between concentration and area for MCs. As shown in Fig. 2, the linearity was very good for all MCs with correlation coefficient ( $r^2$ ) greater than 0.998. The limit of detection in MRM mode was calculated using USEPA procedures found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11) and were below 0.1 µg/L or lower for all the MCs.

Using the above listed MRM parameters, 1 µg/L microcystin standard mixture was easily detected and separated (Fig. 3). The constructed ion chromatogram (1 µg/L) showing the transition from the individual microcystin precursor ion to its corresponding product ions chromatogram and spectra are shown in Fig. 4. By using LC triplequadrupole MS with ESI in Multiple Reaction Monitoring (MRM) mode, the limit of detection and quantitation for all microcystins were determine to be between 0.2 and 1 pg on column, with 5:1 S/N ratio.

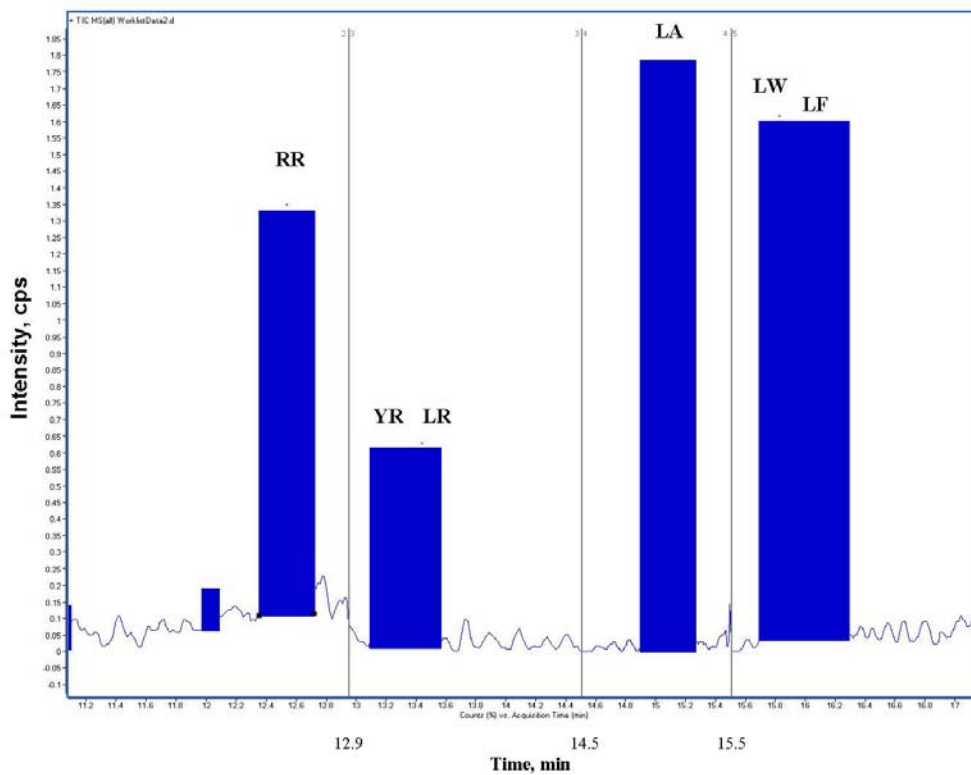
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Fig. 2. Calibration curves of individual MCs ranging from 1-200 µg/L.



