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National Coastal Condition Assessment 2015 Laboratory Operations Manual

Version 2.1 May 2016

NOTICE

The goal of the National Coastal Condition Assessment (NCCA) is to provide a comprehensive assessment of the Nation's freshwater, marine shoreline and estuarine waters. The complete documentation of overall project management, design, methods, and standards is contained in four companion documents, including:

National Coastal Condition Assessment: Quality Assurance Project Plan EPA 841-R-14-005
National Coastal Condition Assessment: Site Evaluation Guidelines EPA 841-R-14-006
National Coastal Condition Assessment: Field Operations Manual EPA 841-R-14-007
National Coastal Condition Assessment: Laboratory Methods Manual EPA 841-R-14-008

This document (*Laboratory Operations Manual*) contains information on laboratory methods for analyses of the samples collected during the National Coastal Condition Assessment (NCCA). It also provides quality assurance objectives, sample handling procedures, and data reporting requirements. Methods described in this document are to be used specifically in work relating to the NCCA 2015. All NCCA Cooperator laboratories must follow the guidelines presented in the document.

With the exception of the requirements in Chapter 4 for evaluating algal toxics, mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. Chapter 4 requires use of a specific kit and supplemental materials manufactured by a single firm.

More details on specific methods for site evaluation, sampling, and sample processing can be found in the appropriate companion document.

The suggested citation for this document is:

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LIST OF ACRONYMS

ADT	analysis decision tree
AFDM	ash-free dry mass
ANC	acid neutralizing capacity
ANS	Academy of Natural Sciences
AQM	absolute quantitation method
ASTM	American Society for Testing and Materials
Avg	Average
BHI	brain heart infusion
BV	biovolume
Ca	Calcium
CAS	Chemical Abstracts Service assigns unique identifiers to chemicals
CCE	calibrator cell equivalents
CEQ	cell equivalent
Chl-*a*	chlorophyll-a
Cl	Chloride
CO_2	carbon dioxide
Ct	threshold cycle
CPR	cardiopulmonary resuscitation
cv	curriculum vitae
DCF	dilution/concentration factor
DDT	dichloro-diphenyl-trichloroethane
DI	de-ionized
DIC	differential interference contrast
DL	detection limit
DNA	Deoxyribo-nucleic Acid
DO	dissolved oxygen
DOC	dissolved organic carbon
DTH	depositional targeted habitat
DW	distilled water
ELISA	enzyme-linked Immunosorbent assay
EMAP	Environmental Monitoring and Assessment Program
ENT	enterococci
EPA	Environmental Protection Agency
ETOH	ethyl alcohol
FOM	Field Operations Manual
g	grams
GEQ	genomic equivalent
GIS	geographic information system
GPS	global positioning device
HCl	hydrogen chloride
HDPE	high density polyethylene
HNO_3	nitric acid
HRP	antibody-Horseradish Peroxidase
H2S	hydrogen sulfide
$H2SO_4$	sulphuric acid
IBD	ionic balance difference

ID	Identification
IM	information management
IPC	internal positive control
ISBN	International Standard Book Number
ISO	International Organization for Standardization
IT IS	Integrated Taxonomic Information System (IT IS)
K	potassium
kg	kilograms
L	Liters
LCR	Labeled Compound Recovery
LCS	Laboratory Control Sample
LFB	Laboratory Fortified Blanks
LFM	Laboratory Fortified Matrices
LIMS	Laboratory Information Management System
LOM	Laboratory Operations Manual
LRL	Laboratory Reporting Limit
mg	milligrams
mg/kg	milligrams per kilogram
Mg	magnesium
mL	milliliters
MDL	method detection limit
Mn	manganese
MPCA	Minnesota Pollution Control Agency
MSDS	Materials Safety Data Sheet
N	nitrogen
Na	sodium
NABS	North American Benthological Society
NALMS	North American Lakes Management Society
NARS	National Aquatic Resource Surveys
NAWQA	National Water Quality Assessment Program
ND	non-detect
NELAC	National Environmental Laboratory Accreditation Conference
NELAP	National Environmental Laboratory Accreditation Program
ng	nanograms
NH_4	ammonium
NIST	National Institute of Standards
NO_2	nitrite
NO_3	nitrate
NRSA	National Rivers and Streams Assessment
NTL	no template control
NTU	Nephelometric Turbidity Units
OD	optical density
ORD	EPA's Office of Research and Development
OSHA	Occupational Safety and Health Administration
OW	EPA's Office of Water
PAH	Polycyclic Aromatic hydrocarbons
PAR	Photosynthetically Active Radiation

PBS	phosphate buffered saline
PCB	polychlorinated biphenyl
PctDIFF	percent difference
PDE	percent disagreement in enumeration
PCR	polymerase chain reaction
PE	performance evaluation
PES	performance evaluation samples
PHab	physical habitat
P-M	Palmer-Maloney (P-M) count
PDE	percent difference in enumeration
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSE	percent sorting efficiency
PT	performance testing
PTD	percent taxonomic disagreement
QA	quality assurance
QAPP	Quality Assurance Project Plan
QA/QC	quality assurance/quality control
QC	quality control
QCCS	Quality Control Check Sample
QMP	Quality Management Plan
qPCR	quantitative polymerase chain reaction
QRG	Quick Reference Guide
RL	reporting limit
RMSE	root mean square error
RO	reverse-osmosis
RPD	Relative Percent Difference
RQM	relative quantitation method
RSD	Relative Standard Deviation
RTH	richest targeted habitat
Sb	antimony
SEG	Site Evaluation Guidelines
SFS	Society of Freshwater Science
SiO_2	silica
SO_4	sulphate
SOPs	Standard Operating Procedures
SPC	sample processing control
S-R	Sedgewick-Rafter count
SRM	standard reference material
SS	salmon sperm
TMB	tetramethylbenzidine
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus
TRANS	transect

TSN	taxonomic serial number
TSS	total suspended solids
TVS	total volatile solids
µg	micrograms
µg/g	micrograms per gram
µg/L	micrograms per liter
UNK	unknown
USGS	United States Geological Survey
WSA	Wadeable Streams Assessment
WQX	Water Quality Exchange

1.0 INTRODUCTION

This manual describes methods for laboratory analyses of the samples to be collected during the National Coastal Condition Assessment (NCCA). The manual includes quality assurance objectives, sample handling specifications, and data reporting requirements.

The NCCA is one of a series of water assessments conducted by States, Tribes, the U.S. Environmental Protection Agency (EPA), and other partners. In addition to coastal waters, the National Aquatic Resource Surveys (NARS) also focuses on rivers and streams, lakes, and wetlands in a revolving sequence. The purpose of these assessments is to generate statistically-valid reports on the condition of our Nation's water resources and identify key stressors to these systems.

The goal of NCCA is to address two key questions about the quality of the Nation's coastal waters:

- What percent of the Nation's coastal waters are in good, fair, and poor condition for key indicators of water quality, ecological health, and recreation?
- What is the relative importance of key stressors such as nutrients and contaminated sediments?

The NCCA is a probability-based survey of our Nation's coastal and estuarine waters, and designed to:

- Assess the condition of the Nation's coastal and estuarine waters at national and regional scales, including the Great Lakes;
- Identify the relative importance of selected stressors to coastal and estuarine water quality;
- Evaluate changes in condition from previous National Coastal Assessments (NCA) starting in 2000; and
- Help build State and Tribal capacity for monitoring and assessment and promote collaboration across jurisdictional boundaries.

EPA selected the sampling locations using a probability based survey design. Sample surveys have been used in a variety of fields (e.g., monthly labor estimates, forest inventory analysis) to determine the status of populations or resources of interest using a representative sample of a relatively few members or sites. Using this survey design allows data from the subset of sampled sites to be applied to the larger target population, and assessments with known confidence bounds to be made.

The NCCA field sampling season will be during the index period of June through the end of September. Field crews will collect a variety of measurements and samples from the statistically selected sampling locations identified by geographical coordinates. The samples are shipped to laboratories to evaluate the indicators identified in Table 1.1. The indicators are similar to those evaluated in previous NCA.

Table 1.1 NCCA: Indicators

Measure/Indicator		Assessment outcome
Water Quality	Dissolved oxygen	Hypoxia/anoxia
	pH Temperature Depth Conductivity (freshwater) Salinity (marine)	Water column characterization
	Secchi/light measurements PAR	Societal value and ecosystem production
	Nutrients: • Dissolved inorganic NO_2 , NO_3 NH_4 ,PO_4; • Total N and P	Nutrient enrichment
	Chlorophyll *a*	
Sediment Quality	Grain size (Silt/Clay content)	Influencing factor for extent and severity for contamination
	Total Organic Carbon (TOC)	Influencing factor for extent and severity for contamination
	Sediment chemistry • 15 metals • 25 PAHs • 20 PCBs • 14 pesticides • 6 DDT metabolites	Risk of biological response to sediment contamination
	Sediment toxicity (10-day static bioassay with *Leptocheirus* or *Hyalella)*	Biological response to sediment exposure
Biological Quality	Whole body fish contaminants • 13 metals (no Sb or Mn) • 20 PCBs • 14 pesticides • 6 DDT metabolites • Optional: PAHs (national lab only)	Environmentally available contaminant exposure
	Benthic community structure	Biological response to site conditions

2.0 GENERAL LABORATORY GUIDELINES

This chapter describes the general laboratory guidelines with an overview to the quality assurance / quality control (QA/QC) requirements. Each of the following chapters describes a different procedure and the relevant QA/QC requirements for that particular procedure. In addition, the Quality Assurance Project Plan (QAPP) provides a comprehensive consolidation of the QA/QC requirements for NCCA 2015.

2.1 Responsibility and Personnel Qualifications

Each laboratory shall train its laboratory personnel in advance in the use of equipment and procedures used for the standard operating procedure (SOP) in which they are responsible. All personnel are responsible for complying with all of the QA/QC requirements that pertain to the samples to be analyzed. Each laboratory follows its institutional or organizational requirements for instrument maintenance. Appendix A identifies the specific documentation that each laboratory must submit to demonstrate its qualifications for performing the analyses.

2.2 Roles and Contact Information

The **EPA Headquarters Project Management Team** consists of the Project Leader, Alternate Project Leaders, Project QA Lead, and Laboratory Review Coordinator. The Team is responsible for overseeing all aspects of the project and ensuring that the laboratories properly adhere to the technical and quality assurance requirements. The Team is the final authority on all decisions regarding laboratory analysis.

The **NARS Information Management (IM) Coordinator** tracks the location of each NCCA sample that involves post-processing. The coordinator will be the labs main point of contact in regards to sample tracking and data submission.

Table 2.1 NCCA: Contact Information

Title*	Name	Contact Information
EPA HQ NCCA Project Lead, Acting	Hugh Sullivan, OW	**sullivan.hugh@epa.gov** 202-564-1763
EPA HQ NCCA Project QA Coordinator	Sarah Lehmann, OW	**lehmann.sarah@epa.gov** 202-566-1379
EPA HQ NCCA Laboratory Review Coordinator	Kendra Forde, OW	**forde.kendra@epa.gov** 202-564-0417
EPA HQ NARS Team Leader	Sarah Lehmann, OW	**lehmann.sarah@epa.gov** 202-566-1379
Information Management Center Coordinator	Marlys Cappaert, SRA International Inc.	**cappaert.marlys@epa.gov** 541-754-4467 541-754-4799 (fax)

*For any technical direction, laboratories under contract to EPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the contacts provided in this table. For any technical information or sample tracking, the laboratories are permitted to contact these persons.

2.3 Sample Tracking

Samples are collected by a large number of different field crews during the index period (May through September). The actual number of sites sampled on a given day will vary widely during this time. Field crews will submit electronic forms when they have shipped samples and the NARS IM Center will input each sample into the NARS IM database. Laboratories can track sample shipment from field crews by accessing the NARS IM database. Participating laboratories will be given access to the NARS IM system, where they can acquire tracking numbers and information on samples that have been shipped to them by field crews (either by overnight shipment for perishable samples or batch shipments for preserved samples). Upon sample receipt, the laboratory must immediately log in to the database and confirm that samples have arrived. Each laboratory will make arrangements with the NARS IM Coordinator, listed above, to ensure access is granted.

When the samples arrive from the field crews, the shipments will include tracking forms (refer to the NCCA FOM). These forms will list the samples included in the shipment. Laboratory personnel must cross check the forms with the samples received to verify that there are not any inconsistencies. If any sample is missing or damaged, contact the NARS IM Coordinator immediately.

2.4 Reporting

All labs must provide data analysis information to the HQ Project Management Team and the NARS IM Center by **March 30, 2016** or as stipulated in contractual agreements. These reports must include the data elements specified for each analytical method in this manual. The submitted filename must use the following naming convention:

- Indicator name (ex: microcystins)
- Date of files submission to NARS IM Center by year, month, and day (ex: 2015_11_01)
- Laboratory name (ex: MyLab)

Combined, the file name would look as follows: Microcystins_2015_11_01_MyLab.xlsx
Before the laboratory submits the batch data to EPA, the analyst who generated the data and an experienced data reviewer independently check and review the data, as follows:

The analyst shall review the data to ensure that:

- Sample preparation information is correct and complete;
- Analysis information is correct and complete;
- The appropriate method and standard operating procedures were followed;
- Analytical results are correct and complete;
- Quality control samples were within established control limits;
- Blanks (where appropriate) were within the appropriate QC limits; and
- Documentation is complete.

The data reviewer shall review the data package to verify that:

- Calibration data (where appropriate) are scientifically sound and appropriate;
- QC samples were within established control limits;
- Qualitative and quantitative results are correct; and
- Documentation is complete.

Accompanying its data submission for each batch, the laboratory shall provide a short narrative that includes the following information:

- Project summary referencing the batch QC identification number, total number of samples in the batch and their sample numbers, and the analytical methodology used for analysis;
- Discussion of any protocol deviations that may have occurred during sample testing;
- Discussion of QC questions or issues that were encountered and the corrective measures taken;
- Definitions of any laboratory QC codes used in the data;
- Summary and discussion of samples that are diluted by the presence of an interference, non-target analyte, or target analyte; and
- QC samples exceeding established control limits or parameters required by laboratory internal analytical SOPs and an explanation of why, if known.

As specified in the QAPP, remaining sample material and specimens must be maintained by the EPA's designated laboratory or facilities as directed by the NCCA 2015 Project Lead. Unless otherwise authorized by the Project Lead, the laboratory shall retain:

- The sample materials, including vials, for a minimum of three (3) years from the date the EPA publishes the 2015 NCCA report. During this time, the laboratory shall maintain the materials at the temperature specified in its laboratory method. The laboratory shall periodically check the sample materials for degradation. Unless the Project Lead arranges for transfer of sample materials to EPA, at the end of the retention period, the laboratory shall follow its internal protocols for disposal.
- Original records, including laboratory notebooks and raw data files (including logbooks, bench sheets, and instrument tracings), for a minimum of ten (10) years from the date that EPA publishes the final report.

The Project Lead is responsible for maintaining the following:

- Deliverables from contractors and cooperators, including raw data, which are permanent as per EPA Record Schedule 258.
- EPA's project records which under Schedule 501 are permanent.

3.0 ALGAL TOXIN (MICROCYSTIN) IMMUNOASSAY PROCEDURE

This chapter describes an immunoassay procedure that measures concentrations of total microcystins in water samples. In applying the procedure, the laboratory uses Abraxis' Microcystins-ADDA Test Kits (Figure 3.1; "kits"). Each kit is an enzyme-linked immunosorbent assay (ELISA) for the determination of microcystins and nodularins in water samples. Microcystins refers to the entire group of toxins, all of the different congeners, rather than just one congener. Algae can produce one or many different congeners at any one time, including Microcystin-LR (used in the kit's calibration standards), Microcystin-LA, and Microcystin-RR. The different letters on the end signify the chemical structure (each one is slightly different), which makes each congener different.

Figure 3.1 Microcystins: Abraxis Test Kit
(Converted from color to grayscale from James, page 3, 2010)

3.1 Summary of the Procedure

The procedure is an adaptation of the instructions provided by Abraxis for determining total microcystins concentrations using its ELISA-ADDA kits.[1] For samples samples with salinity<3.5 parts per thousand (ppt), the procedure's reporting range is 0.15 µg/L to 5.0 µg/L, although, theoretically, the procedure can detect, not quantify, microcystins concentrations as

1 Abraxis, "Microcystins-ADDA ELISA (Microtiter Plate): User's Guide R021412." Retrieved on January 14, 2014 from http://www.abraxiskits.com/uploads/products/docfiles/278_Microcystin%20PL%20ADDA%20users%20R120214.pdf.

low as 0.10 µg/L. For samples with higher concentrations of microcystins, the procedure includes the necessary dilution steps. The procedure also provides additional sample preparation steps for samples with salinities≥3.5 ppt. The results then are adjusted by a factor of 1.75 for a reporting range of 0.263 µg/L to 8.75 µg/L.

3.2 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions, because the kit substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. In addition to the laboratory's usual requirements such as a Chemical Hygiene Plan, the laboratory must adhere to the following health and safety procedures:

1. Laboratory facilities must properly store and dispose of solutions of weak acid.

2. Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).

3. When working with potential hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with the TMB and stopping solution. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

3.3 Definitions and Required Resources (Personnel, Laboratories, and Equipment)

This section provides definitions and required resources for using the procedure.

3.3.1 Definitions

The procedure uses the following terms:

Absorbance (A) is a measure of the amount of light absorbed by a sample at a specific wavelength. A standard statistical curve is used to convert the absorbance value to the concentration value of microcystins.

Brackish and Seawater Samples, for the purposes of the ABRAXIS microcystins test procedure, are samples with salinity greater than or equal to 3.5 parts per thousand (ppt). (EPA is using different definitions for the water chemistry samples.) EPA recognizes that brackish water is usually defined as 0.5 ppt, and seawater as 35 ppt, but for this immunoassay procedure, it is important to use additional steps described in Section 3.5.2 for any sample with salinity greater than or equal to 3.5 ppt. The sample labels provide the salinity levels.

Calibration Range is the assay range for which analysis results can be reported with confidence. For example, assays of undiluted samples with salinities<3.5 ppt range from the reporting limit of 0.15 µg/L to a maximum value of 5.0 µg/L.

Coefficient of Variation (CV): The precision for a sample is reported in terms of the percent CV of its absorbance values. To calculate the %CV, first calculate the standard deviation, *S*, as follows:

$$S = \left[\frac{1}{n-1}\sum_{i=1}^{n}(A_i - \bar{A})^2\right]^{1/2}$$

where *n* is the number of replicate samples, A_i, is the absorbance measured for the i^{th} replicate. Per Section 3.5.4, samples are evaluated in duplicate (i=1 or 2); controls are either evaluated in duplicate or triplicate (i=1, 2, 3). $\bar{A}$ is the average absorbance of the replicates. Then, calculate %CV as:

$$\%CV = \left|\frac{S}{\bar{A}}\right| \times 100$$

Dark or Dimly Lit: Away from sunlight, but under incandescent lighting is acceptable.

Detection Limit is the minimum concentration at which the analyte can be *detected* with confidence. In other words, the outcome can be reported with confidence that it is greater than zero (i.e., present in the sample). The detection limit is less than the reporting limit at which the *measured* value of the analyte can be reported with confidence. Also see "Sample-Specific Detection Limit."

Duplicates are defined as two aliquots of the same sample which are analyzed separately using identical procedures. The results are used to evaluate the precision of the laboratory analyses. Per Section 3.5.4, controls are evaluated in duplicate or triplicate (i.e., three aliquots).

NARS: National Aquatic Resource Surveys. The National Coastal Condition Assessment (NCCA) is part of the NARS program.

NARS Information Management System (NARS IM): The IM system established to support all surveys, including NCCA, in the NARS program. The IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. Freshwater and coastal samples will be collected during the field stage of NCCA.

Relative Standard Deviation (RSD) is the same as the coefficient of variation (%CV). Because many of the plate reader software programs provides the CV in their outputs, the procedure presents the quality control requirement in terms of %CV instead of RSD.

Reporting Limit: A reporting limit is the point at which the measured value of the analyte can be reported with confidence.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method's detection limi. For diluted samples, the sample-specific detection limit will be the product of the method's detection limit and the dilution factor. Typical values for the dilution factor will be 10 or 100.

Seawater Sample: See definition for brackish and seawater samples.

3.3.2 General Requirements for Laboratories

Expertise. To demonstrate its expertise, the laboratory shall provide EPA with one or more of the following:

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
- Documentation detailing the expertise of the organization, including professional certifications for water-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.

Quality assurance and quality control requirements.
To demonstrate its expertise in quality assurance and quality control procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable Standard Operating Procedures (SOPs).
To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NCCA QAPP Certification Page.

3.3.3 Personnel

The procedure refers to the following personnel:

Laboratory Technician: This procedure may be used by any laboratory technician who is familiar with the NCCA Quality Assurance Project Plan, and this procedure in the NCCA Laboratory Operations Manual (which differs from the Abraxis instructions). The laboratory technician also must be familiar with the use of a multichannel pipette and plate readers.

External QC Coordinator is an EPA staff person who is responsible for selecting and managing the "**QC contractor**." To eliminate the appearance of any inherent bias, the QC contractor must be dedicated to QA/QC functions, and thus, must not be a primary laboratory or a field sampling contractor for NCCA. The QC contractor is responsible for complying with instructions from the External QC Coordinator; coordinating and paying for shipments of the performance samples to participating laboratories; comparing immunoassay results from the laboratories; and preparing brief summary reports.

3.3.4 Equipment/Materials

The procedures require the following equipment and information:

- Abraxis ADDA Test Kit, Product #520011 (see items in Section 3.5.2)
- Adhesive Sealing Film (Parafilm) for Micro Plates (such as Rainin, non-sterile, Cat. No. 96-SP-100): Used to cover plates during incubation.
- Data Template – See Figure 3.2
- Distilled or Deionized Water: For diluting samples when necessary.
- ELISA evaluation software
- Glass scintillation, LC, vials (two vials of 20 mL each)
- Glass vials with Teflon-lined caps of size:
  - 20 mL
  - 4 mL (for dilutions)
- Multichannel Pipette & Plastic Tips: A single-channel and an 8-channel pipette are used for this method.
- Norm-ject syringes (or equivalent)
- Paper Towels: For blotting the microtiter plates dry after washing.
- Permanent Marker (Sharpie Fine Point): For labeling samples, bottles, plates and covers.
- Plate Reader (e.g., Metertech Model M965 AccuReader; ChroMate®; or equivalent readers with software to read the microtiter plates and measure absorbances).
- Reagent Reservoirs (e.g., Costar Cat Number 4870): Plain plastic reservoir for reagents that accommodate the use of a multi-channel pipette.
- Test tubes (glass): For dilutions, if needed.
- Timer: For measuring incubation times.
- Vortex Genie: For mixing dilutions.
- Whatman Glass fiber syringe filter (25mm, GF 0.45 µm filter)

Analysis of samples with salinity≥3.5 ppt require additional equipment and supplies, as follows:

- Microcystins-ADDA Seawater Sample Clean-Up Kit (Product #529912) which includes the following supplies:
  - Disposable 5 ¾" glass Pasteur pipettes
  - Disposable 9" glass Pasteur pipettes
  - Glass wool
  - Pasteur pipette bulb
  - Microcystins-ADDA Seawater Sample Treatment Solution
  - Microcystins-ADDA Seawater Sample Clean-up Resin
- 12x75 mm test tubes
- Scoopula
- Micropipettes with disposable plastic tips
- Vortex mixer

3.4 Sample Receipt

Field crews hold the microcystins samples on ice while in the field and then pack the samples in ice for delivery to a central facility ("batching laboratory") or the State's laboratory. The batching and State laboratories freeze the samples upon receipt. Periodically, the batching laboratory ships samples to the microcystins laboratory. The batching and microcystins laboratory may retain the frozen samples for several months before analysis.

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery.

1. Report receipt of samples in the NARS IM sample tracking system (within 24 clock hours). Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM (see Chapter 2 for contact information).

2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
   a. Verify that the sample IDs in the shipment match those recorded on the:
      i. Chain of custody forms when the batching laboratory sends the samples to the microcystins laboratory; or
      ii. Sample tracking form if the field crew sends the shipment directly to the State laboratory.
   b. Record the information in Table 3.1 into NARS IM, including the Condition Code for each sample:
      i. *OK*: Sample is in good condition
      ii. *C*: Sample container was cracked
      iii. *L*: Sample container is leaking
      iv. *ML*: Sample label is missing
      v. *W*: Sample is warm (>8^{o}), record the temperature in the comment field, and perform the assay
   c. If any sample is damaged or missing, contact the EPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).

3. Store samples in the freezer until sample preparation begins.

4. Maintain the chain of custody or sample tracking forms with the samples.

Table 3.1 Microcystins Login: Required Data Elements

FIELD	FORMAT	DESCRIPTION
LAB ID	text	Name or abbreviation for QC laboratory
DATE RECEIVED	MMDDYY	Date sample was received by lab
SITE ID	text	NCCA site id as used on sample label
VISIT NUMBER	numeric	Sequential visits to site (1 or 2)

FIELD	FORMAT	DESCRIPTION	
SAMPLE ID	numeric	Sample id as used on field sheet (on sample label)	
DATE COLLECTED	MMDDYY	Date sample was collected	
CONDITION CODE	text	Condition codes describing the condition of the sample upon arrival at the laboratory.	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		W	Sample is warm (>8°)
		Q	Other quality concerns, not identified above
CONDITION COMMENT	text	Comments about the condition of the sample. If the condition code='W' then provide the temperature	

3.5 Procedure

The following sections describe the sample and kit preparation and analysis.

3.5.1 Sample Preparation: Freeze-Thaw Steps

For each frozen sample (500 mL per sample), the laboratory technician runs it through a freeze-thaw cycle three times to lyse the cells as follows:

1. All cycles: Keep the samples in dark or dimly lit areas (i.e., away from sunlight, but under incandescent lighting is acceptable).

2. First freeze-thaw cycle:
    a. Start with a frozen 500 ml sample.
    b. Thaw the sample to room temperature (approximately 25° C). Swirl the sample to check for ice crystals. At this temperature, no ice crystals should be present in the sample.
    c. Shake well to homogenize the sample, then transfer 10 mL to an appropriately labeled clean 20 mL glass vial.

3. Second freeze-thaw cycle:
    a. Freeze the vial.
    b. Keep the large sample bottle (from the 500 mL initial sample) frozen for future use.
    c. Thaw the sample vial contents to room temperature.

4. Third freeze-thaw cycle:
    a. Freeze the vial.
    b. Thaw the vial contents to room temperature.
    c. Filter the vial contents through a new, syringe filter (0.45 µm) into a new, labeled 20 mL glass scintillation vial. Norm-ject syringes and Whatman Glass fiber

syringe filters (25mm, GF 0.45 µm filter) or other similar alternative are acceptable. Use one new syringe and filter per sample.

3.5.2 Additional Sample Preparation for Samples with Salinity>3.5 parts per thousand

For any sample with salinity of 3.5 parts per thousand (ppt) or greater (the salinity will be marked on sample vials), the laboratory technician needs to perform the following additional steps provided by Abraxis. [2] For all other samples (i.e. with salinity less than 3.5 ppt), the technician skips this section (i.e., Section 3.5.2) and goes directly to kit preparation as described in Section 3.5.3. For samples with salinity 3.5 ppt the technician:

1. Prepares the column as follows:
    a. Place a small amount of glass wool into the top of a 5 ¾" glass Pasteur pipette. Using a 9" glass Pasteur pipette, push the glass wool into to the bottom of the 5 ¾" pipette to form the base of the column. The depth of the glass wool should be approximately 5 mm. Place the column into a 12x75 mm test tube.
    b. Each column will require approximately 1.5 g of Seawater Sample Clean-Up Resin. Calculate and add the appropriate amount of Microcystins-ADDA Seawater Sample Clean-Up Resin to a 20 mL glass vial.
    c. Add distilled or deionized water at an approximately 2:1 ratio to the Microcystins-ADDA Seawater Sample Clean-Up Resin (for example, 10 mL of deionized or distilled water per 5 g of Resin). Shake or vortex.
    d. Pipette the Resin in water solution into the column using the 9" Pasteur pipette. Avoid the formation of air bubbles in the column bed by keeping the tip of the pipette at the surface of the bed being created. Fill the column to the indentation approximately 2 cm from the top of the pipette. This will create an approximately 8 cm column.
    e. Allow the deionized or distilled water to drain from the column. [3]. Lift the tip of the column at least 1 cm above the surface of the water in the tube. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining water out of the column. Avoid allowing the tip of the column to come into contact with the water in the tube to prevent aspiration of water back into the column.
    f. Place the column into an appropriately labeled 4 mL glass vial.

2. Cleans up the sample as follows:
    a. Add 1 mL of the sample to a clean, appropriately labeled 4 mL glass vial. Add 50 µL of Microcystins-ADDA Seawater Sample Treatment Solution. Vortex.

2 Reformatted from Abraxis, "Microcystins in Brackish Water or Seawater Sample Preparation" Retrieved on January 14, 2014 from http://abraxiskits.com/uploads/products/docfiles/385_MCT-ADDA%20in%20Seawater%20Sample%20Prep%20%20Bulletin%20R041112.pdf. Reproduced with permission. Except for Abraxis' solutions labeled as seawater, EPA has removed references to "brackish" and "seawater" which typically are defined as having different cutpoints than 3.5 ppt for salinity.

3 Additional correspondence between EPA and Abraxis notes that this step leaves the resin in the column.

b. Add 375 µL of the treated sample to the top of the column. Allow the sample to drain through the column and collect in the vial.
c. Add a second 375 µL aliquot of the treated sample to the column. Allow to drain through the column.
d. Lift the tip of the column at least 1 cm above the surface of the sample in the vial. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining sample out of the column. Avoid allowing the tip of the column to come into contact with the sample in the vial to prevent aspiration of the sample back into the column.
e. Lower the column back into the vial. Add 500 µL of distilled or deionized water to the top of the column. Allow the rinse to drain through the column and collect with the sample.
f. Lift the tip of the column at least 1 cm above the surface of the sample/rinse in the vial. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining rinse out of the column. Avoid allowing the tip of the column to come into contact with the sample in the vial to prevent aspiration of the sample back into the column.
g. Remove the column and discard (columns are single use only). Cap vial and vortex. The sample can then be analyzed using the Abraxis Microcystins-ADDA ELISA Kit beginning with the next section (3.5.3).

3.5.3 Kit Preparation

The technician prepares the kits using the following instructions:

1. Check the expiration date on the kit box and verify that it has not expired. If the kit has expired, discard and select a kit that is still within its marked shelf life. (Instead of discarding the kit, consider clearly labelling it as expired and keeping it for training activities.)

2. Verify that each kit contains all of the required contents:
    - Microtiter plate
    - Standards (6) referenced in this procedure as follows with the associated concentration:
        - S0: 0 µg/L
        - S1: 0.15 µg/L
        - S2: 0.40 µg/L,
        - S3: 1.0 µg/L
        - S4: 2.0 µg/L
        - S5: 5.0 µg/L
    - Kit Control (KC): 0.75 µg/L
    - Antibody solution
    - Anti-Sheep-HRP Conjugate
    - Wash Solution 5X Concentrate
    - Color Solution
    - Stop Solution
    - Diluent
    - Foil bag with 12 microtiter plate strips

3. If any bottles are missing or damaged, discard the kit. This step is important because Abraxis has calibrated the standards and reagents separately for each kit.

4. Adjust the microtiter plate, samples, standards, and the reagents to room temperature.

5. Remove 12 microtiter plate strips (each for 8 wells) from the foil bag for each kit. The plates contain 12 strips of 8 wells. If running less than a whole plate, remove unneeded strips from the strip holder and place in the foil bag, ziplocked closed, and store in the refrigerator (4-8^{o} C).

6. Prepare a negative control (NC) using distilled water.

7. The standards, controls, antibody solution, enzyme conjugate, color solution, and stop solutions are ready to use and do not require any further dilutions.

8. Dilute the wash solution with deionized water. (The wash solution is a 5X concentrated solution.) In a 1L container, dilute the 5X solution 1:5 (i.e., 100 mL of the 5X wash solution plus 400 mL of deionized water). Mix thoroughly. Set aside the diluted solution to wash the microtiter wells later.

9. Handle the stop solution containing diluted H_2SO_4 with care.

3.5.4 Insertion of Contents into Wells

This section describes the steps for placing the different solutions into the 96 wells. Because of the potential for cross contamination using a shaker table, the following steps specify manual shaking of the kits instead mechanized shaking.

1. While preparing the samples and kit, turn the plate reader on so it can warm up. The plate reader needs a minimum of 30 minutes to warm up.

2. Turn on the computer so that it can control and access the plate reader.

3. Print the template (Figure 3.2) to use as reference when loading the standards, controls, and samples as described in the next step. Templates contain rows, labeled with a marking pen, of strips of 8 wells that snap into the blank frame. (If the laboratory wishes to use a different template, provide a copy to the EPA HQ Laboratory Review Coordinator for approval prior to first use. (See Chapter 2 of the manual for contact information.)

4. Using the 100-µL pipette, add 50 µL, each, of the standards, controls, and samples to the appropriate wells in the plate. Place all six standards (0.00, 0.15, 0.40, 1.00, 2.0 and 5.0 µg/L), the kit control (0.75 µL), and negative control, in pairs, starting in the well in the upper left-hand corner of the kit as shown in Figure 3.2. Verify that the software displays the same template or make any necessary corrections.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S4	NC	U4	U8	U12	U16	U20	U24	U28	U32	U36
B	S0	S4	NC	U4	U8	U12	U16	U20	U24	U28	U32	U36
C	S1	S5	U1	U5	U9	U13	U17	U21	U25	U29	U33	U37
D	S1	S5	U1	U5	U9	U13	U17	U21	U25	U29	U33	U37
E	S2	KC	U2	U6	U10	U14	U18	U22	U26	U30	U34	U38
F	S2	KC	U2	U6	U10	U14	U18	U22	U26	U30	U34	U38
G	S3	KC	U3	U7	U11	U15	U19	U23	U27	U31	U35	U39
H	S3	NC	U3	U7	U11	U15	U19	U23	U27	U31	U35	U39

Key:
S0-S5 = Standards;
KC = Control supplied with Kit (i.e., Kit Control);
NC = Negative Control;
U = Unknown (sample collected by the field crew);

Figure 3.2 Microcystins: Template for samples

5. Add 50 µL of the pink antibody solution to each well using the multi-channel pipettor and a reagent reservoir. Use dedicated reagent reservoirs for each reagent to avoid contamination from one reagent to another.

6. Place the sealing Parafilm over the wells.

7. Manually mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.

8. Place the plate in a dimly lit area (as defined in Section 3.3.1) for 90 minutes.

9. After 90 minutes, carefully remove the Parafilm.

10. Empty the contents of the plate into the sink, pat inverted plate dry on a stack of paper towels, and then wash the wells of the plate three times with 250 µL of washing solution using the multi-channel pipette. After adding the washing solution each time, empty the solution into the sink and use the paper towels as before.

11. Add 100 µL of enzyme conjugate solution to all wells using the multi-channel pipettor.

12. Cover the wells with Parafilm.

13. Manually mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.

14. Place the strip holder in a dimly lit area for 30 minutes.

15. After 30 minutes, remove the Parafilm, decant, and rinse the wells three times again with 250 µL of washing solution as described in step 10.

16. Add 100 µL of color solution to the wells using the multi-channel pipette and reagent reservoir. This color solution will make the contents have a blue hue.

17. Cover the wells with Parafilm.

18. Manually mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.

19. Place the plate in a dimly lit area for 20 minutes.

20. After 20 minutes, remove the Parafilm and add 50 µL of stopping solution to the wells in the same sequence as for the color solution. This will turn the contents a bright yellow color. After adding the stopping solution, read the plate within 15 minutes.

21. Within 15 minutes of adding the stopping solution, use the microplate ELISA photometer (plate reader) to determine the absorbance at 450 nm. The software (i.e., commercial ELISA evaluation program) calculates the absorbance and concentration values of the samples from the calibration curve and the average values for each pair. Use a 4-parameter standard curve fit to determine the concentrations.

22. Dispose of solution in plates in a lab sink. Rinse plates and sink with water to dilute the weak acid present.

23. Perform QC evaluations of the data as follows:
    a. If the following **failures** occur, then the laboratory must reanalyze all samples in the analytical run:
        i. Standard curve with a correlation coefficient, R, of less than 0.99
        ii. Standards S0-S5 must have decreasing absorbance values. First, calculate the average values for each standard. That is, if $\bar{A}_i$ is the absorbance average for S_i, then the absorbance averages must be:
            $$\bar{A}_0 > \bar{A}_1 > \bar{A}_2 > \bar{A}_3 > \bar{A}_4 > \bar{A}_5$$
        iii. The average absorbance of the standard S0 less than 0.8 (i.e., $\bar{A}_0 < 0.8$).
        iv. Two or more negative control sample results report detectable concentrations of microcystins (i.e., values ≥ 0.1 µg/L). If this occurs, then evaluate possible causes (e.g., cross-contamination between samples), and if appropriate, modify laboratory processes before the next analytical run.
        v. Results for control samples of outside the acceptable range of 0.75 +/- 0.185 µg/L. That is, results must be between 0.565 µg/L and 0.935 µg/L.

b. If either, or both, of the following situations occur, then the sample must be reanalyzed (maximum of two analyses,[4] consisting of the original analysis and, if necessary, one reanalysis):
    i. The concentration value registers as HIGH (exceeds the calibration range).[5] Dilute the sample for the reanalysis per Section 3.5.5.
    ii. The %CV > 15% between the duplicate absorbance values for a sample.

24. If the sample has a salinity of 3.5 ppt or greater, then convert the results by multiplying by 1.75. If the assay was non-detected, then the detection limit is 0.175 µg/L. The reporting limit is 0.263 µg/L. The calibration range is 0.263 µg/L to 8.75 µg/L.

25. Record the results, even if the data failed the quality control requirements in #23b, for each well in EPA's data template (see Table 3.2 for required elements). The required entries are for the following columns:
    a. **TYPE** indicates the sample type using one of the following codes: S0-S5 for standards; KC or NC for controls; and U for unknown sample.
    b. **CONC** contains the numeric concentration value. Two special cases:
        i. Non-detected concentrations: If the sample is non-detected, then provide the sample-specific detection limit which is 0.1 µg/L if the sample is undiluted with a salinity<3.5 ppt in the sample. See step 24 for reporting values for samples with salinity ≥3.5 ppt. See Section 3.5.5 for calculating the sample-specific detection limit for a diluted sample.
        ii. If the result shows that it is "HI," this indicates that the sample value is outside of the calibration range and must be diluted and re-run using another analytical run. Leave the CONC column blank and record 'HI' in the DATA FLAG column.
    c. **DATA FLAGS** have codes for the following special cases:
        i. **ND** if the sample was non-detected;
        ii. **J** if the value is detected but at a level below the reporting limit of 0.15 µg/L (for undiluted samples with salinity <3.5 ppt; see step 24 for samples with salinity ≥3.5 ppt);
        iii. **HI** if the concentration value registers as HIGH (exceeds the calibration range).
    d. **QUALITY FLAGS** have codes for the following special cases:
        i. **QCF** if there is a QC failure per step 23 above. The QCF code must be used for all failures to facilitate data analysis.
        ii. **Q** for any other quality issue (describe in **COMMENTS**)
    e. DILUTION FACTOR is only required if the sample was diluted.

[4] In its data analyses, EPA compares the microcystins data values to 10 µg/L, which is the World Health Organization threshold for moderate risk. If a sample is diluted once following the procedures in Section 3.5.5 and the concentration still registers as HIGH, the concentration is recorded as >50 µg/L which is greater than the WHO threshold. EPA does not require additional dilution to obtain a more precise value, but a laboratory may choose to increase the dilution of the sample and report the associated concentration value.
[5] A value of HIGH is not a QA/QC failure, but rather indicates a necessity to find the correct dilution to get it within calibration.

f. **DUP AVG** and **DUP CV** are required for duplicate samples and control samples (use all three values if the controls are used in triplicate).