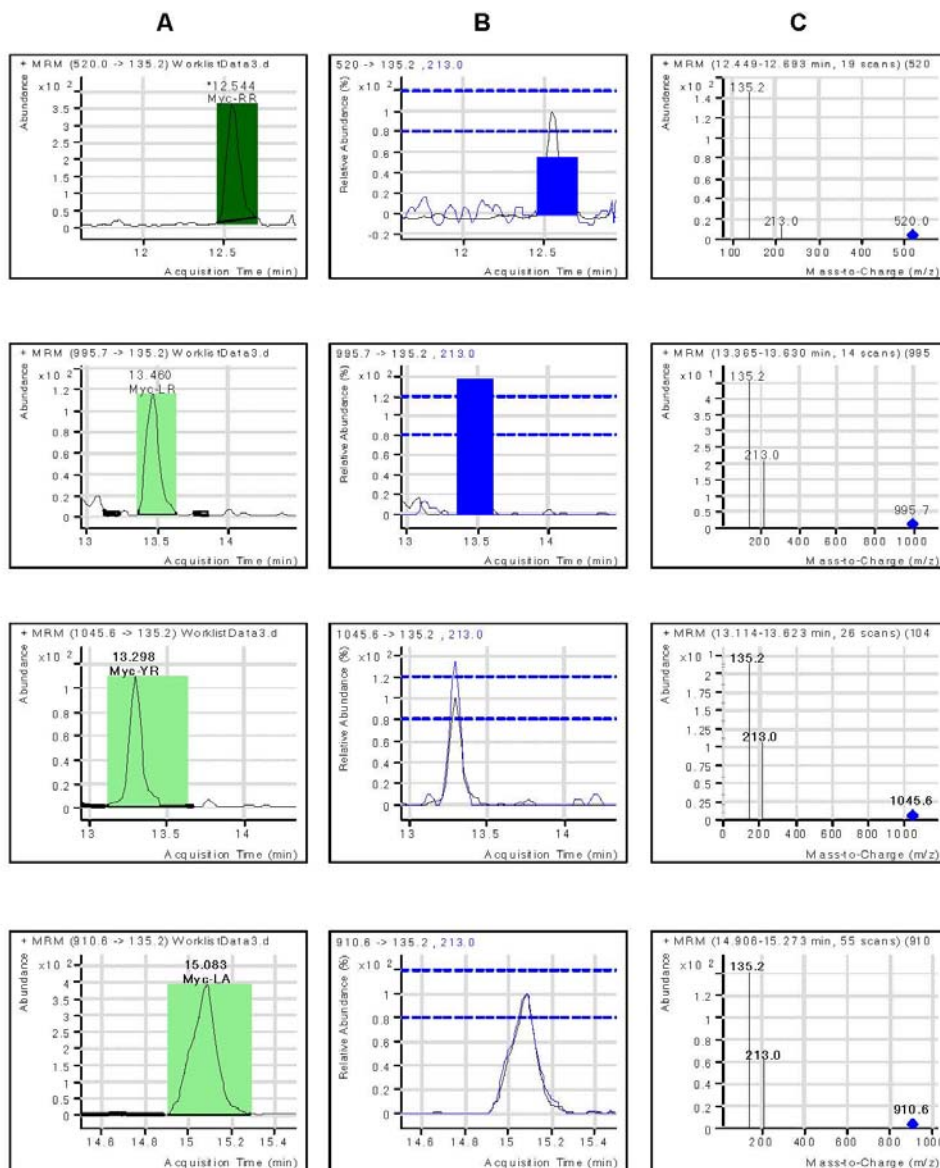
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Fig. 3. Total ion chromatogram of a microcystin standard at 1 pg on column.

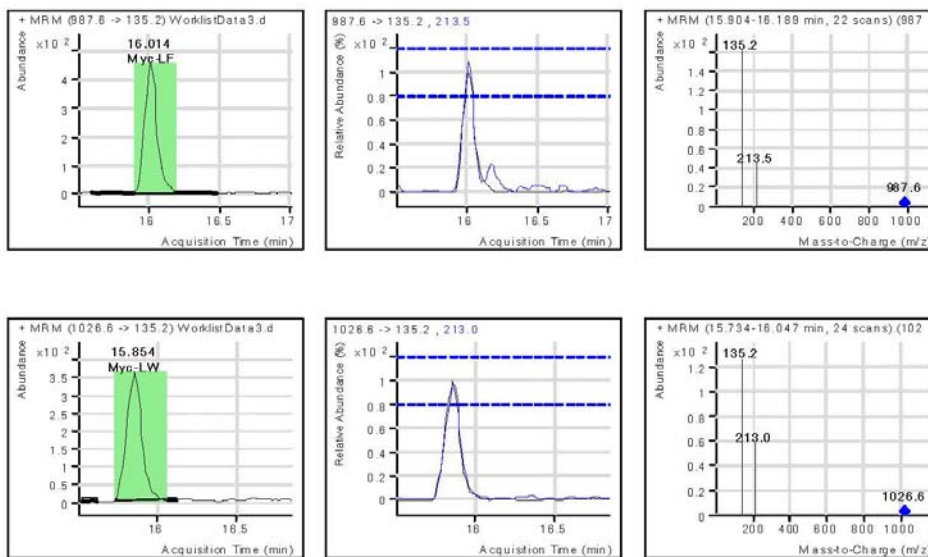


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Fig. 4. Microcystin chromatograms in MRM mode: precursor ion (A), product ion (B) and spectra (C).



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## 5.0 Method validation

### 5.1 Liquid samples method validation

#### 5.1.1. Fresh water solid phase extraction (SPE)

The method was first tested with several types of SPE cartridges (Supelco C8, Oasis HLB, C18 J.T Baker Strata X). Acidified water samples (100 mL), fortified with MCs mixture at 5 µg/L, went thru the SPE procedure detailed earlier in Section 3.3.3. The result from this study shows that J.T Baker C18 cartridges extract all the tested microcystins and Nodularin from the water with acceptable recovery as shown in Table 3.



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Table 3. Microcystin Solid Phase Extraction Results.

Biotxin Compounds	C8-Supelco	C18-JTBaker	C18-HLB Oasis	Strata X
	% Recovery	% Recovery	% Recovery	% Recovery
Myc-YR	114	118	60.5	60.0
Noduralin	78.8	82.8	56.3	50.0
Myc-LW	74.1	139	10.8	8.08
Myc-LF	86.1	157	13.2	ND
Myc-LR	60.8	109	58.8	60.3
Myc-LA	4.99	48.1	18.6	13.2
Myc-RR	151	143	95.4	114

The selected SPE C18 cartridge (J.T.Baker) was used to validate the method. Triplicate river water samples fortified with MCs and NDLN at 5µg/L level (LCS) and triplicate glass fiber filters spiked (FS) with MCs and NDLN at 0.2µg were extracted following the procedure listed in Section 4.3. Recoveries obtained for all tested MCs were ranging from 74.0-125 % and from 73.8-110% for water and filter extracts respectively. NDLN recoveries were ranging from 89.7-113 for both. MC-LW showed lower recoveries in the filter extracts. This loss could be contributed to the sorption of this MC to the wall of the C18 cartridges. Recoveries for all individual MC and the estimated method detection limit (MDL) for MCs calculated from students *t* times standard deviation for water samples (200mL) using this procedure are listed in Table 4.

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Table 4. C18 SPE water (LCS) and filters (FS) method validation.

Microcystin Congeners	LCS1	LCS2	LCS3	Ave% Rec	StDev	%RSD	MDL=t*s tdev µg/L
MCY-RR	102	99.6	102	101.3	1.45	1.43	0.009
MCY-LR	104	103	111	106.0	4.51	4.25	0.005
MCY-YR	114	115	125	118	6.08	5.15	0.015
MCY-LA	92.0	89.9	101	94.3	5.90	6.25	0.013
MCY-LF	85.6	74.0	84.8	81.5	6.48	7.95	0.020
MCY-LW	66.7	72.3	81.9	73.6	7.69	10.4	0.024
Nodularin	100	101	113	104.7	7.51	7.18	0.009

Microcystin Congeners	FS1	FS2	FS3	Ave% Rec	StDev	%RSD	MDL=t*s tdev µg/L
MCY-RR	73.8	79.2	76.0	76.3	2.72	3.56	0.005
MCY-LR	87.2	89.6	86.8	87.9	1.51	1.72	0.016
MCY-YR	103	111	110	108	4.36	4.04	0.021
MCY-LA	82.0	77.0	84.0	81.0	3.61	4.45	0.021
MCY-LF	92.0	84.8	80.8	85.9	5.68	6.61	0.023
MCY-LW	38.8	44.8	52.7	45.4	6.97	15.3	0.027
Nodularin	94.7	89.7	92.0	92.1	2.50	2.72	0.026

## 5.2 Fresh water direct injection

LC-MS/MS triplequadrupole operated in MRM has shown the ability to achieve extremely low detection of MCs (2 pg on column). For this reason a direct injection method was validated on the most common microcystins (MC-RR, MC-LR and MC-YR). A set of nine fortified river water samples (0.5 µg/L) were diluted with methanol to obtain (9:1) water-methanol (v/v). A portion of the sample was filtered through 0.45 µm Gelman filters then directly injected into LC-MS/MS (QqQ). The MDL for water using direct injection was determined to be 0.1 µg/L based on signal-to-noise equivalent to 7:2. The MRM results obtained in Table 5 shows the mean recoveries were 104, 97.0 and 95.4 % for MC RR, -LR and -YR, respectively, with RSD< 11%.

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Table 5. Direct injection results from 0.2 µg/L fortified water samples.