Table 3.2 Microcystins: Required Data Elements

STAGE	FIELD	FORMAT	DESCRIPTION	
LOGIN	LAB ID	Character	Name or abbreviation for QC laboratory	
	DATE RECEIVED	MMDDYY	Date sample was received by lab	
	SITE ID	Character	NCCA site ID code as recorded on sample label or tracking form (blank if standard or control)	
	VISIT NUMBER	Numeric	sequential visits to site (1 or 2) (blank if standard or control)	
	SAMPLE ID	Numeric	6-digit Sample ID number as recorded on sample jar or tracking form (blank if standard or control)	
	DATE COLLECTED	MMDDYY	Date sample was collected (blank if standard or control)	
	CONDITION CODE	Character	Sample condition upon arrival at the laboratory (blank if standard or control)	
			Flag	Definition
			Blank or N	Not a sample (blank, standard, or control)
			OK	Sample is in good condition
			C	Sample container is cracked
			L	Sample or container is leaking
			ML	Sample label is missing
			W	Sample iswarm (>8°)
	CONDITION COMMENT	Character	Comments about the condition of the sample. If the condition code='W' then provide the temperature	
ANALYSIS	TECHNICIAN	Character	Name or initials of technician performing the procedure	
	ANALYSIS DATE	MMDDYY	Date when samples are inserted into the wells per Section 3.5.4	
	ANALYSIS TIME	24-hour time	Time when 1st sample is inserted into the wells per Section 3.5.4	
	KIT EXPIRE DATE	MMDDYY	Expiration date on kit box	
	KIT ID	Character	Kit identification code. If one does not exist, assign a unique code to each kit.	
	R2	Numeric	R^2 from curve fit to the average absorbance values for the standards. Value is between 0 and 1.	
	TYPE	Character	Type of solution being tested in the well	
			Code	Definition
			KC	Kit Control
			NC	Negative Control
			S0,S1, S2,S3, S4, S5	Standard

STAGE	FIELD	FORMAT	DESCRIPTION	
			U	Sample of unknown concentration
	LOCATION	Character	Location of well in the kit (e.g., B5 would be the fifth well from the left in the second row B)	
	SALINITY	Numeric	If the sample vial has the salinity marked on the vial, record the value in units of parts per thousand. Otherwise, leave blank.	
	CONC	Numeric	Concentration or sample-specific detection limit of contents of well in µg/L. Sample-specific detection limit should be 0.1 µg/L for a sample with salinity <3.5 ppt which hasn't been diluted. (Detection limit is 0.175 µg/L for samples with salinity ≥3.5 ppt)	
	ABSORBANCE	Numeric	Absorbance value	
	DILUTION FACTOR	Numeric	10, 100, etc for number of times the sample was diluted. If not diluted, leave blank or record 1	
	CV_ABSORB	Numeric	Calculated %CV of duplicate values of absorbance for a sample. Only calculated for TYPE=U, KC, or NC. Enter %CV. Value is between 0 and 100%.	
	AVG_ABSORB	Numeric	Calculated average of absorbance values for a sample. Only provided for TYPE=U, KC, NC, or SC. Average value of the original sample and its duplicate (or replicates for KC and NC).	
	AVG_CONC	Numeric	Calculated average of concentration values for a sample. Substitute for any value below the reporting limit.	
	DATA FLAG (if appropriate)	Character	Data qualifier codes associated with specific identifications of voucher samples. These codes provide more information than those used when reporting receipt of samples. A technician may use alternative or additional qualifiers if definitions are provided as part of the submitted data package (e.g., as a separate worksheet page of the data submission file).	
			Flag	Definition
			ND	Concentration below detection.
			HI	Result indicated a high concentration (i.e., outside calibration range)
			J	Concentration above detection but below reporting limit.
	QUAL_FLAG	QCF/Q	QCF	QC failure
			Q	Other quality concerns, not identified above
	COMMENTS	Character	Explanation for data flag(s) (if needed) or other comments.	

3.5.5 Dilutions (if needed)

Dilutions if needed are prepared as follows (using clean glass tubes):

1. 1:10 dilution
    a. Add 900 µL of distilled water to a clean vial. (Note: Dilutions may also be made using the kit's diluent rather than distilled water.)
    b. Pipette 100 µL from the sample into the vial. (To provide more accurate dilutions and less chance of contaminating the diluent, add the diluent to the vial before the sample.)
    c. Mix by vortexing.
    d. Multiply final concentration and Abraxis' detection limit by 10 to obtain the sample-specific detection limit.. For example, for a sample with salinity<3.5 ppt, Abraxis' detection limit is 0.1 µg/L and the sample-specific detection would be 1.0 µg/L for a 1:10 dilution.

2. 1:100 dilution
    a. Add 3.96 mL of distilled water to a clean, appropriately labeled glass vial. (Note: Dilutions may also be made using the kit's diluent rather than distilled water.)
    b. Vortex the sample to mix thoroughly, then pipette 40 µL from the sample and add to the water (or diluent) in the appropriate labeled vial. Vortex.
    c. Multiply the final concentration and Abraxis' detection limit by 100 to obtain the sample-specific detection limit. For example, for a sample with salinity<3.5 ppt, Abraxis' detection limit is 0.1 µg/L and the sample-specific detection would be 10 µg/L for a 1:100 dilution.

3. Other dilutions can be calculated in the same manner as #1 and #2 if needed.

3.6 Quality Measures

This section describes the quality assurance and quality control measures used to ensure that the data will meet NCCA's requirements.

3.6.1 Assistance Visits

Assistance visits are intended to familiarize EPA with actual procedures being implemented by different laboratories; and to ensure a clear and consistent understanding of procedures and activities by both EPA and the laboratories. If EPA decides to conduct an assistance visit, a qualified EPA scientist or contractor will administer a checklist based upon the steps described in this chapter. EPA will develop, review and approve the checklist prior to conducting an assistance visit.

3.6.2 QC Samples

The External QC Coordinator will instruct the QC contractor to provide one or two identical sets of freshwater and/or seawater performance test samples to all participating laboratories. If the laboratory will assay both freshwater and seawater samples, then it will receive both sets (i.e.,

freshwater and seawater). Each set will contain five samples to test the expected range of concentrations in the NCCA samples.

For the contract laboratory, the QC contractor will provide the first set to be run with the first set of samples and a second set to be run at the midpoint of the assigned samples. If available, a third set will be run with the final batch of samples. Because most state laboratories will have relatively few samples that can be analyzed using a single kit, the QC contractor will send only one set to each state laboratory.

Each laboratory will run the QC samples following the same procedures used for the other samples. The External QC Coordinator will compare the results and assess patterns in the data (e.g., one laboratory being consistently higher or lower than all others). Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any deviations from the Method or unique laboratory practices that might account for differences between the laboratory and others. With this additional information, the External QC Coordinator will determine an appropriate course of action, including no action, flagging the data, or excluding some or all of the laboratory's data.

3.6.3 Summary of QA/QC Requirements

Table 3.3 provides a summary of the quality control requirements described in Sections 3.5 and 3.6.

Table 3.3 Microcystins: Sample analysis quality control activities and objectives

Quality Control Activity	Description and Requirements	Corrective Action
Kit – Shelf Life	Is within its expiration date listed on kit box.	If kit has expired, then discard or clearly label as expired and set aside for training activities.
Kit - Contents	All required contents must be present and in acceptable condition. This is important because Abraxis has calibrated the standards and reagents separately for each kit.	If any bottles are missing or damaged, discard the kit.
Calibration	All of the following must be met: Standard curve must have a correlation coefficient of ≥0.99; Average absorbance value, $\bar{A}_0$, for S0 must be ≥0.80; and Standards S0-S5 must have decreasing average absorbance values. That is, if $\bar{A}_i$ is the average of the absorbance values for S_i, then the absorbance average values must be: $\bar{A}_0 > \bar{A}_1 > \bar{A}_2 > \bar{A}_3 > \bar{A}_4 > \bar{A}_5$	If any requirement fails: Results from the analytical run are not reported. All samples in the analytical run are reanalyzed until calibration provides acceptable results. At its discretion, the lab may consult with EPA for guidance on persistent difficulties with calibration.
Kit Control	The average concentration value of the duplicates (or triplicate) must be within the range of 0.75 +/- 0.185 µg/L. That is, the	If either requirement fails: Results from the analytical run are not reported

Quality Control Activity	Description and Requirements	Corrective Action
	average must be between 0.565 µg/L and 0.935 µg/L.	The lab evaluates its processes, and if appropriate, modifies its processes to correct possible contamination or other problems. The lab reanalyzes all samples in the analytical run until the controls meet the requirements.
Negative Control	The values for the negative control replicates must meet the following requirements: All concentration values must be < 0.15 µg/L (i.e., the reporting limit; and one or more concentration results must be nondetectable (i.e., <0.10 µg/L)	
Sample Evaluations	All samples are run in duplicate. Each duplicate pair must have %CV≤15% between its absorbance values.	If %CV of the absorbances for the sample>15%, then: Record the results for both duplicates using different start dates and/or start times to distinguish between the runs.. Report the data for both duplicate results using Quality Control Failure flag "QCF"; and re-analyze the sample in a new analytical run. No samples are to be run more than twice. If the second run passes, then the data analyst will exclude the data from the first run (which will have been flagged with "QCF"). If both runs fail, the data analyst will determine if either value should be used in the analysis (e.g., it might be acceptable to use data if the CV is just slightly over 15%).
Results Within Calibration Range	All samples are run in duplicate. If both of the values are less than the upper calibration range (i.e., ≤ 5.0 µg/L for undiluted samples with salinity<3.5 ppt; ≤ 8.75 µg/L for undiluted samples with salinity ≥3.5 ppt), then the requirement is met.	If a result registers as "HIGH", then record the result with a data flag of "HI." If one or both duplicates register as 'HIGH,' then the sample must be diluted and re-run. No samples are to be run more than twice. The lab reports both the original and diluted sample results.
External Quality Control Sample	External QC Coordinator, supported by QC contractor, provides 1-2 sets of identical samples to all laboratories and compares results.	Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any deviations from the Method or unique laboratory practices that might account for differences between the laboratory and others. With this additional information, the External QC

Quality Control Activity	Description and Requirements	Corrective Action
		Coordinator will determine an appropriate course of action, including no action, flagging the data, or excluding some or all of the laboratory's data.

3.7 Sample and Record Retention

The laboratory shall retain:

1. The sample materials, including vials, for a minimum of 3 years from the date the EPA publishes the final report. During this time, the laboratory shall freeze the materials. The laboratory shall periodically check the sample materials for degradation.

2. Original records, including laboratory notebooks and the reference library, for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

3.8 References

Abraxis, "Microcystins-ADDA ELISA (Microtiter Plate)," Product 520011, R021412, Undated. Retrieved January 2014 from http://www.abraxiskits.com/uploads/products/docfiles/278_Microcystin%20PL%20ADDA%20users%20R120214.pdf.

Abraxis, "Microcystin-ADDA ELISA Kit, Detailed Procedure," Undated. Retrieved January 2014 from http://www.abraxiskits.com/uploads/products/docfiles/253_PN520011FLOW.pdf.

Abraxis, "Microcystins in Brackish Water or Seawater Sample Preparation" Undated. Retrieved on January 2014 from http://abraxiskits.com/uploads/products/docfiles/385_MCT-ADDA%20in%20Seawater%20Sample%20Prep%20%20Bulletin%20R041112.pdf.
Loftin, K.A., et al., "Comparison of Two Cell Lysis Procedures for Recovery of Microcystins in Water Samples from Silver Lake in Dover, Delaware, with Microcystin Producing Cyanobacterial Accumulations," in USGS Open-File Report 2008 -1341. 2008. Retrieved April 2013 from http://pubs.usgs.gov/of/2008/1341/pdf/of2008_1341.pdf.

James, R., et al., "Environmental Technology Verification Report: Abraxis Microcystin Test Kits: ADDA ELISA Test Kit; DM ELISA Test Kit; Strip Test Kit," in Environmental Technology Verification System Center 2010. Retrieved March 2013 from http://nepis.epa.gov/Adobe/PDF/P100EL6B.pdf

Kamp, L. (Abraxis) "Re: question about instructions for brackish water or seawater"; Email to M. Smith (EPA). June 23, 2015.

4.0 BENTHIC MACROINVERTEBRATES

This chapter describes the steps for identifying benthic macroinvertebrate organisms in samples collected in coastal waters and the Great Lakes during the 2015 National Coastal Condition Assessment (NCCA). Field crews preserve samples in the field with formalin and ship them to a central holding facility or directly to the laboratory. Because NCCA samples generally have fewer than 400 organisms, this procedure requires the laboratory to fully sort and identify all organisms in the sample. If, upon initial inspection, a sample appears likely to have more than 400 organisms, contact the EPA HQ Laboratory Review Coordinator (see contact information in Chapter 2) for processing instructions. (EPA may require use of the subsampling procedures such as those described in the Laboratory Operations Manual for the 2013-2014 National Rivers and Streams Assessment (NRSA)).[6]

In the following discussion, Sections 4.1, 4.2, and 4.3 summarize the procedure; health and safety concerns; and definitions and required resources. Section 4.4 provides the steps for acknowledging sample receipt. Section 4.5 provides the steps for preparing and picking organisms from the sample. Sections 4.6 – 4.8 provide the steps for the taxonomy identification; data entry; and sample and record retention. Sections 4.9 and 4.10 describe EPA's external review of laboratory operations and quality measures. Section 4.11 identifies references used in developing the procedure. Attachment 4.1 provides an example of a taxonomic bench sheet.

4.1 Summary of Method

The procedure describes the steps for picking and identifying organisms from sediment samples. This section provides a summary of the procedure and quality control measures.

The sorter evenly distributes each sample across a tray(s) and then picks all organisms from the sample. During the identification step, a taxonomist identifies all organisms to the target taxonomic levels for the survey and discards materials that do not meet the identification criteria. For each species or lowest identifiable taxonomic level, the taxonomist includes at least one representative organism in the laboratory's reference collection for NCCA 2015.

As part of the quality control measures, a second taxonomist will re-identify a subset (usually 10%) of the samples to quantify enumeration and taxonomic precision, or consistency, as percent difference in enumeration (PDE) and percent taxonomic disagreement (PTD), to help target corrective actions, and ultimately to help minimize problems during data analysis.

4.2 Health and Safety Warnings

In addition to the laboratory's requirements, persons using this procedure must abide by the following health and safety procedures:

6 USEPA, 2013, National Rivers and Streams Assessment 2013-14: Laboratory Operations Manual EPA 841-B-12-010.

1. Wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear / goggles).

2. When working with potential hazardous chemicals (e.g. Rose Bengal) or biological agents (benthic organisms and sediments), avoid inhalation, skin contact, eye contact, or ingestion. If skin contact occurs, remove clothing immediately and wash / rinse thoroughly. Wash the affected skin areas thoroughly with large amounts of soap and water.

4.3 Definitions and Required Resources (Laboratory, Personnel, and Equipment)

This section provides definitions and required resources for using this procedure. Section 4.3.1 defines the terms used throughout the procedure. Section 4.3.2 describes the expertise required for each laboratory using the procedure. Section 4.3.3 describes the roles and responsibilities of the personnel involved in the procedure. Section 4.3.4 identifies the equipment necessary to apply the procedure in preparing, sorting, and identifying benthic macroinvertebrate organisms in samples.

4.3.1 Definitions

The procedure uses the following throughout the document:

Dissecting microscope: Microscope configured to allow low magnification of three-dimensional objects that are larger or thicker than the compound microscope can accommodate.

Distinct taxa: Data analysts use the number of distinct (i.e., unique) taxa within a given sample to evaluate the richness associated with the sample location. The distinctness attribute is assessed sample by sample, and not across all samples. To facilitate the data analyses, the database includes an additional variable ("flag") that is used for the first identification of a particular taxon in a sample. Section 4.6 provides the steps used to identify which taxa are flagged.

Good quality digital photograph: Good quality means that other taxonomists can readily identify the taxon from one or multiple photographs and the library can readily locate the photographs. To ensure that the photographs meet these objectives, the image must be:

- Taken through the microscope at a high enough resolution so that the key diagnostic features are distinguishable and clear. Include all features that would be necessary for an experienced taxonomist to identify the specimen, this may require multiple photographs and at different magnifications.

- Positioned so that it includes:

  - Only one taxon in the photo. If necessary, the laboratory may edit (e.g., crop) the digital photograph and save the file with a new filename as specified below. Both the original and edited files must be included in the digital library.
  - A scale bar or measurements in an appropriate location to indicate the size of the specimen.
  - One specimen that lies flat on the surface instead of tilted (to the extent practicable).

- Saved using a format that preserves the image in the highest resolution possible.

- Saved with a filename that is consistent within the digital library and shall include the following elements in the order listed below:
  - NCCA2015
  - Laboratory name (or abbreviation)
  - Sample number
  - Taxa name
  - Magnification (if applicable, otherwise indicate no magnification as "1x")
  - Date (format YYYYMMDD) that the photograph was taken.
  - Appendage of "e" if the photograph was edited (e.g., cropped).

  For example, on September 8, 2015, laboratory ABC identified the specimen in sample 1234 to be a *Capitella capitata* and took a digital photograph at a resolution of 40x and then cropped the photograph to eliminate extraneous material. The filenames of the original and edited photographs would be: NCCA2_ABC_1234_ capitella capitata_40x_20150908.gif and NCCA2_ABC_1234_ capitella capitata_40x_20150908e.gif.

Elutriate: Circulate water over the sample in order to wash away the lighter or finer particles of the detritus.

Inorganic material: Material that is not capable of further decay (e.g., gravel, sand, silt)

Integrated Taxonomic Information System (ITIS): Database with standardized, reliable information on species nomenclature and their hierarchical taxonomic classification.

NARS: National Aquatic Resource Surveys. The National Coastal Condition Assessment (NCCA) is part of the NARS program.

NARS Information Management (IM) System: The IM system established to support all surveys, including NCCA, in the NARS program. The IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. The samples are collected during the field stage of NCCA.

Organic material: Material derived from living organisms that is capable of further decay (e.g., leaves, sticks, algae).

Percent sorting efficiency (PSE): Number of organisms recovered by sorter (A) compared to the combined (total) number of recoveries by the sorter (A) and independent sorter (B) for a sample (sorter B sorts through pickate and counts only organisms missed by Sorter A).

$$PSE = \frac{A}{A+B} \times 100 \quad (1)$$

Percent disagreement in enumeration (PDE): measure of taxonomic precision comparing the number of organisms, n_1, counted in a sample by the primary taxonomist with the number of organisms, n_2, counted by the internal or external QC taxonomist.

$$PDE = \frac{|n_1 - n_2|}{n_1 + n_2} \times 100 \quad (2)$$

Percent taxonomic disagreement (PTD): measure of taxonomic precision comparing the number of agreements (positive comparisons, $comp_{pos}$) of the primary taxonomist and internal or external QC taxonomists. In the following equation, N is the total number of organisms in the larger of the two counts.

$$PTD = \left[1 - \frac{comp_{pos}}{N}\right] \times 100 \quad (3)$$

Pickate: This is the remaining material left from the tray after the sorter has removed all benthic macroinvertebrates. This could include small stones, sticks or leaves, etc.

Primary laboratory: The laboratory that 1) sorts the sample; and 2) provides the first identification of benthic macroinvertebrates in the sample.

Secondary laboratory: The laboratory selected by the External QC Coordinator. It provides an independent identification of the benthic macroinvertebrates in the sample. The secondary laboratory must provide QC taxonomists who did not participate in the original identifications for the sample.

Target taxonomic levels: Target taxonomic levels for the NCCA is typically species (lowest practical level). NCCA excludes meiofauna (due to being smaller than 0.5 mm) from identifications. Additional exceptions include Oligochaeta (Class) and Chironomidae (Family) in samples from marine, polyhaline and mesohaline regions **ONLY**.

Taxonomic Bench Sheet: Form used by the laboratory to record information about the sample during the identification procedure.

Taxonomic Serial Number (TSN): stable and unique identifier that the Integrated Taxonomic Information System (ITIS), Encyclopedia of Life, and/or Catalogue of Life

couples with each scientific name to serve as the "common denominator" for accessing information. ITIS numbers are preferred, but when they are not available, secondary sources are acceptable.

a)

4.3.2 Laboratory

The procedure may be used by any laboratory that demonstrates competency in analytical work and quality procedures as documented by any one or more of the following::

1. Analytical work: To demonstrate its expertise, the laboratory shall provide EPA with one or more of the following:
    a. Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
    b. Memorandum describing experience with analyses that are the same or similar to the requirements of this method.
    c. Dated copy of relevant Accreditation or Certification (NELAC, ISO, state, etc.) for the laboratory and/or its experts who will perform and/or oversee the analyses. The accreditation must be for the entirety of analysis that the laboratory will be performing.
    d. Memorandum that describes the laboratory's participation in round robin studies and/or performance studies.
    e. Report of findings from an on-site technical assessment or audit.

2. Quality procedures.
    a. To demonstrate its expertise in quality assurance and quality control procedures, the laboratory shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable Standard Operating Procedures (SOPs).
    b. To demonstrate its ongoing commitment, the person in charge of quality issues for the laboratory shall sign the NCCA 2015 QAPP Certification Page.

3. Reporting standardized data. To demonstrate its expertise, the laboratory shall provide EPA with a memorandum that confirms that the laboratory has a computerized Laboratory Information Management System (LIMS) routinely used to track samples and record laboratory results. The memorandum also shall confirm that the laboratory will use LIMS to record and report results from the procedure.

4.3.3 Personnel

The procedure may be used by any person who has received training in processing and identification of benthic macroinvertebrates. For purposes of this procedure, EPA assumes that the following personnel are responsible for performing specific duties:

Internal Taxonomy QC Officer provides oversight of daily operations, sample processing, monitors QC activities at the laboratory to determine conformance, and conducts performance and systems audits of the procedures. The laboratory must retain documentation for the qualifications for the Internal Taxonomy QC Officer meeting the following requirements. The laboratory must provide, or otherwise make available, this documentation to EPA upon request. The Internal Taxonomy QC Officer is an experienced taxonomist who:

1. Demonstrated an initial enumeration and identification proficiency (as measured by PDE≤5% and PTD≤15%.
2. Maintains enumeration and identification proficiency in periodic QC checks (i.e., 1 in 10 samples with a minimum of one sample checked).

External QC Coordinator is an EPA staff person. Because the assigned duties are primarily administrative in nature, the External QC Coordinator is not required to have laboratory experience, although such experience would be preferable.

External QC Taxonomists, are selected by the External QC Coordinator (after consultation with EPA experts), and have demonstrated expertise and experience to be used as a quasi "gold standard" for taxonomic evaluations.

Taxonomists are trained, and have considerable experience, in identifying benthic macroinvertebrates, i.e., taxonomy. It is also important that the taxonomist maintains contact with other taxonomists through professional societies and other interactions, and keeps up with the pertinent literature, since systematics and species identifications change over time. EPA prefers, but does not require, that the freshwater taxonomists are certified by the Society of Freshwater Science (SFS). Each laboratory must submit the resume or *curriculum vitae* for the taxonomists who identify benthic macroinvertebrates for the NCCA samples to the EPA Project QC Officer.

Sorters are laboratory technicians who have basic training in laboratory procedures. An "experienced" sorter is one that has achieved ≥90% sorting efficiency in 5 consecutive samples.

4.3.4 Equipment/Materials

The procedure requires the following equipment and materials for sample preparation (subsampling), sorting, and taxonomic identifications.

4.3.4.1 Sample Preparation (Subsampling) and Sorting Equipment/Materials

- U.S. 35 sieve (500 µm)
- Round buckets
- Standardized, possibly, gridded screen (40 Mesh (380-µm openings, T304 stainless steel wire, 34GA (0.010"))
- 6-cm scoop
- White plastic or enamel pan (6" x 9") for sorting
- Teaspoon

- Permanent ink pen (e.g Pigma Micron® pen)
- Dropper
- Fine-tipped forceps (watchmaker type, straight and curved)
- Vials with caps or stoppers
- Sample labels for vials
- 70-80% ethanol
- Stereo zoom microscope (6-10X magnification)

4.3.4.2 Taxonomy Identification Equipment/Materials

- Stereo dissecting microscope with fiber optics light source (50-60X magnification)
- Compound microscope (10, 40, and 100X objectives, with phase-contrast capability)
- Digital camera with high resolution capability mounted on a microscope
- Petri dishes
- Microscope slides (1" x 3" flat, precleaned)
- Cover slips (appropriately sized)
- CMCP-10 (or other appropriate mounting medium)
- Permanent ink pen (e.g Pigma Micron® pen)
- Dropper
- Fine-tipped forceps (watchmaker type, straight and curved)
- Vials with caps or stoppers
- Sample labels for vials
- 70 - 80% non-denatured ethanol in plastic wash bottle
- Taxonomic Bench Sheet (Attachment 4.1 provides an example)
- Hand tally counter

4.4 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel should start the following login steps within 24 clock hours of receiving a delivery.

1. Record receipt of samples in the NARS IM system (within 24 clock hours) and the laboratory's Information Management System (LIMS). Assign the appropriate chronological bench number to each sample. Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM (see Chapter 2 for contact information).

2. Inspect each jar THE SAME DAY THEY ARE RECEIVED:
   a. Add 70-80% formalin to the jar, if necessary (i.e., to cover the contents completely).
   b. Verify that the site identification and sample number on the label also appear on the chain of custody form in the shipment.
   c. Notify the EPA HQ Laboratory Review Coordinator (see contact information in Chapter 2) if any jars were broken and/or there are discrepancies between the custody form and jars.

3. Store the sample containers at room temperature until sorting begins. If the sample will be stored for a long time before sorting, replace the formalin with ethanol for better preservation of the organisms.

4. Maintain the chain-of-custody form with the samples; it will be needed if the samples are transported to any other location (e.g., for taxonomic identification, external QC evaluation).

5. Verify that the login information includes the required data elements in Table 4.1. After completing all required elements, provide the information to the data entry personnel.

Table 4.4.1 Benthics Macroinvertebrates Login: Required Data Elements

FIELD	FORMAT	DESCRIPTION	
LAB NAME	Character	Name of lab	
LAB ID (optional)	Character	Lab sample id	
DATE RECEIVED	MMDDYY	Date sample was received by lab	
SITE ID	Character	NCCA site identification code as used on sample label	
VISIT NUMBER	Numeric	Sequential visits to site (1 or 2, if specified on label)	
SAMPLE ID	Numeric	Sample number as used on field sheet (on sample label)	
DATE COLLECTED	Date	Date sample was taken	
SALINITY	Numeric	Salinity: Value is provided on the sample label	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NP	Not enough preservative used
		Q	Other quality concerns, not identified above (explain in COND_COMMENTS)
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)	

4.5 Sample Preparation and Picking Organisms

This section describes the steps for the sorter in preparing the sample and picking organisms.

1. Remove the lid from the sample container and remove the internal sample label.

2. Carefully decant the formalin from the sample container by pouring the fluid through a sieve (U.S. 35) into a separate container. Inspect the mesh of the sieve for any organisms and return any organisms found to the sample container so they can be included in the sample sort process.

3. Remove sieved organisms from the sample container and place into a sorting tray.

4. Sort all samples under a minimum of 6x (maximum of 10x) dissecting microscope. Remove the macroinvertebrates from the detritus with forceps. In general, do not remove:
    - Empty snail or bivalve shells
    - Organisms of water surface-dwelling or strict water column[2] arthropod taxa, and meiofauna.
    - Incidentally-collected terrestrial taxa.
    - Fragments such as legs, antennae, gills, wings, or tails.

   For Oligochaeta, attempt to remove only whole organisms or fragments that include the head. In other words, do not remove fragments without the head.
    - In case of uncertainties, place the organism in the sort vial for the taxonomist to make the final determination.

5. Place picked organisms of the same type into a single set of jars and vials containing 70-80% ethanol.

6. This QC step is performed if: 1) the sorter (sorter A) has not reached 90% proficiency in 5 consecutive samples (referred to as the "proficiency QC check" below); or 2) this sample is the 1 in 10 sample QC check for experienced sorters (referred to as the "periodic QC check" below). For this step, a second sorter (sorter B):
    - Performs QC checks using the same power microscope as the sorter;
    - Extracts any missed organisms found in the pickate from Sorter A and places them into the sample vial, or other suitable sample vial;
    - Notes the number of organisms missed; and
    - Adds that number to the final count of the sample.
    - Calculates the PSE for the sample (see Section 4.3.1 for definition; equation 1). If the PSE is:
        - <90% and the sample is the:
            - Proficiency QC check, a second sorter must check the next 5 samples until the original sorter has PSE≥90% for 5 consecutive samples.
            - Periodic QC check, then a second sorter examines the original sorter's samples since the last QC check for missed organisms. The original sorter must again demonstrate proficiency by achieving a PSE≥90% in 5 consecutive samples.
        - ≥90% and the sample is the:
            - Proficiency QC check, the sample counts towards the 1 in 5 consecutive samples used to establish proficiency.
            - Periodic QC check, no corrective action is required.
    - Records the results from the QC step. The laboratory must record the results from all QC steps, even if they exceed the frequency required by this step. The laboratory must provide the sorter QC results to EPA upon request.

[2]Strict water column taxa are those that do not have at least one life stage that is benthic (i.e., bottom-dwelling).

7. Remove the remaining material left on the sorting pan (i.e. material such as sticks, organic debris) and place it in a separate container with preservative (70-80% ethanol). Label the container "Pickate," on both internal and external labels.

8. Label the vials and jars of sorted organisms and material using permanent ink (e.g., using a Pigma Micron® pen). Internal sample labels should be made of cotton rag paper or an acceptable substitute.

9. Retain the vials and materials for the time period specified in Section 4.8.

10. Thoroughly clean all sample preparation and sorting equipment and make sure all equipment is free of organisms prior to sorting the next sample.

4.6 Taxonomic Identification

The taxonomist performs the following steps in identifying the benthic macroinvertebrate organisms:

1. Upon receipt of a set of sample vials from the sorter:
    a. Compare all site identification codes and sample numbers on the form with those entered on the labels of samples, and resolve any discrepancies with the sorter.
    b. Determine if any vials are broken. For any broken vial, attempt to recover as much of the sample as possible. Describe the damage in the LAB_COMMENTS field in the database.
    c. Maintain the chain-of-custody form with the sample vials; it will be needed to return/store them.

2. Empty one sample vial at a time into a small Petri dish. Add 70-80% ethanol to keep the organisms covered. Remove the internal sample label and complete the top portion of a Taxonomic Bench Sheet (for an example, see Attachment 4.1), using the information from the label. Depending on the type of organisms, select the appropriate step:
    a. For all *Chironomidae* organisms, extract the organisms from the Petri dish.
        i. Prepare slide mounts using CMCP-10 (or CMC-9, CMC-10, or other media) and applying a coverslip. All organisms must be visible, which generally means a maximum of 10-20 organisms per slide. Label the slides with the same sample identification code or log-in number as the ethanol organisms.
        ii. If the laboratory prefers to use another method than slide mounting, the EPA External QC Coordinator will grant a waiver if the following applies:
            1) The request is for a laboratory located at a single location. For example, EPA would *not* consider the combined qualifications of a prime contract laboratory and its subcontract laboratories. Instead, for whichever laboratories met the requirements, EPA would evaluate and grant (or deny) a waiver for the prime contract laboratory separate from each of its subcontractor laboratories.

2) The request for a waiver must identify and describe a minimum of three studies. For each study, the external QC evaluation must demonstrate that the laboratory met or exceeded the NCCA QC requirements (i.e., PDE≤5% and PTD≤15%) for its *Chironomidae* organisms.
3) The laboratory agrees to mount the organisms on slides if it fails one of the periodic (NCCA) external QC evaluations, as follows:
    a. It must mount all *Chironomidae* organisms in samples processed since the previous external QC evaluation (i.e., for which it met the PDE and PTD requirements).
    b. It must continue to mount all *Chironomidae* organisms for the unprocessed samples.

b. For all other organisms, remove similar organisms to other dishes (keep these covered with 70-80% ethanol).

3. View the sample to ensure that all necessary diagnostic characters have been observed, according to the taxonomic key or other literature using:
    a. A stereo dissecting microscope for organisms in dishes.
    b. A compound microscope for slides of *Chironomidae* and *Oligochaeta* organisms

4. Identify organisms to the lowest practical taxonomic level (species is the target for all organisms with the exception of meiofauna, (due to being smaller than 0.5 mm). Additional exceptions include Oligochaeta (Class) and Chironomidae (Family) in samples from marine, polyhaline and mesohaline regions **ONLY**. If a laboratory or individual taxonomist is having trouble reaching species for a taxonomic group (not for an individual organism which might be damaged or otherwise difficult to identify), the lab must contact the NCCA project lead for guidance. Add any necessary data qualifiers (see list provided with Required Data Elements in Table 4.2).
    a. Enter the Taxonomic Serial Number (TSN) as it appears in the column "Unique Identifier" of the taxa list provided by EPA.
    b. Note whether the identification of a group of organisms is distinct (Distinct=Y/N) from other organisms in the same sample as follows:
        i. If the organisms can be identified to the target level, then Distinct="Y."
        ii. If an organism cannot be identified to the target level then assign values as follows:
            1) If at least some of the organisms in the sample can be identified to the target level, then:
                a. Distinct="Y" for organisms identified at the target level; and
                b. Distinct="N" for organisms that were identified at a higher taxonomic level (e.g., family) that may contain a target level taxa already identified in a given sample (e.g., genus).
                c. An example would be, if some organisms from a sample are identified to *Macoma*, but other organisms in the sample could only be identified to *Tellinidae* and/or

Veneroida, then *Macoma* would be distinct, but *Tellinidae* and/or *Veneroida* would not be Distinct.

2) If none of the organisms in the sample could be identified at the target level, then:
    a. Distinct=”Y” for organisms identified at the lowest taxonomic level (e.g., family); and
    b. Distinct=”N” for organisms identified at a higher level (e.g., order).
    c. For example, if a taxonomist can identify a number of *Veneroida* (Order) families, but a number of the organisms could not be taken past *Veneroida*, then the individual families would be distinct, but the order would not be distinct.

Record the identifications. For example, using the taxonomic bench sheet in Attachment 4.1, record the identification in the Column labeled "taxon." Enter the number of larvae, pupae, and adults, or total count (e.g. mollusks), if appropriate life history column does not apply, of each taxon under the appropriate columns.

iii. If the target taxonomic level cannot be achieved due to immature or damaged organisms this should be noted in the data file in the QA_FLAG field (e.g., QA_FLAG=IM). Table 4.2 provides other codes for the QA_FLAG field.

iv. If damaged organisms can be identified, they are counted ONLY if the:
    1) Fragment includes the head, and, in the case of arthropods, the thorax;
    2) Oligochaetes have a sufficient number of segments in the head;
    3) Mollusk shell (bivalve or gastropod) is occupied by a organism;
    4) Organism is the sole representative of a taxon in the sample.

v. If a unique taxon is determined for which the appropriate taxonomic level is not available in the literature and there are other taxa in that taxonomic level:
    1) Provide good quality digital photographs of the organism to outside experts for identification; and
    2) Include the tentative identification in the database with a data qualifier code of QA_FLAG='UN' so that these organisms can be distinguished from other organisms in the data analysis.
    3) When the outside expert identifies the organism, update the database with the correct identification.

5. Compare taxa names from the taxa list provided by EPA to the names used for the identifications. Check the non-matches for the following common problems and correct them.
    a. Abbreviations
    b. Extra information identifiers (e.g., sp., spp., , nr., cf., genus 1, w/ hair chaetae)
    c. Extra character (e.g., "?", "Acentrella ?turbida", blank space)
    d. The word "probably" or "prob" (e.g., "Microcylloepus prob. similis")
    e. Double names (e.g., Callibaetis callibaetis)
    f. Common misspellings
    g. Tribes/subfamilies/subgenus sometimes may not appear
    h. Species with incorrect genus (Hydatopsyche betteni)
    i. Split level taxonomy (e.g., Cricotopus/Orthocladius)
    Invalid name (e.g., taxonomic change, synonym; Sphaeriidae vs. Pisiidae)

6. Complete the identification by entering the totals for each developmental stage and the total number of each taxon in the cells at the bottom of the sheet. Cross-check to be sure the totals were summed correctly.

7. Provide the data to the Internal Taxonomic Officer for another review to confirm that the identifications use the same nomenclature as the taxa list provided by EPA and the laboratory's reference collection.

8. Make two copies of the bench sheet or computer file used to record the identifications. They are distributed as follows: 1) the project file; and 2) EPA's External QC Coordinator.

9. Prepare a list of primary and secondary technical literature used in completing the identifications. Provide complete citations in bibliographic format, including authors' names, date of publication, title of document, name of journal or publisher, volume and page numbers, or ISBN number, as appropriate. These citations will be kept on file with the Internal Taxonomic QC Officer, who will periodically review the reference collection to ensure that it is complete.

10. Verify that the reference collection contains at least one organism that represents each genus (or lowest taxonomic level) identified from all sample. For any missing references, choose an appropriate organism(s) from the sample to represent a taxon name in the master taxa list:
    a. Place the physical specimen in the reference library.
    b. Place two labels in the sample container to identify: organisms placed in the reference collection, and those in the non-reference organisms.
    c. Obtain a good quality representative digital photographs of the specimen (see instructions in Section 4.3.1).

11. If the Internal Taxonomy QC Officer selects the sample for a QC check, the Internal Taxonomy QC Officer re-counts and re-identifies the organisms in the sample following the same steps above for the original taxonomist. One in 10 of the taxonomist's samples must be checked. The Internal Taxonomy QC Officer records the independent verifications on a bench sheet or computer file. The Internal Taxonomy QC Officer will also supply a list of taxa that were found to be problematic during their QC sorting check, which can be submitted in an Excel or Word document format. (If the Internal Taxonomy QC Officer performs the QC check more frequently, then all QC data must be submitted.)

12. Carefully return the rest of the organisms to the original sample vial, fill with 70-80% ethanol, and cap tightly.

13. Re-package the samples and slide-mounted organisms carefully, and sign and date the chain-of-custody form. Return or store the samples according to laboratory protocols and requirements in Section 4.8.

14. Verify that all required data elements in Table 4.2 have been recorded by the taxonomist and Internal Taxonomy QC Officer. If the results were recorded on paper, provide the Taxonomic Bench Sheet to the data entry personnel.

Table 4.2 Benthic Macroinvertebrates Taxonomic Identification: Required Data Elements

FIELD	FORMAT	DESCRIPTION
LAB NAME	Character	Name of lab

FIELD	FORMAT	DESCRIPTION	
LAB ID (optional)	Character	Lab sample id	
DATE RECEIVED	Date	Date sample was received by lab	
SITE ID	Character	NCCA site identification code as used on sample label	
VISIT NUMBER	Numeric	Sequential visits to site (1 or 2, if specified on label)	
SAMPLE ID	Numeric	Sample number as used on field sheet (on sample label)	
DATE COLLECTED	Date	Date sample was taken	
DATE TAXON	Date	Date that the taxonomist started identifying organisms in the sample	
ANALYST NAME	Character	Name of taxonomist or Internal Taxonomy QC Officer (if record provides results of QC check)	
QC VERIFICATION	Character	Y if the record provides the results from the QC check	
FAMILY	Character	Taxonomic family	
SUBFAMILY	Character	Taxonomic subfamily	
TRIBE	Character	Taxonomic tribe	
GENUS GROUP	Character	Taxonomic genus group (e.g., *thienemannimyia*)	
GENUS	Character	Taxonomic genus	
SPECIES	Character	Taxonomic species	
TSN	Numeric	Taxonomic Serial Number as defined by "UniqueIdentifier" in taxa list provided by EPA. If taxon is not in this list, provide citation for reference used to identify organism in CITATION field	
LAB TIN (OPTIONAL)	Character	Lab taxa ID number	
TAXANAME	Character	Unique taxon name in the taxa list provided by EPA	
ABUNDANCE LARVAE	Numeric	Number of individual larvae or immature bugs	
ABUNDANCE PUPAE	Numeric	Number of individual pupae	
ABUNDANCE ADULT	Numeric	Number of individual adults	
ABUNDANCE TOTAL	Numeric	Total number of individuals	
DISTINCT	Character	Distinct taxa in sample (y/n) (See description in Section 4.6)	
CITATION	Character	Citation for reference used to identify organism, if taxon not present in taxa list provided by EPA database	
QA FLAG (if appropriate)	Character	QA/QC flag (lab may use its own flags, if defined in QA_COMMENTS field or provided to NARS IM team)	
		Flag	Definition
		DD	Damaged Organism, poor condition or fragments
		IM	Immature
		IN	Indeterminate (explain in QA_COMMENTS field)
		NP	Not enough preservative used
		NT	Not able to meet target level for identification (may be used with other codes, or explain in QA_COMMENTS field)
		S	Sample shipping problem (explain in QA_COMMENTS field)

FIELD	FORMAT	DESCRIPTION	
		UN	Unknown. Identification is tentative. Organism has been sent to expert taxonomist for definitive identification.
		Q	Other quality concerns, not identified above
QA_COMMENTS	Character	Explanation for QA FLAG (if needed)	
LAB COMMENTS	Character	General laboratory analysis comments	

4.7 Data Entry

Tables 4.1 and 4.2 identify the required data elements that the sorting and taxonomic laboratories must provide to EPA, preferably in EPA's data template, available separately from EPA. In addition, the laboratory must provide the resume or *curriculum vitae* for each taxonomist who identifies benthic macroinvertebrates for the NCCA samples. The resume or *cv* for each taxonomist is submitted once to EPA's External QC Coordinator.

4.8 Sample and Record Retention

The laboratory shall retain:

1. The sample materials, including vials, slides, and sorting residuals, for a minimum of 3 years from the date the EPA publishes the final report. During this time, the laboratory shall store the materials in a cool location away from sunlight. The laboratory shall periodically check the sample materials for degradation and refill jars and vials with 70-80% ethanol if necessary.

2. Original records, including laboratory notebooks and the reference library, for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

4.9 External Taxonomic Quality Control

EPA requires that all NCCA laboratories ("primary laboratories") participate in the External Taxonomic Quality Control Evaluation. Each taxonomist must participate in the QC evaluation, even if the taxonomist is under subcontract with, or consulting for, another firm.

In contrast to the internal QC evaluation in Section 4.6 that verify adherence to the procedures and ensures in-laboratory consistency between taxonomists, the purpose of the external QC evaluation is to ensure consistency between laboratories and taxonomists. To achieve this objective, EPA compares the primary laboratory results to those from a second laboratory, considered a quasi "gold standard" for taxonomic evaluations.

The External QC Coordinator, who is an EPA staff member, is responsible for selecting and managing the "QC contractor." To eliminate the appearance of any inherent bias, the QC contractor must be dedicated to QA/QC functions, and thus, must not be a primary laboratory or a field sampling contractor for NCCA. The QC contractor is responsible for complying with instructions from the External QC Coordinator; obtaining and managing the secondary laboratory; coordinating and paying for shipments of the QC samples between locations; comparing sample identifications by different laboratories; facilitating reconciliation teleconferences; and preparing brief summary reports.

The External QC Coordinator will arrange for the QC contractor to conduct a minimum of two QC evaluations. To the extent practicable, the External QC Coordinator and QC contractor will schedule batch evaluations evenly throughout the project period.

Each QC evaluation consists of the following steps:

1. In consultation with the QC contractor, the External QC Coordinator determines an appropriate time to conduct the evaluation based upon the total number of samples assigned to the laboratory, the delivery schedule, processing schedule, and the following constraints:
    a. Availability of samples from other laboratories. For example, if three state laboratories are each processing less than 30 samples, the External QC Coordinator might combine their samples into one batch for the QC evaluation.
    b. If a primary laboratory is responsible for processing 100 samples or more for the NCCA, the External QC Coordinator will split their samples into several batches (e.g., each 50 to 100 samples) so that EPA can evaluate and correct performance on an ongoing basis.

2. The External QC Coordinator provides the QC contractor with a list of laboratories and processed samples. Sample identification includes the site identification code, sample number, and taxonomist who performed the identifications.

3. The QC contractor randomly selects 10% of the samples from each NCCA laboratory, subject to the following constraints:
    a. If the primary laboratory received fewer than 30 samples, then the QC contractor randomly selects three samples for the evaluation.
    b. For each taxonomist identified on the list, the QC contractor ensures that the selection includes one or more of his/her samples.
    c. The External QC Coordinator may elect to provide an initial evaluation of the national laboratory by selecting a small batch from the samples that the laboratory completed in the first 2-3 months.

4. The QC contractor provides a list of the QC samples, and instructions, to the External QC Coordinator and each primary laboratory participating in the evaluation. Although the External QC Coordinator and QC contractor may tailor the instructions for the participating taxonomists' preferences, the instructions are likely to specify the following:

a. Pack and ship the QC samples to the central holding facility designated by the QC contractor. Instructions are likely to require that the:
    i. Shipments contain chain-of-custody documentation for all slides and containers.
    ii. Containers (e.g., slides, vials) include the site identification code and sample number.
    iii. Containers cannot be marked in any way that might identify the taxonomic classification for any organism.
    iv. The number of taxa in a vial or container should be based on practical considerations (e.g., size of animals and amount of ethanol needed for preservation, amount of ethanol allowed in a single shipment to meet DOT shipping requirements).
b. Track the QC samples using forms provided by the QC contractor.
c. Email a spreadsheet with the data for the QC samples to the External QC Coordinator. (EPA requires that all labs use its spreadsheet template for recording the taxonomic data.)

5. The QC contractor reviews the condition of the QC samples (e.g., verifies that the containers do not identify taxon for any organism) and ships the samples to the secondary laboratory along with instructions and the EPA template for reporting data.

6. Within 24 hours of receipt, the secondary laboratory:
    a. Notifies the QC contractor that it has received the samples;
    b. Faxes or emails any additional receipt records, including discrepancies, within 24 hours; and
    c. Completes any other instructions from the QC contractor.

7. The secondary laboratory:
    a. Re-identifies and re-counts following the procedures in the Method, except does not:
        i. Develop a reference library.
        ii. Photograph organisms unless the taxa are identified for reconciliation discussion.
        iii. Perform any internal QC checks.
    b. Records the required data elements in Section 4.7.
    c. Enters the data using EPA's spreadsheet template for the taxonomic data.
    d. Emails the completed spreadsheet to the QC contractor.

8. The QC contractor compares the original taxonomic results (i.e., data) generated by the primary laboratory to the taxonomic results generated by the secondary laboratory for each sample. As part of this evaluation, the QC contractor calculates PDE and PTD using the equations in Section 4.3.1 and compares their values to the QC requirements in the Section 4.10.

9. If any samples exceed the PDE or PTD limits in Section 4.10, the QC contractor consults with the External QC Coordinator to determine if reconciliation calls are necessary to

resolve differences. The External QC Coordinator may decide that a reconciliation call is unnecessary if there appears to be an obvious explanation for differences, few samples are affected, or other reasons.

10. The QC contractor schedules and facilitates reconciliation teleconferences with EPA and the laboratories.
    a. In preparation for the teleconferences:
        i. The QC contractor instructs the secondary laboratory to photograph representative specimens for each taxon identified for discussion.
        ii. The QC contractor provides the participants with a spreadsheet that includes:
            1. List of samples and taxon identifications for discussion;
            2. Relevant data from the primary and secondary laboratories; and
            3. PDE and PTD values.
        iii. The primary and secondary laboratories provide participants with the relevant reference (or citation) and photograph for each taxonomic identification for the discussion.
        iv. The QC contractor emails a meeting announcement for a convenient time for all participants. The email identifies instructions for accessing the External QC Coordinator's toll-free teleconference line.
    b. Within a week after the teleconference, the QC contractor sends an email to the External QC Coordinator and other teleconference participants that summarizes:
        i. Agreements to use common nomenclature for discrepancies;
        ii. Commitments to reevaluate identifications by reexamining samples;
        iii. Application of changes that are appropriate for all samples, not just the QC samples (e.g., common nomenclature)
        iv. Items that will not be resolved for some reason (e.g., sample degraded during shipment).

11. After completing the reconciliation calls, the participants complete the following steps:
    a. Secondary laboratory:
        i. Reexamines samples as deemed necessary during the reconciliation call
        ii. Updates its database with changes to:
            1. QC samples per reexamination and other items in the QC contractor email; and
            2. Non-QC samples as appropriate (e.g., nomenclature changes apply to all samples, not just QC samples).
        iii. Provides database to QC contractor.
    b. QC contractor confirms that the secondary laboratory (i.e., its subcontractor) completed its assignments before allowing the secondary laboratory to move to the next step.
    c. Secondary laboratory stores its original records, including laboratory notebooks and the reference library, for a minimum of 10 years from the date that EPA publishes the final report.
    d. Secondary laboratory and QC contractor follow steps 4 and 5 above to return the samples to the primary laboratory.

e. After receiving the samples (and tracking per step 4), the primary laboratory:
    i. Reexamines samples as deemed necessary during the reconciliation call;
    ii. Updates its database with changes to:
        1. QC samples per reexamination and other items in the QC contractor email; and
        2. Non-QC samples as appropriate (e.g., nomenclature changes apply to all samples, not just QC samples)
    iii. Provides the revised database to the External QC Coordinator (not the QC contractor). It also confirms that it has completed all relevant items identified in the QC contractor's email summary of the teleconferences (from Step 10.b).
f. QC contractor provides EPA with a report or memorandum that:
    i. Identifies the participating laboratories, with the following information about each laboratory:
        1. Laboratory name
        2. Address
        3. Contact person (name, telephone, and email)
    ii. Quantifies the taxonomic precision (PDE and PTD) as they were prior to the reconciliation call;
    iii. Assesses data acceptability;
    iv. Highlights taxonomic problem areas;
    v. Identifies any discrepancies for which the External QC Coordinator determined that a reconciliation teleconference was not necessary;
    vi. Identifies primary and secondary laboratory commitments to change its identifications or provide additional review of any organisms; and
    vii. Provides recommendations for improving precision for other samples not included in the QC evaluation.

12. After review, the External QC Coordinator:
    a. Submits the report, and draft technical direction with next steps for the laboratory, to the EPA staff managing or coordinating with the primary laboratory.
    b. Determines if significant differences within the batch of QC samples warrant re-identification of samples by the primary laboratory and a second QC evaluation by the secondary laboratory. If deemed necessary, EPA will instruct the primary laboratory to include the samples for review with the next batch of QC samples.

As an additional verification on the generation of the data, EPA may conduct assistance visits at the laboratories. If EPA decides to conduct an assistance visit, a qualified EPA scientist or contractor will administer a checklist based upon the steps described in this chapter. The objective of the visit would be to:

- Confirm the laboratory is properly implementing the steps in the method.
- Assist with questions from laboratory personnel.
- Suggest corrections if any errors are made.

4.10 Quality Assurance/Quality Control (QA/QC)

Equation 4.1 Percent sorting efficiency (PSE)
Number of organisms found by the sorter (A) compared to the combined (total) number of found by the sorter (A) and the number recovered by the QC Officer (B) from Sorter A's pickate for a sample. PSE should be ≥90%.

$$PSE = \frac{A}{A+B} \times 100$$

Equation 4.2 Percent disagreement in enumeration (PDE)
Measure of taxonomic precision comparing the number of organisms, n_1, counted in a sample by the primary taxonomist with the number of organisms, n_2, counted by the internal or external QC taxonomist. PDE should be ≤5%.

$$PDE = \frac{|n_1 - n_2|}{n_1 + n_2} \times 100$$

Equation 4.3 Percent taxonomic disagreement (PTD)
Measure of taxonomic precision comparing the number of agreements (positive comparisons, $comp_{pos}$) of the primary taxonomist and internal or external QC taxonomists. In the following equation, N is the total number of organisms in the larger of the two counts. PTD should be ≤15%.

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N}\right)\right] \times 100$$

Table 4.3 Benthic Macroinvertebrates: Measurement Data Quality Objectives

Variable or Measurement	Precision	Accuracy
Sort and Pick	90% [a]	90% [a]
Identification	85% [b]	95% [c]

NA = not applicable; [a] As measured by PSE; [b] As measured by (100%-PTD); [c] As measured by (100%-PDE)

Table 4.4 Benthic Macroinvertebrates: Laboratory quality control

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
SAMPLE PROCESSING AND SORTING			
Sample pickate examined by another sorter	10% of all samples (minimum of 1)	PSE ≥ 90%	If < 90%, examine all residuals of samples by that sorter and retrain sorter

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
	completed per sorter		
IDENTIFICATION			
Duplicate identification by Internal Taxonomy QC Officer	1 in 10 samples per taxonomist,	PTD ≤15%	If PTD >15%, reidentify all samples completed by that taxonomist since last meeting the acceptance criteria, focusing on taxa of concern
Independent identification by outside, expert, taxonomist	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxa	Record both tentative and independent IDs
External QC	10% of all samples completed per laboratory	PDE ≤ 5% PTD ≤ 15%	If PDE > 5%, implement recommended corrective actions. If PTD > 15%, implement recommended corrective actions.
Use of widely/commonly accepted taxonomic references by all NCCA labs	For all identifications	All keys and references used by each lab must be on bibliography prepared by one or more additional NCCA labs or in The taxa list provided by EPA. This requirement demonstrates the general acceptance of the references by the scientific community.	If a lab proposes to use other references, the lab must obtain prior permission from External QC Officer before submitting the data with the identifications based upon the references.
Prepare reference collection	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Internal Taxonomy QC Officer periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate
DATA VALIDATION			
Taxonomic "reasonableness" checks	All data sheets	Taxa known to occur for coastal waters or Great Lakes.	Second or third identification by expert in that taxon

4.11 References

Epler, J.H. 2001. Identification manual for the larval chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. Special Publication SJ2001-SP13. North Carolina Department of Environment and Natural Resources, Raleigh, NC, and St. Johns River Water Management District, Palatka, FL. 526 pp.

Merritt, R.W., K.W. Cummins, and M.B. Berg (editors). 2008. An Introduction to the Aquatic Insects of North America, 4rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.
Stribling, J.B., S.R. Moulton, and G.T. Lester. 2003. "Determining the quality of taxonomic data." Journal of the North American Benthological Society 22(4):621-631.

USEPA. 2012. National Rivers and Streams Assessment 2013-2014: Laboratory Operations Manual. EPA-841-B-12-010. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Attachment 4.1: Benthic Macroinvertebrates: Taxonomy Bench Sheet (example)

Laboratory Information		Sample Information	
Project ID		Sample ID	
Station Name		Site ID	
Station Location		Date Collected	
Station Number		Field Crew ID	

Taxonomist Name_____
Date 1st Organism Identified in Sample:_____ **QC Check?** Y / N

TSN (Use # in UniqueIdentifier from taxa list provided by EPA)	Taxon	Distinct (Y/N)	Counts of Organisms in the Taxon:				Cumulative Number of Organisms in Sample	Data Qualifier (Codes in Table 4.2)
			Total (any stage)	Larvae	Pupae	Adults		

Comments:

5.0 WHOLE BODY FISH PROCESSING AND CONTAMINANT ANALYSIS

This chapter describes fish processing and analysis requirements for whole body fish samples. The purpose is to determine concentrations of contaminants in fish samples collected in the 2015 NCCA and related studies. The laboratory shall perform analysis to determine the lipid content, concentrations of metals, mercury, pesticides, and PCBs found in fish within coastal waters and Great Lakes. EPA also may require the national contract laboratory to analyze the samples for PAHs; however, EPA will not require the State laboratories to analyze for them.

At each sampling site, the Field Operations Manual (FOM) instructs the crews to collect five fish of the same species (or 10 sea urchins of any species) and similar size for each sample. The crew, or EPA's batch laboratory, then ships the fish specimens on dry ice to the laboratory.

In the following discussion, Sections 5.1, 5.2, and 5.3 summarize the procedure; health and safety concerns; and definitions and required resources. Section 5.4 provides the steps for acknowledging sample receipt. Section 5.5 provides the steps for creating whole fish composites. Sections 5.6 – 5.7 provide the minimum requirements that the laboratory must meet in performing the contaminant analyses and the required data elements. Section 5.8 describes EPA's external review of laboratory operations and other quality measures. Section 5.9 identifies references used in developing the procedure.

5.1 Summary of the Procedure

This chapter describes the fish processing and contaminant determination of whole fish samples collected for EPA's 2015 National Coastal Condition Assessment (NCCA). To ensure consistent and uncontaminated fish preparation activities across all samples, it is important that all NCCA participating laboratories adhere to the fish preparation procedures described in Section 5.5. The procedure is an adaption of instructions developed for fish tissue preparation for the National Rivers and Streams Assessment. As described in Section 5.6 the laboratory may choose to use any method that meets EPA's specifications for contamination measurements unless contractually bound to use specific methods (note, those methods must still meet EPA's specifications for contamination measurements)..

5.2 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions. In addition to the laboratory's usual requirements such as a Chemical Hygiene Plan, the laboratory must adhere to the following health and safety procedures:

1. Laboratory facilities must properly store and dispose of solutions of weak acid.

2. Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).

3. When working with potential hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with acid. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

4. When operating grinding equipment, the laboratory personnel must exercise caution.

5.3 Definitions and Required Resources (Personnel, Laboratories, and Equipment)

This section provides definitions and required resources for using the procedure.

5.3.1 Definitions

The procedure uses the following terms:

Detection Limit is the minimum concentration at which the analyte can be *detected* with confidence. In other words, the outcome can be reported with confidence that it is greater than zero (i.e., present in the sample). Also see "Sample-Specific Detection Limit."

Duplicates are defined as two aliquots of the same sample which are analyzed separately using identical procedures. The results are used to evaluate the precision of the laboratory analyses.

Fish Composite: Each composite consists of all parts of the fish including the head, skin, internal organs, muscle, and bones. For sea urchins, it includes only the gonad tissue because it is essentially the only tissue present. Unless otherwise specified, references to "fish" include "sea urchins." With the exception of sea urchins, NCCA does not provide support for analyses of any other invertebrates such as crustacean (e.g., lobster, crabs).

NARS: National Aquatic Resource Surveys. The National Coastal Condition Assessment (NCCA) is part of the NARS program.

NARS Information Management System (NARS IM): The IM system established to support all surveys, including NCCA, in the NARS program. The IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. Freshwater and coastal samples will be collected during the field stage of NCCA.

Non-routine sample: A non-routine sample is any sample that does not meet the definition of a routine sample. See Section 5.5.1 for more information.

Percent Recovery: Recovery is measured by comparing the concentrations of a sample split into two parts; and one part is spiked with a known concentration value. C_s is the

concentration measured in the spiked part; *C* is the concentration measured in the unspiked part; and *s* is the known concentration amount for the spike. The following equation is used to calculate the percent recovery:

$$\%Rs = \frac{C_s - C}{s} \times 100$$

Relative Standard Deviation (RSD): The precision at each concentration is reported in terms of the RSD. To calculate the RSD, first calculate the standard deviation, *S*, as follows:

$$S = \left[\frac{1}{n-1}\sum_{k=1}^{n}(C_s - \bar{C})^2\right]^{1/2}$$

where *n* is the number of replicate samples, *C*, is the concentration measure for the kth sample, and $\bar{C}$ is the average concentration of the replicate samples. Then, RSD is calculated as:

$$RSD = \left|\frac{S}{\bar{C}}\right| \times 100$$

Reporting Limit: A reporting limit is the point at which the measured value of the analyte can be reported with confidence.

Routine sample: A routine composite sample consists of individual adult fish of a single species that meet EPA's length requirement (Length of smallest fish in the composite must be at least 75% of the length of the longest fish),, and sufficient number of fish to meet target mass of 300 grams. See Section 5.5.1 for more information.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method's detection limit. For diluted samples, the sample-specific detection limit will be the product of the method's detection limit and the dilution factor. Typical values for the dilution factors will be 10 or 100.

Spiked Sample: See Percent Recovery definition for purpose of spiked samples.

TOCOR: Task Order Contracting Officer's Representative is EPA's contact person for laboratories under contract to EPA.

5.3.2 General Requirements for Laboratories

Competency: To demonstrate its competency, the laboratory shall provide analyte and matrix specific information to EPA. EPA will accept one or more of the following as a demonstration of competency:

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.

- Documentation detailing the competency of the organization, including professional certifications for fish-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.

Also, the lab must provide a demonstration of competency with fish samples in achieving the method detection limits, accuracy, and precision targets.

Quality assurance and quality control requirements.
To demonstrate its expertise in quality assurance and quality control procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NCCA QAPP Certification Page.

5.3.3 Personnel

The procedure refers to the following personnel:

Laboratory Technician: This procedure may be used by any laboratory technician who is familiar with the NCCA Quality Assurance Project Plan, and this procedure in the NCCA Laboratory Operations Manual.

External QC Coordinator is an EPA staff person who is responsible for selecting and managing the "**QC contractor**." To eliminate the appearance of any inherent bias, the QC contractor must be dedicated to QA/QC functions, and thus, must not be a primary laboratory or a field sampling contractor for NCCA. The QC contractor is responsible for complying with instructions from the External QC Coordinator; coordinating and paying for shipments of the performance samples to participating laboratories; comparing immunoassay results from the laboratories; and preparing brief summary reports.

5.3.4 Equipment/Materials

The procedures require the following equipment and information:

- Scale
- Powder-free nitrile gloves
- Tape measure
- 5% nitric acid
- Deionized water (DI water)
- Grinding equipment
- Glass containers
- Jars

5.4 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery. The laboratory must inspect the samples promptly on receipt. As samples arrive, the laboratory must:

1. Log the samples into the National Aquatic Resource Survey Information Management system (NARS-IM) within 24 clock hours. Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM (see Chapter 2 for contact information).

2. Check that each shipping container has arrived undamaged. Check the temperature of one of the samples in the cooler using a thermometer that reads to at least -20 ºC (i.e., the expected temperature of frozen samples), or an infra-red (IR) temperature "gun" and record the reading. Record the condition and temperature of the sample in the database using the codes in Table 5.1.

3. Compare the information on the label on each individual fish specimen to the sample tracking form for each composite and verify that each specimen was included in the shipment and is properly wrapped and labeled. The crew labels each fish specimen using the sample identification code and appends a specimen identification code. For example, if the sample number is "NCCA15-1111," then the crew might label specimen "A" as "NCCA15-1111.A." Record the number of fish in each sample.

4. Weigh each sample (i.e., all fish specimens collectively), record the weight in the database, and confirm that the sample meets the weight requirements of 140 grams (g) for a routine sample. If the sample weight is less than the required minimum, contact EPA for instructions, which are likely to involve preparing fewer aliquots for possibly fewer types of analyses than originally intended (e.g., perhaps EPA might eliminate the pesticides analysis for the sample).

5. Verify that all required data elements, per Table 5.1, have been recorded. If any elements are missing, then enter them into the database.

6. Transfer the samples to the freezer for long-term storage. Except during processing and analysis stages, the samples must be stored frozen to less than or equal -20 °C.

7. Notify the EPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 5.1 Whole Body Fish Login: Required Data Elements

Variable	Type	Description
SITE_ID	Character	Site identification code
SAMPLE	Character	Sample number
DATE_COLLECT	Date	Date that the field crew collected the sample

Variable	Type	Description		
	ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory (fish should be frozen).	
	NUMBER_FISH	Numeric	Number of fish in the sample	
	SAMPLE_WT	Numeric	Total weight of sample (all fish)	
	CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
			Flag	Definition
			OK	Sample is in good condition
			C	Sample wrapping is cracked
			L	Sample or container is leaking
			ML	Sample label is missing
			NF	Sample is not at proper temperature
			Q	Other quality concerns, not identified above
	COND_COMMENT	Character	Explanation for Q FLAG (if needed)	

5.5 Whole Fish Preparation and Homogenization Procedures

This section describes the whole fish preparation and homogenization procedures. As described in Section 5.5.1, if a laboratory determines that a sample is non-routine, the laboratory contacts the EPA HQ NCCA Laboratory Review Coordinator (Chapter 2 provides contact information) for additional instructions before continuing with the compositing and homogenization procedures in Section 5.5.2. Section 5.5.3 describes rigorous equipment cleaning and rinsate collection steps used before the compositing and homogenization steps in Section 5.5.4.

5.5.1 Sample Classification: Routine or Non-Routine

Each sample is either a "routine" composite sample, or a "non-routine" composite sample, based on the following definitions:

- *Routine sample* – A routine composite sample consists of individual adult fish of a single species that meet EPA's length and other requirements. For example, the species must be one of the target species identified in Appendix B of this LOM. The laboratory homogenizes the fish to prepare one composite sample.
- *Non-routine sample* – A non-routine sample is any sample that does not meet the definition of a routine sample. When field crews collect non-routine samples, depending on the circumstances, EPA will provide instructions for processing, or possibly destroying, the non-routine samples. These instructions also may include discarding some of the fish in the composite sample based on size before proceeding with homogenizing. For non-routine composites, the laboratory homogenizes only the designated specimens, i.e., those that EPA identifies by specimen number.

Note: Non-routine samples do not include samples from an incorrect sampling location, an unnecessary duplicate sample, or inappropriate fish species. EPA does not plan on using these "invalid" samples, so it is imperative that the sample preparation laboratory not process any sample without specific instructions from EPA. Therefore, laboratories shall retain such samples in frozen storage until EPA determines the appropriate course of action, which may include processing the sample. If the status of any composite sample in the instructions is not clear, the laboratory must contact EPA and wait for clarification.

5.5.2 Fish Examination and Preparation

This section describes the steps for fish examination and preparation.

1. Put on powder-free nitrile gloves (if not already gloved) before unpacking individual fish specimens. For sea urchins, wear thick rubber gloves to provide protection from the urchin spines. As samples are unpacked and unwrapped, inspect each fish carefully for any damage (e.g., tears in the skin or punctures in the gut). Document any damage in comments per Table 5.2.

2. The field crews measured the total length of each fish specimen in the field and recorded those lengths on the sample tracking form. Because of the importance of length measurements, EPA requires laboratories to perform a second series of measurements of the length for each fish. Because it may be difficult to reproduce the field measurements of fish length when the specimens are still partially frozen, begin processing the specimens in the following steps:
    a. Lay them out in order by specimen number (e.g., the portion of the sample ID after the decimal point)
    b. Allow them to partially thaw to the point that each specimen can be laid relatively flat.
    c. Using the length data on the sample tracking form (or the relative length order data in the fish sample processing instructions spreadsheet), confirm that the specimen ID for the longest specimen recorded on the tracking form is the same as the specimen ID on the label of the longest specimen. Repeat this relative length comparison for each of the other specimen IDs to ensure that the length orders based on the recorded lengths in the sample tracking form are consistent with the specimen IDs on the individual fish labels. This check is important for confirming that the field crews attached the correct label to each fish in the composite sample.
    d. Record the required data elements per Table 5.2 for the length of each species.

8. Weigh each fish to the nearest gram (wet weight) prior to any sample processing. In the database, record the required weight data elements per Table 5.2 for each specimen.

9. Identify and record the species of each fish specimen. Confirm that the species is one of the target species listed in Appendix B of this LOM.

10. Determine if the sample is routine or non-routine (per classification definitions in Section 5.5.1) and record its classification and any applicable fish code from Table 5.3. Return

any non-routine sample to the freezer and contact the EPA HQ NCCA Laboratory Review Coordinator for processing instructions (see Chapter 2 for contact information).

11. Verify that all required data elements, per Tables 5.2 and 5.3, have been recorded. If any elements are missing, then enter them into the database.

12. Rinse each fish with deionized water and remove any adhering slime as a precautionary measure to treat for possible contamination from sample handling in the field. Use HDPE wash bottles for rinsing fish and for cleaning homogenization equipment and utensils. Do **NOT** use Teflon® wash bottles for these procedures.

13. Return to freezer for storage until ready to homogenize the sample. If the laboratory intends to proceed directly to homogenization, then allow the sample to partially thaw while cleaning the equipment as described in the next section.

Table 5.2 Whole Body Fish: Data Elements for Each Fish Specimen

Variable	Type	Description
SITE_ID	Character	Site identification code
SAMPLE	Character	Sample number
SPECIMEN_ID	Character	Identification code assigned to a single fish
SPECIES	Character	Species of fish
FISH_WT	Numeric	Weight of fish
WT_UNIT	Character	Units of fish weight (kg, lb)
FISH_LEN	Numeric	Length of fish
LEN_UNIT	Character	Units of fish length (cm, in)
COMMENT	Character	Comment about condition of fish or other observations

Table 5.3 Whole Body Fish: Data Elements from Examination of Each Sample

Variable	Type	Description		
SITE_ID	Character	Site identification code		
SAMPLE	Character	Sample number		
SAMPLE_CLASS	Character	Sample classification: Routine or Non-routine		
FISH CODE	Character	Codes describing any deviations from the FOM criteria for fish collection for each sample		
		Flag	Definition	
		SP	Not all specimens are of the same species	
		LE	Not all specimens lengths are within 75% of longest fish	
		NS	Specimen number is fewer than minimum of 5 or greater than 20 maximum	

Variable	Type	Description		
			WT	Mass does not meet minimum of 140 grams *
			LL	Longest fish exceeds 400 mm maximum length
			LS	Shortest fish below 100 mm minimum length
			Q	Other quality concerns, not identified above

* Field crews are required to collect a minimum of 300 grams, but the minimum required for laboratory analyses is 140 grams.

5.5.3 Equipment Cleaning and Rinsate Collection

This section describes the rigorous cleaning required to protect against cross-contamination of samples. To verify that the cleaning procedures are effective, EPA requires the collection of rinsate samples as described below.

1. Before processing any sample, thoroughly clean all of the homogenization equipment. Disassemble the homogenization equipment (i.e., blender, grinder, or other device) and thoroughly **clean all surfaces and parts** that contact the sample. Similarly, **clean all knives, cutting boards, and other utensils used**. The cleaning steps are as follows:
    a. Wash with a detergent solution (phosphate- and scent-free) and warm tap water
    b. Rinse three times with warm tap water
    c. Rinse three times with deionized (DI) water
    d. Rinse with acetone
    e. Rinse three times with DI water
    f. Rinse with (not soak in) 5% nitric acid
    g. Rinse three times with DI water
    h. Allow the components to air dry
    i. Reassemble the homogenization equipment

2. Once per batch (i.e., once per maximum of 20 samples), collect rinsate samples for use in assessing any equipment contamination. To minimize the number of project samples that might be affected by cross contamination, collect the normal rinsate samples on the first day that samples in a batch of 20 are processed. Ideally (not required), the laboratory will vary the point at which the rinsates are collected on that first day over the course of the project (e.g., between the 1st and 2nd samples for one batch, the 2nd and 3rd samples for another batch, etc.). Prior to reassembling the homogenization equipment, use the following steps to prepare enough rinsate samples for the relevant QA/QC activities:
    a. Prepare each **hexane rinsate sample** by pouring a 100-mL portion of pesticide-grade hexane over all parts of homogenization equipment, including the cutting boards and knives, and collect it in a clean glass container. Place an additional 100-mL aliquot of clean hexane in a similar glass container for use as a solvent blank. Allow the solvent to evaporate from the equipment. Per QA/QC requirements, the laboratory will analyze the rinsate and solvent blank for the

Polychlorinated biphenyls (PCBs), pesticides, and Polycyclic Aromatic hydrocarbons (PAHs) selected for NCCA analysis.

b. Once the hexane has evaporated, prepare **each DI water rinsate** using 250 mL of DI water. Collect the DI water rinsate in a clean glass or HDPE container. Place a second aliquot of DI water in a separate similar clean container for use as a blank. Acidify these two samples to pH < 2 with nitric acid. Per QA/QC requirements, the laboratory will analyze the rinsate and blank samples for metals and mercury.
c. Store the rinsates and blanks at a cold, not freezing, temperature (<6 ºC).

5.5.4 Compositing and Homogenization Procedure

This section describes the steps for a "batch" homogenization method that uses the entire homogenized volume of all fish specimens to prepare the composite. In contrast to an "individual" method that would combine equal weights of tissue from each specimen, the batch homogenization method uses the complete specimens regardless of each individual specimen's proportion to one another. The steps are as follows:

1. Change gloves *between* samples. The technician may use the same gloves in handling all fish *within* a given sample.

2. Partially thaw samples for ease of grinding during homogenization.

3. For sea urchins, prepare the sea urchin for compositing by cracking open the shell of each sea urchin in the sample. From all of the sea urchins in the sample, extract and composite only the gonad tissue. (The gonad tissue is essentially the only tissue present in sea urchins.)

4. Process each sample using a size-appropriate homogenization apparatus (e.g., automatic grinder or high-speed blender). If difficulties arise with the samples sticking to equipment, try the following:
    a. Chill the grinder briefly with a few small pieces or pellets of dry ice.
    b. Add pellets of dry ice to the specimens as they enter the grinder.

5. Mix the specimens thoroughly until completely homogenized as evidenced by a final composite sample of soupy composition with uniform color and texture. Visible chunks or pieces of skin, bone, or tissue (e.g., liver tissue has red bits) will hinder extraction and digestion and, therefore, are NOT acceptable.

6. Grind the sample a second time, using the same grinding equipment. It is not necessary to clean the grinding equipment between grinding cycles of the same sample. This second grinding should proceed more quickly. The final sample must have a soupy composition with uniform color and texture. If there are obvious differences in color or texture, grind the entire sample a third time.

7. Prepare the sample aliquots for each type of analysis (e.g., mercury, PCBs) and place any remaining sample materials in a separate jar. Table 5.4 provides target mass weights needed for each type of analysis. When filling jars, leave sufficient space, at least 20%,

at the top of each jar to allow for expansion of the tissue as it freezes. *Jars filled beyond 80% capacity may break when freezing.* Wipe off the outside of the jars to remove any residue or moisture. Label each container and place inside one heavy-weight food-grade self-sealing plastic freezer bag to avoid sample loss due to breakage. Freeze the tissue aliquots at -20 ºC, and maintain samples in the freezer until analysis.

8. For one sample in every batch (same batch as specified for the rinsate samples collected in Section 5.5.3), the laboratory conducts triplicate analyses of the lipid content to confirm that the grinding has resulted in an homogeneous sample. As with the collection of rinsate samples, the laboratory performs the homogeneity testing on the first day on which samples in a batch of 20 are processed. However, the sample chosen for homogeneity testing must be one that yields enough tissue mass to support the added mass needed for triplicate lipid aliquots (15 to 30 g).

    a. The laboratory selects one sample processed on the first day of every batch that will provide well over 140 g of total tissue mass.

    b. From that sample, place three 5- to 10-g aliquots in clean glass or plastic containers of suitable size and label as appropriate.

    c. Calculate the mean lipid content (in percent), the standard deviation (SD), and the relative standard deviation (RSD) as follows:

$$\text{mean } \%\,\text{lipids} = \frac{\sum_{i=1}^{3} (\%\,\text{lipids})_i}{3}$$

$$\text{SD} = \sqrt{\frac{\sum_{i=1}^{3} (\%\,\text{lipids}_i - \text{mean lipids})^2}{2}}$$

$$\text{RSD} = \frac{\text{SD}}{\text{mean}}$$

    d. If the RSD of the triplicate results is:
        - Less than or equal to the QC criterion, then the homogenization effort is judged to be sufficient for all samples in that QC batch.
        - Otherwise, corrective action consists of regrinding all of the aliquots from each composite sample in the affected batch until meeting the QC criterion. This may entail retrieving all sample aliquots (see Table 5.4) from the freezer, allowing them to partially thaw, homogenizing them again, determining new lipids results, and performing a new homogenization QC determination. New sample containers are required for any rehomogenized samples. Also, follow the steps in Section 5.5.3 for cleaning the equipment between each composite sample in rehomogenizing the samples.

e. For this sample analyzed in triplicate, record the lipid content measured in the first analysis.

9. Before homogenizing the next sample, clean the **grinding equipment and all other sample preparation equipment** using the procedures described in Section 5.5.3.

Table 5.4. Whole Body Fish: Initial Aliquot Requirements

Analysis	Target Mass	Sample Jar Requirements
Mercury	5 - 10 g	50-mL HDPE straight-sided jar with **foil-lined lid**, or conical HDPE tube with snap top
Metals other than mercury	5 - 10 g	50-mL HDPE straight-sided jar with **foil-lined lid**, or conical HDPE tube with snap top
PCBs	30 - 35 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid
Pesticides	30 - 35 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid
PAHs (only by EPA request)	30 – 35 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid
Lipids	10 - 15 g	Laboratory's choice, as this aliquot will be used in-house to determine the lipid content of the sample
Maximum*	140 g	

*In the event that insufficient fish tissue mass exists to prepare the required number of aliquots, contact EPA for instructions.

5.6 Contaminant Analysis: Requirements

The laboratory shall perform analysis of the homogenized composites to determine the lipid content, concentrations of metals, mercury, pesticides, and PCBs. EPA also may require the national contract laboratory to analyze the samples for PAHs; however, EPA will not require the State laboratories to analyze for them. With the exception of sea urchins, NCCA does not provide support for analyses of any other invertebrates such as crustaceans (e.g., lobster, crabs).

After preparing the fish composites as described in Section 5.5, laboratories may choose to use any analysis method, including those in Table 5.5, that measures contaminants to the levels of the method detection limits identified in Table 5.6. In addition, the method must meet the target precision of 30% and the target accuracy as follows:

- Metals: 20%
- Organics (PCBs, pesticides, and PAHs): 35%

The laboratory must store the fish samples frozen at a maximum of -20° C and complete the analyses within one year.[7]

Table 5.5 Whole Body Fish: Analytical Methods

Analysis	Extraction	Methods that Meet the QA/QC Requirements (any method that meets the QA/QC requirements is acceptable)
Metals (except Mercury)	Any method using microwave assisted digestion[8]	EPA Method 6020A[9]
Mercury		EPA Method 245[10]
PCBs, Pesticides, PAHs	EPA Method 3540C[11]	EPA Method 8270[12]
Percent Lipids	Any method using hexane	EPA Method 9071B[13]

Table 5.6 Whole Body Fish: Lipids and Required Contaminants

Type	UNITS	Parameter	CAS Number	PCB Number (where applicable)	MDL Target
LIPID	% Wet Weight	% LIPID			
METAL	µg/wet g (mg/L)	Aluminum	7429-90-5		10.0
		Arsenic	7440-38-2		2.0
		Cadmium	7440-43-9		0.2
		Chromium	7440-47-3		0.1
		Copper	7440-50-8		5.0
		Iron	7439-89-6		50.0
		Lead	7439-92-1		0.1

[7] NCCA allows for a 1-year holding time because of the sheer volume of sample collected in a short amount of time. Generally, EPA recommends different holding times, see for example Appendix J "Recommended procedures for preparing whole fish composite homogenate samples" in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 (Fish Sampling and Analysis)*, 3rd Edition, 2000. EPA #823-B-00-007. Retrieved from http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/upload/2009_04_23_fish_advice_volume1_v1cover.pdf.

[8] For example, see Method 3150A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils," retrieved from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf.

[9] For example, Method 6020A "Inductively Coupled Plasma-Mass Spectrometry" retrieved from http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/6020a.pdf.

[10] For example, Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved from http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_245_7.pdf.

[11] For example, see Method 3540C "Soxhlet Extraction" retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3540c.pdf.

[12] For example, Method 8270D "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS) retrieved from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8270d.

[13] Method 9171B "n-Hexane Extractable Material (HEM) for Sludge, Sediment, And Solid Samples," retrieved from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/9071b.pdf.

Type	UNITS	Parameter	CAS Number	PCB Number (where applicable)	MDL Target
		Mercury	7439-97-6		0.01
		Nickel	7440-02-0		0.5
		Selenium	7782-49-2		1.0
		Silver	7440-22-4		0.3
		Tin	7440-31-5		0.05
		Vanadium	7440-62-2		1.0
		Zinc	7440-66-6		50.0
PCB	ng/wet g (µg/L)	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	2051-24-3	209	2.0
		2,4'-Dichlorobiphenyl	34883-43-7	8	2.0
		2,2',3,4',5,5',6-Heptachlorobiphenyl	35065-29-3	180	2.0
		2,2',3,3'4,4',5,6-Octachlorobiphenyl	52663-78-2	195	2.0
		2,2',3,4 ,5,5',6-Heptachlorobiphenyl	52663-68-0	187	2.0
		2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	128	2.0
		2,2',3,3'4,4',5-Heptachlorobiphenyl	35065-30-6	170	2.0
		2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138	2.0
		2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153	2.0
		2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206	2.0
		2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105	2.0
		2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101	2.0
		2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118	2.0
		2,3,3',4,6'-Pentachlorobiphenyl	38380-03-9	110	2.0
		3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	126	2.0
		2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44	2.0
		3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	77	2.0
		2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52	2.0
		2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66	2.0
		2,2',5-Trichlorobiphenyl	37680-65-2	18	2.0
		2,4,4'-Trichlorobiphenyl	7012-37-5	28	2.0
PEST	ng/wet g (µg/L)	2,4'-DDD	53-19-0		2.0
		2,4'-DDE	3424-82-6		2.0
		2,4'-DDT	789-02-6		2.0
		4,4'-DDD	72-54-8		2.0
		4,4'-DDE	72-55-9		2.0
		4,4'-DDT	50-29-3		2.0
		Aldrin	309-00-2		2.0
		Alpha-BHC	319-84-6		2.0
		Beta-BHC	319-85-7		2.0
		Delta-BHC	319-86-8		2.0
		Alpha-Chlordane	5103-71-9		2.0
		Gamma-Chlordane	5566-34-7		2.0
		Dieldrin	60-57-1		2.0

Type	UNITS	Parameter	CAS Number	PCB Number (where applicable)	MDL Target
		Endosulfan I	959-98-8		2.0
		Endosulfan II	33213-65-9		2.0
		Endosulfan Sulfate	1031-07-8		2.0
		Endrin	72-20-8		2.0
		Endrin Aldehyde	7421-93-4		2.0
		Endrin Ketone	53494-70-5		2.0
		Heptachlor	76-44-8		2.0
		Heptachlor Epoxide	1024-57-3		2.0
		Hexachlorobenzene	118-74-1		2.0
		Lindane	58-89-9		2.0
		Mirex	2385-85-5		2.0
		Cis-Nonachlor	5103-73-1		2.0
		Oxychlordane	26880-48-8		2.0
		Trans-Nonachlor	39765-80-5		2.0
PAHs*		Acenaphthene	83-32-9		2.0
		Acenaphthylene	208-96-8		2.0
		Anthracene	120-12-7		2.0
		Benz(a)anthracene	200-280-6		2.0
		Benzo(b)fluoranthene	205-99-2		2.0
		Benzo(k)fluoranthene	207-08-9		2.0
		Benzo(g,h,i)perylene	191-24-27-2		2.0
		Benzo(a)pyrene	50-32-8		2.0
		Benzo(e)pyrene	192-97-2		2.0
		Biphenyl	92-54-4		2.0
		Chrysene	218-01-9		2.0
		Dibenz(a,h)anthracene	53-70-3		2.0
		Dibenzothiophene	132-65-0		2.0
		2,6-Dimethylnaphthalene	581-42-0		2.0
		Fluoranthene	205-99-2		2.0
		Fluorene	86-73-7		2.0
		Indeno(1,2,3-c,d)pyrene	193-39-5		2.0
		1-Methylnaphthalene	90-12-0		2.0
		2-Methylnaphthalene	91-57-6		2.0
		1-Methylphenanthrene	832-69-9		2.0
		Naphthalene	91-20-3		2.0
		Perylene	198-55-0		2.0
		Phenanthrene	85-01-8		2.0
		Pyrene	129-00-0		2.0
		2,3,5-Trimethylnaphthalene	2245-38-7		2.0

* EPA also may require the national contract laboratory to analyze the samples for PAHs; however, EPA will not require the State laboratories to analyze for them.