Toxins	LCS1	LCS2	LCS3	LCS4	LCS5	LCS6	LCS7	LCS8	LCS9	Avg	StDev
MC-RR	106	100	102	98.8	104	110	110	110	96.8	104	5.09
MC-LR	88.0	85.1	90.2	87.2	93.9	107	104	101	116	97.0	10.6
MC-YR	100	88.4	96.5	94.0	103	106	95.0	83.5	92.4	95.4	7.00

This validated method was tested by analyzing split contaminated water samples received as part of a Round Robin study organized by Florida Department of Environmental Protection (EPA). Twelve laboratories throughout the United States participated in this exercise. Three types of water samples were received:

1) water from natural bloom (2007) occurred in Lake Munson (M) which was caused by cyanobacteria, *Microcystis aeruginosa*, 2) water sample containing toxin produced by cultures from University of Texas laboratory (T) and 3) a microcystin standard (S) diluted in water. Each laboratory was provided with 10 blind water samples. Either three or four replicates were provided to each laboratory for each sample type. Laboratories were required to hold the samples in the dark at 4°C for no more than one week before analysis. Two different extractions were performed on the water samples for comparison:

- a) An aliquot of round robin water sample (200mL) was first filtered thru 0.45 µm glass fibers filters then extracted with SPE cartridge. The filters were sonicated and both SPE and filters were extracted according to the procedure outlined in Section 4.3.
- b) The second extraction consisted of 1 mL of methanol added to 9 mL of the round robin water sample sonicated for 45 min, centrifuged for 30 min and a portion of the methanolic solution was filtered thru Gelman filters and directly injected into LC-MS/MS. Results from both type of extraction are listed in Tables 6-8.

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Table 6. Comparison of direct injection (sonication) vs. SPE and filters from Round Robin standard (S).

Sonication	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	2.80	2.96	2.96	2.91	0.09	3.18
Myc-YR						
Total MCs	2.80	2.96	2.96	2.91	0.09	3.18

SPE	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	0.37	0.49	0.55	0.47	0.09	19.4
Myc-YR						
Total MCs	0.37	0.49	0.55	0.47	0.09	19.4

Filters	S-1	S-2	S-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	1.28	1.27	1.17	1.24	0.06	5.12
Myc-YR						
Total MCs	1.28	1.27	1.17	1.24	0.06	5.12

Total MC	S-1	S-2	S-3
Sonication	2.80	2.96	2.96
SPE + filters	1.65	1.76	1.72

Table 6 contains the results obtained from the analysis of round robin sample (S) showing the presence of low levels of MC-LR (~2 ppb). The combined results obtained from SPE and filters correlate well with the direct injection results. The average MC-LR value obtained from sonication was 2.90 ppb compared to 1.71 ppb obtained with SPE and filters, the difference could be contributed to losses during sample preparation.

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Table 7. Comparison of direct injection vs. SPE and filters from University of Texas culture (T).

Sonication	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	65.0	60.6	63.1	62.9	2.21	3.51
Myc-YR						
Total MCs	65.0	60.6	63.1	62.9	2.21	3.51

SPE	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	0.51					
Myc-LR	37.5	40.0	39.6	39.0	1.37	3.51
Myc-YR						
Total MCs	38.0	40.0	39.6	39.2	1.1	2.7

Filters	T-1	T-2	T-3	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR						
Myc-LR	11.69	7.54	6.27	8.50	2.84	33.4
Myc-YR						
Total MCs	11.69	7.54	6.27	8.5	2.84	33.4

Total MC	T-1	T-2	T-3
Sonication	65.0	60.6	63.1
SPE + filters	49.7	47.5	45.9

Microcystins	T-1	T-2	T-3
Demethyl-RR	6.20	5.65	7.61
Demethyl-LR	16.9	16.5	18.0

Table 7 shows the presence of mainly MC-LR in the Texas culture samples. With an average of 62.9 ppb for the direct injection compare to 48.1 ppb obtained by SPE extraction. The RSD values were below 5% for all replicates. Desmethylated

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microcystin (demethyl-MC) RR and LR were also found in these samples with an average value of 5.82 and 17.13 ppb, respectively.

Table 8. Comparison of direct injection vs. SPE and filters from Lake Munson (M) natural bloom.