5.7 Data Entry

Tables 5.1 (Section 5.4), 5.2 (Section 5.5), 5.3 (Section 5.5), and 5.7 (below) identify the required data elements that laboratories must provide to EPA, preferably in EPA's data template, available separately from EPA.

Table 5.7 Whole Body Fish: Data Elements for Each Sample

Variable	Type	Description		
SITE_ID	Character	Site identification code or type of QC sample (e.g., LAB BLANK)		
SAMPLE	Character	Sample number, LCS, QCCS, Blank, Matrix Spike, or Rinsate		
REPEAT	Numeric	Duplicate or Triplicate (otherwise blank)		
DATE_COLLECT	Date	Date that the field crew collected the sample		
	ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory (fish should be frozen).	
	NUMBER_FISH	Numeric	Number of fish in the sample	
	SAMPLE_WT	Numeric	Total weight of sample (all fish)	
	SAMPLE_CLASS	Character	Sample classification: Routine or Non-routine	
	CONDITION CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
			Flag	Definition
			OK	Sample is in good condition
			C	Sample wrapping is cracked
			L	Sample or wrapping is leaking
			ML	Sample label is missing
			NF	Sample is not at proper temperature
	COND_COMMENT	Character	Explanation for Q FLAG (if needed)	
	FISH CODE	Character	Codes describing any deviations from the criteria for fish collection for each sample	
			Flag	Definition
			SP	Not all specimens are of the same species
			LE	Not all specimens lengths are within 75% of longest fish
			NS	Specimen number is fewer than minimum of 5 or greater than 20 maximum
			WT	Mass does not meet minimum of 500 grams

Variable	Type	Description		
			LL	Longest fish exceeds 400 mm maximum length
			LS	Shortest fish below 100 mm minimum length
			Q	Other quality concerns, not identified above
PARAMETER	Character	Analyte name		
CAS_NO	Character	CAS Registry number corresponding to the analyte		
LABNAME	Character	Laboratory name (abbreviation)		
METHOD	Character	Laboratory method used		
ANALYST	Character	Last name or initials of person who performed the analysis		
REVIEWER	Character	Last name or initials of the person who provided a separate independent review of the data		
INSTRUMENT	Character	Identification of instrument used for the analysis – provide enough information to identify the particular instrument in the laboratory		
DATE PREPARED	Date	Date that the sample homogenization started		
DATE ANALYSIS	Date	Date that the sample analysis started		
QC_BATCH_LOT	Character	Unique laboratory quality control lot numbers assigned to the batch of samples. The lot number must associate each batch of field samples to the appropriate rinsates, laboratory control sample, matrix spike, laboratory duplicate, and method blank samples.		
HOLDING TIME	Y/N	Analysis performed within holding time		
MATRIX	Character	Fish		
MDL	Numeric	Lab method detection limit (based upon lab's historical data)		
LRL	Numeric	Lab reporting limit (based upon lab's historical data)		
DILUTION	Numeric	Dilution of sample (blank or 1 if no dilution)		
RECOVERY	Numeric	Only for appropriate QC samples		
RESULT	Numeric	Concentration value		
REASON	Character	Reason for qualification in RESULT_QUAL (usually blank)		
RESULT_QUAL	Character	Data qualifier (usually blank)		
UNIT	Character	Unit of measurement for RESULT, MDL, and RL		
QC_CODE	Character	Apply laboratory defined QC codes and describe in the comments field. Provide set of laboratory's code as part of the case narrative		
COMMENT	Character	Explain situation that created QC code, or any unusual aspects of the analysis		

5.8 Quality Measures

This section describes the quality assurance and quality control measures used to ensure that the data will meet NCCA's requirements.

5.8.1 Assistance Visits

Assistance visits are intended to familiarize EPA with actual procedures being implemented by different laboratories; and to ensure a clear and consistent understanding of procedures and activities by both EPA and the laboratories. If EPA decides to conduct an assistance visit, a qualified EPA scientist or contractor will administer a checklist based upon the steps described in this chapter.

5.8.2 QC Samples

Once or twice during the performance period, the External QC Coordinator will provide one or two identical sets of QC samples to all participating laboratories. Each set will contain up to five QC samples. As determined by the External QC Coordinator, the QC samples may be synthetic; aliquots of additional samples collected at NCCA sites; or reference samples obtained from an organization such as the National Institute of Standards. Each laboratory will run the QC samples following the same procedures used for the other samples. The External QC Coordinator will compare the results to the expected value and determine consistency between laboratories (e.g., determine if one laboratory is consistently higher or lower than all others). Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any unique laboratory practices that might account for differences between the laboratory and others. The contractor shall analyze the external QC samples using the same procedures as those for the field samples.

5.8.3 Summary of QA/QC Requirements

QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. The laboratory must conduct QC analyses for each batch of samples. Each batch shall consist of no more than 20 samples. Unique laboratory quality control lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate measures such as laboratory control sample, matrix spike, laboratory duplicate, and method blank samples. Also, each laboratory QC samples (i.e., preparation and instrument blanks, laboratory control sample (LCS), spike/duplicate, etc.) must be give a unique sample identification. Table 5.8 provides a summary of the quality control requirements.

Table 5.8 Whole Body Fish: Quality control activities

Quality Control Activity	Description and Requirements	Corrective Action
Demonstrate competency for analyzing fish samples with the required methods	Demonstration of competency with fish samples in achieving the method detection limits. accuracy, and precision targets	EPA will not approve any laboratory for NCCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can demonstrate competency for its NCCA samples.

Quality Control Activity	Description and Requirements	Corrective Action
Check condition of sample when it arrives.	Sample issues, such as punctures or rips in wrapping; missing label; temperature; adherence to holding time requirements; sufficient volume for test. All samples should arrive at the laboratory in a frozen state.	Assign appropriate condition code identified in Table 5.1.
Store sample appropriately. While stored at the laboratory, the sample must be kept at a maximum temperature of -20° C.	Check the temperature of the freezer per laboratory's standard operating procedures.	Record temperature of sample upon arrival at the laboratory. If at any other time, samples are warmer than required, note temperature and duration in comment field.
Determine if all fish meet the criteria	Evaluate if the sample contains fish of the same species and are similar in size (within 75%), and provides enough material to run the analysis	Contact the EPA HQ NCCA Laboratory Review Coordinator* for a decision on fish selection and/or chemical analysis.
Analyze sample within holding time	The test must be completed within the holding time (i.e., 28 days for mercury; 6 months for other metals; and 1 year for all others). If the original test fails, then the retest also must be conducted within the holding time.	Perform test, but note reason for performing test outside holding time. EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
Perform once at the start of each batch to evaluate the labeled compound recovery (LCR) in a Laboratory Control Sample (LCS). This tests the performance of the equipment.	Control limits for recovery cannot exceed 100±20%.	First, prepare and analyze one additional LCS. If the second blank meets the requirement, then no further action is required. If the second LCS fails, then determine and correct the problem before proceeding with any sample analyses.
Perform once at the start of each batch to evaluate the entire extraction and analysis process using a Method Blank	Control limits cannot exceed the laboratory reporting level (LRL).	First, prepare and analyze one additional blank. If the second blank meets the requirement, then no further action is required. If the second blank fails, then determine and correct the problem (e.g., homogenization, reagent contamination, instrument calibration, or contamination introduced during filtration) before proceeding with any sample analyses. Reestablish statistical

Quality Control Activity	Description and Requirements	Corrective Action
		control by analyzing three blank samples. Report values of all blanks analyzed.
Check calibration immediately before and immediately after the sample batch is run (abbreviated as QCCS for quality control check sample)	Results must be ±10% of each other or as specified in method criteria	If calibration fails before analysis, recalibrate and reanalyze QCCS until it passes. If check fails after all samples in the batch have been analyzed, verify the QCCS reading. If the QCCS reading fails a second time, then reanalyze all samples in the batch and report both sets of results. For the first run, include a data qualifier that indicates that the QCCS reading taken immediately following the first run failed. For the second run, include a data qualifier that indicates that it is the second set and whether the QCCS reading immediately following that second run passed. No sample is to be analyzed more than twice.
Evaluate rinsates for first sample in each batch. This evaluation is a surrogate for assessing cross-contamination.	Results must be below the LRL.	If first rinsate is above LRL, analyze rinsate from a second sample. If second rinsate sample also has results above the LRL, then assign a data qualifier to all samples in the batch for the parameters with results above the LRL in the rinsates. Also, improve procedures for cleaning all surfaces, knives, and homogenization equipment between samples.
Compare lipids in triplicate for the first sample in each batch. This evaluation is a surrogate for assessing homogenization.	Substitute the LRL for any value below the LRL before calculating the RSD. If the RSD of the triplicate results is ≤20%, then the homogenization effort is judged to be sufficient for all samples in the batch.	If the RSD could not be achieved, then regrind all samples in the batch one or more times as described in Section 5.5
Compare results of one laboratory duplicate sample or matrix spike duplicate sample for each batch	Results must be within the target precision goal in Table 5.8.1 (30% for all analytes).	If both results are below LRL, then conclude that the test has passed. Otherwise, prepare and analyze a split from different sample in the batch. If the second result is within the target precision goal (see Table 5.8.1) of the original sample, then

Quality Control Activity	Description and Requirements	Corrective Action
		report the data and findings for both QC samples. However, if the two results differ by more than the target precision goal, review precision of QCCS measurements for batch; check preparation of split sample; etc. and report evaluation and findings in the case narrative. Consult with the EPA HQ NCCA Laboratory Review Coordinator* to determine if reanalysis of the entire batch (at the laboratory's expense) is necessary. If no reanalysis is necessary, report and quantify all samples in batch. If reanalysis is necessary, then report all QC sample and the 2nd analysis of the batch. If the second set also is unacceptable, then assign a data code to each sample in the batch.
Compare results of one matrix spike sample per batch to evaluate performance in matrix	Evaluate performance after the first 3 batches. Ideally, control limits for recovery will not exceed the target accuracy goal (Table 5.8.1), but this may not be realistic for all parameters with this matrix.	If both results are below LRL, then conclude that the test has passed for the batch. Otherwise, if any results are not within the target accuracy goal for the 3 batches, within 2 working days, contact the EPA HQ NCCA Laboratory Review Coordinator* to discuss method performance and potential improvements. Continue to perform the test for every batch. Report the results from the original analysis, the matrix spike, matrix spike duplicate, and %recovery.
Maintain the required MDL identified in the Section 5.6	Evaluate for each sample	If MDL could not be achieved, then provide dilution factor or QC code and explanation in the comment field.
Use consistent units for QC samples and field samples	Verify that all units are provided in wet weight units and consistently within each indicator type as follows: Metals in µg/g or ppm. PCBs, pesticides, and PAHs in ng/g or µg/L.	If dry units are reported for any sample (QC or field), reanalyze the sample and report only the reanalysis results. If it is not possible to provide the results in wet units, then assign a QC code and describe the reason for dry

Quality Control Activity	Description and Requirements	Corrective Action
		units in the comments field of the database.
Maintain completeness	Completeness objective is 95% for all parameters.	Contact EPA HQ NCCA Laboratory Review Coordinator* immediately if issues affect laboratory's ability to meet completeness objective.

*Chapter 2 provides contact information for the EPA HQ NCCA Laboratory Review Coordinator. Laboratories under contract to EPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the Laboratory Review Coordinator.

5.9 Sample and Record Retention

The laboratory shall retain:

1. The sample materials, including vials, for a minimum of 3 years from the date the EPA publishes the final report. During this time, the laboratory shall freeze the materials. The laboratory shall periodically check the sample materials for degradation.

2. Original records, including laboratory notebooks and the reference library, for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

5.10 References

All references are from U.S. Environmental Protection Agency:

Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 (Fish Sampling and Analysis), 3rd Edition, 2000. Appendix J "Recommended procedures for preparing whole fish composite homogenate samples". EPA #823-B-00-007. Retrieved from http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/upload/2009_04_23_fish_advice_volume1_v1cover.pdf.

Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved from http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_245_7.pdf.

Method 3150A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils," retrieved from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf.

Method 6020A "Inductively Coupled Plasma-Mass Spectrometry" retrieved from http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/6020a.pdf.
Method 8270D "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS) retrieved from Method 8270D "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry.

Method 9171B "n-Hexane Extractable Material (HEM) for Sludge, Sediment, And Solid Samples," retrieved from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/9071b.pdf.

6.0 SEDIMENT CONTAMINANT, GRAIN SIZE, AND TOC ANALYSES

This chapter describes the analysis requirements for sediment samples. The purpose is to determine concentrations of contaminants, grain size, and total organic carbon (TOC) in sediment samples collected in the 2015 NCCA and related studies. The laboratory shall perform analysis to determine the moisture content, concentrations of metals, mercury, pesticides, and PCBs found in sediments in coastal waters and Great Lakes.

At each sampling site, the Field Operations Manual (FOM) instructs the crews to collect sediment samples. The field crew then ships the samples on wet ice to either its own state laboratory or EPA's batching laboratory. Once the samples arrive, the laboratory will freeze the samples for the contaminant analyses and refrigerate the grain size and TOC samples.

In the following discussion, Sections 6.1, 6.2, and 6.3 summarize the procedure; health and safety concerns; and definitions and required resources. Section 6.4 provides the steps for acknowledging sample receipt. Sections 6.5 – 6.6 provide the minimum requirements that the laboratory must meet in performing the contaminant analyses and the required data elements. Section 6.7 describes EPA's external review of laboratory operations and other quality measures. Section 6.8 identifies references used in developing the procedure.

6.1 Summary of the Procedure

This chapter describes the contaminant, grain size, and TOC determination of sediment samples collected for EPA's 2015 National Coastal Condition Assessment (NCCA). As described in Section 6.5, unless otherwise contractually bound by other requirements, the laboratory may choose to use any method that meets EPA's specifications for contamination measurements.

6.2 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions. In addition to the laboratory's usual requirements such as a Chemical Hygiene Plan, the laboratory must adhere to the following health and safety procedures:

1. Laboratory facilities must properly store and dispose of solutions of weak acid.

2. Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).

3. When working with potential hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with acid. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

6.3 Definitions and Required Resources (Personnel, Laboratories, and Equipment)

This section provides definitions and required resources for using the procedure.

6.3.1 Definitions

The procedure uses the following terms:

Detection Limit is the minimum concentration at which the analyte can be *detected* with confidence. In other words, the outcome can be reported with confidence that it is greater than zero (i.e., present in the sample). Also see "Sample-Specific Detection Limit."

Duplicates are defined as two aliquots of the same sample which are analyzed separately using identical procedures. The results are used to evaluate the precision of the laboratory analyses.

NARS: National Aquatic Resource Surveys. The National Coastal Condition Assessment (NCCA) is part of the NARS program.

NARS Information Management System (NARS IM): The IM system established to support all surveys, including NCCA, in the NARS program. The IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. Freshwater and coastal samples will be collected during the field stage of NCCA.

Percent Recovery: Recovery is measured by comparing the concentrations of a sample split into two parts; and one part is spiked with a known concentration value. C_s is the concentration measured in the spiked part; C is the concentration measured in the unspiked part; and s is the known concentration amount for the spike. The following equation is used to calculate the percent recovery:

$$\%Rs = \frac{C_s - C}{s} \times 100$$

Relative Percent Difference (RPD): Relative percent difference compares the matrix spike (S) and the matrix spike duplicate (D) using the following equation:

$$RPD = \frac{|S - D|}{(S + D)/2} \times 100$$

Reporting Limit: A reporting limit is the point at which the measured value of the analyte can be reported with confidence.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method's detection limit. For diluted samples, the sample-specific detection limit will be the product of the method's detection limit and the dilution factor. Typical values for the dilution factors will be 10 or 100.

Spiked Sample: See Percent Recovery definition for purpose of spiked samples.

TOC: Total Organic Carbon

TOCOR: Task Order Contracting Officer's Representative is EPA's contact person for laboratories under contract to EPA.

6.3.2 General Requirements for Laboratories

Competency. To demonstrate its competency, the laboratory shall provide analyte and matrix specific information to EPA. EPA will accept one or more of the following as a demonstration of competency:

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
- Documentation detailing the competency of the organization, including professional certifications for water-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.
- Demonstration of competency with sediment samples in achieving the method detection limits, accuracy, and precision targets.

Quality assurance and quality control requirements.
To demonstrate its competency in quality assurance and quality control procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NCCA QAPP Certification Page.

6.3.3 Personnel

The procedure refers to the following personnel:

Laboratory Technician: This procedure may be used by any laboratory technician who is familiar with the NCCA Quality Assurance Project Plan, and this procedure in the NCCA Laboratory Operations Manual.

External QC Coordinator is an EPA staff person who is responsible for selecting and managing the "**QC contractor**." To eliminate the appearance of any inherent bias, the QC contractor must be dedicated to QA/QC functions, and thus, must not be a primary laboratory or a field sampling contractor for NCCA. The QC contractor is responsible for complying with instructions from the External QC Coordinator; coordinating and paying for shipments of the performance samples to participating laboratories; comparing immunoassay results from the laboratories; and preparing brief summary reports.

6.3.4 Equipment/Materials

The analytical methods, selected by the laboratory, specify the required equipment.

6.4 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery. The laboratory must inspect the samples promptly on receipt. As samples arrive, the laboratory must:

1. Log the samples into the National Aquatic Resource Survey Information Management system (NARS-IM) within 24 clock hours. Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM (see Chapter 2 for contact information).

2. Check that each shipping container has arrived undamaged. Check the temperature of one of the samples in the cooler using a thermometer that reads from 21 ºC (i.e., room temperature) down to -20 ºC or lower (i.e., the expected temperature of frozen samples), or an infra-red (IR) temperature "gun" and record the reading. Field crews ship sediment samples on wet ice; the batch laboratory freezes the sample and ships with dry ice. Record the condition and temperature of the sample in the database using the codes in Table 6.1.

3. Verify that all required data elements, per Table 6.1, have been recorded. If any elements are missing, then enter them into the database.

4. Transfer the samples to the freezer for long-term storage. Except during processing and analysis stages, the samples must be stored frozen to less than or equal -20 °C.

5. Notify the EPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 6.1 Sediment Chemistry, Grain Size, and TOC Login: Required Data Elements

Variable	Type	Description	
SITE_ID	Character	Site identification code	
SAMPLE	Character	Sample number	
DATE_COLLECT	Date	Date that the field crew collected the sample	
	ANALYSIS_TYPE	Character	Contaminant, TOC, or GRAIN SIZE
	ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory
	CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control

Variable	Type	Description		
			Flag	Definition
			OK	Sample is in good condition
			C	Sample container is cracked
			L	Sample or container is leaking
			ML	Sample label is missing
			Q	Other quality concerns, not identified above
	COND_COMMENT	Character	Explanation for Q FLAG (if needed)	

6.5 Laboratory Analysis: Requirements

The laboratory shall perform analysis of the sediment samples to determine the moisture content, grain size, and concentrations of TOC, metals, mercury, pesticides, PAHs, and PCBs.

Table 6.2 identifies the storage requirements. Laboratories may choose to use any analysis method, including those in Table 6.2, which measures the parameters to the levels of the method detection limits identified in Table 6.3. In addition, the contaminant analysis method must meet the precision and accuracy targets of 30% and 20%, respectively. For each batch of contaminant samples, precision is assessed using the relative percent difference (RPD) between the matrix spike (MS) and the matrix spike duplicate (MSD); and accuracy by the average percent recovery (%Rs) between the matrix spike and matrix spike duplicate. Section 6.3.1 provides the equations used to calculate the RPD and %Rs. The precision and accuracy targets for each batch of TOC are both 10% and determined by the RPD of one sample and its duplicate (for precision) and the analysis of Certified Reference Material (CRM; for accuracy). The grain size target precision is 10% as determined using a Laboratory Control Sample (LCS) (accuracy is not applicable).

Table 6.2 Sediment Chemistry, Grain Size, and TOC: Analytical Methods

Storage Requirements	Type	Methods that Meet the QA/QC Requirements (any method that meets the QA/QC requirements is acceptable)
Freeze samples with maximum of -20° C	Metals (except Mercury)	Extraction: EPA Method 3051A Analysis: EPA Method 6020A[14]

14 For example, see:

- Method 3051A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, And Oils" retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf; and
- Method 6020A "Inductively Coupled Plasma-Mass Spectrometry" retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6020a.pdf.

	Mercury	EPA Method 245.7[15]
	PCBs, Pesticides, PAHs	Extraction: EPA Method 3540C Analysis: EPA Method 8270D[16]
	TOC	Lloyd Kahn Method[17]
Refrigerate at 4° C (do not freeze)	Grain Size	Any method that reports the determination as %silt and meets QA/QC requirements

Table 6.3 Sediment Chemistry, Grain Size, and TOC: Required Parameters

Type	UNITS	Parameter	CAS Number	PCB Number (where applicable)	MDL Target
	% sand and % silt/clay	Grain Size	not applicable		0.05%
	mg/kg	Total Organic Carbon (TOC)	not applicable		0.01%
METAL	dry weight µg/g (ppm)	Aluminum	7429-90-5		1500
		Antimony	7440-36-0		0.2
		Arsenic	7440-38-2		1.5
		Cadmium	7440-43-9		0.05
		Chromium	7440-47-3		5.0
		Copper	7440-50-8		5.0
		Iron	7439-89-6		500
		Lead	7439-92-1		1.0
		Manganese	7439-96-5		1.0
		Mercury	7439-97-6		0.01
		Nickel	7440-02-0		1.0
		Selenium	7782-49-2		0.1
		Silver	7440-22-4		0.3
		Tin	7440-31-5		0.1
		Vanadium	7440-62-2		1.0
		Zinc	7440-66-6		2.0
PCB	dry weight ng/g (ppb)	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	2051-24-3	209	1.0
		2,4'-Dichlorobiphenyl	34883-43-7	8	1.0
		2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170	1.0

15 For example, see Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved June 27, 2014 from http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_245_7.pdf.

16 For example, see:

- Method 3540C "Soxhlet Extraction" retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3540c.pdf; and
- Method 8270D "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS) retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8270d.pdf.

17 For example, the "Lloyd Kahn Method" developed by Lloyd Kahn at EPA Region II and retrieved from www.nj.gov/dep/srp/guidance/rs/lloydkahn.pdf.

Type	UNITS	Parameter	CAS Number	PCB Number (where applicable)	MDL Target
		2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187	1.0
		2,2',3,4',5,5',6-Heptachlorobiphenyl	35065-29-3	180	1.0
		2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	128	1.0
		2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138	1.0
		2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153	1.0
		2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206	1.0
		2,2',3,3',4,4',5,6-Octachlorobiphenyl	52663-78-2	195	1.0
		2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105	1.0
		2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101	1.0
		2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118	1.0
		2,3,3',4,6'-Pentachlorobiphenyl	38380-03-9	110	1.0
		3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	126	1.0
		2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44	1.0
		3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	77	1.0
		2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52	1.0
		2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66	1.0
		2,2',5-Trichlorobiphenyl	37680-65-2	18	1.0
		2,4,4'-Trichlorobiphenyl	7012-37-5	28	1.0
PEST	dry weight ng/g (ppb)	2,4'-DDD	53-19-0		1.0
		2,4'-DDE	3424-82-6		1.0
		2,4'-DDT	789-02-6		1.0
		4,4'-DDD	72-54-8		1.0
		4,4'-DDE	72-55-9		1.0
		4,4'-DDT	50-29-3		1.0
		Aldrin	309-00-2		1.0
		Alpha-BHC	319-84-6		1.0
		Beta-BHC	319-85-7		1.0
		Delta-BHC	319-86-8		1.0
		Alpha-Chlordane	5103-71-9		1.0
		Gamma-Chlordane	5566-34-7		1.0
		Dieldrin	60-57-1		1.0
		Endosulfan I	959-98-8		1.0
		Endosulfan II	33213-65-9		1.0
		Endosulfan Sulfate	1031-07-8		1.0
		Endrin	72-20-8		1.0
		Endrin Aldehyde	7421-93-4		1.0
		Endrin Ketone	53494-70-5		1.0
		Heptachlor	76-44-8		1.0
		Heptachlor Epoxide	1024-57-3		1.0
		Hexachlorobenzene	118-74-1		1.0
		Lindane	58-89-9		1.0
		Mirex	2385-85-5		1.0
		Cis-Nonachlor	5103-73-1		1.0

Type	UNITS	Parameter	CAS Number	PCB Number (where applicable)	MDL Target
		Oxychlordane	26880-48-8		1.0
		Trans-Nonachlor	39765-80-5		1.0
PAHs	dry weight ng/g (ppb)	Acenaphthene	83-32-9		10
		Acenaphthylene	208-96-8		10
		Anthracene	120-12-7		10
		Benz(a)anthracene	200-280-6		10
		Benzo(b)fluoranthene	205-99-2		10
		Benzo(k)fluoranthene	207-08-9		10
		Benzo(g,h,i)perylene	191-24-27-2		10
		Benzo(a)pyrene	50-32-8		10
		Benzo(e)pyrene	192-9		10
		Biphenyl	92-54-4		10
		Chrysene	218-01-9		10
		Dibenz(a,h)anthracene	53-70-3		10
		Dibenzothiophene	132-65-0		10
		2,6-Dimethylnaphthalene	581-42-0		10
		Fluoranthene	205-99-2		10
		Fluorene	86-73-7		10
		Indeno(1,2,3-c,d)pyrene	193-39-5		10
		1-Methylnaphthalene	90-12-0		10
		2-Methylnaphthalene	91-57-6		10
		1-Methylphenanthrene	832-69-9		10
		Naphthalene	91-20-3		10
		Perylene	198-55-0		10
		Phenanthrene	85-01-8		10
		Pyrene	129-00-0		10
		2,3,5-Trimethylnaphthalene	2245-38-7		10

6.6 Data Entry

Table 6.4 identifies the required data elements that laboratories must provide to EPA, preferably in EPA's data template, available separately from EPA. If the laboratory applies its own QC codes, the data transmittal should define the codes.

Table 6.4 Sediment Chemistry, Grain Size, and TOC: Data Elements for Each Sample

Variable	Type	Description
SITE_ID	Character	Site identification code or type of QC sample (e.g., LAB BLANK)
SAMPLE	Character	Sample number, LCS, QCCS, Blank, Matrix Spike, or CRM
ANALYSIS_TYPE	Character	Contaminant, TOC, or GRAIN SIZE
REPEAT	Numeric	Duplicate
DATE_COLLECT	Date	Date that the field crew collected the sample

Variable	Type	Description	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		VT	Volume not sufficient for testing
		VR	Volume not sufficient for a retest, if required
		Q	Other quality concerns, not identified above
COND_COMMENT	Character	Explanation for Q FLAG (if needed)	
PARAMETER	Character	Analyte name	
CAS_NO	Character	CAS Registry number	
LABNAME	Character	Laboratory name (abbreviation)	
METHOD	Character	Laboratory method used	
ANALYST	Character	Last name or initials of person who performed the analysis	
REVIEWER	Character	Last name or initials of the person who provided a separate independent review of the data	
INSTRUMENT	Character	Identification of instrument used for the analysis – provide enough information to identify the particular instrument in the laboratory	
DATE PROCESSED	Date	Date that the analysis started	
QC_BATCH_LOT	Character	Unique laboratory quality control lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate laboratory control sample, matrix spike, laboratory duplicate, method blank, and CRM samples.	
HOLDING TIME	Y/N	Analysis performed within holding time	
MATRIX	Character	Sediment (Water also is a permissible value if the laboratory analyzes a very liquid sediment sample as water)	
MDL	Numeric	Lab method detection limit (based upon lab's historical data)	
LRL	Numeric	Lab reporting limit (based upon lab's historical data)	
MOISTURE	Numeric	Moisture in the sample (value used by lab to convert wet units to dry)	
MOIST_UNIT	Character	Unit used to report moisture (% or mg/kg)	
DILUTION	Numeric	Dilution of sample (blank or 1 if no dilution)	
RECOVERY	Numeric	Only for appropriate QC samples	
RESULT	Numeric	Concentration value	
REASON	Character	Reason for qualification in RESULT_QUAL (usually blank)	
RESULT_QUAL	Character	Data qualifier (usually blank)	
UNIT	Character	Unit of measurement for RESULT, MDL, and RL	
QC_CODE	Character	Apply laboratory defined QC codes and describe in the comments field. Provide set of laboratory's code as part of the case narrative	

Variable	Type	Description
COMMENT	Character	Explain situation that created QC code, or any unusual aspects of the analysis

6.7 Quality Measures

This section describes the quality assurance and quality control measures used to ensure that the data will meet NCCA's requirements.

6.7.1 Assistance Visits

Assistance visits are intended to familiarize EPA with actual procedures being implemented by different laboratories; and to ensure a clear and consistent understanding of procedures and activities by both EPA and the laboratories. If EPA decides to conduct an assistance visit, a qualified EPA scientist or contractor will administer a checklist based upon the steps described in this chapter.

6.7.2 QC Samples

Once or twice during the performance period, the External QC Coordinator will provide one or two identical sets of QC samples to all participating laboratories. Each set will contain up to five QC samples. As determined by the External QC Coordinator, the QC samples may be synthetic; aliquots of additional samples collected at NCCA sites; or reference samples obtained from an organization such as the National Institute of Standards. Each laboratory will run the QC samples following the same procedures used for the other samples. The External QC Coordinator will compare the results to the expected value and determine consistency between laboratories (e.g., determine if one laboratory is consistently higher or lower than all others). Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any unique laboratory practices that might account for differences between the laboratory and others. The contractor shall analyze the external QC samples using the same procedures as those for the field samples.

6.7.3 Summary of QA/QC Requirements

QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. The laboratory must conduct QC analyses for each batch of samples. Each batch shall consist of no more than 20 samples. Unique laboratory quality control lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate measures such as laboratory control sample, matrix spike, laboratory duplicate, and method blank samples. Also, each laboratory QC samples (i.e., preparation and instrument blanks, laboratory control sample (LCS), spike/duplicate, etc.) must be given a unique sample identification. Table 6.5 provides a summary of the quality control requirements.

Table 6.5 Sediment Chemistry, Grain Size, and TOC: Quality control activities for samples

Activity	Evaluation	Corrective Action
Demonstrate competency for analyzing sediment samples to meet the performance measures	Demonstration of competency with sediment samples in achieving the method detection limits, accuracy, and precision targets.	EPA will not approve any laboratory for NCCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can demonstrate competency for its NCCA samples.
Check condition of sample when it arrives.	Sample issues such as cracked container; missing label; sufficient volume for test.	Assign appropriate condition code identified in Table 6.4.
Store sample appropriately. While stored at the laboratory, the sample must be kept at a temperature ≤-20° C except jars for grain analyses are refrigerated at 4°C.	Check the temperature of the refrigerator/freezer and refrigerator per laboratory's standard operating procedures.	Record temperature of sample upon arrival at the laboratory. If at any other time, samples are warmer than required, note temperature and duration in comment field. Data analyst will consider temperature deviations in evaluating the data. He/she will flag the deviations and determine whether the data appear to be affected and/or the data should be excluded from the analyses.
Analyze sample within holding time	The test must be completed within the holding time of 1 year. If the original test fails, then the retest also must be conducted within the holding time.	Perform test, but note reason for performing test outside holding time. EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
Perform once at the start of each batch to evaluate the labeled compound recovery (LCR) in a Laboratory Control Sample (LCS). This tests the performance of the equipment.	Control limits for recovery cannot exceed 100±20%.	First, prepare and analyze one additional LCS. If the second blank meets the requirement, then no further action is required. If the second LCS fails, then determine and correct the problem before proceeding with any sample analyses.
Perform once at the start of each batch to evaluate the entire extraction and analysis process using a Method Blank	Control limits cannot exceed the laboratory reporting level (LRL).	First, prepare and analyze one additional blank. If the second blank meets the requirement, then no further action is required. If the second blank fails, then determine and correct the problem (e.g., contamination, instrument calibration) before proceeding with any sample analyses. Reestablish statistical

Activity	Evaluation	Corrective Action
		control by analyzing three blank samples. Report values of all blanks analyzed.
Check calibration immediately before and immediately after the sample batch (abbreviated as QCCS for quality control check sample)	Results must be ±10% of each other or as specified in method criteria	If calibration fails before analysis, recalibrate and reanalyze QCCS until it passes. If check fails after all samples the batch have been analyzed, verify the QCCS reading. If the QCCS reading fails a second time, then reanalyze all samples in the batch and report only the set of results associated with the acceptable QCCS reading. Also report all QCCS readings for the batch.
Compare results of one laboratory duplicate sample (for TOC) or matrix spike duplicate sample (for contaminants) for each batch (not required for grain size)	Results must be within the target precision goal in Section 6.5.	If both results are below LRL, then conclude that the test has passed. Otherwise, prepare and analyze a split from different sample in the batch. If the second result is within the target precision goal (see Section 6.5) of the original sample, then report the data and findings for both QC samples. However, if the two results differ by more than the target precision goal, review precision of QCCS measurements for batch; check preparation of split sample; etc. and report evaluation and findings in the case narrative. Consult with the EPA HQ NCCA Laboratory Review Coordinator to determine if reanalysis of the entire batch (at the laboratory's expense) is necessary. If no reanalysis is necessary, report and quantify all samples in batch. If reanalysis is necessary, then report all QC sample and the 2nd analysis of the batch. If the second set also is unacceptable, then assign a data code to each sample in the batch.
Compare results of one matrix spike sample per batch to evaluate performance in matrix	Evaluate performance after the first 3 batches; and then every subsequent batch. Ideally, control limits for recovery will not	If both the original and duplicate results are below LRL, then conclude that the test has passed for the batch. Otherwise, if any

Activity	Evaluation	Corrective Action
(not required for TOC and grain size)	exceed the target accuracy goal, but this may not be realistic for all parameters with this matrix.	results are not within the target accuracy goal for the first 3 batches, within 2 working days, contact the EPA HQ NCCA Laboratory Review Coordinator to discuss method performance and potential improvements. After achieving acceptable results or EPA's permission to continue, perform the test for every subsequent batch. For each batch, report the results from the original analysis and its duplicate and their RPD for TOC; the matrix spike, matrix spike duplicate, RPD and %recovery for contaminants.
Compare results of TOC Certified Reference Material once per each batch	Value must be within 10% of the certified value.	If value is outside the acceptable range, analyze a second CRM. If the second CRM also is measured outside the acceptable range, then determine and correct the problem (e.g., contamination, instrument calibration) before reanalyzing all samples in the batch.
Maintain the required MDL identified in Section 6.5	Evaluate for each sample	If MDL could not be achieved, then provide dilution factor or QC code and explanation in the comment field.
Participate in External Quality Control	Evaluate QC samples provided by the External QC Coordinator	Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any deviations from the Method or unique laboratory practices that might account for differences between the laboratory and others. With this additional information, the External QC Coordinator will determine an appropriate course of action, including no action, flagging the data, or excluding some or all of the laboratory's data.
Maintain completeness	Completeness objective is 95% for all parameters.	Contact EPA HQ NCCA Laboratory Review Coordinator immediately if issues affect

Activity	Evaluation	Corrective Action
		laboratory's ability to meet completeness objective.

*Chapter 2 provides contact information for the EPA HQ NCCA Laboratory Review Coordinator. Laboratories under contract to EPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the Laboratory Review Coordinator.

6.8 Sample and Record Retention

The laboratory shall retain:

1. The sample materials, including vials, for a minimum of 3 years from the date the EPA publishes the final report. During this time, the laboratory shall freeze the materials used in the contaminant analyses and refrigerate those used for the grain size and TOC. The laboratory shall periodically check the sample materials for degradation.

2. Original records, including laboratory notebooks and the reference library, for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

6.9 References

All references are from U.S. Environmental Protection Agency:

Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved June 27, 2014 from http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_245_7.pdf.

Method 3051a "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, And Oils" retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf.

Method 3150A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils," retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf.

Method 3540C Method 3540C "Soxhlet Extraction" retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3540c.pdf.

Method 6020A “Inductively Coupled Plasma-Mass Spectrometry” retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6020A.pdf.

Method 8270D “Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS) retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8270D.pdf.

Method 9171B “n-Hexane Extractable Material (HEM) for Sludge, Sediment, And Solid Samples,” retrieved June 27, 2014 from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/9071b.pdf.

7.0 WATER CHEMISTRY AND CHLOROPHYLL *A*

This chapter describes the analysis requirements for water quality samples. The purpose is to determine concentrations of water quality parameters and chlorophyll *a* in water quality samples collected in the 2015 NCCA and related studies. The laboratory shall perform analysis to determine levels of ammonia (NH_3), nitrate (NO_3), nitrate-nitrite (NO_3-NO_2), total nitrogen (TN), total phosphorous (TP) and ortho-phosphate (PO_4) (also called soluble reactive phosphorus (SRP), pH, conductivity and chlorophyll *a* found in coastal waters and Great Lakes. In addition, the laboratory shall measure chloride (Cl) and sulfate (SO_4) levels in Great Lakes samples.

In the following discussion, Sections 7.1, 7.2, and 7.3 summarize the procedure; health and safety concerns; and definitions and required resources. Section 7.4 provides the steps for acknowledging sample receipt. Sections 7.5 – 7.6 provide the minimum requirements that the laboratory must meet in performing the analyses and the required data elements. Section 7.7 describes EPA's external review of laboratory operations and other quality measures. Section 7.8 identifies references used in developing the procedure.

7.1 Summary of the Procedure

This chapter describes the analysis of ammonia, nitrate-nitrite, total nitrogen, total phosphorous and ortho-phosphate, nitrate, pH, conductivity and chlorophyll *a,* and chloride samples collected for EPA's 2015 National Coastal Condition Assessment (NCCA). As described in Section 7.5, unless otherwise contractually bound by other requirements, the laboratory may choose to use any method that meets EPA's specifications for contamination measurements.

7.2 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions. In addition to the laboratory's usual requirements such as a Chemical Hygiene Plan, the laboratory must adhere to the following health and safety procedures:

1. Laboratory facilities must properly store and dispose of solutions of weak acid.

2. Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).

3. When working with potential hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with acid. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

7.3 Definitions and Required Resources (Personnel, Laboratories, and Equipment)

This section provides definitions and required resources for using the procedure.

7.3.1 Definitions

The procedure uses the following terms:

Cl: Chloride

Detection Limit is the minimum concentration at which the analyte can be *detected* with confidence. In other words, the outcome can be reported with confidence that it is greater than zero (i.e., present in the sample) Also see "Sample-Specific Detection Limit."

Duplicates are defined as two aliquots of the same sample which are analyzed separately using identical procedures. The results are used to evaluate the precision of the laboratory analyses.

NARS: National Aquatic Resource Surveys. The National Coastal Condition Assessment (NCCA) is part of the NARS program.

NARS Information Management System (NARS IM): The IM system established to support all surveys, including NCCA, in the NARS program. The IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. Freshwater and coastal samples will be collected during the field stage of NCCA.

NH_3: Ammonia

NO_3: Nitrate

NO_3-NO_2: Nitrate-nitrite

Percent Recovery: Recovery is measured by comparing the concentrations of a sample split into two parts; and one part is spiked with a known concentration value. C_s is the concentration measured in the spiked part; C is the concentration measured in the unspiked part; and s is the known concentration amount for the spike. The following equation is used to calculate the percent recovery:

$$\%Rs = \frac{C_s - C}{s} \times 100$$

Relative Standard Deviation (RSD): The precision at each concentration is reported in terms of the RSD. To calculate the RSD, first calculate the standard deviation, S, as follows:

$$S = \left[\frac{1}{n-1}\sum_{k=1}^{n}(C_s - \bar{C})^2\right]^{1/2}$$

where n is the number of replicate samples, C, is the concentration measure for the k[th] sample, and $\bar{C}$ is the average concentration of the replicate samples. Then, RSD is calculated as:

$$RSD = \left|\frac{S}{\bar{C}}\right| \times 100$$

Reporting Limit: A reporting limit is the point at which the measured value of the analyte can be reported with confidence.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method's detection limit. For diluted samples, the sample-specific detection limit will be the product of the method's detection limit and the dilution factor. Typical values for the dilution factors will be 10 or 100.

SO_4: Sulfate.

Spiked Sample: See Percent Recovery definition for purpose of spiked samples.

SRP: Soluble Reactive Phosphorus (also called orthophosphate)

TN: Total nitrogen

TP: Total phosphorous

7.3.2 General Requirements for Laboratories

Expertise. To demonstrate its competency/expertise, the laboratory shall provide EPA with performance data demonstrating their proficiencies in analyzing water quality samples. In addition, the laboratory must provide one or more of the following:

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
- Documentation detailing the expertise of the organization, including professional certifications for water-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.

Quality assurance and quality control requirements.
To demonstrate its expertise in quality assurance and quality control procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), Laboratory Quality Assurance Manuals, QAPPs, and applicable Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NCCA QAPP Certification Page.

7.3.3 Personnel

The procedure refers to the following personnel:

> **Laboratory Technician**: This procedure may be used by any laboratory technician who is familiar with the NCCA Quality Assurance Project Plan, and this procedure in the NCCA Laboratory Operations Manual.

7.3.4 Equipment/Materials

The analytical method, selected by the laboratory, identifies the necessary equipment.

7.4 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery. For each sampled site, the lab will receive the following samples on wet ice:

- One 250 ml amber bottle labeled 'CHEM' for water chemistry analyses
- A filter in a 50 ml tube for chlorophyll *a* labeled 'CHLA'

Additionally, as a separate batch shipment the lab will receive 250 ml bottles labeled 'NUTS' for dissolved nutrients analyses (either from the crews or from an EPA batching laboratory). Crews and the batch lab will maintain these samples frozen but will ship overnight on wet ice.

The laboratory technician must inspect the samples promptly on receipt and:

1. Log the samples into the National Aquatic Resource Survey Information Management system (NARS-IM) within 24 clock hours. Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM (see Chapter 2 for contact information).

2. Check that each shipping container has arrived undamaged. Check the temperature of one of the samples in the cooler using a thermometer that reads to at least -20 ºC (i.e., the expected temperature of frozen samples), or an infra-red (IR) temperature "gun" and record the reading. Temperature of the wet ice shipments should be 4 ºC or at less. .Record the condition and temperature of the sample in the database using the codes in Table 7.1.

3. Verify that all required data elements, per Table 7.1, have been recorded in the NARS IM database. If any data elements are missing, then enter them into the database.

4. Transfer the samples for storage as follows:

a. Water chemistry aliquots are prepared following the requirements in Section 7.5 and then are stored in a refrigerator at 4° C.
b. Chlorophyll-*a* filters to the freezer for no more than 30 days before analysis. Except during processing and analysis stages, the filter must be stored frozen to less than or equal -20 °C ± 2°.
c. Dissolved nutrient samples are prepared following the requirements in Section 7.5 and then are stored in a refrigerator at 4° C.

5. Notify the EPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 7.1 Water Chemistry Login: Required Data Elements

Variable	Type	Description	
SITE_ID	Character	Site identification code	
SAMPLE	Character	Sample number	
DATE_COLLECT	Date	Date that the field crew collected the sample	
ANALYSIS_TYPE	Character	Water Chemistry or Chlorophyll α or Nutrients	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory (CHEM, CHLA and NUTS sample will be on wet ice);	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NF	Sample is not at proper temperature
		Q	Other quality concerns, not identified above
COND_COMMENT	Character	Explanation for Q FLAG (if needed)	

7.5 Preparation of Water Chemistry Aliquots

Figure 7.1 presents the sample preparation processing steps for the water chemistry indicators, including filtering and acidifying.
For the dissolved nutrient (NUTS) sample, the laboratory technician:

1. Thaws the frozen sample.
2. Splits the sample into two aliquots as shown in figure 7.1.
3. Adds ultra-pure acid (H_2SO_4, depending on the analytes, see Table 7.2) to one of the two aliquots. Caps the bottle tightly and inverts the bottle several times to mix.
4. Stores all aliquots in a refrigerator at 4°C.

For the unfiltered, water chemistry (CHEM) sample, the laboratory technician

1. Thaws the frozen sample.
2. Splits the sample into two aliquots as shown in figure 7.1.
3. Adds ultra-pure acid (H_2SO_4,) to one aliquot of the unfiltered, CHEM sample. Caps the bottle tightly and inverts the bottle several times to mix.
4. Stores all aliquots in a refrigerator at 4°C.

If the dissolved nutrient sample is compromised in some way, the laboratory technician will filter a new sample from the water chem (CHEM) sample as follows:

1. Uses 0.4µm pore size polycarbonate filters for all filtration.
2. Rinses vacuum filter funnel units thoroughly with reverse-osmosis (RO) or de-ionized (DI) water (ASTM Type II reagent water) five times before each use and in between samples. After placing a filter in the funnel unit, run approximately 100 mL of RO or DI water through the filter, with vacuum pressure, to rinse the filter. Discard the rinse water.
3. Places the appropriate sample bottle under the funnel unit and filter sample directly into the bottle. If a new filter is needed, remove the sample bottle, and rinse the new filter with 100 mL of RO or DI water before continuing.
4. After all filtered and unfiltered aliquots are collected, adds ultra-pure acid (H_2SO_4, depending on the analyte, see Table 7.2) to the sample in the aliquot container. Cap tightly and invert the bottle several times to mix.
5. Stores all aliquots in a refrigerator at 4°C.

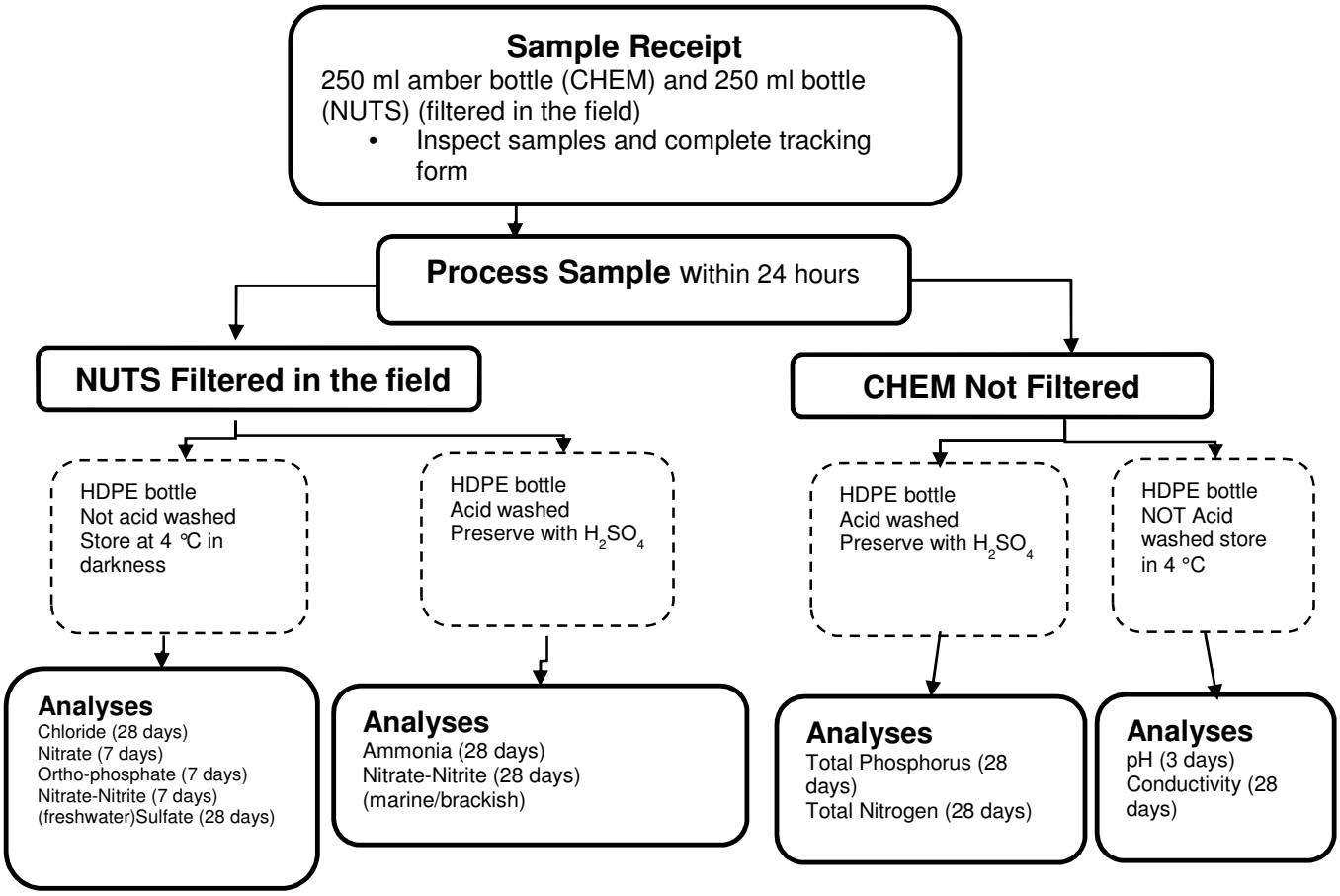

Figure 7.1 Water Chemistry and Dissolved Nutrient Samples: Receipt and Holding Times

Table 7.2 Water chemistry: acid preservatives added for various indicators

	Preservatives
	H_2SO_4 Used for:
Indicators	
	NH_4
	Total N
	Total P
	NO_2-NO_3

7.6 Water Chemistry and Chlorophyll *a* Analysis: Requirements

The laboratory shall perform analysis of the samples to determine the ammonia (NH_3), chloride and sulfate (Great Lakes only), nitrate-nitrite (NO_3-NO_2), total nitrogen (TN), total phosphorous (TP) and ortho-phosphate, nitrate (NO_3), and chlorophyll *a*. As an alternative to specifying laboratory methods for sample analysis, NCCA uses a performance-based approach that defines a set of laboratory method performance requirements for data quality as shown in Table 7.3. Method performance requirements for this project identify the reporting limit, precision, and accuracy objectives for each parameter. NCCA is designating the reporting limit as the lowest

value that the laboratory needs to quantify (as opposed to just detecting the parameter in the sample), and is the value of the lowest non-zero calibration standard that the laboratory must use. EPA has set the value to double the long-term method detection limit (LT-MDL), following guidance presented in Oblinger, Childress et al. (USGS, 1999)18.

NCCA expresses precision and accuracy objectives in both absolute and relative terms following Hunt and Wilson (1986). The transition value is the value at which performance objectives for precision and accuracy switch from absolute (≤ transition value) to relative (> transition value). For pH, the objectives are established for samples with lower, midrange and higher pH levels.

For duplicate samples, NCCA estimates the precision as the pooled standard deviation (calculated as the root-mean square) of all samples at the lower concentration range, and as the pooled percent relative standard deviation of all samples at the higher concentration range. For standard samples (of known concentration), precision is estimated as the standard deviation of repeated measurements across batches at the lower concentration range, and as percent relative standard deviation of repeated measurements across batches at the higher concentration range. Accuracy is estimated as the difference between the mean measured value and the target value of a performance evaluation and/or internal reference samples at the lower concentration range measured across sample batches, and as the percent difference at the higher concentration range.

Table 7.4 summarizes the analytical methods used at the NCCA central laboratory (EPA ORD-Corvallis). Other participating laboratories may use alternative analytical methods for each target analyte as long as they can satisfactorily demonstrate the alternative method is able to achieve the performance requirements as listed in Table 7.3. Appendix A identifies the information that the laboratory should provide to the NCCA Laboratory Review Coordinator to use in determining whether the laboratories meet the necessary requirements.

18 If a laboratory has questions related to meeting the -LT-MDL, they may contact the NCCA Laboratory Review Coordinator to discuss concerns.

Table 7.3 Water Chemistry and Chlorophyll-*a*: Laboratory Method Performance Requirements

Parameter	Units	Potential Range of Samples[1]	Method Detection Limit Objective[2]	Transition Value[3]	Precision Objective[4]	Accuracy Objective[5]
Ammonia (NH_3)	mg N/L	0 to 17	0.01 marine (0.7 µeq/L) 0.02 freshwater	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Chloride (Cl)	mg Cl/L	0 to 5,000	0.20 (6 µeq/L)	1	± 0.10 or ±10%	± 0.10 or ±10%
Conductivity	µS/cm at 25˚C	1-66,000	1.0	20	±2 or ±10%	±2 or ± 5%
Nitrate-Nitrite (NO_3-NO_2)	mg N/L	0 to 360 (as nitrate)	0.01 marine 0.02 freshwater	0.10	± 0.01 or ±10%	± 0.01 or ±10%
pH (Laboratory)	Std Units	3.5-10	N/A	5.75, 8.25	≤5.75 or ≥ 8.25 = ±0.07; 5.75-8.25 = ±0.15	≤5.75 or ≥ 8.25 =±0.15; 5.75-8.25 = ±0.05
Total Nitrogen (TN)	mg N/L	0.1 to 90	0.01	0.10	± 0.01 or ±10%	± 0.01 or ±10%
Total Phosphorous (TP) and ortho-Phosphate	mg P/L	0 to 22 (as TP)	0.002	0.02	± 0.002 or ±10%	± 0.002 or ±10%
Nitrate (NO_3)	mg N/L	0. to 360	0.01 marine (10.1 µeq/L) 0.03 freshwater	0.1	± 0.01 or ±5%	± 0.01 or ±5%
Sulfate (SO_4)	mg/L	0 to 5000	0.5 freshwater (10.4 ueq/L)	2.5	±0.25 or ±10%	±0.25 or ±10%
Chlorophyll-*a*	µg/L in extract	0.7 to 11,000	1.5	15	± 1.5 or ±10%	± 1.5 or ±10%

[1] Estimated from samples analyzed at the EPA Western Ecological Division-Corvallis laboratory between 1999 and 2005

[2] The method detection limit is determined as a one-sided 99% confidence interval from repeated measurements of a low-level standard across several calibration curves.

[3] Value for which absolute (lower concentrations) vs. relative (higher concentrations) objectives for precision and accuracy are used.

[4] For duplicate samples, precision is estimated as the pooled standard deviation (calculated as the root-mean square) of all samples at the lower concentration range, and as the pooled percent relative standard deviation of all samples at the higher concentration range. For standard samples, precision is estimated as the standard deviation of repeated measurements across batches at the lower concentration range, and as

percent relative standard deviation of repeated measurements across batches at the higher concentration range.

For pH precision, the looser criteria applies to mid-range samples. For NCCA, that is less of a concern than the ability to measure more acidic or basic samples accurately and precisely.

[5] Accuracy is estimated as the difference between the measured (across batches) and target values of performance evaluation and/or internal reference samples at the lower concentration range, and as the percent difference at the higher concentration range.

Table 7.4 Water Chemistry and Chlorophyll-*a*: Analytical Methods Used by Central Laboratory, EPA ORD-Corvallis)

Analyte	Summary of Method[19]	References[20]	WRS SOP[21]
Nitrate+Nitrite, as N	Ion Chromatography (freshwater samples) OR FIA automated colorimetric (cadmium reduction for brackish samples)	EPA 300.6; SW-846 9056A; APHA 4110B EPA 353.2 APHA 4500-NO_3-N-E Lachat 10-107-04-1-C	WRS 36A.0 (April 2011 WRS 40A.5 (May 2011)
Ammonia, as N	FIA automated colorimetric (salicylate, dichloroisocyanurate)	Lachat 10-107-06-3-D	WRS 30A.4 (April 2011)
Total nitrogen (TN)	Persulfate Digestion; FIA Automated Colorimetric Analysis (Cadmium Reduction, sulfanilamide)	EPA353.2 (modified) APHA 4500-N-C (modified) ASTM WK31786 U.S. EPA (1987) Lachat 10-107-04-1-C (modified)	WRS 34A.5 (April 2011)
Total phosphorus (TP) and ortho-Phosphate	Persulfate Digestion; Automated Colorimetric Analysis (molybdate, ascorbic acid)	APHA 4500-P-E USGS I-4650-03 U.S. EPA (1987) Lachat 115-01-1-B (modified)	WRS 34A.5 (April 2011)
Nitrate, chloride, sulfate	Ion Chromatography (Great Lakes samples only)	EPA 300.6; SW-846 9056A; APHA 4110B	WRS 40A.5 (May 2011)
Chlorophyll-a (Chl-a)	Extraction 90% acetone analysis by fluorometry	EPA 445.0 , EPA 446.0	WRS 71A.3 (April 2011)
pH (lab)	Automated, using ManSci PC-Titrate w/ Titra-Sip autotitrator and Ross combination pH electrode. Initial pH determination for ANC titration	EPA 150.6 (modified)	WRS 16A.0 (April 2011)

[19] FIA=Flow injection analysis. AAS=Atomic Absorption Spectrometry

[20] U.S. EPA, 1987. *Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry.* EPA/600/4-87/026. U.S. Environmental Protection Agency, Office of Research and Development, Washington D.C. APHA= American Public Health Association (*Standard Methods*). ASTM=American Society of Testing and Materials.

[21] WRS= Willamette Research Station. References are to laboratory SOP being used at central laboratory. Available upon request from the EPA HQ Laboratory Review Coordinator.

Analyte	Summary of Method[19]	References[20]	WRS SOP[21]
Specific conductance @ 25°C	Electrolytic, Man-Tech TitraSip automated analysis OR manual analysis, electrolytic	EPA 120.6	WRS 16A.0 (April 2011) WRS 11A.4 (April 2011)

7.7 Data Entry

Table 7.5 identifies the required data elements that laboratories must provide to EPA, preferably in EPA's data template, available separately from EPA.

Table 7.5 Water Chemistry and Chlorophyll-*a*: Data Elements for Each Sample

Variable	Type	Description	
SITE_ID	Character	Site identification code or type of QC sample (e.g., LAB BLANK)	
SAMPLE	Character	Sample number, LCS, QCCS, Blank, Matrix Spike, or CRM	
ANALYSIS_TYPE	Character	Contaminant	
REPEAT	Numeric	Duplicate	
DATE_COLLECT	Date	Date that the field crew collected the sample	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NF	Sample is not at proper temperature
		Q	Other quality concerns, not identified above
COND_COMMENT	Character	Explanation for Q FLAG (if needed)	
PARAMETER	Character	Analyte name	
CAS_NO	Character	CAS Registry number	
LABNAME	Character	Laboratory name (abbreviation)	
METHOD	Character	Laboratory method used	
ANALYST	Character	Last name or initials of person who performed the analysis	
REVIEWER	Character	Last name or initials of the person who provided a separate independent review of the data	
INSTRUMENT	Character	Identification of instrument used for the analysis – provide enough information to identify the particular instrument in the laboratory	
DATE PROCESSED	Date	Date that the analysis started	
QC_BATCH_LOT	Character	Unique laboratory quality control lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate laboratory control sample, matrix spike, laboratory duplicate, method blank, and CRM samples.	
HOLDING TIME	Y/N	Analysis performed within holding time	

Variable	Type	Description
MATRIX	Character	Water
MDL	Numeric	Lab method detection limit (based upon lab's historical data)
LRL	Numeric	Lab reporting limit (based upon lab's historical data)
DILUTION	Numeric	Dilution of sample (blank or 1 if no dilution)
RESULT	Numeric	Concentration value
REASON	Character	Reason for qualification in RESULT_QUAL (usually blank)
RESULT_QUAL	Character	Data qualifier (usually blank)
UNIT	Character	Unit of measurement for RESULT, MDL, and LRL
QC_CODE	Character	Apply laboratory defined QC codes and describe in the comments field. Provide set of laboratory's code as part of the case narrative
COMMENT	Character	Explain situation that created QC code, or any unusual aspects of the analysis

7.8 Quality Measures

This section describes the quality assurance and quality control measures used to ensure that the data will meet NCCA's requirements. QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. The laboratory must conduct QC analyses for each batch of samples. Each batch shall consist of no more than 20 samples. Unique laboratory quality control lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate measures such as laboratory control sample, matrix spike, laboratory duplicate, and method blank samples. Also, each laboratory QC samples (i.e., preparation and instrument blanks, laboratory control sample (LCS), spike/duplicate, etc.) must be give a unique sample identification. Table 7.5 provides a summary of the quality control requirements.

Table 7.5 Water Chemistry and Chlorophyll-*a*: Quality control activities for water quality samples

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Demonstrate competency for analyzing water samples to meet the performance measures	All	Demonstration of past experience with water samples in achieving the method detection limits	Once	See Appendix A	EPA will not approve any laboratory for NCCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
					demonstrate competency for its NCCA samples.
Check condition of sample when it arrives.	All	Sample issues such as cracked container; missing label; temperature; adherence to holding time requirements; sufficient volume for test.	Once	No sample issues or determination that sample can still be analyzed	Lab determines if the sample can be analyzed or has been too severely compromised (e.g., contamination). Assign appropriate condition code identified in Table 7.1.
Store sample appropriately.	All	Check the temperature of the refrigerator per laboratory's standard operating procedures.	Record temperature of sample upon arrival at the laboratory. Check temperature of the refrigerator/freezer where samples are stored at least daily if using a continuous temperature logger and twice daily (once at beginning of the day and once at the end) not using a continuous logger.	While stored at the laboratory, the sample must be kept at a maximum temperature of 4° C (for aliquots except chlorophyll *a*) and -20° C for the chlorophyll *a* sample.	If at any time samples are warmer than required, note temperature and duration (either from the continuous temperature log or from the last manual reading) in comment field. Lab will still perform test. EPA expects that the laboratory will exercise every effort to maintain samples at the correct temperature.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Analyze sample within holding time	All			The test must be completed within the holding time specified in the analytical method.	Perform test in all cases, but note reason for performing test outside holding time. EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
Analyze Laboratory/ Reagent Blank	All		Once per day prior to sample analysis	Control limits ≤ MDL	Prepare and analyze new blank. Determine and correct problem (e.g., reagent contamination, instrument calibration, or contamination introduced during filtration) before proceeding with any sample analyses. Reestablish statistical control by analyzing three blank samples.
Analyze Filtration Blank	All dissolved analytes	ASTM Type II reagent water processed through filtration unit	Prepare once per week and archive Prepare filter blank for each box of 100 filters, and examine the results before any other filters are used from that box.	Measured concentrations <MDL	Measure archived samples if review of other laboratory blank information suggest source of contamination is sample processing.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Determine LT-MDL Limit for Quality Control Check Sample (QCCS)	All	Prepared so concentration is four to six times the LT-MDL objective	Once per day	Target LT-MDL value (which is calculated as a 99% confidence interval)	Confirm achieved LRL by repeated analysis of LT-MDL QCCS. Evaluate affected samples for possible re-analysis.
Analyze Calibration QCCS	All		Before and after sample analyses	±10% or method criteria	Repeat QCCS analysis. Recalibrate and analyze QCCS. Reanalyze all routine samples (including PE and field replicate samples) analyzed since the last acceptable QCCS measurement.
Analyze Laboratory Duplicate Sample	All		One per batch	Control limits < precision objective	If results are below LRL: Prepare and analyze split from different sample (volume permitting). Review precision of QCCS measurements for batch. Check preparation of split sample. Qualify all samples in batch for possible reanalysis.

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
Analyze Standard Reference Material (SRM)	When available for a particular indicator		One analysis in a minimum of five separate batches	Manufacturers certified range	Analyze standard in next batch to confirm suspected inaccuracy. Evaluate calibration and QCCS solutions and standards for contamination and preparation error. Correct before any further analyses of routine samples are conducted. Reestablish control by three successive reference standard measurements that are acceptable. Qualify all sample batches analyzed since the last acceptable reference standard measurement for possible reanalysis.
Analyze Matrix Spike Samples	Only prepared when samples with potential for matrix interferences are encountered		One per batch	Control limits for recovery cannot exceed 100±20%	Select two additional samples and prepare fortified subsamples. Reanalyze all suspected samples in batch by the method of

QC Sample Type and Description	Indicators	Description	Frequency	Acceptance Criteria	Corrective Action
					standard additions. Prepare three subsamples (unfortified, fortified with solution approximately equal to the endogenous concentration, and fortified with solution approximately twice the endogenous concentration).
Use consistent units for QC samples and field samples	All	Verify that all units are provided consistently within each indicator.	Data reporting	For each indicator, all field and QC samples are reported with the same measurement units	If it is not possible to provide the results in consistent units, then assign a QC code and describe the reason for different units in the comments field of the database.
Maintain completeness	All	Determine completeness	Data reporting	Completeness objective is 95% for all indicators (useable with or without flags).	Contact EPA HQ NCCA Laboratory Review Coordinator* immediately if issues affect laboratory's ability to meet completeness objective.

*Chapter 2 provides contact information for the EPA HQ NCCA Laboratory Review Coordinator. Laboratories under contract to EPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the Laboratory Review Coordinator.

7.9 Sample and Record Retention

The laboratory shall retain:

1. The sample materials for a minimum of 1 year after collection. During this time, the laboratory shall store the materials cold (e.g., 4 ° C) and in darkness. The lab shall retain the sample materials from the 1 year point until the EPA publishes the final report at ambient temperatures.
2. Original records, including laboratory notebooks for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

7.10 References

Hunt, D.T.E. and A.L. Wilson. 1986. ***The Chemical Analysis of Water: General Principles and Techniques***. 2nd ed. Royal Society of Chemistry, London, England.

USEPA, 1987. ***Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry.*** EPA/600/4-87/026. U.S. Environmental Protection Agency, Office of Research and Development, Washington D.C.

USEPA. 1997. ***Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices – 2nd Edition***. EPA No. 600-R-97-072. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC, retrieved June 30, 1997 from http://www.epa.gov/microbes/documents/marinmet.pdf.

USEPA. September 1997. Method 353.4 "Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis, Revision 2.0", retrieved June 30, 2014 from http://www.epa.gov/microbes/documents/m353_4.pdf.

USGS. 1999. "New reporting procedures based on long-term method detection levels and some considerations for interpretations of water-quality data provided by the U.S. Geological Survey National Water Quality Laboratory." Open-File Report: 99-193 by Childress, Oblinger, *et al.*, retrieved June 30, 2014 from http://pubs.usgs.gov/of/1999/0193/report.pdf.

Youden, W.J. 1969. Ranking laboratories by round-robin tests. In *Precision Measurement and Calibration*. H.H. Ku, ed. NBS Special Publication 300, Vol. 1. U.S. GPO Washington, D.C.

8.0 SEDIMENT TOXICITY TESTING

This chapter describes the analysis requirements for sediment toxicity testing. The purpose is to assess the toxicity of sediment samples collected in the 2015 NCCA and related studies.

At each sampling site, the Field Operations Manual (FOM) instructs the crews to collect sediment samples. The field crew then ships the samples on wet ice to the laboratory. If EPA uses a batching laboratory, it will refrigerate the samples, before shipping on wet ice to the analysis laboratory.

In the following discussion, Sections 8.1, 8.2, and 8.3 summarize the procedure; health and safety concerns; and definitions and required resources. Section 8.4 provides the steps for acknowledging sample receipt. Sections 8.5 – 8.6 provide the minimum requirements that the laboratory must meet in performing the analyses and the required data elements. Section 8.7 describes EPA's external review of laboratory operations and other quality measures. Section 8.8 identifies references used in developing the procedure.

8.1 Summary of the Procedure

This chapter describes toxicity testing of sediment samples collected for EPA's 2015 National Coastal Condition Assessment (NCCA). As described in Section 8.5, unless otherwise contractually bound by other requirements, the laboratory may choose to use any method that meets EPA's specifications.

8.2 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions. In addition to the laboratory's usual requirements such as a Chemical Hygiene Plan, the laboratory must adhere to the following health and safety procedures:

1. Laboratory facilities must properly store and dispose of solutions of weak acid.

2. Laboratory personnel must wear proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves).

3. When working with potential hazardous chemicals (e.g., weak acid), laboratory personnel must avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin and mucous membranes with acid. If skin contact occurs, remove clothing immediately. Wash and rinse the affected skin areas thoroughly with large amounts of water.

8.3 Definitions and Required Resources (Personnel, Laboratories, and Equipment)

This section provides definitions and required resources for using the procedure.

8.3.1 Definitions

The procedure uses the following terms:

Replicates are defined as two or more aliquots of the same sample which are analyzed separately using identical procedures. The results are used to evaluate the precision of the laboratory analyses.

NARS: National Aquatic Resource Surveys. The National Coastal Condition Assessment (NCCA) is part of the NARS program.

NARS Information Management System (NARS IM): The IM system established to support all surveys, including NCCA, in the NARS program. The IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. Freshwater and coastal samples will be collected during the field stage of NCCA.

%CONT_SURV: Average percentage of organisms that survived in the replicate test chambers over the percent survival in control.

%REP_SURV: Percentage of organisms that survived in the test chamber for each set of replicates.

8.3.2 General Requirements for Laboratories

Expertise. To demonstrate its expertise, the laboratory shall provide EPA with performance data demonstrating their proficiencies in analyzing water quality samples. In addition, the laboratory must provide one or more of the following:

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
- Documentation detailing the expertise of the organization, including professional certifications for water-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.

Quality assurance and quality control requirements.
To demonstrate its expertise in quality assurance and quality control procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NCCA QAPP Certification Page.

Preparation for the work

To demonstrate its preparation for the work, the laboratory shall provide documentation that it has complied with the following control analyses prior to the start of any work.

1. The laboratory shall ensure that the water source for the overlying water has been demonstrated to support survival, growth, and reproduction of the test organisms. The laboratory shall provide information on how the laboratory maintains the quality of the water used for the tests.
2. The laboratory shall ensure that the clean sediment is appropriate for the control tests. The laboratory shall provide information about the sediment chemistry analysis and explanation of how the control sediment was selected
3. The laboratory shall ensure that the organisms are healthy for the tests. The laboratory shall provide the source of the organisms; historic information about the culturing; and procedures for evaluating the condition and age of the organism and water quality upon arrival. If the laboratory intends to purchase the organisms (i.e., instead of in-house culturing), identify the commercial source; its shipping arrangements (e.g., test organisms are shipped in well-oxygenated water in insulated containers to maintain temperature during shipment); and evaluation upon arrival at the laboratory (e.g., measuring temperature and dissolved oxygen of the water in the shipping containers to determine if the organisms might have been subjected to low dissolved oxygen or temperature fluctuations).
4. The laboratory shall complete a "non-toxicant" test of each new chamber before using the chamber for NCCA samples. A "new" chamber is one that the laboratory has not previously used for any sediment toxicity testing for any client (e.g., replacement glassware). Ideally, although EPA is not requiring it, the laboratory will test freshwater and marine samples in wholly separate chambers.

   Test requirements: The test chambers contain control sediment (sometimes called the negative control) and clean overlying water for the amphipod species to be tested. Survival of the test organisms will demonstrate whether facilities, water, control sediment, and handling techniques are adequate to achieve acceptable species-specific control survival. For the test to be acceptable, survival at 10 days must equal or exceed the survival requirements in QA/QC specifications in Section 8.7.

8.3.3 Personnel

The procedure refers to the following personnel:

Laboratory Technician: This procedure may be used by any laboratory technician who is familiar with the NCCA Quality Assurance Project Plan, and this procedure in the NCCA Laboratory Operations Manual.

External QC Coordinator is an EPA staff person who is responsible for selecting and managing the "**QC contractor**." To eliminate the appearance of any inherent bias, the QC contractor must be dedicated to QA/QC functions, and thus, must not be a primary laboratory or a field sampling contractor for NCCA. The QC contractor is responsible for complying with instructions from the External QC Coordinator; coordinating and paying

for shipments of the performance samples to participating laboratories; comparing results from the laboratories; and preparing brief summary reports.

8.3.4 Equipment/Materials

The analytical method, selected by the laboratory, identifies the necessary equipment.

8.4 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery. The laboratory must inspect the samples promptly on receipt. As samples arrive, the laboratory must:

1. Log the samples into the National Aquatic Resource Survey Information Management system (NARS-IM) within 24 clock hours. Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM (see Chapter 2 for contact information).

2. Check that each shipping container has arrived undamaged. Check the temperature of one of the samples in the cooler using a thermometer that measures temperatures between 0 ºC (refrigerated samples are typically 4 °C) and 30 °C (ambient room temperature is typically less than 26 °C), or an infra-red (IR) temperature "gun" and record the reading. Field crews and the batching laboratory will ship sediment samples on wet ice. Record the condition and temperature of the sample in the database using the codes in Table 8.1.

3. Verify that all required data elements, per Table 8.1, have been recorded. If any elements are missing, then enter them into the database.

4. Transfer the samples to the refrigerator until ready for toxicity testing. Except during processing and analysis stages, the samples must be stored at 4°C.

5. Notify the EPA immediately about any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 8.1 Sediment Toxicity Login: Required Data Elements

FIELD	FORMAT	DESCRIPTION
LAB ID	Character	Name or abbreviation for laboratory
TYPE	Character	Control or NCCA Sample
DATE RECEIVED	MMDDYY	Date sample was received by lab; leave blank for control
SITE ID	Character	NCCA site id as used on sample label; leave blank for control
VISIT NUMBER	Numeric	Sequential visits to site (1 (or blank) or 2); leave blank for control
SAMPLE ID	Numeric	Sample id as used on field sheet (on sample label); leave blank for control
DATE COLLECTED	MMDDYY	Date sample was collected; leave blank for control

FIELD	FORMAT	DESCRIPTION	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory (it should arrive on wet ice).	
CONDITION CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory; leave blank for control	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NF	Sample is not at proper temperature
		VT	Volume not sufficient for testing (VT)
		VR	Volume not sufficient for a retest, if required
		HT	Received outside holding time
		Q	Other quality concerns, not identified above
COND_COMMENT	Character	Explanation for Q FLAG (if needed)	

8.5 Toxicity Testing: Requirements

The laboratory shall perform toxicity testing of sediment samples. Laboratories may choose to use any analysis method using the required organisms of *Hyalella azteca* (freshwater) or *Leptocheirus plumulosus* (marine). The laboratory's method must meet the quality requirements in Section 8.7, including mean survival of the control's treatments must remain greater than or equal to 80% and 90%, respectively. It is essential that the contractor require that all of its laboratory technicians use the same procedures and meet the required quality elements. At a minimum, the laboratory must:

1. Perform the procedures using the 10-day tests. Possible methods include those described in the following documents:
   a. Marine: Test Method 100.4 in EPA 600/R-94/025[22] or ASTM E1367-03[23]
   b. Freshwater: Test Method 100.1 in EPA 600/R-99/064[24] or ASTM E1706[25]

2. Test the following number of replicates for each sample and control:
   a. Marine: 5 replicates with 20 organisms per replicate
   b. Freshwater: 4 replicates with 10 organisms per replicate

[22] Chapter 11 in *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*, June 1994, retrieved from http://water.epa.gov/polwaste/sediments/cs/upload/marinemethod.pdf.
[23] American Society for Testing and Materials (ASTM). 2008. E1367-03 "Standard Guide for Conducting 10-Day Static Sediment Toxicity Tests With Marine and Estuarine Amphipods." *Annual Book of Standards, Water and Environmental Technology*, Vol. 11.05, West Conshohocken, PA.
[24] Section 11 in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Second Edition, March 2000, retrieved from http://water.epa.gov/polwaste/sediments/cs/upload/freshmanual.pdf.
[25] ASTM 2009 E1706. "Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates."

3. Test no more than 10 samples and one control within each batch.

4. Use the following organisms for the tests:
    a. Marine: *Leptocheirus plumulosus*
    b. Freshwater: *Hyalella azteca*

5. Select organisms for each batch of tests that are:
    a. From the same culture;
    b. Cultured at the same temperature as will be used for the tests;
    c. (optional) EPA would prefer but does not require that the organisms are cultured in the same water as that used for testing.

6. Use a water source (for the overlying water) demonstrated to support survival, growth, and reproduction of the test organisms.
    a. For marine sediments, 175 mL of sediment and 800 mL of overlying seawater
    b. For freshwater sediments, 100mL of sediment and 175mL of overlying freshwater

7. Use clean sediment for control tests.

8. Implement the following for exposure/feeding
    a. For marine sediments, exposure is static (i.e., water is not renewed), and the animals are not fed over the 10 d exposure period
    b. For freshwater, exposure is renewed (i.e., 2 volumes a day) and the animals are fed over the 10 day exposure period

9. Follow the following procedure for homogenization/sieving: Water above the sediment is not discarded, but is mixed back into the sediment during homogenization. Sediments should be sieved for marine samples (following the 10 day method) and the sieve size should be noted. For freshwater samples, they should not be sieved to remove indigenous organisms unless there is a good reason to believe indigenous organisms may influence the response of the test organism. For freshwater samples, large indigenous organisms and large debris can be removed using forceps and if sediments must be sieved, the samples should be analyzed before and after sieving (e.g., pore-water metals, DOC, and AVS) to document the influence of sieving on sediment chemistry (note sieve size).

Additional details are provided in the summary tables 8.2 and 8.3.

Table 8.2 Test Conditions for Conducting 10-d Sediment Toxicity Tests for marine sediments

Parameter	Conditions
1. Test type:	Whole sediment toxicity test, static
2. Temperature:	25℃ for L. plumulosus
3. Salinity	20‰
4. Light quality:	Wide-spectrum fluorescent lights
5. Illuminance:	500 – 1000 lux
6. Photoperiod:	24L:0D
7. Test chamber:	1 L glass beaker or jar with ~10 cm I.D.

8. Sediment volume:	175 mL (2 cm)
9. Overlying water volume:	800 mL
10. Renewal of overlying water:	None
11. Size and life stage of amphipods:	L. plumulosus: 2-4 mm (no mature males or females)
12. Number of organisms per chamber:	20 per test chamber
13. Number of replicate chambers/treatment:	5 (required)
14. Feeding:	None
15. Aeration:	Water in each test chamber should be aerated overnight before start of test and throughout the test aeration at rate that maintains ≥90% saturation of dissolved oxygen concentration
16. Overlying water:	Clean sea water, natural or reconstituted water
17. Overlying water quality:	Temperature daily; pH, ammonia, salinity, and DO at test start and end.
18. Test duration:	10 d
19. Endpoints:	Survival
20. Test acceptability:	Minimum mean control survival of 90%

Table 8.3 Test Conditions for Conducting 10-d Sediment Toxicity Tests for freshwater sediments

Parameter	Conditions
1. Test type:	Whole-sediment toxicity test with renewal of overlying water
2. Temperature:	23°± 1°C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illuminance:	100 to 1000 lux
5. Photoperiod:	16L:8D
6. Test chamber:	300 mL high-form beaker
7. Sediment volume	100 mL
8. Overlying water volume:	175 mL
9. Renewal of overlying water:	2 volume additions/d; continuous or intermittent (*e.g.*, 1 volume addition every 12 h)
10. Age of organisms:	7- to 14-d old at the start of the test (1- to 2-d range in age)
11. Number of organisms/ chamber:	10
12. Replicate chambers/treatment:	4 required
13. Feeding:	YCT food, fed 1.0 mL daily (1800 mg/L stock) to each test chamber.
14. Aeration:	None unless DO in overlying water drops below 2.5 mg/L
15. Test duration:	10 d
16. Endpoint:	Survival
17. Test acceptability:	Min. mean control survival of 80%.

8.6 Data Entry

Tables 8.3 and 8.4 identify the required data elements describing the test conditions and outcomes for the replicates and batches. Laboratories must provide the data elements to EPA, preferably in EPA's data template, available separately from EPA.