Sonication	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	57.3	71.9	72.7	70.7	68.2	7.28	10.7
Myc-LR	63.6	84.2	79.4	78.8	76.5	8.93	11.7
Myc-YR	1.80	1.50	1.50	1.90	1.68	0.21	12.3
Total MCs	123	158	154	151	147	15.9	10.9

SPE	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	60.3	67.3	60.3	72.1	65.0	5.77	8.88
Myc-LR	58.5	81.5	70.0	80.4	72.6	10.8	14.8
Myc-YR	0.19	0.79	1.25	0.65	0.72	0.44	60.6
Total MCs	119	150	132	153	138	16.0	11.6

Filters	M-1	M-2	M-3	M-4	Avg (ppb)	StDev (ppb)	RSD (%)
Myc-RR	9.88	11.4	16.7	10.1	12.0	3.21	26.7
Myc-LR	8.73	7.08	8.96	9.08	8.46	0.93	11.0
Myc-YR	0.35	0.54	0.74	0.54	0.54	0.16	29.4
Total MCs	19.0	19.0	26.4	19.7	21.0	3.62	17.2

Total MC	M-1	M-2	M-3	M-4
Sonication	123	158	154	151
SPE+filters	138	169	158	173

Microcystins	M-1	M-2	M-3	M-4
Demethyl-RR	60.0	74.2	74.2	72.6
Demethyl-LR	56.5	72.5	68.1	68.8

MC-RR, MC-LR and MC YR were found in Munson Lake samples with an average of 68.2, 76.5 and 1.68 ppb, respectively. The total microcystins obtained from direct

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injection of the four replicates were practically the same as the one obtained from SPE and filters combined and were 151 and 173 ppb, respectively. Desmethyl-MC-RR and desmethyl-MC-LR were also found in the samples with an average of 70.2 and 66.5 ppb, respectively (Table 8).

In summary, the total concentration of microcystins obtained from direct injection was slightly better compared to the combined MCs obtained from SPE and filters extractions. The method showed excellent precision by comparing replicate results. Only this LC-MS/MS technique was able to detect and report the presence of desmethylated variants compared to other participating laboratory methods. Since desmethylated-MC standards were not available at the time, the desmethyl-MCs values were estimated using the methylated congeners response factor.

### 5.3 Biota samples (fish and mussels)

California coastal mussels (M), oysters (O), Rainbow Trout filets and livers were used for method validation. Samples (2-5 g fresh weight) were polytronned, homogenized, fortified with 5 ng/g microcystins mixture standard and extracted with methanol-water (90:10) using the sonication procedure listed in Section 4.3. Recovery experiments were performed using replicate samples. The results showed that all tested MCs were extracted with high degree of efficiency using sonication technique (Table 9). Recoveries obtained from mussels ranged from 79.9-104 % with percent RSD<15 (n=8). The average microcystin recovery for oysters was 102 % with average standard deviation of ±14.9. The mean recoveries were 106 % for fish fillet (n=4) and 85.7 % for fish liver (n=3). The % RSD was below 11 % for both.

Table 9. Recoveries of microcystins by sonication in various matrices: mussel (M), oyster (O), fish fillet (FF) and fish liver (FL).

	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8
MC-RR	112	115	125	118	85.4	92.5	94.0	89.2
MC-LR	82.9	77.5	81.8	114	114	107	115	114
MC-YR	72.0	87.3	97.9	116	109	117	121	115
MC-LA	73.5	72.9	73.6	80.3	82.5	74.5	75.6	106
MC-LW	75.4	74.3	83.8	92.0	81.9	74.3	79.0	85.6
MC-LF	82.8	80.7	89.1	96.1	85.9	71.5	78.1	89.2

The method detection limit (MDL) calculated from student's *t* times standard deviation for mussels (n=8) determine to be ≤ 1 ng/g using MRM (Table 10).

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Table 10. Determination of microcystin MDL in tissue (n=8).

Microcystins	Avg (% Rec)	StDev	RSD (%)	MDL=t* StDev
MCY-RR	104	15.2	14.6	0.91
MCY-LR	101	16.8	16.7	1.01
MCY-YR	104	17.3	16.5	1.04
MCY-LA	79.9	11.1	13.9	0.67
MCY-LF	80.8	6.27	7.77	0.38
MCY-LW	84.2	7.62	9.05	0.46

	O-1	O-2	O-3	O-4	FF-1	FF-2	FF-3	FF-4	FL-1	FL-2	FL-3
MC-RR	106	103	105	93.3	114	106	99.1	101	81.6	77.0	83.8
MC-LR	79.1	74.8	107	117	120	122	108	103	78.4	84.4	82.4
MC-YR	80.1	79.0	115	118	123	119	109	108	89.0	83.2	87.0
MC-LA	102	101	103	110	108	107	110	107	88.6	76.4	84.8
MC-LW	118	116	101	88.8	125	111	112	103	79.8	68.8	85.4
MC-LF	120	118	103	90.3	110	106	105	104	78.4	84.4	82.4



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## 6. QUALITY CONTROL

Quality control checks are routinely performed in the WPCL operations. These checks may be increased or modified to meet the needs of a particular analysis or project.