Table 8.3 Sediment Toxicity Replicates: Laboratory method performance requirements

FIELD	FORMAT	DESCRIPTION
LAB ID	Character	Name or abbreviation for laboratory
TYPE	Character	Control or NCCA Sample
SAMPLE ID	Numeric	Sample id as used on field sheet (on sample label); leave blank for control
RETEST	Y or blank	Y for yes if the sample is being retested; blank if original test or control
CHAMBER ID	Character	Identification code for test chamber
BATCH ID	Character	Identification code for batch
REPLICATE	Numeric	Replicate number: 1-5 for marine; 1-4 for freshwater
TEST TYPE	Character	Marine or Freshwater
ORGANISM	Character	Leptocheirus plumulosus (marine) or Hyalella azteca (freshwater)
NO_SURVIVED	Numeric	Number of organisms that survived out of 20 (marine) and 10 (freshwater)
%REP_SURV	Numeric	Percentage of organisms that survived in the test chamber for the replicate
REP_COMMENT	Character	Any comments about the test procedures or any abnormalities
%CONT_SURV	Numeric	Optional Field: Average percentage of organisms that survived in the replicate test chambers over the percent survival in control.

Table 8.4 Laboratory method performance requirements for sediment toxicity batches

FIELD	FORMAT	DESCRIPTION
BATCH ID	Character	Identification code for batch
BATCH_SAMPLES	Numeric	Number of NCCA samples in the batch (integer≤10) excluding the control
TEST TYPE	Character	Marine or Freshwater
ORGANISM	Character	Leptocheirus plumulosus (marine) or Hyalella azteca (freshwater)
CONTROL	Character	Source of control sediment
START_DATE	MMDDYY	Date that the laboratory starts the test procedure for the batch
END_DATE	MMDDYY	Date that the laboratory ends the test procedure for the batch
%SURV	Numeric	%Survival for the sample (or control) calculated using the %REP_SURV
BATCH_PASS	P/F	Indicate if the batch passed (P) or failed (F) the QA/QC requirements (e.g., control achieved required survival rates)
QC_CODE	Character	Laboratory assigned code for QC issues with the sample
QC_DESCRIPTION	Character	Description of conditions associated with the QC_CODE
SURV_COMMENT	Character	Any comments about the test procedures or any abnormalities

8.7 Quality Measures

This section describes the quality assurance and quality control measures used to ensure that the data will meet NCCA's requirements.

8.7.1 Assistance Visits

Assistance visits are intended to familiarize EPA with actual procedures being implemented by different laboratories; and to ensure a clear and consistent understanding of procedures and activities by both EPA and the laboratories. If EPA decides to conduct an assistance visit, a qualified EPA scientist or contractor will administer a checklist based upon the steps described in this chapter.

8.7.2 QC Samples

Once or twice during the performance period, the External QC Coordinator will provide one or two identical sets of QC samples to all participating laboratories. Each set will contain up to five QC samples. As determined by the External QC Coordinator, the QC samples may be synthetic; aliquots of additional samples collected at NCCA sites; or reference samples obtained from an organization such as the National Institute of Standards. Each laboratory will run the QC samples following the same procedures used for the other samples. The External QC Coordinator will compare the results to the expected value and determine consistency between laboratories (e.g., determine if one laboratory is consistently higher or lower than all others). Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any unique laboratory practices that might account for differences between the laboratory and others. The contractor shall analyze the external QC samples using the same procedures as those for the field samples.

8.7.3 Summary of QA/QC Requirements

QC protocols are an integral part of all analytical procedures to ensure that the results are reliable and the analytical stage of the measurement system is maintained in a state of statistical control. The laboratory must conduct QC analyses for each batch of samples. Each batch shall consist of no more than 10 samples. Unique laboratory quality control lot numbers must be assigned to each batch of samples. The lot number must associate each batch of field samples to the appropriate measures such as laboratory control samples. Table 8.5 provides a summary of the quality control requirements.

Table 8.5 Quality control activities for sediment toxicity samples

Activity	Evaluation	Corrective Action
Laboratory demonstrates competency for conducting sediment toxicity analyses	EPA will review SOPs, lab certifications, past performance results, etc. as part of the lab verification process.	EPA will not approve any laboratory for NCCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can

Activity	Evaluation	Corrective Action
		demonstrate competency for its NCCA samples.
Check condition of sample when it arrives.	Sample issues, such as cracked or leaking container; missing label; temperature; adherence to holding time requirements; insufficient volume for test.	Assign appropriate condition code identified in Table 8.1.
Sample storage	All samples: 4 °C at arrival at the laboratory (temperature recorded at arrival) and while stored at the laboratory.	Record temperature upon arrival at the laboratory. Check temperature of the refrigerator where samples are stored at least daily if using a continuous temperature logger and twice daily (beginning and end of day) if the lab does not have a continuous logger. If refrigerator is warmer than required, note temperature and duration (either from the continuous temperature log or from the last manual reading) in comment field. Lab will still perform test. EPA expects that the laboratory will exercise every effort to maintain samples at the correct temperature.
Holding Time	The test must be completed within 8 weeks after sample collection. If the original test fails, then the retest also must be conducted within the 8 weeks after sample collection.	Perform test, but note reason for performing test outside holding time. EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
Check that the organisms are healthy before starting the test	Unhealthy organisms may appear to be discolored, or otherwise stressed (for example, greater than 20 percent mortality for the 48 hours before the start of a test).	Don't start test using unhealthy organisms.
Maintain conditions as required in Section 8.3.	Check conditions (e.g., temperature, DO) each test day. Record conditions in bench sheet or in laboratory database.	Note any deviations in comments field (Table 8.1). In extreme cases, conduct a new toxicity test for all samples

Activity	Evaluation	Corrective Action
		affected by the adverse conditions.
Control survival rates	For a test of a batch of samples to be considered valid, the control's mean survival in *Hyalella* and *Leptocheirus* treatments must remain ≥80% and ≥90%, respectively.	Data template includes a field to record if a test passed or failed the control requirements. If a test fails, retest all samples in the batch. Report both the original and retest results. If both tests fail, submit data to EPA for further consideration. Include comments in the data template noting any particular factors that may have caused the test to fail twice.

*Chapter 2 provides contact information for the EPA HQ NCCA Laboratory Review Coordinator. Laboratories under contract to EPA must contact the Task Order's Contracting Officer's Representative (TOCOR) instead of the Laboratory Review Coordinator.

8.8 Sample and Record Retention

The laboratory shall retain:

1. The sample materials, including vials until March 31, 2016 which will allow EPA with time to review the data and contact the laboratory with any questions about the samples. Until this time, the laboratory shall refrigerate the sediment samples. The laboratory shall periodically check the sample materials for degradation.

2. Original records, including laboratory notebooks, for a minimum of 10 years from the date that EPA publishes the final report.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

8.9 References

American Society for Testing and Materials (ASTM). 2008. E1367-03 "Standard Guide for Conducting 10-Day Static Sediment Toxicity Tests With Marine and Estuarine Amphipods." Annual Book of Standards, Water and Environmental Technology, Vol. 11.05, West Conshohocken, PA.

ASTM. 2009. E1706. "Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates.

United Stated Environmental Protection Agency (USEPA). 1994. Chapter 11 in Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods, retrieved on March 13, 2014 from http://water.epa.gov/polwaste/sediments/cs/upload/marinemethod.pdf.

USEPA. 2000. Section 11 in Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, Second Edition, retrieved on March 13, 2014 from http://water.epa.gov/polwaste/sediments/cs/upload/freshmanual.pdf.

9.0 FISH TISSUE FILLET (GREAT LAKES)

Laboratory Methods incorporated in EPA OST Manuals/QAPP.

10.0 MERCURY IN FISH TISSUE PLUGS

10.1 Summary of the Procedure

This procedure is applicable to the analysis of mercury in fish tissue plugs. The method is performance based. Laboratories may use any method that meets the requirements below to analyze the fish tissue samples (for example, EPA Method 1631). Example SOPs are provided in Appendix D of this LOM.

10.2 General Requirements for Laboratories

Competency. To demonstrate its competency, the laboratory shall provide EPA with performance data demonstrating their proficiencies in analyzing water quality samples. In addition, the laboratory must provide one or more of the following:

- Memorandum that identifies the relevant services that the laboratory provided for the National Aquatic Resource Surveys in the past five years.
- Documentation detailing the expertise of the organization, including professional certifications for water-related analyses, membership in professional societies, and experience with analyses that are the same or similar to the requirements of this method.

Also, the lab must provide a demonstration of past experience with fish tissue samples in applying the laboratory SOP in achieving the method detection limit.

Quality assurance and quality control requirements.
To demonstrate its expertise in quality assurance and quality control procedures, the organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include Quality Management Plans (QMP), QAPPs, and applicable Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment, the person in charge of quality issues for the organization shall sign the NCCA QAPP Certification Page.

10.2.1 Personnel

Laboratory Technician: This procedure may be used by any laboratory technician who is familiar with the NCCA Quality Assurance Project Plan, and this procedure in the NCCA Laboratory Operations Manual.

10.2.2 Equipment/Materials

The analytical method, selected by the laboratory, identifies the necessary equipment.

10.3 Sample Receipt

Because EPA initiates tracking procedures designed to recover any missing shipment, the laboratory personnel responsible for tracking samples must start the following login steps within 24 clock hours of receiving a delivery.

1. Report receipt of samples in the NARS IM sample tracking system (within 24 clock hours). Alternatively, for shipments with a large number of samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARS-IM (see Chapter 2 for contact information).

2. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
   a. Verify that the sample IDs in the shipment match those recorded on the:
      i. Chain of custody forms when the batching laboratory sends the samples to the microcystins laboratory; or
      ii. Sample tracking form if the field crew sends the shipment directly to the State laboratory.
   b. Record the information in Table 10.1 into NARS IM, including the Condition Code for each sample:
      i. *OK*: Sample is in good condition
      ii. *C*: Sample container was cracked
      iii. *L*: Sample container is leaking
      iv. *ML*: Sample label is missing
      v. *VT*: Volume not sufficient for testing
      vi. *W*: Sample is warm (>8^{o}), record the temperature in the comment field, and perform the assay
      vii. *Q*: other quality concerns, not identified above.
   c. If any sample is damaged or missing, contact the EPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Chapter 2 of the Manual).

3. Store samples in the freezer until sample preparation begins.

4. Maintain the chain of custody or sample tracking forms with the samples.

Table 10.1 Fish Tissue Plugs Login: Required Data Elements

FIELD	FORMAT	DESCRIPTION
LAB ID	text	Name or abbreviation for QC laboratory
DATE RECEIVED	MMDDYY	Date sample was received by lab
SITE ID	text	NCCA site id as used on sample label
VISIT NUMBER	numeric	Sequential visits to site (1 or 2)
SAMPLE ID	numeric	Sample id as used on field sheet (on sample label)
DATE COLLECTED	MMDDYY	Date sample was collected
CONDITION CODE	text	Condition codes describing the condition of the sample upon arrival at the laboratory.

FIELD	FORMAT	DESCRIPTION	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		VT	Volume or mass not sufficient for testing (VT)
		W	Sample is warm (>8°)
		Q	Other quality concerns, not identified above
CONDITION COMMENT	text	Comments about the condition of the sample. If the condition code='W' then provide the temperature	

10.4 Quality Measures

This section describes the quality assurance and quality control measures used to ensure that the data will meet NCCA's requirements. Tables 10.2 and 10.3 provide a summary of the measurement data quality objectives and quality control requirements.

10.4.1 Assistance Visits

Assistance visits are intended to familiarize EPA with actual procedures being implemented by different laboratories; and to ensure a clear and consistent understanding of procedures and activities by both EPA and the laboratories. If EPA decides to conduct an assistance visit, a qualified EPA scientist or contractor will administer a checklist based upon the steps described in this chapter.

10.4.2 QC Samples

Once or twice during the performance period, the External QC Coordinator will provide one or two identical sets of QC samples to all participating laboratories. Each laboratory will run the QC samples following the same procedures used for the other samples. The External QC Coordinator will compare the results to the expected value to determine whether the values are within expected ranges. The contractor shall analyze the external QC samples using the same procedures as those for the field samples.

Table 10.2 Measurement data quality objectives

Variable or Measurement	MDL	Quantitation Limit
Mercury	0.47 ng/g	5.0 ng/g

Table 10.3 Quality Control

Activity	Evaluation/Acceptance Criteria	Corrective Action
Demonstrate competency for analyzing fish samples to meet the performance measures	Demonstration of past experience with fish tissue samples in applying the laboratory SOP in achieving the method detection limit	EPA will not approve any laboratory for NCCA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can demonstrate competency for its NCCA samples.
Check condition of sample when it arrives.	Sample issues, such as punctures or rips in wrapping; missing label; temperature; adherence to holding time requirements; sufficient volume for test. All samples should arrive at the laboratory frozen.	Assign an appropriate condition code.
Store sample appropriately. While stored at the laboratory, the sample must be kept at a maximum temperature of -20° C.	Check the temperature of the freezer per laboratory's standard operating procedures.	Record temperature of sample upon arrival at the laboratory. If at any other time, samples are warmer than required, note temperature and duration in comment field.
Analyze sample within holding time	The test must be completed within the holding time (i.e., 1 year). If the original test fails, then the retest also must be conducted within the holding time.	Perform test, but note reason for performing test outside holding time. EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
Maintain quality control specifications from selected method/SOP (that meets the measurement data quality objectives)	Data meet all QC specifications in the selected method/SOP.	If data do not meet all QC requirements, rerun sample or qualify data. If the lab believes the data are to be qualified without rerunning sample, the lab must consult with the EPA Survey QA Lead before proceeding.
Maintain the required MDL	Evaluate for each sample	If MDL could not be achieved, then provide dilution factor or QC code and explanation in the comment field.
Use consistent units for QC samples and field samples	Verify that all units are provided in wet weight units and consistently	If it is not possible to provide the results in the same units as most other analyses, then assign a QC code and describe the reason for different units in the comments field of the database.
Maintain completeness	Completeness objective is 95% for all parameters.	Contact the EPA Survey QA Lead immediately if issues affect

Activity	Evaluation/Acceptance Criteria	Corrective Action
		laboratory's ability to meet completeness objective.

11.0 FECAL INDICATOR: ENTEROCOCCI

Laboratory methods incorporated into EPA ORD Manuals/QAPP.

12.0 ALGAL TOXINS, RESEARCH INDICATOR

See Appendix C for USGS Organic Geochemistry Research Laboratory (OGRL) Standard Operating Procedures as modified for use in NCCA 2015 relating to the Algal Toxins Research Indicator.

- **Appendix C.1 OGRL-SOP-5400 (as modified for use in NCCA 2015)**: Analysis of Cyanotoxins and Algal Toxins in Fresh and Marine Surface Water, Accumulations, and Blooms (Internal Standard Calibration of Standard Addition) – LCTX
- **Appendix C.2 OGRL-SOP-4520**: Sequential Freeze/Thaw Cell-Lysis Procedure for Total and Dissolved Algal Toxin Analysis of Water Samples
- **Appendix C.3 OGRL-2010**: Data and Information Backup for all OGRL Instruments

APPENDIX A: LABORATORY REMOTE EVALUATION FORMS

Email the completed and signed forms to Kendra Forde (forde.kendra@epa.gov).
Questions: Contact Kendra Forde at forde.kendra@epa.gov or 202-566-0417.

NCCA 2015 Document Request Form – Chemistry Laboratories

EPA and its state and tribal partners will conduct the 2015 National Coastal Condition Assessment. NCCA is a survey of the nation's coastal waters and Great Lakes. It is designed to provide statistically valid regional and national estimates of the condition of coastal waters and the Great Lakes. Consistent sampling and analytical procedures ensure that the results can be compared across the country.

As part of the 2015 NCCA, the Quality Assurance Team has been requested to conduct a technical assessment to verify quality control practices in your laboratory and its ability to perform chemistry analyses under this project. Our review will be assessing your laboratory's ability to receive, store, prepare, analyze, and report sample data generated under EPA's 2015 NCCA.

The first step of this assessment process will involve the review of your laboratory's certification and/or documentation. Subsequent actions may include (if needed): reconciliation exercises and/or a site visit. All labs will need to complete the following forms:

All laboratories will be required to complete the following forms and check the specific parameter in which your laboratory will be conducting an analysis for the 2015 NCCA:

- ☐ Water Chemistry and chlorophyll *a* (all of the analytes identified in the LOM and QAPP)
- ☐ Microcystin
- ☐ Mercury in Fish Tissue Plugs
- ☐ Sediment Chemistry
- ☐ Grain Size
- ☐ Total Organic Carbon (TOC)

If your lab has been previously approved within the last 5 years for the water chemistry indicator:

- ☐ A *signature* on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for chemistry labs conducting analyses for the 2015 NCCA.
- ☐ A signature on the Quality Assurance Project Plan (QAPP) and the Laboratory Operations Manual (LOM) Signature Form indicates that you will follow both the QAPP and the LOM.

If you have not been approved within the last 5 years through the laboratory verification process for the water chemistry indicator, in order for us to determine your ability to participate as a laboratory in the NCCA, we are requesting that you submit the following documents (if available) for review:

- ☐ Documentation of a successful *quality assurance audit* from a prior National Aquatic Resource Survey (NARS) that occurred within the last 5 years.
- ☐ Documentation showing participation in a previous NARS for Water Chemistry for the same parameters/methods.

Additionally, we request that all labs provide the following information in support of your capabilities, (these materials are required if neither of the two items above are provided):

- ☐ A copy of your laboratory's *accreditations and certifications* if applicable (i.e. NELAC, ISO, state certifications, NABS, etc.).
- ☐ An updated copy of your laboratory's *QAPP* and Laboratory Quality Assurance Manuals
- ☐ *Standard Operating Procedures* (SOPs) for your laboratory for each analysis to be performed (if not covered in 2015 NCCA LOM).
- ☐ Documentation attesting to experience running all analytes for the 2015 NCCA, including chlorophyll *a*.

Laboratory Signature Form – Chemistry Laboratories

I _____ certify that the laboratory, located in _____, will abide by the following standards in performing the following data analysis and reporting for the 2015 National Coastal Condition Assessment (NCCA).
This applies to the _____ chemistry indicator.

1.) Use procedures identified in the 2015 NCCA Laboratory Operations Manual (or equivalent). If using equivalent procedures, please provide the procedures and obtain approval from EPA.
2.) Read and abide by the 2015 NCCA Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOPs).
3.) Have an organized IT tracking system in place for recording sample tracking and analysis data.
4.) Provide Quality Control (QC) data for internal QC check, on a quarterly basis.
5.) Provide data using the template provided on the NARS Sharefile.
6.) Provide data results in a timely manner. This will vary with the type of analysis and the number of samples to be processed. Sample data must be received no later than May 1, 2016 or as otherwise negotiated with EPA.
7.) Participate in a laboratory technical assessment or audit if requested by EPA NCCA staff (this may be a conference call or on-site audit).
8.) Agree to analyze for all parameters specified in the LOM for the appropriate indicator(s) identified above, including Chlorophyll-*a,* for water chemistry.

Signature _____ Date _____

NCCA 2015 Document Request Form - Biology Laboratories

EPA and its state and tribal partners will conduct the 2015 National Coastal Condition Assessment. NCCA is a survey of the nation's coastal waters and Great Lakes. It is designed to provide statistically valid regional and national estimates of the condition of coastal waters and the Great Lakes. Consistent sampling and analytical procedures ensure that the results can be compared across the country.

As part of the 2015 NCCCA, the Quality Assurance Team has been requested to conduct a technical assessment to verify quality control practices in your laboratory and its ability to perform biology analyses under this project. Our review will be assessing your laboratory's ability to receive, store, prepare, analyze, and report sample data generated under EPA's 2015 NCCA.

The first step of this assessment process will involve the review of your laboratory's certification and/or documentation. Subsequent actions may include (if needed): reconciliation exercises and/or a site visit.

All laboratories will be required to complete the following forms and check the specific parameter in which your laboratory will be conducting an analysis for the 2015 NCCA:

- ☐ Mercury in Fish Plugs
- ☐ Benthic Macroinvertabrates
- ☐ Sediment Toxicity

If your laboratory has been previously approved within the last 5 years for the specific parameters:

- ☐ A *signature* on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for biology laboratories conducting analyses for the 2015 NCCA.
- ☐ A signature on the Quality Assurance Project Plan (QAPP) and the Laboratory Operations Manual (LOM) Signature Form indicates you will follow both the QAPP and the LOM.

If you have not been approved within the last 5 years through the laboratory verification process for the specific parameters, in order for us to determine your ability to participate as a lab in the NCCA, we are requesting that you submit the following documents (if available) for review:

- ☐ Documentation of a successful *quality assurance audit* from a prior National Aquatic Resource Survey (NARS) that occurred within the last 5 years.
- ☐ Documentation showing participation in previous NARS for this particular indicator.

Additionally, we request that all labs provide the following information in support of your capabilities, (these materials are required if neither of the two items above are provided):

- ☐ A copy of your laboratory's *accreditations and certifications* if applicable (i.e. NELAC, ISO, state certifications, NABS, etc.).

- ☐ Documentation of NABS (or other) *certification* for the *taxonomists* performing analyses (if applicable).
- ☐ An updated copy of your Laboratory's *QAPP* and Laboratory Quality Assurance Manuals.
- ☐ *Standard Operating Procedures* (SOPs) for your lab for each analysis to be performed (if not covered in 2015 NCCA LOM).

Laboratory Signature Form – Biology Laboratories

I _____ certify that the laboratory
located in _____, will abide by the following standards in performing biology data analysis and reporting for the 2015 National Coastal Condition Assessment (NCAA).
This applies to the _____ biological indicator.

Use procedures identified in the 2015 NCCA Lab Operations Manual (or equivalent). If using equivalent procedures, please provide the procedures and obtain approval from EPA.
Read and abide by the 2015 NCCA Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOPs).
Have an organized IT tracking system in place for recording sample tracking and analysis data.
Use taxonomic standards outlined in the 2015 NCCA Laboratory Operations Manual.
Participate in taxonomic reconciliation exercises during the field and data analysis season, which include conference calls and other laboratory reviews.
Provide Quality Control (QC) data for internal QC checks, including for sorting, on a monthly basis.
Provide data using the template provided on the NARS Sharefile.
Provide data results in a timely manner. This will vary with the type of analysis and the number of samples to be processed. Sample data must be received no later than May 1, 2016 or as otherwise negotiated with EPA. Samples results for independent taxonomic QC described in the LOM and QAPP must be provided to EPA prior to final datasets to allow for reconciliation to take place.
Participate in a Laboratory technical assessment or audit if requested by EPA NCCA staff (this may be a conference call or on-site audit).
Agree to utilize taxonomic nomenclature and hierarchical established for NCCA 2015.

Signature _____ Date _____

APPENDIX B: TARGET FISH SPECIES FOR WHOLE FISH ANALYSES

Table B.1 Northeast region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

NORTHEAST REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**	**FISH PLUG LIST***
Ictaluridae	*Ameiurus catus*	White catfish	Primary
	Ictalurus punctatus	Channel catfish	Primary
Moronidae	*Morone americana*	White perch	Primary
Paralichthyidae	*Paralichthys dentatus*	Summer flounder	Primary
Pleuronectidae	*Pseudopleuronectes americanus*	Winter flounder	Primary
Sciaenidae	*Cynoscion regalis*	Gray weakfish	Primary
	Sciaenops ocellatus	Red drum	Primary
Sparidae	*Stenotomus chrysops*	Scup	Primary
NORTHEAST REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**	**FISH PLUG LIST***
Achiridae	*Trinectes maculatus*	Hogchoaker	
Anguillidae	*Anguilla rostrata*	American eel	Secondary
Atherinopsidae	*Menidia menidia*	Atlantic silverside	
Batrachoididae	*Opsanus tau*	Oyster toadfish	
Ephippidae	*Chaetodipterus faber*	Atlantic spadefish	
Moronidae	*Morone saxatilis*	Rock fish	Secondary
Mugulidae	*Mugil cephalus*	Black mullet	
Pomatomidae	*Pomatomus saltatrix*	Bluefish	Secondary
Sciaenidae	*Bairdiella chrysoura*	Silver perch	
	Menticirrhus saxatilis	Northern kingfish	
Serranidae	*Centropristis striata*	Black sea bass	
Triakidae	*Mustelus canis*	Smooth dogfish	
Triglidae	*Prionotus carolinus*	Northern searobin	
	Prionotus evolans	Striped searobin	

** Indicates whether species also occurs in the primary or secondary fish plug list*

Table B.2 Southeast region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

SOUTHEAST REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**	**FISH PLUG LIST***
Ariidae	*Ariopsis felis*	Hardhead sea catfish	Primary
	Bagre marinus	Gafftopsail sea catfish	Primary
Paralichthyidae	*Paralichthys albigutta*	Gulf flounder	Primary
	Paralichthys dentatus	Summer flounder	Primary
	Paralichthys lethostigma	Southern flounder	Primary
Sciaenidae	*Cynoscion arenarius*	Sand weakfish (or seatrout)	Primary
	Cynoscion nebulosus	Speckled trout	Primary
	Cynoscion regalis	Gray weakfish	Primary
	Leiostomus xanthurus	Spot croaker	Primary
Sparidae	*Lagodon rhomboides*	Pinfish	
SOUTHEAST REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**	**FISH PLUG LIST***
Cichlidae	*Tilapia mariae*	Spotted tilapia	
Haemulidae	*Haemulon aurolineatum*	Tomtate	
Sciaenidae	*Bairdiella chrysoura*	Silver perch	
	Menticirrhus americanus	Southern kingfish	
Serranidae	*Centropristis striata*	Black sea bass	

** Indicates whether species also occurs in the primary or secondary fish plug list*

Table B.3 Gulf region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

GULF REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Ariidae	*Ariopsis felis*	Hardhead sea catfish	Primary
	Bagre marinus	Gafftopsail sea catfish	Primary
Paralichthyidae	*Paralichthys albigutta*	Gulf flounder	Primary
	Paralichthys dentatus	Summer flounder	Primary
	Paralichthys lethostigma	Southern flounder	Primary
Sciaenidae	*Cynoscion arenarius*	Sand weakfish (or seatrout)	Primary
	Cynoscion nebulosus	Speckled trout	Primary
	Cynoscion regalis	Gray weakfish	Primary
	Leiostomus xanthurus	Spot croaker	Primary
	Micropogonias undulatus	Atlantic croaker	Primary
	Sciaenops ocellatus	Red drum	Primary
Sparidae	*Lagodon rhomboides*	Pinfish	
GULF REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Carangidae	*Caranx hippos*	Crevalle jack	
	Chloroscombrus chrysurus	Atlantic bumper	
Diodontidae	*Chilomycterus schoepfii*	Burrfish	
Gerreidae	*Eucinostomus gula*	Silver jenny	
Haemulidae	*Orthopristis chrysoptera*	Pigfish	
Ictaluridae	*Ictalurus furcatus*	Blue catfish	
Lepisosteidae	*Lepisosteus oculatus*	Spotted gar	
Lutjanidae	*Lutjanus griseus*	Gray snapper	
Sciaenidae	*Pogonias cromis*	Black drum	
Serranidae	*Diplectrum formosum*	Sand perch	
Triglidae	*Prionotus scitulus*	Leopard searobin	

** Indicates whether species also occurs in the primary or secondary fish plug list*

Table B.4 Western region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

WESTERN REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Atherinopsidae	*Atherinops affinis*	Topsmelt silverside	
Cottidae	*Leptocottus armatus*	Pacific staghorn sculpin	Primary
	Oligocottus rimensis	Saddleback sculpin	
Cynoglossidae	*Symphurus atricaudus*	California tonguefish	
Embiotocidae	*Cymatogaster aggregata*	Shiner perch	Primary
	Embiotoca lateralis	Striped seaperch	Primary
Gasterosteidae	*Gasterosteus aculeatus*	Three-spined stickleback	
Paralichthyidae	*Paralichthys californicus*	California flounder	Primary
	Citharichthys sordidus	Pacific sanddab	Primary
	Citharichthys stigmaeus	Speckled sanddab	
Pleuronectidae	*Isopsetta isolepis*	Butter sole	
	Parophrys vetulus	English sole	Primary
	Psettichthys melanostictus	Pacific sand sole	
	Platichthys stellatus	Starry flounder	Primary
Sciaenidae	*Genyonemus lineatus*	White croaker	Primary
Serranidae	*Paralabrax nebulifer*	Barred sand bass	Primary
	Paralabrax maculatofasciatus	Spotted sand bass	Primary
WESTERN REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Echinodermata/ Toxopneustidae	*Tripneustes gratilla* (Hawaii ONLY)	Collector urchin	
Batrachoididae	*Porichthys notatus*	Plainfin midshipman	
	Porichthys myriaster	Specklefin midshipman	

Chimaeridae	*Hydrolagus colliei*	Spotted ratfish	
Embiotocidae	*Amphistichus argenteus*	Barred surfperch	Secondary
Paralichthyidae	*Xystreurys liolepis*	Fantail sole	
Pleuronectidae	*Pleuronichthys guttulatus*	Diamond turbot	Secondary
	Microstomus pacificus	Dover sole	Secondary
	Lepidopsetta bilineata	Rock sole	
	Lyopsetta exilis	Slender sole	
Sciaenidae	*Umbrina roncador*	Yellowfin croaker	

** Indicates whether species also occurs in the primary or secondary fish plug list.*

Table B.5 Great Lakes primary and secondary target species - whole body fish tissue collection (Ecofish)

GREAT LAKES PRIMARY ECOFISH TARGET SPECIES			
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**	**FISH PLUG LIST***
Catostomidae	*Moxostoma macrolepidotum*	Shorthead redhorse	Primary
Centrarchidae	*Ambloplites rupestris*	Rock bass	Primary
	Lepomis gibbosus	Pumpkinseed	Primary
	Lepomis macrochirus	Bluegill	Primary
	Micropterus dolomieu	Smallmouth bass	Primary
	Pomoxis annularis	White crappie	
	Pomoxis nigromaculatus	Black crappie	
Cottidae	*Cottus bairdii*	Mottled sculpin	
	Cottus cognatus	Slimy sculpin	
Cyprinidae	*Couesius plumbeus*	Lake chub	
	Cyprinus carpio	Common carp	Primary
	Pimephales notatus	Bluntnose minnow	
Esocidae	*Esox lucius*	Northern pike	Primary
	Esox masquinongy	Muskellunge	Primary
Gasterosteidae	*Gasterosteus aculeatus*	Three-spined stickleback	
Gobiidae	*Neogobius melanostomus*	Round goby	
	Proterorhinus marmoratus	Tubenose goby	
Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	Primary
	Ictalurus punctatus	Channel catfish	Primary
	Noturus flavus	Stonecat	
Gadidae	*Lota lota*	Burbot	Primary
Moronidae	*Morone americana*	White perch	Primary
	Morone chrysops	White bass	Primary
Osmeridae	*Osmerus mordax*	American/ rainbow smelt	
Percidae	*Gymnocephalus cernuus*	Ruffe	
	Perca flavescens	Yellow perch	Primary
	Percina caprodes	Logperch	
	Sander canadensis	Sauger	
	Sander vitreus	Walleye	Primary
Percopsidae	*Percopsis omiscomaycus*	Trout-perch	
Salmonidae	*Coregonus artedi*	Cisco/ lake herring	
	Coregonus clupeaformis	Lake whitefish	Primary
	Oncorhynchus gorbuscha	Pink salmon	
	Oncorhynchus kisutch	Coho salmon	Primary
	Oncorhynchus mykiss	Rainbow trout	Primary
	Oncorhynchus tshawytscha	Chinook salmon	Primary
	Salvelinus namaycush	Lake trout	Primary
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	Primary
GREAT LAKES SECONDARY ECOFISH TARGET SPECIES			
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**	**FISH PLUG LIST***
Catostomidae	*Catostomus catostomus*	Longnose sucker	
	Catostomus commersonii	White sucker	Secondary
	Moxostoma anisurum	Silver redhorse	
Centrarchidae	*Micropterus salmoides*	Largemouth bass	
Clupeidae	*Alosa pseudoharengus*	Alewife	
	Dorosoma cepedianum	American gizzard shad	
Cyprinidae	*Cyprinella spiloptera*	Spotfin shiner	
	Luxilus cornutus	Common shiner	
	Notropis stramineus	Sand shiner	
Esocidae	*Esox niger*	Chain pickerel	

Fundulidae	*Fundulus diaphanus*	Banded killifish	
	Fundulus majalis	Striped killifish	
Ictaluridae	*Ameiurus melas*	Black bullhead	
Salmonidae	*Prosopium cylindraceum*	Round whitefish	
	Salmo trutta	Brown trout	Secondary
	Salvelinus fontinalis	Brook trout	
	Salvelinus fontinalis x namaycush	Splake	

** Indicates whether species also occurs in the primary or secondary fish plug list*

APPENDIX C: ALGAL TOXINS RESEARCH INDICATOR STANDARD OPERATING PROCEDURES

Appendix C.1

Title:	Identifier:	Revision:	Effective Date:
Analysis of Cyanotoxins and Algal Toxins in Fresh and Marine Surface Water, Accumulations, and Blooms (Internal Standard Calibration or Standard Addition) – LCTX (As modified for NCCA 2015)	*OGRL-SOP-5400*	**2**	**8/31/2015**

USGS
science for a changing world

APPROVALS FOR USE

Author's Name (Print):	Author's Signature:	Date:
Keith A. Loftin		8/31/2015
Project Director's Name (Print) *Michael T. Meyer*	Project Director's Signature	Date: 8/31/2015

Organic Geochemistry Research Laboratory (OGRL)

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Analysis of Cyanotoxins and Algal Toxins in Fresh Surface Water, Accumulations, and Blooms (Standard Addition)

NOTE: Laboratory personnel may produce paper copies of this procedure printed from the controlled document file. However, it is their responsibility to ensure that they are trained on and utilizing the current version of this procedure. The procedure author may be contacted if text is unclear.

This is a direct inject analytical method developed for the separation, detection, and quantitation of cyanotoxins and algal toxins in fresh and marine surface water, and cyanobacterial accumulations and blooms by standard addition. Separation and detection of algal toxins is made using multiple reaction monitoring (MRM) mode of a liquid chromatography triple quadrupole mass spectrometer (LC/MS/MS). Quantitation is accomplished by either internal standard calibration curve or single point standard addition described in this SOP at a level equivalent to 1.0 µg/L. Standard addition can be used exclusively or when matrix effects are greater than +/- 20% (28.3% RSD) of spiked concentration. Samples analyzed by this procedure at minimum should be filtered which would be analogous to a dissolved algal toxin concentration or lysed and filtered which would be analogous to a total algal toxin concentration.

1.0 SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the preparation, separation, detection, and quantitation for 14 cyanotoxins and algal toxins by liquid chromatography tandem mass spectrometry (LC/MS/MS) at the USGS Organic Geochemistry Research Laboratory (OGRL) in Lawrence, KS. The LCTX procedure applies to the following compounds in water:

anatoxin-a (ANAA), cylindrospermopsin (CYLS), domoic acid (DMAC), microcystin-HtYR (MCHtYR), microcystin-LA (MCLA), microcystin-LF (MCLF), microcystin-LR (MCLR), microcystin-LW (MCLW), microcystin-LY (MCLY), microcystin-RR (MCRR), microcystin-WR (MCWR), microcystin-YR, (MCYR), nodularin-R (NODR), and okadaic acid (OKAC). Simetone is used as an internal standard and L-phenylalanine is used to differentiate anatoxin-a from environmental phenylalanine since they have similar MRM transitions (isobaric compounds) and elute near each other chromatographically.

1.3 The minimum reporting limit (MRL) and minimum detection level (MDL) is matrix and compound dependent. However, the MRL to date has ranged from 0.10 µg/L (0.10 ppb) to 0.30 µg/L (0.30 ppb) based on a 100 µL injection depending on toxin.

2.0 TRAINING

The Project Director is responsible for ensuring that all who perform the function(s) described in this SOP for the OGRL are familiar with the objectives of and properly trained in its procedures. In addition, lab technicians using this procedure must document that they have read and understand this procedure in their training folder.

3.0 DEFINITIONS

3.1 Liquid Chromatography (LC) — An analytical instrument that relies on the interaction of an analyte with a solid stationary phase contained in a column and a liquid mobile phase as it passes through the analytical column (column) carrying the analyte.

3.2 Triple Quadrupole Mass Spectrometer (MS/MS)—An analyte detector that can determine the mass of selected fragments and fragments of fragments. This detector is typically used in conjunction with a chromatographic technique.

3.3 LC/MS/MS—A hyphenated technique where a liquid chromatograph is used for analyte separation is connected to a tandem mass spectrometer as the detector.

3.4 Chromatogram—The data that is acquired from the LC/MS/MS.

3.5 Analyte—The compound of interest.

3.6 Internal Standard— A standard (preferably an isotope labeled version of the analyte(s) of interest when possible) that is spiked into all samples, blanks and calibration samples. This compound should not be present in the environment and is used to correct for variation in analytical processes or techniques.

3.7 Reagent Water—treated water (18.2 MΩ/cm, < 1 ppb Total Organic Carbon (TOC)) generated by the laboratory system at the OGRL.

3.8 Stock Standard—a known concentration of an individual compound dissolved in a known volume of solvent. Target concentration is usually 100 µg/mL but can be greater if sufficient standard is available with adequate solubility.

3.9 LCTX Working Standard Mix— a reagent water spiked with a known concentration of all cyanotoxins and algal toxins that are determined by this method. This does not include the internal standard, simetone.

3.10 Analytical column--A stainless steel column containing a solid, stationary phase used to aid in separation on the LC.

3.11 Mobile phase—The solvent or combination of solvents that carries the analyte through the analytical column that aid in separation on the LC.

3.12 CAS#--Reference number assigned by Chemical Abstract Services to a chemical.

3.13 SOP—Standard operating procedure.

3.14 MeOH—Methanol, LC/MS grade or better.

3.15 ACN—Acetonitrile, LC/MS grade or better.

3.16 Formic Acid—Concentrated formic acid, usually 90% or greater.

3.17 THF-Tetrahydrofuran, analytical grade or better.

3.18 LCTX—an acronym for the liquid chromatography/triple quadrupole mass spectrometer method of cyanotoxins and algal toxins**.**

3.19 PPE---Personal Protective Equipment

3.20 Electrospray positive mode (ES +) —An ionization mode of positive polarity used by the tandem mass spectrometer to aid in fragmentation of positive ions.

3.21 Electrospray negative mode (ES -) —An ionization mode of negative polarity used by the tandem mass spectrometer to aid in fragmentation of negative ions.

3.22 Multiple Reaction Monitoring (MRM) — The scan type used for detection and quantitation of a parent and corresponding daughter fragment of an analyte.

3.23 Processed Sample—For purposes of this SOP, this term means that a sample has at minimum been filtered (Dissolved Cyanotoxin Analysis) or lysed and filtered (Total Cyanotoxin Analysis).

4.0 PERSONNEL HEALTH AND SAFETY

Note: This SOP is to be used in conjunction with an approved Chemical Hygiene Plan. Also, consult the Chemical Hygiene Plan for information on and use of all PPE including nitrile gloves, safety glasses, and a lab coat should be worn especially when making stock standard solutions.

4.1 Acetontrile, methanol, or tetrahydrofuran should not come in contact with skin or eyes, be inhaled, or be swallowed. Contact lenses should not be worn when working with these chemicals. Should contact occur, immediately wash with water. To prevent inhalation, use a fume hood with a suitable face velocity and cover containers before transporting. If a person breathes large amounts of any of these chemicals, move the exposed person to the fresh air at once. If any of these chemicals has been swallowed, get medical attention immediately by calling 911.

4.2 Care should be taken when working with THF, being a cyclic ether, there is concern for peroxide formation. **Do not evaporate THF to dryness!** THF is typically shipped with an inhibitor to prevent peroxide formation. There is no need to remove the inhibitor as part of processes conducted in this SOP. Use as is.

4.3 Cyanotoxins and algal toxins, by their very nature, are naturally occurring poisons that must be handled with care. The compounds covered in this SOP have a variety of indications when exposure occurs and relevant concentrations are not well defined for humans. However, in lieu of human acute and chronic toxicity information, mouse bioassays have been used to set suggested exposure thresholds. The World Health Organization has also suggested guidelines for some toxins with respect to drinking water and recreational exposure and US EPA has published health advisory thresholds in finished drinking water for anatoxin-a, cylindrospermopsin, and microcystin-LR.

4.4 Leaks may occur in fittings due to the high operating pressure of the LC. Safety goggles should be worn to protect eyes from splash.

4.5 The column compartment is hot and precautions should be taken before handling columns or touching the walls of column compartment.

4.6 The spray chamber of the MS/MS is very hot, with temperatures in excess of 650°C, and must be allowed to cool before touching.

5.0 APPARATUS AND INSTRUMENTATION

5.1 Analytical balance—capable of accurately weighing 0.0500 g ± 0.0001 g.

5.2 Top loading balance—capable of accurately weighting 5.0 g ± 0.1 g

5.3 Auto pipettes--10-to 10,000-µL, variable-volume auto pipettes with disposable plastic tips (Rainin, Woburn, MA, or equivalent).