### 6.1 QA Samples

Internal quality assurance samples (fortified samples and duplicates, appropriate reference materials, duplicate samples, and method or procedural blanks) will be analyzed with each set or every twenty analyses being performed. These internal quality assurance analyses are conducted for the parameters being monitored by that analytical procedure. In addition, the compounds contained in the quality assurance sample will be representative of those compounds being monitored.

Accuracy is measured by calculating percent recovery for laboratory control spikes (fortified reagent sample), matrix spikes (fortified samples) and when available, certified reference materials (CRMs or SRMs). Accuracy is also determined for CRMs by comparing the analysis results with the certified (consensus) or reference (non-certified) values. CRM results are acceptable if they are within 65-135% of the 95<sup>th</sup> percentile confidence interval of the consensus values for certified materials.

Precision is measured by calculating the relative percent difference (RPD) for analytes from duplicate analysis of samples, fortified samples and fortified blanks.

The results of all QA analyses and the percent recoveries for fortified samples and reference materials will be calculated and documented.

### 6.2 Duplicate Samples

One duplicate sample and/or a matrix spike duplicate or laboratory control spike duplicate will be analyzed for each set of twenty samples analyzed. The relative percent difference for each constituent is calculated as follows:

$$RPD = \{(D_1 - D_2) / [(D_1 + D_2) / 2]\} \times 100$$

Where, RPD = Relative Percent Difference

D<sub>1</sub> = First Sample Value

D<sub>2</sub> = Second Sample Value (duplicate)

The results of all duplicate determinations and the calculated relative percent difference will be reported with the data sets. For RPD, use a control limit of 25 percent unless otherwise specified by a project specific QAPP.

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If either sample value is less than the MDL, the notation of "ND" (not detected) will be reported. If the precision falls outside the control limits, the analysis results will be reported with the appropriate data qualifier.

### 6.3 Fortified Matrix (MS/MSD) Sample Analyses

When required, matrix spike and matrix spike duplicate analyses will be conducted at a rate of five percent. The spike will be added prior to any digestion, extraction, or distillation steps as a check on the sample preparation and analysis. An amount of analyte will be added to the sample that is five to ten times the reporting limit for the analyte of interest. Recovery values are calculated as follows:

$$\text{Recovery} = [(D_a - D) / D_s] \times 100$$

Where, Recovery = Percent Recovery

$D_a$  = Analysis value of fortified sample  
 $D$  = Analysis value of sample without spike  
 $D_s$  = Amount of spike added

Recovery values for fortified samples must be greater than 50 percent except where a specific method (SOP) or project specific QAPP require a different acceptable range. Exceptions shall be noted in the project specific data quality objectives. When a specific method and analyte require a different acceptable recovery range, as determined by actual spike recovery runs, the acceptable range shall be noted in the Standard Operating Procedure for that method. If the recovery falls outside of the acceptable recovery range, the analysis results will be qualified or rejected. If the results are rejected, the batch of samples associated with the rejected results may need to be re-analyzed. When sample concentrations are less than the MDL, the value of "0" will be used as the sample result concentration for purposes of calculating spike recoveries. All fortified sample results will be reported with the data package.

If the percent recovery for matrix spike is unacceptable, there might be an interference due to the matrix. The sample will be diluted to lower the interference and re-analyzed. If matrix interference is determined to be the cause of unacceptable recoveries, the data will be qualified.

### 6.4 Method Blanks

Method blanks will be analyzed at a minimum of once for every batch of samples. Blank concentrations should not exceed the reporting limit for the analyte. If blank values exceed the reporting limit, the source of the contamination should be investigated and corrected, and the results associated with the contaminated blank re-analyzed or qualified. All blank analysis results will be reported with the data package.

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## 6.5 Laboratory Control Samples

While reference materials are not available for all analytes, a way of assessing the accuracy of an analytical method is still required. Laboratory control samples (LCSs) provide an alternate method of assessing accuracy. An LCS is a specimen of known composition prepared using contaminant-free reagent water or an inert solid spiked with the target analyte at the midpoint of the calibration curve or at the level of concern. The LCS must be analyzed using the same preparation, reagents, and analytical methods employed for regular samples.

SOP Section Approval: \_\_\_\_\_ Date: \_\_\_\_\_

SOP Final Approval: \_\_\_\_\_ Date: \_\_\_\_\_

SOP QA Officer Approval: \_\_\_\_\_ Date: \_\_\_\_\_