5.4 Mechanical vortex mixer.

5.5 Data acquisition system—computer and printer compatible with all systems.

5.6 Instrument Software – LC/MS/MS software used for acquisition and data reduction supplied by LC/MS/MS manufacturer.

6.0 CHEMICALS AND REAGENTS

6.1 Mobile phase A, 0.1 % formic acid in reagent water.

6.2 Mobile phase B, 0.1 % formic acid in a mixture of 50/50 (v/v) MeOH to ACN.

6.3 Active and passive needle rinse solution for LC—Mobile phase B.

6.4 Stock solutions of analytes– See attachment A.

6.5 Stock internal standard solution, simetone, as received from Chem Services, inc. dissolved in methanol.

6.6 An aqueous 5% tetrasodium ethylene diamine tetraacetic acid (EDTA) solution made in reagent water is added to samples to minimize metal chelation. Volume is dependent on data quality (e.g. higher metals content requires more EDTA.

7.0 PROCEDURE

Note: Deviations from SOPs must be recorded in an appropriate instrument or work log. Include the name of the person recording the deviation, date it occurred and type of deviations, and whether the deviation was corrected (if applicable).

7.1 Preparation of 100 µg/mL individual stock standard solutions of cyanotoxins and algal toxins.

7.1.1 It is critical that all work with concentrated standards be conducted in a properly functioning fume hood. Remove all other items from hood that are not necessary for the work of making the stock cyanotoxin and algal toxin standards prior to initiation of stock standard preparation. Place a sign on the hood before beginning work with toxins for other personnel to stay out of this hood until the sign is removed. The sign should read “Stay out until further notice! Cyanotoxin work in progress. Contact: “your name”, office number, and phone number with questions. This will be in effect for 24 hours from the conclusion of toxin work with concentrated standards and decontamination of surfaces with 50 % aqueous ethanol solution or 50 % aqueous isopropanol solution. All materials including paper towels, gloves, pipettes, and used pipette tips should be left in the hood for 24 hours also before being bagged, tied off, and disposed in the dumpster. Pipettes can be wiped down with 50 % isopropanol or ethanol solutions.

7.1.2 When working with toxins always where nitrile gloves, appropriate safety glasses. A lab coat is recommended or wash your hands and arms with soap and water upon conclusion of work or at breaks.

7.1.3 The mass of toxin received from the distributor may be difficult to observe in the vial since standards usually only have 25 to 250 µg of material. This typically results in a thin film that is clear to offwhite. All solutions therefore must be initially made in the original vial. Target volume of stock standards is 0.25 to 1.0 mL with the appropriate solvent. See attachment A for individual stock standard concentration and the appropriate solvent or solvent mixture. Final individual stock standard solution storage can be in screw cap vials from supplier. For those standards arriving with crimp caps and septa the individual stock standard solutions will need to be transferred to separate screw cap LC/MS/MS grade vials.

7.1.4 Unless a certificate of analysis is available regarding standard purity assume 100 % purity for now. There are few certified reference materials available for these toxins and there is not an independent testing lab to confirm purity separate from the manufacturer. Aliqouts of standards will be evaluated for purity and the final concentrations will be corrected at that point. (Usually purity is corrected for when making the standards, but correction is not possible in this case since purity is unknown until measured).

7.1.5 Add appropriate volume of diluents as listed in Attachment A for a given mass of toxin and vortex capped vial for a couple of minutes. **Keep standards covered in the dark as much as possible when not working with them!** Allow standards to sit at room temperature in the dark for approximately 5 minutes and re-vortex capped vials for approximately 2 minutes. Keep standards at room temperature for 5 more minutes. Record the stock standard concentration, lot number from the manufacturer, name of the preparer and the date prepared in the working standards notebook.

7.2 Preparation of 100 µg/L LCTX Working Standard Mix.

7.2.1 Add 50 µL of each 100 µg/mL toxin standard to a labeled 123 mL amber glass bottle. Weigh in 49.3 g of reagent water to the bottle. Cap and invert bottle. (**Note: Since this is a research method, the number of standards added to the mix may change over time. The mass of reagent water to add will decrease by the same volume as the total volume of toxin solution added.).** Each working standard mix should have a lot number connected to the individual 100 µg/mL individual stock standards. The specific information should be recorded in the working standards notebook (i.e. LCTX-WSM-001).

7.2.2 Divide the 100 µg/L LCTX Working Standard Mix into subaliqouts by placing 1.5 mL of the 100 µg/L LCTX Working Standard Mix into labeled screw capped LC vials. Make 10 LC vials at a time since they will last for quite a while. Each vial label should be labeled with a lot number that ties it back to the original 100 µg/L LCTX Working Standard Mix (i.e. LCTX-WSM-001a). Keep the remainder of the 100 µg/L LCTX Working Standard Mix in the original bottle (7.2.1) and remove from freezer for use only when the 1.5 mL aliquots have been used up and make 10 more subaliqouts. Store all 100 µg/L LCTX Working Standard Mixes in the appropriate standards freezer until use.

7.3 Preparation of the 1.23 mg/mL Stock Internal Standard Solution of Simetone.

7.3.1 Weigh 123 mg of Simetone (more if purity is not 100%) into 100mL of MeOH. Mix until simetone completely dissolved. Store in Stock Standards Freezer until needed.

7.4 Preparation of 1.23 mg/L Intermediate Internal Standard of Simetone.

7.4.1 Dilute 1 mL of 1.23 mg/mL Stock Internal Standard Simetone Solution with 999 mL of MeOH. Mix and store in the freezer when not in use.

7.5 Preparation of 0.123 mg/L Working Internal Standard of Simetone.

7.5.1 Dilute 10 mL of the 1.23 mg/L Intermediate Internal Standard of Simetone with 90 mL of reagent water. Mix and store in the freezer when not in use.

7.6 **Preparation of LCTX Internal Standard (LCTX ISTD)**.

7.6.1 Dilute 2.5 mL of the 0.123 mg/L Working Internal Standard with 2.5 mL of reagent water. Label as LCTX ISTD.

7.7 Preparation of Check Standards, Blanks, and Samples for LCTX Analytical Run.

7.7.1 Obtain current LCTX run sheet from Project Management Office or off the computer in ResLab (OGRL Computer Network). Each analytical run should consist of the following: Check Standards (2)- 1.0 µg/L, Blanks after each Check Standard and every standard addition (SA) sample, and unspiked (A) and spiked samples (SA-Standard Addition), and duplicate unspiked and spiked samples (at least 1 duplicate for every 10 samples). There should be 15 to 25 samples per analytical run. For every sample there will be two vials—one containing sample and LCTX ISTD solution and the other containing sample and LCTX SA solution.

7.7.2 Make labels for all Check Standards, Blanks, and Samples as indicated by the run sheet. Remember to make labels for the standard addition samples. All labels except for the Blanks go on microvials. Only two Blank labels will be needed per analytical run and those labels will be placed on 2 mL screw capped LC vials. Labels should have the sample name, LCTX Run number, date of preparation, and initials of personnel preparing the analytical run. Initials should be cross-walked to full names in log book.

7.7.3 Apply labels to all vials and organize by analytical run.

7.7.4 Preparation of Blanks

7.7.4.1 Place 2 mL of reagent water into a labeled 2 mL screw capped LC vial. There should be a total of two vials with Blank solution per analytical run.

7.7.5 Preparation of Samples

7.7.5.1 Invert all samples 3 times before pipetting.

7.7.5.2 Pipette 1.5 mL of filtered sample into a glass LC/MS/MS vial and cap.

7.8 Make sure the Source of the MS/MS is clean. If source is not cleaned and you are not trained ask your supervisor for training.

7.9 Mobile Phase Preparation for LC

7.9.1 Preparation of Mobile Phase A: 0.1 % Formic acid in reagent water.

7.9.1.1 Add 2 mL of concentrated formic acid to 2 L of reagent water. Cap and invert 3 times. pH will be approximately 2.6 to 2.7 typically.

7.9.1.2 Place on channel A of the LC.

7.9.2 Preparation of Mobile Phase B: 0.1 % Formic acid in 50/50 Methanol/Acetonitrile.

7.9.2.1 Add 2 mL of concentrated formic acid to 1 L of LC/MS/MS grade or better methanol and 1 L of LC/MS/MS grade or better acetonitrile. Cap and invert 3 times.

7.9.2.2 Place on channel B of the LC.

7.9.3 Preparation of Mobile Phase C: Reagent Water.

7.9.3.1 Add 2 L of reagent water into a 2 L mobile phase bottle.

7.9.3.2 Place on channel C of the LC.

7.9.4 Preparation of Mobile Phase D: 50/50 Methanol/Acetonitrile.

7.9.4.1 Add 1 L of LC/MS/MS grade or better methanol to 1 L of LC/MS/MS grade or better acetonitrile in a 2 L mobile phase bottle.

7.9.4.2 Place on channel D of the LC.

7.10 Make sure all frits, guard cartridge, and analytical column are in place for LCTX. Analytical column is a Waters Corp. Atlantis T3 analytical column. A Waters Corp. Atlantis dC18 analytical column can also be substituted resulting in slight changes in analyte retention time. The guard cartridge is a Waters Atlantis dC18 cartridge.

7.11 Prime the LC pumps by opening the purge valves, setting flow rate at 2 to 5 mL/min proportion at 25% for each of the 4 mobile phases. Let prime for at least 5 minutes. Change flow to 95% A and 5% B to reflect starting conditions of separation for 5 minutes. When priming is finished, reduce flowrate back to initial flowrate conditions of LCTX method (usually around 0.7 mL/min), and close purge valve LC pump.

7.12 This is a performance based method and is suitable for any bioinert LC/MS/MS system as long as quality control criteria are met. SOP is written currently for an Agilent 1260 bioinert LC/6460 triple quadrupole mass spectrometer with a jet stream source attached. Multimode sources are suitable as well when used in electrospray mode only. The LCTX method is adapted from Loftin et al., 2008 and Graham et al., 2010.

7.12.1 Defragment partitioned hard drive of instrument computer weekly.

7.12.2 Open Agilent MassHunter Acquisition software.

7.12.3 Open the current LCTX Project

7.12.4 Open an old LCTX worklist from a previous run and resave with the current analytical run number and date.

7.12.5 Enter the correct sample names and save the batch (see appendix B for example layout). Check that the correct acquisition method is being used.

7.12.6 Recheck worklist for typographical errors. Resave if any changes.

7.12.7 Place vials in appropriate position in autosampler tray (as shown in appendix B unless project scientists requests a change). Blanks go in vial 1 and vial 2 slots, internal standard solution in vial 3, and standard addition solution is 100 µg/L calibration standard. Place QC, calibration standards, and samples in order in well plate trays starting with P1-A1 according to worklist.

7.13 Equilibration of LC/MS/MS

7.13.1 Open the LCTX method.

7.13.2 Start the worklist in multiple vial mode.

7.13.3 Run the first 3 injections of the worklist as 1 µg/L control standards. If using a new column, then may need to run up to 6 injections to equilibrate column.

7.13.4 Evaluate retention time stability, peak shape and abundance. Values should be within 60 seconds, consistent peak shape based on historical data, and within 30% of historical abundance, respectively.

7.13.5 If data is not consistent, then begin troubleshooting which may include:

7.13.5.1 Check that LC backpressure is within typical ranges.

7.13.5.2 Make sure purge valve is closed.

7.13.5.3 Check for leaks.

7.13.5.4 Check that spray from electrospray needle is positioned correctly and has a concentric spray.

7.13.5.5 Infuse a standard in the MS/MS to check MS/MS performance.

7.13.5.6 Notification of supervisor as needed and remedial action to correct instrument performance.

7.14 Submission of Worklist.

7.14.1 If control standards data looks comparable between injections, then proceed with worklist. Control standards should be within +/- 20% of expected concentration or abundance.

7.14.2 Verify periodically that internal standard, blanks, controls, and standard addition samples look appropriate. Confirm that peak shapes and retention times are consistent compared to historical analysis runs (e.g. retention times within 1 minute of historical value unless method needs to be modified with approval from supervisor. If not, remedy the problem following the troubleshooting steps in Section 7.13.5.

7.15 Post Run Instrument Clean-Up.

7.15.1 The last line of the worklist should include a blank injection using the LCTX clean method. This will use mobile phases C and D to clean any residual traces due to sample matrix out of the column under the clean conditions which are at a LC higher temperature. No acid modifier is added to mobile phases C and D for proper column storage.

7.16 Data Reduction with Agilent MassHunter Quantitation software

7.16.1 Once data has been acquired, open the Agilent MassHunter Quantitation software.

7.16.2 Create a new Batch and load newly acquired data from worklist into the batch.

7.16.3 Load the appropriate LCTX quant method.

7.16.4 Edit the LCTX quant method to update retention times and MRM ratios as necessary using a mid to upper range calibration standard. Check integration of all compounds.

7.16.5 Save method with batch folder and process calibration data. Use linear or quadratic curve fits. 1/x weighting is permissible. R^2 values should be 0.98 or greater. Save when done.

7.16.6 Quantitate all samples

7.16.7 Evaluate calibration data, make sure blanks are blank below the minimum reporting level (MRL) of the method, duplicates and control standards are within +/- 20% (28.3% RSD) of expected concentration or abundance.

7.16.8 For Standard Addition Calculations, use the standard addition LCTX quant method or export the results table into a spreadsheet program such as Microsoft Excel.

7.16.9 When quantitation is complete, have a supervisory chemist provide quality control of the data set as described in Section 10.

7.16.10 Reanalysis of samples is necessary when quality control or instrument performance renders the data outside of acceptable QC metrics as established in Section 10 of the SOP, Table 5.11.1 of the NCCA 2015 QAPP and best professional scientific judgement by a supervisory chemist. When using standard addition for quantitation, check if concentrations prior to correction for dilution are greater than 2.5 µg/L. If so, dilute the original sample and reanalyze by standard addition as described in this SOP.

7.16.11 If any samples exhibit data quality issues confer with a supervisory chemist for evaluation of problem.

7.16.12 When data quality is deemed acceptable then store an electronic data analysis report for record keeping.

7.17 Refer to the 2010 OGRL SOP on backing up data for data archival.

8.0 REFERENCES

8.1 2010 Backing up Data

8.2 Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. Chorus, I.; Bartram, J.; Eds., Spon Press: London, 1999.

8.3 Graham, J.L., Loftin, K.L., Meyer, M.T., Ziegler, A.C. 2010. Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States, *Environ. Sci. Technol.*, 44, 7361-7368.

8.4 Loftin, K.A., Meyer, M.T., Rubio, F., Kamp, L., Humphries, E., Whereat, E. 2008. Comparison of two cell lysis procedures for recovery of microcystins in water samples from

Silver Lake in Dover, Delaware, with microcystin producing cyanobacterial accumulations. USGS OFR 2008-1341, 9 p.

9.0 RECORDS AND ARCHIVAL

The person performing this SOP is responsible for submitting the following records to be archived to the Project Documents Archival manager or stored in the appropriate location in the laboratory (usually next to instrument computer).

9.1 Instrument Maintenance Log

9.2 Worklist Log

9.3 Tune files Log

9.4 Computer data files for each sample and control are stored, copied, backed up, and archived according to OGRL-SOP-2010.

10.0 QUALITY CONTROL

10.1 Supervisory chemist visually reviews QC data for each run or uses software to identify excursions from permissible results described in Sections 7.16.5 and 7.16.7, and MRLs listed in Table 5.11.1 of the NCCA 2015 QAPP.

10.1.1 Analytical control is maintained by the use of carryover blanks (COB), laboratory duplicates (DUP), and Control Standards (CCV).

10.1.2 Standard addition (spiked sample duplicate) results provide checks for and qualify matrix dependent shifts in retention times and Multiple Reaction Monitoring (MRM) ratios, and provide a basis for sample matrix-corrected results when responses deviate from expected (e.g. +/- 20% or 28.3% RSD).

10.1.3 Target analytes will be quantitated by internal standard curve unless matrix effects are greater than +/- 20%. Larger deviations will trigger reanalysis and quantitation by standard addition.

11.0 ATTACHMENTS

11.1 Appendix A: Analyte List

11.2 Appendix B: Instrument Worklist Example

11.3 Appendix C: LCTX LC/MS/MS method

11.4 Appendix D: LCTX Clean LC/MS/MS method

12.0 REVISIONS TO THIS SOP

Rev. 1 9/29/2008 Initial version

Rev. 2 8/31/2015

Appendix A: Analyte List

Toxin	Type	CAS#	Stock Concentration (µg/mL)	Diluent (solvent)
anatoxin-a	Cyanotoxin	64285-06-9	100	Water
cylindrospermopsin	Cyanotoxin	143545-90-8	100	Methanol
domoic Acid	Algal toxin	14277-97-5	100	Methanol
microcystin-HiLR	Cyanotoxin	NA	100	Methanol
microcystin-HtYR	Cyanotoxin	NA	100	Methanol
microcystin-LA	Cyanotoxin	96180-79-9	100	Methanol
microcystin-LF	Cyanotoxin	154037-70-4	100	Methanol
microcystin-LR	Cyanotoxin	101043-37-2	100	Methanol
microcystin-LW	Cyanotoxin	111755-37-4	100	Methanol
microcystin-RR	Cyanotoxin	111755-37-4	100	Methanol
microcystin-WR	Cyanotoxin	NA	100	Methanol
microcystin-YR	Cyanotoxin	101043-37-2	100	Methanol
nodularin-R	Cyanotoxin	118399-22-7	100	Methanol
okadaic acid	Algal toxin	78111-17-8	100	Methanol
L-phenylalanine	Amino acid	63-91-2	100	Water

Appendix B: Example Instrument Run Sheet Layout

Worklist Number	**Sample ID**	**Sample Type**	**Injection Volume (µL)**	**Standard**
1	1 µg/L LCTX Standard Mix a	Column Equilibration Sample	1	100 µg/L LCTX Standard Mix
2	1 µg/L LCTX Standard Mix b	Column Equilibration Sample	1	100 µg/L LCTX Standard Mix
3	1 µg/L LCTX Standard Mix c	Column Equilibration Sample	1	100 µg/L LCTX Standard Mix
4	Blank 1	Instrument Blank	0	Blank
5	0.001 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	100	0.10 µg/L LCTX Standard Mix
6	0.010 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	1	1 µg/L LCTX Standard Mix
7	0.030 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	3	1 µg/L LCTX Standard Mix
8	0.050 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	5	1 µg/L LCTX Standard Mix
9	0.080 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	8	1 µg/L LCTX Standard Mix
10	0.10 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	10	1 µg/L LCTX Standard Mix
11	0.25 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	25	1 µg/L LCTX Standard Mix
12	0.50 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	50	1 µg/L LCTX Standard Mix
13	0.75 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	75	1 µg/L LCTX Standard Mix
Worklist Number	**Sample ID**	**Sample Type**	**Injection Volume (µL)**	**Standard**

14	1 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	1	100 µg/L LCTX Standard Mix
15	5 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	5	100 µg/L LCTX Standard Mix
16	8 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	8	100 µg/L LCTX Standard Mix
17	10 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	10	100 µg/L LCTX Standard Mix
18	25 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	25	100 µg/L LCTX Standard Mix
19	50 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	50	100 µg/L LCTX Standard Mix
20	75 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	75	100 µg/L LCTX Standard Mix
21	99 µg/L LCTX Standard Mix	Internal Standard Curve Calibration	99*	100 µg/L LCTX Standard Mix
22	Blank 2	Instrument Blank	0	Blank
23	NCC-439871 A	Sample	99	
24	NCC-439872 A	Sample	99	
25	NCC-439873 A	Sample	99	
26	NCC-439874 A	Sample	99	
27	NCC-439875 A	Sample	99	
28	NCC-439876 A	Sample	99	
29	NCC-439877 A	Sample	99	
30	NCC-439878 A	Sample	99	
Worklist Number	**Sample ID**	**Sample Type**	**Injection Volume (µL)**	**Standard**
31	NCC-439880 A	Sample	99	
32	NCC-439881 A	Sample	99	

33	NCC-439871 L	Instrument Duplicate	99	
34	NCC-439881 SA	Spiked Sample Duplicate	99 - sample 1 - 100 µg/L LCTX Standard Mix	
35	Blank 3	Instrument Blank	0	Blank
36	1 µg/L LCTX Standard Mix CC1	Calibration Check	1	100 µg/L LCTX Standard Mix
37	Blank 4	Instrument Blank	0	Blank
38	NCC-439882 A	Sample	99	
39	NCC-439883 A	Sample	99	
40	NCC-439884 A	Sample	99	
41	NCC-439885 A	Sample	99	
42	NCC-439886 A	Sample	99	
43	NCC-439887 A	Sample	99	
44	NCC-439888 A	Sample	99	
45	NCC-439889 A	Sample	99	
46	NCC-439890 A	Sample	99	
47	NCC-439891 A	Sample	99	
48	NCC-439882 L	Instrument Duplicate	99	
49	NCC-439891 SA	Spiked Sample Duplicate	99 - sample 1 - 100 µg/L LCTX Standard Mix	
50	Blank 5	Instrument Blank	0	Blank
Worklist Number	**Sample ID**	**Sample Type**	**Injection Volume (µL)**	**Standard**
51	1 µg/L LCTX Standard Mix CC2	Calibration Check	1	100 µg/L LCTX Standard Mix
52	Blank 6	Instrument Blank	0	Blank

1 Only 99 µL is injected to accommodate a 1 µL stacked injection of internal standard (simetone) for 100 µL injection loops. 100 µL of standard mix can be injected on larger injection loops.

2 L = instrument sample duplicate

3 SA = Spiked Sample Duplicate. Final concentration can be modified by changing the injection volume of the standard spiked. This example shows a 1.0 µg/L equivalent final concentration.

Appendix C.2

Title:	Identifier:	Revision:	Effective Date:
Sequential Freeze/Thaw Cell-Lysis Procedure for Total and Dissolved Algal Toxin Analysis of Water Samples	*OGRL-SOP-4520*	**2**	**1/18/2016**

APPROVALS FOR USE		
Author's Name (Print): *Keith A. Loftin*	Author's Signature:	Date: *01/18/16*
Project Director's Name (Print) *Mike T. Meyer*	Project Director's Signature	Date: **01/22/16**
Organic Geochemistry Research Laboratory (OGRL)		

STANDARD OPERATING PROCEDURE

PROCESSING WATER SAMPLES FOR ALGAL TOXIN ANALYSIS

Table of Contents

Processing Water Samples for Microcystin Analysis

NOTE: Laboratory personnel may produce paper copies of this procedure printed from the controlled document file. However, it is their responsibility to ensure that they are trained on and utilizing the current version of this procedure. The procedure author may be contacted if text is unclear.

1.0 SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the sequential freeze/thaw cell-lysis process for preparing water samples for algal toxin analysis at the USGS Organic Geochemistry Research Group (OGRL) Laboratory in Lawrence, KS.

1.2 *Algal toxins* are toxins produced and released by phytoplankton. These algal blooms can be extremely toxic to many different species of birds and mammals (including humans).

1.3 This Standard Operating Procedure (SOP) describes the cell-lysis process of freezing and thawing water samples as a means to lyse the algal cells and release of algal toxins for analysis.

2.0 TRAINING

The OGRL Director or designee is responsible for ensuring that all who perform the functions described in this SOP for the OGRL are familiar with the objectives of and properly trained in its procedures. In addition, one must document that they have read and understand this procedure in their training folder.

3.0 DEFINITIONS

3.1 *Total Algal Toxins*—For purposes of this SOP, this term refers cell-lysis of all phytolankton in a given water sample followed by filtration to remove particulates.

Cell-lysis results in intracellular algal toxins transferred to the dissolved phase of the water sample.

3.2 *Dissolved Algal Toxins*—For purposes of this SOP, this term refers to filtration to remove particulates of a given water sample. Given that this sample is filtered in the absence of artificial (laboratory induced) cell-lysis, the algal toxins measured in the water sample do not represent intracellular algal toxins, but dissolved-phase algal toxins from naturally lysed algae.

3.3 *Frozen Water Sample*—A water sample that has been placed overnight in a freezer and is frozen completely through.

3.4 *Thawing Water Sample*—A water sample that has been removed from a freezer to thaw protected from light by aluminum foil.

3.5 *Thawed Water Sample*—A water sample that contains no ice and is composed only of liquid.

3.6 *Filtering*—The process of forcing a sample through a filter to remove particulates.

3.7 *Sample ID*—Each sample in a defined project will have a unique ID that is generally five digits long with a letter.

3.8 *Project Code*—This is the three digit code noted on all sample labels. It is unique and informs the lab employees which project the sample is a part of.

3.9 *Project Title*—This is the title of the project. It will generally include information such as the purpose of the study and who is concerned with the results. An example would be 'EPA Lake Assessment.'

3.10 *Reslab*—This is the name of the shared network used by all members of the Organic Geochemistry Research Laboratory.

4.0 PERSONNEL HEALTH AND SAFETY

4.1 **Note:** This SOP is to be used in conjunction with an approved Chemical Hygiene Plan. Also, consult the Chemical Hygiene Plan for information on and use of all personal protective equipment (PPE).

4.2 **Toxins:** The nature of this work can expose an individual to algae and algal toxins if appropriate standard safety protocols are not followed. Notify supervisor when initiating work with environmental samples that may contain toxins and as always report any safety incidences at the earliest opportunity to the laboratory safety officer.

4.3 Always where gloves, at minimum safety glasses, work in the hood when possible and to the extent necessary. Do not ingest, inhale, get in eyes, or contact with skin. If contact with skin made then wash with copious amounts of soap and water. If eye contact made immediately use the eyewash station to rinse eyes then seek medical attention as necessary. For ingestion or inhalation, seek appropriate medical attention. The toxins are not known to be volatile, but can be aerosolized.

5.0 EQUIPMENT AND SUPPLIES

Descriptions of commonly used pieces of equipment, their advantages, and their limitations are listed below.

5.1 *Nitrile Gloves*- Required for handling all environmental samples potentially containing toxins.

5.2 *Freezer Space*- Space should be set aside for the water samples in a freezer with a temperature range less than or equal to –20˚C (+/-5 ˚C).

5.3 *Refrigerator Space*- Space should be set aside for the water samples in a refrigerator or the walk-in with a temperature range between 2˚C and 10˚C.

5.4 *Aluminum Foil*- Foil is used for covering the sinks full of thawing samples. **Algal toxins have been reported as light sensitive, it is necessary to cover all of the samples at all times!**

5.5 *Vacuum Filtration*-The process by which particulates are removed from samples by vacuum.

5.6 *Syringe Filtration*-The process by which particulate are removed from samples by use of syringe and filter.

5.7 *Clear LC/MS Screw Top Vials*- These are 2mL clear glass vials. One vial is needed for each sample. A fine tip permanent marker is used to print pertinent information onto the labeling sticker, which is attached to the vial.

5.8 *Screw Top Cap*- This blue cap is used to seal the clear screw top vial.

5.9 *1000 mL Beaker*- This beaker is used to collect unwanted water sample.

5.10 *Permanent Marker*- Used for labeling the 1000mL dump beaker.

5.11 *Labeling Tape*- Colored tape that is used to label the 1000mL beaker.

5.12 *Labeling Stickers*- Used for labeling the screw top vials during the filtration process.

5.13 *Fine Tip Permanent Marker*- Used for writing on labeling stickers during the filtration process.

5.14 *10mL pipette and pipette tips*- Used for transferring 10mL of the unfiltered sample to the syringe filter.

5.15 *Empty Chromacol Cardboard Box*- This box is used to store the chromacol vials in a freezer after processing is complete. A spreadsheet that includes a list of the vials being stored is placed inside the box. A label is also necessary on the outside of the box.

5.16 *Empty Vial File or Tray*- An item used to store all clear screw top vials belonging to a designated IMN run. It is labeled and stored in a freezer for future analysis and archival/storage.

5.17 *Clear tape*- This is used to affix printed labels to the chromacol vials.

5.18 *1 oz. amber glass bottle* – a sample storage container used for frozen storage/archival of sample filtrate.

6.0 PROCEDURE FOR FREEZE/THAW CYCLING

Note: Deviations from SOPs must be recorded in an appropriate instrument or work log. HDPE or Teflon sample bottles may be used in place of amber glass for selected projects at the initiation of a study. Additionally, different filter procedures may be used prior to the initiation of a study. These deviations from this SOP are not

acceptable after a study is initiated. Deviations to this procedure are subject to approval by the principle investigator.

6.1 If sample is to be processed for Dissolved Algal toxin analysis only skip to section 7.0. If sample is to be processed for both Total and Dissolved Algal toxin, then the sample will need to be homogenized by inversion of the sample at least 3 times. Split the homogenized sample in half and label each sample appropriately. Designate 1 bottle as Total and the other Dissolved. Take the sample split for Total Algal toxin analysis through the remaining Freeze/Thaw procedure starting with step 6.2. Skip to Section 7.0 of this SOP to begin processing for Dissolved Algal toxin analysis. Any glass container that will be frozen should be no more than half full of sample.

6.2 It is important to have as much communication between all personnel involved in the project as possible.

6.2.1 At the beginning of every shift, read through the “Log Note” left from the students who last worked on the project (see section 10.3.1.3).

6.2.2 Speak with the student login assistant and find out if any new samples have been received for the afternoon.

6.3 If there are new samples that have been logged in, ask the login assistant for the physical location of the samples. Also, find out if the samples have undergone the first freeze or freeze/thaw cycle (sometimes samples are stored frozen before shipment to OGRL and may or may not thaw during shipment).

6.4 Create a “Processing Spreadsheet” for the new samples (see section 9.1).

6.5 Each morning all samples from the freezer and refrigerator are thawed in an empty sink for the day.

6.5.1 Cover all samples with aluminum foil while in sink and do not have samples touching each other to allow air to circulate between the bottles.

6.6 Print out the “Sample Checklist” (see section 10.1) and note where all the samples are located in the cycling process.

6.6.1 To make the checklist easy to read, choose a different colored pen/highlighter to mark: the thawing samples, the samples that have been sent to the freezer for the next freeze cycle and the samples ready for filtration.

6.6.1.1 If the sample has just completed its first, then add 1 line by permanent marker to the lid. Repeat with a second line for the completion of the second freeze/thaw cycle, and a third line when the third freeze/thaw cycle is complete. Record dates of each freeze/thaw step in the spreadsheet for each sample.

6.6.1.2 If the sample has just completed its third thaw, it is ready for filtration and then vialing (see section 7.0). These samples will be kept in the refrigerator before filtration begins. If samples will not be filtered within 24 hours then do not do the third thaw until ready for filtration.

6.7 Make sure that all samples are accounted for and all spreadsheets are updated on the computer spreadsheet (see section 9.0).

6.8 All samples that are still thawing will be kept overnight in a refrigerator (Thawing time is very dependent on sample volume).

6.9 Samples that are completely thawed out will follow the sample procedure outlined in sections 6.5.

6.10 All spreadsheets must be updated and printed out for storage in the project binder located in the Project Management office (see section 9.0)

7.0 PROCEDURE FOR FILTERING/VIALING

7.1 One of two filtration techniques (vacuum filtration or syringe filtration) will be used on a set of project samples as indicated by the principle investigator or the project management office. Filter type and mesh size can be modified by the principle investigator to meet project needs, but changes should be recorded in sample spreadsheet.

7.2 Vacuum filtration

7.2.1 Get a clean 47 mm diameter 100 mL two-piece glass funnel and funnel clamp for each sample.

7.2.2 Assemble the filter assembly with a 0.7 micron, 47 mm diameter glass fiber filter in between the two filter pieces and clamp together.

7.2.3 Attach filter assembly to a clean 250 to 1000 mL side arm vacuum flask.

7.2.4 Connect vacuum flask with vacuum tubing to house vacuum.

7.2.5 Invert the capped sample bottle vigorously at least 3 times to homogenize sample.

7.2.6 Add approximately 30 mL of sample to funnel and apply vacuum until filter is dry.

7.2.7 All filtrate should be stored in clean glass vials for freezing. Glass vials should not be more than half full.

7.2.8 Make sure all vials have appropriate identifying information (e.g. sample ID, data, personnel, and “TF” for total and filtered or “DF” for dissolved and filtered.

7.2.9 Transfer 1 mL of filtered sample to a labeled 2 mL screw capped vial.

7.2.10 Store the remaining filtrate in

7.2.11 When finished filtering update all spreadsheets (see section 9.0).

7.3 Syringe Filtration

7.3.1 Attach an unused 25 mm, 0.7 micron glass fiber membrane syringe filter to an unused 10 mL HDPE syringe with luer lock fitting after removing syringe plunger.

7.3.2 Lay syringe plunger on a clean chemwipe.

7.3.3 Add 10 mL of sample to syringe barrel with syringe filter in place.

7.3.4 Replace removed syringe plunger back into syringe barrel and filter 1 mL of sample directly into labeled, glass 2 mL LC/MS vial. Cap vial.

7.3.5 Filter remaining sample, to larger 1 oz. amber glass bottle.

8.0 REFERENCES

Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring, and management. Eds. I. Chorus, J. Bartram, Spon Press: London, 1999.

Graham, J.L., Loftin, K.A., Meyer, M.T., Ziegler, A.C., 2010, Cyanotoxins mixtures and taste-and-odor compounds in cyanobacterial blooms from the midwestern United States, Environmental Science and Technology, 44, 7361-7368.

9.0 RECORDS AND ARCHIVAL

The person performing this SOP is responsible for submitting the following records to be archived to the Project Office Manager.

9.1 PROCESSING SPREADSHEETS (All spreadsheets are maintained in "ResLab" on the network.)

9.1.1 The processing sheets are important because they allow OGRL staff to keep track of the freeze/thaw cycling for each sample. They also indicate when each sample was filtered/vialed and the current location.

9.1.2 The template for this spreadsheet should include: the project code, sample ID, date received, number of bottles processed with this sample ID, a section to fill-in the date for each freeze and thaw (repeated three times), date the sample was filtered/vialed, storage location, initials of student and a notes section.

9.2 Liquid Chromatography Tandem Mass Spectrometry and Enzyme-Linked Immunosorbent Assay Run Sheets

9.2.1 The Project Management office and the Principle Investigator should be notified as samples are ready for analyses so run sheets may be populated and samples analyzed as appropriate.

10.0 QUALITY CONTROL

10.1 SAMPLES CHECKLIST

10.1.1 The samples checklist is created in the morning and about an hour before the personnel will leave for the day. After completing the checklist, personnel will update the Processing Spreadsheets with the new information.

10.1.1 To create the checklist, search through each Processing Spreadsheet to find which samples have not yet been filtered/vialed. Copy and paste the entire row of the sample's processing information onto the checklist and keep adding samples.

10.1.2 **Samples not completely thawed.** When a sample is still somewhat or totally frozen, mark the sample ID on the Samples Checklist with a colored highlighting marker. *Place the sample back in the sink to thaw or in the refrigerator for overnight storage as is appropriate.*

10.1.3 **Samples that are thawed and ready to freeze.** When a sample is completely thawed and ready to enter the next freeze cycle, mark the sample ID on the Samples Checklist with a colored highlighting marker (a different color than the marker used in section 8.2.3.3.1.1). *Mark a new line on the top of the sample bottle and place it into the freezer.*

10.1.4 **Samples that are thawed and ready for filtration.** When a sample is completely thawed and ready for filtration and vialling, mark the sample ID on the Samples Checklist with a colored highlighting marker (a different color than the markers used in sections 8.2.3.3.1.1 and 8.2.3.3.2.1). *Set the bottle aside under a cover and filter/vial.*

10.2 CHECKLIST FOR FILTRATION

10.2.1 The Checklist for Filtration helps the personnel accurately complete all filtration steps. While filtering a sample, check off each step in the process.

10.2.2 The checklist should include: the project code, sample ID, each step of filtration/vialing and the initials of the student.

10.3 **NOTES**

10.3.1.1 After cleanup at the end of the work shift it is necessary for personnel to communicate their progress on the project to the Project Management Office and Principle Investigator.

11.0 ATTACHMENTS

No attachments

12.0 REVISIONS TO THIS SOP

No revisions

1/18/2016 reviewed.

Appendix C.3

Title: *Data and Information Backup for all OGRL Instruments*	Identifier: *OGRL-2010*	Revision: **5**	Effective Date: **1/12/16**

15.1.1.1 APPROVALS FOR USE

Author's Name (Print): *Keith A. Loftin*	Author's Signature:	Date: *1/12/16*
Project Director's Name (Print) *Michael T. Meyer*	Project Director's Signature	Date: **1/12/16**

15.1.2 Organic Geochemistry Research Group (OGRG)

STANDARD OPERATING PROCEDURE

DATA AND INFORMATION BACKUP FOR HP GCMS, LCMS, AND HPLC INSTRUMENTS

Table of Contents

Data and Information Backup for all OGRL instruments

NOTE: Laboratory personnel may produce paper copies of this procedure printed from the controlled document file. However, it is their responsibility to ensure that they are trained on and utilizing the current version of this procedure. The procedure author may be contacted if text is unclear.

1.0 SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the process Data and Information Backup for instruments for the USGS Organic Geochemistry Research Laboratory (OGRL) in Lawrence, KS.

2.0 TRAINING

The Project Director is responsible for ensuring that all who perform the function(s) described in this SOP for the OGRL are familiar with the objectives of and properly trained in its procedures. In addition, one must document that they have read and understand this procedure in their training folder.

3.0 DEFINITIONS

3.1 Computer—PC that is used to operate and control OGRL instruments.

3.2 External Backup Drive—external data storage drive used for transferring information from instrument computer's hard drive to an external flash drive.

4.0 PERSONNEL HEALTH AND SAFETY

Note: This SOP is to be used in conjunction with an approved Chemical Hygiene Plan. Also, consult the Chemical Hygiene Plan for information on and use of all PPE.

4.1 Obey and follow all Safety Regulations when entering the Laboratory.

5.0 EQUIPMENT AND SUPPLIES

Descriptions of commonly used pieces of equipment, there advantages and their limitations are listed below.

5.1 External Backup Drive — Each instrument is currently equipped with this device.

6.0 PROCEDURE

Note: Deviations from SOPs must be recorded in an appropriate instrument or work log.

6.1 Each instrument is equipped with an external backup drive to archive instrument methods, worklists, and data folders (hereafter referred to as data).

6.2 Data is manually archived weekly during scheduled instrument downtime.

6.3 Data archive is then backed up onto the USGS KS WSC network drive and also maintained on the external backup drive. The USGS KS WSC network drive has a redundant mirror site in case of network failure.

6.4 Over time given the operation of the instruments large quantities of data stored in files on the instrument computer hard drive will have to be permanently removed from the instrument computer (e.g. when 75% of computer's memory is consumed). Each instrument is backed up using the same general procedure. If possible perform backups when the instrument computer is not in operation.

6.5 Printed copies of instrument sequences and analytical methods are also maintained at each instrument.

7.0 REFERENCES

No references are cited in this SOP.

8.0 RECORDS AND ARCHIVAL

The person performing this SOP is responsible for submitting the following external drives to be archived to the Project Documents Archival manager.

9.0 QUALITY CONTROL

No quality control measures have been defined for this procedure.

10.0 ATTACHMENTS

There are no attachments to this SOP.

11.0 REVISIONS TO THIS SOP

6/6/00- Initial Version
2/15/02- Revisions 2 , Added Section 11.0
6/4/03-Reviewed, no changes
1/12/04 – Reviewed, no changes
1/12/16 – Reviewed, changed archive procedures from tape drive back up to external flash drive storage.

APPENDIX D: EXAMPLE SOPS FOR MERCURY IN FISH TISSUE PLUG ANALYSES

Eurofins Document Reference	EFGS-SOP-137-R02	**Revision**	2
Effective Date	6/17/2013	**Status**	Final
Historical/Local Document Number	FGS-SOP-137.02		
Local Document Level	Level 3		
Local Document Type	SOP		
Local Document Category	NA		

Prepared by	Ryan Nelson
Reviewed and Approved by	Dave Wunderlich and Patrick Garcia-Strickland

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Approvals:

Prepared by: _____ Date: 6/17/13

Approved by: _____ Date: 6/17/2013

Approved by: _____ Date: 6/18/13

[redacted]

1 Revision Log:

Revision: 06	Effective Date: This version	
Section	**Justification**	**Changes**
Cover	Required change	Changed company name from Frontier Global Sciences to Eurofins Frontier Global Sciences.
All	Formatting requirement per LOM SOP-LAB-201	Reformatted document to new corporate specifications.
13.1, 13.2	Required	Added hardware and software components
14.8	Required	Updated mercury standard prep
14.9	Required	Updated standard and reagent documentation procedures
15.2 – 15.4	Required	Updated calibration information
16.7	Required	Added instrument maintenance and troubleshooting

2 Reference:

2.1 EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, 2002.

2.2 Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, April 1995 with January 1996 revisions.

2.3 Bloom, N.S.; and Tsalkitzis, E. Standard Operating Procedure FGS-012 Determination of Total Mercury in Aqueous Media (Modified EPA Method1631). Frontier GeoSciences Inc., Quality Assurance Manual 1995.

2.4 Bloom, N.S.; Ultra-Clean Sample Handling, Environmental Lab 1995, March/April, 20.

2.5 Bloom, N.S.; Horvat M., and Watras C.J. Results of the International Mercury Speciation Intercomparison Exercise. Wat. Air Soil Pollut. 1995, 80, 1257.

2.6 Bloom, N.S.; Crecelius, E.A. Determination of Mercury in Seawater at Sub-nanogram per Liter Levels.Mar.Chem.1983, 14, 49.

2.7 Bloom, N.S.; Crecelius, E.A. Distribution of Silver, Lead, Mercury, Copper, and Cadmium in Central Puget Sound Sediments Mar. Chem 1987, 21, 377-390.

2.8 Bloom, N.S.; Fitzgerald, W.F. Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection. Anal. Chem. Acta. 1988, 208, 151.

2.9 Cossa, D.; Couran, P. An International Intercomparison Exercise for Total Mercury in Seawater. App.Organomet. Chem.1990, 4, 49.

2.10 Fitzgerald, W.F.; Gill, G.A. Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis. Anal. Chem. 1979, 15, 1714.

2.11 Gill, G.A.; Fitzgerald, W.F. Mercury Sampling of Open Ocean Waters at the Picogram Level Deep Sea Res.1985, 32, 287.

2.12 EPA Method 30.B, Determination of total vapor phase mercury emissions from coal-fired combustion sources using carbon sorbent traps.

2.13 Chemical Hygiene Plan, Eurofins Frontier Global Sciences, current version.

2.14 National Environmental Laboratory Accreditation Conference, NELAC Standard September 8, 2009.

2.15 Department of Defense Quality Systems Manual for Environmental Laboratories, prepared by DoD Environmental Quality Workgroup, Final Version 4.2, October 2010.

3 Cross Reference:

Document	Document Title
SOP FGS-003	Pipette Verification, Calibration and Maintenance
SOP FGS-007	Cleaning of Sampling Equipment and Bottles
SOP FGS-008	Ultra Clean Aqueous Sample Collection
SOP FGS-012	Oxidation of Aqueous Samples for Total Mercury Analysis
SOP FGS-061	Gold Trap Construction
SOP FGS-094, App F	Standard Operating Procedure Training Record
SOP FGS-099	Waste Disposal Procedure for Client Sample Waste
SOP FGS-121	Determination of Total Mercury by Flow Injection AFS (Mod 1631E)
SOP FGS-155	Calibration of Volumetric Dispensers

4 Purpose:

4.1 This SOP is designed to ensure that all reproducible traceable procedures in EPA 1631 are followed in the standardization of the total mercury analyzers and in the analysis of samples for total mercury, as well as to establish the limits wherein data will be considered acceptable.

5 Scope:

5.1 This Standard Operating Procedure (SOP) describes a method for the determination of total mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold vapor atomic fluorescence spectrometry (CVAFS).

5.2 This method is designed for the determination of mercury in the range of 0.5-40 ng/L (ppt). Application may be extended to higher levels by selection of a smaller sample size, as long as the instrument value (intensity) remains within the calibration curve.

5.3 The Control Limits are established from EPA 1631E.

6 Basic Principles:

6.1 For analysis of aqueous samples, an aliquot of oxidized sample is neutralized with hydroxylamine-hydrochloride (NH_2OH-HCl) to destroy free halogens, and added to a bubbler.

6.2 Stannous chloride ($SnCl_2$) is added to the bubbler to reduce the Hg(II) to volatile Hg(0), and the bubblers are sealed with Keck clips. Blanked gold traps are placed at the end of soda-lime pre-traps. The bubbler is purged with nitrogen (N_2) for 20 minutes. All gas that flows into the bubbler should only leave the system through the soda-lime pre-trap and then the gold trap.

6.3 The gaseous mercury amalgamates to the gold traps, which are removed and individually placed in the analytical train. The gold trap is heated, thus releasing the mercury into the argon gas stream flowing into the instrument.

7 Reference Modifications:

7.1 There were no significant modifications to this method.

8 Definitions:

8.1 Analytical Duplicate (AD): A representative sample (that yielded a result within the calibration curve) is analyzed a second time during the analytical run. The second analysis should be at the same aliquot as the original.

8.2 Analytical Run – The continuous analysis of one or more batches during the same 12 hour-shift. Each analytical day requires a minimum five-point calibration curve, ICV, at least 3 IBLs, and CCV/CCB every ten runs. An analytical day must conclude with a CCV/CCB.

8.3 Analytical Spike and Analytical Spike Duplicate (AS/ASD): A representative sample is selected and spiked, with a dilution of the primary source, during the analytical run, at a target concentration of 1-5X the ambient concentration of the sample. These QC samples are used to indicate sample matrix effects on the analyte of interest. Non-detectable samples are spiked at 1 – 5 x of the MRL/PQL.

8.4 Batch: 20 client samples or less grouped for preparation. See Quality Assurance Section for batch requirements.

8.5 Calibration Standards (CAL) – a series of standards that will be used to calibrate the instrument, made from a primary source stock standard. A calibration blank plus at least five different concentrations are required, beginning with one at PQL concentration.

8.6 Certified Reference Material (CRM) – a standard of known composition that is certified by a recognized authority and representing a sample matrix. It is used to verify the accuracy of a method.

8.7 Continuing Calibration Blank (CCB): An instrument blank that is used to monitor the ambient blank concentration after the Continuing Calibration Verification (CCV).

8.8 Continuing Calibration Verification (CCV): An aliquot of standard from the same source as the calibration standard, at a value of 20ng/L (2.0ng in ~100mL bubbler water). This standard is analyzed after every 10 analytical runs, and determines whether the instrument is maintaining calibration.

8.9 Continuing Demonstration of Capability (CDOC)

8.10 Control Limit (CL) – the limit of the range of acceptability for the quality control samples

8.11 Equipment Blank (EB): Reagent water processed through the sampling devices and placed in a sample container prior to using the equipment to collect samples and used to demonstrate that the sampling equipment is free from contamination.

8.12 Field Blanks (FB): A sample of reagent water placed in a sample container in the field and used to demonstrate that samples have not been contaminated by sample collection or transport activities. EPA-1631E recommends the analysis of at least one field blank per 10 samples collected at the same site at the same time. Analyze the blank immediately before analyzing the samples in the batch.

8.13 Initial Calibration Verification (ICV): A standard that is prepared from a secondary source stock standard with a value of 15ng/L (1.5ng in ~100mL bubbler). This standard is run immediately following the calibration curve and verifies instrument calibration. It is always followed by the IBLs.

8.14 Initial Blank Level (IBL): An instrument blank that is used to demonstrate the ambient blank concentration of the instrument. One per bubbler is needed at the beginning of the analytical run.

8.15 Initial Demonstration of Capability (IDOC).

8.16 Laboratory Control Sample (LCS and LCSD) or Quality Control Sample (QCS): A sample (and duplicate) containing a known concentration of mercury that is used to monitor complete method performance. The preferred LCS is a matrix matched Certified Reference Material (CRM), but a blank spike meets the requirement also. In LIMS, the LCS is always referred to as a Blank Spike (BS), whether it is matrix matched or not.

8.17 Limit of Detection (LOD) – equal to MDL and verified on a quarterly/annual basis, depending on the preparation, by spiking within three times the established LOD and showing a positive result on the instrument.

8.18 Limit of Quantitation (LOQ) – equal to PQL and verified on a quarterly/annual basis, depending on the preparation, by spiking within 2 times the LOQ and showing a recovery between 70 – 130%.

8.19 LIMS: Laboratory Information Management System. Computer software used for managing samples, standards, and other laboratory functions.

8.20 May: This action, activity, or procedural step is optional.

8.21 May Not: This action, activity, or procedural step is prohibited .

8.22 Matrix Spike (MS) and Matrix Spike Duplicate (MSD): A representative sample is selected and spiked with a dilution of the primary source at a known concentration. The MS and MSD are run through the entire analytical process just as the samples are. These QC samples will indicate sample matrix effects on the analyte of interest.

8.23 Method Blank (MBLK) or Preparation Blank (PB): For waters, reagent water that is prepared and analyzed in a manner identical to that of samples. For digested solids, preparations blanks consist of the same reagents used to digest the samples, in the same volume or proportion and are carried through the complete sample preparation and analytical procedure. Boiling chips are used as a blank matrix for solids. Preparation blanks are referred to as BLK in LIMS.

8.24 Method Detection Limit (MDL): A limit derived from 40 CFR, Part 136, Appendix B. This method produces a defined value that is the minimum concentration that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero from a given matrix.

8.25 Method Duplicates/Method Triplicates (MD/MT): A second or third separate sample dilution, taken from the same source sample, prepared and analyzed in the laboratory separately. An MSD may be used as a duplicate.

8.26 Reagent water: 18 MΩ minimum, reagent water starting from a pre-purified (distilled, Reverse Osmosis, etc.) source.

8.27 Must: This action, activity, or procedural step is required.

8.28 Ongoing Precision and Recovery (OPR): A dilution of a secondary source resulting in an instrumental concentration of 5.0 ng/L mercury.

8.29 PM: Project Manager.

8.30 Practical Quantitation Limit (PQL), Method Reporting Limit (MRL): The minimum concentration that can be reported quantitatively. The PQL is often described as 1-10 times higher than MDL. Eurofins Frontier defines the PQL as the lowest concentration that can achieve 70-130% recovery for 10 replicate sample preparations. In LIMS, the PQL is referred to as the MRL.

8.31 Primary Source: The stock standard used to make the calibration standard. Procedural Method: A method where standards and samples are run through the analytical procedure exactly the same. By NELAC definition, this SOP is a procedural method.

8.32 Secondary Source: The stock standard used to make the OPR standard.

8.33 Shall: This action, activity, or procedure is required.

8.34 Should: This action, activity, or procedure is suggested, but not required.

8.35 Stock Standard Solution (SSS) – a standard of analyte that is purchased from a certified source for the preparation of working standards.

8.36 Total mercury: As defined by this method, all bromine monochloride-oxidizable mercury forms and species found in aqueous solutions. This includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg(P), and several tested covalently bound organomercurials (i.e. CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of mercury bound within microbial cells may require additional preparation steps (i.e. UV oxidation, or oven digestion).

8.37 Travel or Trip Blank (TB): A sample of reagent water placed in a sample container in the laboratory and used to demonstrate that samples have not been contaminated by transport activities.

9 Interferences:

9.1 Gold and iodide are known interferences. At a mercury concentration of 2.5 ng/L and at increasing iodide concentrations from 30 to 100 mg/L, test data have shown that mercury recovery will be reduced from 100 to 0 percent. At iodide concentrations greater than 3 mg/L, the sample should be pre-reduced with $SnCl_2$ (to remove brown color immediately prior to analysis) and additional or more concentrated $SnCl_2$ should be added to the bubbler containing sample. If samples containing iodide concentrations greater than 30 mg/L are analyzed, it may be necessary to clean the analytical system with 4N HCl after the analysis.

9.2 Water vapor has the potential to create recovery interferences. To prevent interference from water, ensure that soda-lime pre-traps and gold traps remain dry.

9.3 The presence of high concentrations of silver and/or gold can cause $SnCl_2$ to precipitate out of solution and adhere to the bubbler walls. High concentrations of these metals can sometimes be found in the matrix spike samples from the digestion sets that are shared with the trace metals group. When analyzing digestates where the matrix spike samples have been spiked with silver or gold, the matrix-spiked samples must not be used for mercury analysis. Instead, an alternate matrix spike and matrix spike duplicate (MS/MSD) should be prepared and analyzed. If this is not possible, an Analytical Spike/Analytical Spike Duplicate (AS/ASD) must be analyzed on the ambient sample.

10 Safety Precautions, Pollution Prevention and Waste Handling:

10.1 Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan. This includes, but is not limited to, laboratory coat, safety goggles and nitrile gloves under clean gloves.

10.2 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the MSDS (Material Safety Data Sheets) for each chemical they are working with.

10.2.1 Note: Use particular caution when preparing and using BrCl, as it releases extremely irritating, corrosive fumes similar in effect to free chlorine. Always handle this reagent in an approved fume hood

10.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease-causative agents. Eurofins Frontier will reimburse the expense of Hepatitis A and B immunizations for any laboratory staff member who desires this protection.

10.4 Hydrochloric acid: Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion. Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. For more information see MSDS.

10.5 See Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP) for general information regarding employee safety, waste management, and pollution prevention.

10.6 Pollution prevention information can be found in the current Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP), which details and tracks various waste streams and disposal procedures.

10.7 All laboratory waste is accumulated, managed, and disposed of in accordance with all federal, state, and local laws and regulations. Any waste generated by this procedure should be disposed of according to SOP FGS-099 "Waste Disposal Procedure for

Client Sample Waste," which provides instruction on dealing with laboratory and client waste.

11 Personnel Training and Qualifications:

11.1 An analyst must perform an initial demonstration of capability (IDOC) that includes four replicates of a secondary source before being qualified to analyze samples without supervision. Continuing DOC will be maintained and monitored via performance on CRMs and other QC samples, as well as obtaining acceptable results on proficiency testing exercises.

11.2 The analyst/laboratory technician must have read this SOP and other relevant SOPs and have the training documented on the applicable form(s). The analysis may be questioned on SOP by supervisor(s) and/or trainers.

11.3 Training is documented by the employee and supervisor, and is kept on file in the QA Office. The employee must read, understand, and by signing the training document, agree to perform the procedures as stated in all Standard Operating Procedures (SOPs) related to this method.

11.4 Reading of the SOP must be documented on the correct form such as "Standard Operating Procedure Training Record," Appendix F in FGS-094, the last page of this SOP, Appendix A "Standard Operating Procedure Training Record" or a similar document."

11.5 All employees must also, on a yearly basis, read the Quality Manual (QM), and complete the yearly Ethics training.

11.6 All training documents including IDOCs, CDOCs, SOP reading, Initial QA orientation, and Ethics training are stored by the Quality Assurance Manager in the employees training file for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

11.7 Chemical Safety Training, Compressed Gas Training, Chemical Hygiene Plan documentation, and Shipping of Hazardous goods, are stored by the Health and Safety Officer for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

12 Sample Collection, Preservation, and Handling:

12.1 Aqueous samples are collected in rigorously cleaned fluoropolymer (e.g. Teflon) or PETG bottles and caps (as described in FGS-007 "Cleaning of Sampling Equipment and Bottles for Mercury Analysis"). Certified clean glass bottles with fluoropolymer lids may be used if mercury is the only analyte of interest.

12.1.1 Aqueous samples are preserved upon receipt with 0.2N BrCl that has tested low in mercury. Samples are typically preserved to 1% BrCl v/v, but may require further oxidation due to high levels of organic matter or mercury. Refer to FGS-012 "Oxidation of Aqueous Samples for Total Mercury Analysis" for oxidation of aqueous samples. Samples requiring greater than 10% BrCl must have a method blank prepared at the time of preservation. Preservation levels should be limited to 1%, 2%, 3%, 5%, 10%, and 100%.

12.1.2 Preservation levels other than 1% are written on the LIMS label of the sample bottle. Preservation levels are also documented in the LIMS bench sheet by adjusting the initial and final volumes. For example, a sample preserved at 2 % BrCl must say "2" on the LIMS label, and have an initial volume of 100mL and a final volume of 102mL in the bench sheet.

12.2 All samples should be collected utilizing clean techniques, so as not to cross-contaminate samples with mercury. See FGS-008 "Ultra Clean Aqueous Sample Collection" and EPA Method 1669 for aqueous sample techniques.

13 Apparatus and Equipment:

13.1 LIMS – Element, version 5.85 or higher; Computer – Windows XP, 7 or 8

13.2 *Tekran 2500 Atomic Fluorescence Spectrophotometer (AFS) or equilivalent: A high sensitivity AFS Detector (IDL<1pg) with a required wavelength of 253.7 nm and associated software.*

13.3 Flow meter/needle valve: A unit capable of controlling and measuring gas flow to the cold vapor generator at 200-500 mL/min.

13.4 Teflon Fittings: Connections between components and columns are made using Teflon FEP tubing and Teflon friction fit tubing connectors.

13.5 Soda-Lime pre-trap: A 10cm x 0.9cm diameter Teflon tube containing 2-3 g of reagent grade, non-indicating 8-14 mesh soda-lime ($Ca(OH)_2$+NaOH) aggregates, packed between portions of silanized glass wool. This trap is purged of mercury by placing it on the output of a clean cold vapor generator and purging it with ~3-5% HCl and ~600 µL of $SnCl_2$ for approximately 20 minutes with N_2 at 40 mL/min.

13.6 Cold-vapor generator (bubbler): A 150 mL, tall, flat-bottom borosilicate flask with standard taper 24/40 neck, fitted with a sparger having a coarse glass frit which extends to within 0.2 cm of the flask bottom.

13.7 Gold Traps: Made from 12 cm lengths of 6 mm OD quartz tubing, with a 4-way crimp 3.0 cm from one end. The tube is filled with approximately 2.5 cm of 20/40 mesh gold-coated quartz sand, the end of which is then plugged with quartz wool. Gold-coated sand traps are heated to 450-500°C (the coil should have a barely visible red glow when the room is darkened) with a coil consisting of 75 cm of 24-gauge nichrome wire at a potential of 10 VAC. Potential is applied and finely adjusted with an auto-transformer. Refer to SOP FGS-061 regarding the construction of gold traps used for total mercury analysis.

13.8 Agilent Integrator Recorder or equivalent: Any multi-range chart recorder or integrator with 0.1-5.0 mV input and variable speeds is acceptable. Data capture software may also be used.

13.9 Pipettes: Calibrated variable pipettes with a range of 5 µL – 10 mL. Used to make solutions and sample dilutions. Pipettes are to be calibrated weekly according to SOP FGS-003 and FGS-155.

14 Reagents and Standards:

All reagents, except those made daily, must be entered into LIMS

14.1 Reagent Water: 18-MΩ ultra pure deionized water starting from a pre-purified (distilled, R.O., etc.) source is used. To remove any remaining trace metals and organics, an activated carbon cartridge is placed between the final ion exchange bed and the 0.2-µm filter. Reagent water used in the mercury lab is checked weekly for total mercury concentrations, and must test below 0.25ng/L.

14.2 Hydrochloric Acid (HCl): Concentrated (36-38% weight basis). Must be trace-metal purified and reagent grade. HCl is typically monitored through performance of the BrCl. Sometimes it will be necessary to test the HCl directly. To do so, add 1 mL, using a calibrated pipette, of HCl to approximately 100mL of purged bubbler water. Enter 1mL as aliquot in the Excel spreadsheet. Do not prep blank correct. Analyze one replicate per bottle. This reagent should test below 5.0 ng/L. This solution is considered stable until the expiration date on the bottle, set by the manufacturer.

14.3 0.2N Bromine Monochloride (BrCl):

14.3.1 37.5 g of KBr is added to a 2.5-L bottle of concentrated HCl (pre-analyzed and found to be below 0.25 ng/L Hg). The bottle is then inverted in a fume hood to mix the acid and KBr. The solution then sits overnight allowing for the KBr to be dissolved.

14.3.2 27.5 g of $KBrO_3$, certified to be low in Hg, is slowly added to the acid. When all of the $KBrO_3$ has been added, the solution should have gone from yellow to red to orange.

14.3.3 Loosely cap the bottle, and allow to sit for 30 minutes in a fume hood before tightening the lid. Once capped invert bottle to make sure all of the solids goes into solution. **CAUTION: This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl) which are released from the bottle. Add the $KBrO_3$ SLOWLY and in a well operating fume hood**.

14.3.3.1 To test the BrCl, add 1 mL, using a calibrated pipette, of the BrCl to a prep blank vial containing approximately 4 mL reagent water. Add 200 µL Hydroxylamine-HCl to the vial; pour the entire contents into a bubbler containing approximately 100 mL of purged water. Assume a 100 mL aliquot in the Excel spreadsheet. This reagent must test below 0.20ng/L. Do not prep blank correct. Analyze one replicate per bottle.

14.3.3.2 The expiration time for this reagent is set by default to six months in LIMS. There is no suggested holding time in EPA method 1631E, therefore the holding time can be extended, as long as the primary reagent has not expired. The mercury concentration of the BrCl is monitored through the preparation of water preparation blanks.

14.4 Hydroxylamine hydrochloride: dissolve 300g of NH_2OH-HCl in reagent water and bring the volume up to 1L. This solution may be purified by the addition of 1mL $SnCl_2$ solution and purging overnight at 500mL/min with mercury-free N_2. The working reagent is a 25% solution that is made by adding one part reagent water to one part 50% hydroxylamine hydrochloride. This reagent must test below 0.25ng/L.

14.4.1 To test the Hydroxylamine-HCl (NH_2OH-HCl), add 1 mL of the 50% reagent, using a calibrated pipette, to approximately 100 mL of purged bubbler water.

Assume a 100 mL aliquot in the Excel spreadsheet. This reagent must test below 0.20 ng/L. Do not prep blank correct. Analyze one replicate per bottle.

14.4.2 The expiration time for this reagent is set by default to six months in LIMS. There is no suggested holding time in EPA method 1631E; therefore the holding time can be extended, as long as the primary reagent has not expired.

14.5 Stannous Chloride ($SnCl_2$): Weigh out 500 g $SnCl_2$ using a calibrated balance that also has been verified for the day. Dissolve with three 100 mL aliquots of concentrated HCl and transfer to a 1L I-CHEM glass bottle, which contains approximately 300 mL of reagent water. Bring this solution up to approximately 1 L of volume and purge overnight with mercury-free N_2 at 500 mL/min to remove all traces of mercury. Store tightly capped. The working reagent is a 25 % solution that is made by adding one part reagent water to one part 50 % stannous chloride.

14.5.1 To test the Stannous Chloride ($SnCl_2$), add 1 mL of the 50% reagent, using a calibrated pipette, to approximately 100 mL of purged bubbler water. Assume a 100 mL aliquot in the spreadsheet. This reagent must test below 0.20 ng/L. Do not prep blank correct. Analyze one replicate per bottle.

14.5.2 The expiration time for this reagent by default is set to six months in LIMS. There is no suggested holding time in EPA method 1631E; therefore the holding time can be extended, as long as the primary reagent has not expired.

14.6 Argon Grade 4.7 or better (ultra high-purity grade): Argon that has been further purified by the removal of mercury using a gold trap that is located in line between the gas output and the analyzer gas input.

14.7 Nitrogen Grade 4.5 (standard laboratory grade): Nitrogen that can be further purified of mercury using a gold trap that is located in line between the gas output and bubbler

14.8 Preparation of Total Mercury Standard Solutions:

14.8.1 Mercury standard solutions are prepared in ultra clean volumetric glassware and gravimetrically calibrated pipettes. Resulting solutions must be stored in glass or Teflon bottles and preserved to at least 2 % BrCl. All working standards must be tested prior to use.

14.8.1.1 New working standards and standard dilutions are tested prior to use. Three reps of the new standard are analyzed in the same run as three reps of the current NIST 1641D standard. Analyze 200 µl of the NIST 1641D and assume 100 ml in the bubbler. The mean percent recovery of the three standards should be ±5 % (95-105 %) of the true value and also within 5 % of the average NIST 1641D recovery (e.g. If the average of NIST 1641D recovery is 97 %, the range for the standard is 95-102 %). If the standard does not test within this control limit, it is retested. If it still does not meet the control limit, it is discarded and remade, unless otherwise approved by the Quality Assurance Officer. NOTE: When making serial dilutions to create various standard levels; the lowest concentration may be used to test any of the higher concentration steps (for example: if a 10ng/mL calibration standard is created from a 1000ng/mL spiking standard, only the 10ng/mL standard requires testing.

If the 10ng/mL standard passes, then both standards are considered to be passing within the control limits.)

14.8.2 Total Mercury Stock Standard Solution (Stock): Certified mercury standard purchased from High Purity Standards (1000 µg/mL (1 000 000 ng/mL) primary source) or Absolute Standards (100 µg/mL (100 000 ng/mL) secondary source), or any equivalent standard.

14.8.3 Total Mercury Spiking Standard Solutions (Spiking Standard): Spiking standards are made from either the primary or secondary sources.

14.8.3.1 To make standards, use an ultra clean volumetric flask and a calibrated pipette. Add reagent water until flask is about half full. Add 2 % 0.2N BrCl and the specific spike volume noted below (these volumes may be changed as long as ratio and resulting concentration remains the same). Bring up to the mark with reagent water and mix well prior to testing. When spiking samples, no more than 200 µL of any spiking standard is added to the sample to minimize effects on volume. It is also recommended that staff pipette no less than 25 µL. If possible, minimize headspace during standard storage. Expiration date is currently set at 6 months or when the stock standard expires, whichever is shorter.

14.8.3.2 100,000 ng/mL Spiking Standard: Made from the Primary Stock Standard (High Purity, or equivalent vendor). Dilute 10 mL of the stock standard to 100 mL of reagent water containing 2 % BrCl. (Can also be made by preserving Secondary Stock Standard to 2% BrCl).

14.8.3.3 10,000 ng/mL Spiking Standard: If made from the Primary Stock Standard (High Purity, or equivalent vendor). Dilute 1.0 mL of the stock standard to 100 mL of reagent water containing 2 % BrCl. If made from Secondary Stock Standard, dilute 10mL of stock standard to 100mL with reagent water containing 2% BrCl.

14.8.3.4 1,000 ng/mL Spiking Standard: If made from the Primary Stock Standard (High Purity, or equivalent vendor). Dilute 0.250 mL of the stock standard to 250 mL RO water containing 2 % BrCl. If made from Secondary Stock Standard dilute 2.5mL of stock standard to 250mL with RO water containing 2% BrCl.

14.8.3.5 100 ng/mL Spiking Standard: Made from a stock standard or dilution of a stock standard with a concentration of 100,000 ng/mL. Dilute 0.100 mL of the 100,000 ng/mL dilution to 100 mL of reagent water containing 2 % BrCl. Expiration date is currently set at 3 months or when the stock standard expires, whichever is shorter.

14.8.4 Calibration Standard (10 ng/mL): Must be made from a dilution of the Primary Stock Standard (High Purity, or equivalent vendor). Typically made by diluting 0.5mL of a 10,000 ng/mL Primary Spiking Standard to 500 mL of reagent water containing 2 % BrCl. Transfer to glass or Teflon bottle. The calibration standard is considered stable for three months or until the stock standard expires.

14.8.5 Calibration Standard (1 ng/mL): Must be made from a dilution of a Primary Stock Standard. Typically made by diluting 1.0mL of a 100 ng/mL Primary Spiking Standard to 100mL with Reagent water containing 2% BrCl.

14.8.6 Initial Calibration Verification (ICV): A 10 ng/mL ICV solution is prepared using the Secondary Stock Standard (Absolute Standards, or equivalent vendor). Use 0.100 mL (100 µL) of the Secondary Stock Standard to 1000 mL Milli-Q containing 2 % BrCl. Transfer to one 1000 mL glass or Teflon bottle. The ICV standard is considered stable for three months or until the stock standard expires. It is recommended to alternate expiration date with the CAL standard.

14.8.7 Continuing Calibration Verification (CCV): For CCV analysis, use 200 µL of the 10 ng/mL CAL standard (documented in LIMS as SEQ-CAL3). The True Value is 20 ng/L.

14.8.8 Certified Reference Material (CRM) for Total Mercury in Water: A 1.5679 mg/L solution (1.557 mg/kg at a density of 1.007 g/mL) is prepared by adding a 5.0 mL of CRM NIST 1641d (from ampoule) into a 1000 mL flask containing of reagent water. This solution is diluted to 1000 mL, and an additional 10 mL of 0.2N BrCl is added, resulting in a final volume of 1010 mL. Preparing the solution in this manner makes a 1:200 dilution of the stock CRM. This solution is considered stable for one year, or until the stock standard expires. Results are corrected for the additional 1 % BrCl in the analysis Excel spreadsheet and in LIMS.

14.8.9 Ongoing Precision and Recovery (OPR) for "Strict" 1631E: A 5.0 ng/L solution is prepared by adding 100 µL of the 100 ng/mL secondary spiking standard into 2000 mL reagent water. An additional 1 % BrCl (20 mL) of BrCl is added, so that the final volume is 2020 mL. This standard is analyzed at 100 mL at the instrument, and preparation blank corrected exactly in the same manner as samples

14.9 Documentation of Standards and Reagents:

14.9.1 Standards and Reagents are documented in LIMS upon receipt or creation. A LIMS generated label is affixed to each standard and reagent that has the name of the solution, the person who prepared or received it, the date it was prepared or received, and the expiration date.

14.9.2 Each bottle of standard must be labeled with the following: the date of receipt or creation, the initials (or name) of who entered the standard into LIMS, the concentration and analyte, the expiration date and the LIMS ID. This information must also appear on the certificate of analysis of stock standards.

14.9.3 Stock standards and CRMs are logged into LIMS upon receipt by Shipping and Receiving (S&R) or the Quality Assurance department (QA). These do not require testing, provided there is a Certificate of Analysis on file in QA. When receiving a solid CRM, QA shall generate a work order in LIMS for total solids analysis.

14.9.4 For all standards, LIMS documentation must include the following: a description of the standard, department, expiration date of the standard (not to exceed the expiration of the parent standard), the name of the person who made (or

received) the standard or reagent, the date it was prepared (or received), final volume, a reference date (date entered into LIMS), concentration units (µg/mL), the vendor and vendor lot. The solvent lot is used to document the Lot Number or LIMS ID of the BrCl that was used. In the comments section, the analyst must enter the sequence and applicable results for documentation of standard testing. Other notes may be entered in here as well. The correct parent standard must be noted, as well as the amount used. Analytes are entered individually from the list. LIMS will calculate the true value of the standard based on the amount of the parent used and the final volume. Click the appropriate radio button under Standard type. A Spike Mix is a standard that is used in a bench sheet, and a Calibration standard is a standard used only in sequences. A Reference Standard is a Certified Reference Material (CRM). The standard must not be used until it has passed control limits and is approved by the mercury supervisor, mercury laboratory manager, or QA for use.

14.9.4.1 If the new standard is a calibration standard, a separate standard ID must be created for each calibration point based on the final concentration in the sequence (example: THg CAL1 0.10 ng or THg CAL2 0.50 ng). These are given the same expiration as the standard they are made from, and will need to be generated every three months as each new working calibration standard is made and tested.

14.9.4.2 To generate new "CAL" standards in LIMS, go to the Laboratory drop down menu and select Standards. Open the current CAL1 standard and click "Copy". Update the appropriate information, including the Prepared Date, Expiration Date, Prepared By, and the Reference Date. For these standards, which are to be used in the sequence, the final volume is equal to the assumed aliquot in the bubbler (100 mL). Check that the vendor lot is correct. Remove the old (expired) parent standard. Choose the new parent standard, and enter the amount of standard added to the bubbler for that calibration point. All depleted or expired standards are moved into the Expired Standards Department once they are no longer being used.

14.9.4.3 Each bottle of standard must be labeled with the following: the date of receipt or creation, the initials (or name) of who entered the standard into LIMS, the concentration and analyte, the expiration date and the LIMS ID. This information must also appear on the certificate of analysis of stock standards.

14.9.5 Neat reagents are logged into LIMS with a unique identifier upon receipt by Shipping and Receiving Department and given a default expiration of 3 years, unless otherwise noted by the manufacturer.

14.9.6 Working reagents are prepared by the analyst, logged into LIMS and assigned a unique identifier. Reagents entered into LIMS must have the information listed in section 14.9.2. In addition the parent neat reagents are added by their unique identifier and the amount of each reagent is entered. It is not necessary to enter analytes from the list for reagents. The Solvent Lot is not applicable to working reagents. The radio button must be clicked to Reagent. If the reagent

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requires testing, it must test clean prior to using. All reagents used during analysis and prep should be added to bench sheet.

14.9.7 Depleted or expired standards and reagents are segregated and removed from use.

15 Calibration:

15.1 The analyst should label the strip chart/integrator printout with the corresponding dataset ID as well as print and sign their name. For strip chart printouts, the analyst should label the baseline ratios accordingly (usually X=1 and X=20) and label with the analysis day start time and strip chart drum speed (usually 1 mm/min). The analyst should note the end time as well. If using an integrator, the date and time should be checked and corrected if necessary.

15.2 The calibration sequence determines the range of sample concentrations that are reportable. The calibration sequence starts with a 5-point curve using the total mercury calibration standard solution. The five points are: *0.05ng (0.50 ng/L),* 0.10 ng (1.00 ng/L), 0.50 ng (5.00 ng/L), 2.00 ng (20.00 ng/L), and 4.00 ng (40.0 ng/L). An ICV/OPR and IBLs (one for every bubbler used are analyzed immediately following the standard curve.

15.2.1 Using the 10 ng/mL calibration standard, add 5 µL, 10 µL, 50 µL, and 200 µL to the bubblers sequentially from the left to right. Add 300 µL $SnCl_2$ to the bubblers and seal bubbler tops using Keck Clips.

15.2.2 Place blanked gold traps securely at the end of soda-lime traps (pinched section of gold trap closest to the soda-lime trap). Purge bubblers with N_2 for a minimum of 20 minutes.

15.2.3 Attach individual gold traps to the analytical train and burn in sequential order. Peaks produced should be labelled, as well as recorded in the Excel spreadsheet in real time.

15.3 For the second round, add *400 µL* of the 10 ng/mL mercury calibration standard to the first bubbler. Add 50µL of the *10 ng/mL* ICV(OPR) standard to the second bubbler *(5.0 ng/L). The third and fourth bubblers are used for the first and second IBLs and nothing should be added to these bubblers.* To ensure that nothing is added, keep it sealed with a Keck Clip. Add 300 µL $SnCl_2$ to all bubblers except the fourth and seal bubbler tops with Keck Clips.

15.4 For the third round, use the first and second bubbler to finish the IBLs needed for 1631. The third and fourth bubbler can be used for the first portion of the batch. If the curve does not pass or needs to be investigated any batch portions analyzed in this round will need to be reanalyzed.

15.5 Once the instrument is calibrated and the ICV/IBLs are analyzed and judged to be in control, the instrument is operational. The sample concentrations must fall within the range of the calibration standards or be diluted and reanalyzed.

15.6 The purge efficiency of the bubbler system is 100 % and is independent of volume at the volumes used in this method. Calibration of this system is typically performed using units of mass. For purposes of working in concentration, the volume is assumed to be 100 mL.

15.7 This completes the instrument calibration for total mercury analysis.

16 Procedure:

16.1 *When analyzing on the Tekran 2600, follow the procedure in EFGS-121 while still adhering to the QA/QC criteria of this method.*

16.2 Pre-analysis and Organization:

16.2.1 Prior to analyzing samples it is imperative to reference LIMS for all project specific information, such as QC requirements, suggested dilutions, project manager information, and specifics regarding spike levels.

16.2.2 The analyst should then locate samples and check the work order in LIMS for notes about specific project requirements.

16.2.3 The analyst should compare the sample IDs to the work order and see that the samples are accounted for, and notify the project manager of any discrepancies in analysis required, sample identification, etc.

16.2.4 All mercury analyses receive a unique dataset identifier. This is comprised of the instrument type and number, the date and the calibration number for that day. The format is as follows: THg8-091218-1, where "THg "refers to a total mercury analysis; "8" refers to the analyzer number 8; 091218 refers to the date (December 18, 2009 in the YYMMDD format); and "1" refers to the first calibration of the day.

The sequence number is assigned by LIMS when the data gets imported into LIMS. The alpha-numeric code is based on the following format: 3B02001, where the 3 refers to the year (2013), the "B" is the month (A= January, B=February…L=December), "02" is the day of the month (February 2nd) and the final 3 digits is the nth sequence created on that particular year/month/day combination.

16.2.5 In general, the analyst should organize their samples in the order listed on the bench sheet. The first samples analyzed should be the preparation blanks, then the LCS if analyzing solid samples, followed by actual samples. If possible, run total and dissolved samples side by side to facilitate verification that total concentration is greater than dissolved concentration. See QA section.

16.2.6 All samples specified as being *High QA* should be analyzed prior to any Standard QA projects that are being analyzed on the same instrument on the same day. However, if concentrations are known, analyze samples with low concentrations prior to samples with high concentrations

16.3 Instrument Start Up:

16.3.1 Begin blanking gold traps. To do this, attach one trap at a time to the analytical train and burn to the instrument. Ensure the Argon is flowing at appropriate levels (~25-40 mL/min). The pinched portion of the gold trap should be on the left (closest to the analytical trap). Continue to burn traps in sequential order.

16.3.2 Rinse out the bubbler three times with reagent water and fill with about 100 mL of reagent water. Using a pre-purged pipette, add 3-5 mL HCl. Initially add 600 µL of $SnCl_2$.

16.3.3 Prepare one soda-lime trap for each bubbler. To prepare soda-lime traps, hold soda-lime between two glass wool plugs in a Teflon tube. Cap the tubes with Teflon plugs and attach to the bubbler. Once the soda-lime traps have been attached, the bubbler system (soda lime trap and bubbler water/acid/ $SnCl_2$) must purge for a minimum of 20 minutes before beginning the instrument calibration sequence.

16.4 Analyzing Aqueous Samples:

16.4.1 All aqueous samples should be preserved with BrCl according to FGS-012 at least 24 hours prior to analysis. In the event a sample requires further oxidation prior to analysis, additional BrCl is added and the sample should not be analyzed for at least 12 additional hours. In special cases where rush turn-around-time is required and an oxidation period of less than 24 hours may be used, a heated oven digestion procedure can be utilized.

16.4.2 While bubbling and burning the standard curve, the analyst should prepare a minimum of three BrCl method blanks (BLK) at 1% BrCl. Add 1 mL BrCl and 200 µL hydroxylamine hydrochloride (NH_2OH-HCl) to each bubbler. The aliquot is assumed to be 100 mL. Any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

16.4.3 After the instrument calibration sequence, preparation blanks and the LCS/LCSD are analyzed.

16.4.4 All known field, equipment, and trip blanks should be analyzed before any other sample types, usually after the BLKs. Aliquots of 100 mL should be analyzed, provided there is adequate collected sample volume. Sample aliquot sizes of 125 mL can be analyzed upon request by the project manager.

16.4.5 For all waters, select the appropriate dilution (refer to LIMS, historical data, etc.).

16.4.5.1 For sample aliquots of 25 µL to 10.0 mL, use calibrated pipettes to dispense the aliquots directly into bubbler. Due to minimal amounts of BrCl in aliquots of 10 mL or less, NH_2OH-HCl is not added. It is highly recommended that the analyst should not pipette less than 25 µL. A dilution of the sample should be made to allow a larger aliquot to be analyzed.

16.4.5.2 For sample aliquots greater that 10 mL, gravimetrically weigh out the selected volume (±0.2 g) into a clean 125 mL Teflon bottle. Once quantity is weighed out, neutralize BrCl with 200 µL NH_2OH-HCl no more than five minutes prior to adding the sample to bubblers. The sample should turn from a yellowish color to a clear/cloudy solution, depending on the matrix.

16.4.6 If the material is a seawater or highly dense liquid, it may be necessary to account for the density if the aliquot is gravimetrically determined. Density

checks can be performed at the time of analysis to determine if further determinations are necessary.

16.4.7 The procedure for analysis is similar to that of the calibration. Samples to be analyzed are pipetted or poured into the bubbler (one sample per bubbler) along with 300µL $SnCl_2$. Bubbler tops are sealed with Keck Clips to ensure nominal sample leakage. Blanked gold traps are securely placed at the end of the soda-lime trap. Purge bubblers with N_2 for a minimum of 20 minutes, remove gold traps, and sequentially place in the analytical train. Burn individual traps to analyzer, labeling resulting peaks with corresponding sample in real time.

16.4.7.1 Sample IDs, aliquot volume, BrCl percentage (group ID), peak height/peak area, and dilution factor (if applicable) associated with each sample should be entered into the THg Waters Template Excel spreadsheet.

16.4.7.2 While purging one set of samples, the analyst should begin preparing the next round of water samples in the same fashion to maximize efficiency.

16.5 End of analysis close-down procedure:

16.5.1 Turn off gas flow.

16.5.2 Carryout all end of day cleaning and restocking tasks.

16.6 The analytical data is compiled into an Excel file. The data is then copied and pasted into an Excel template that is LIMS compatible.

16.7 Maintenance and Troubleshooting

16.7.1 ISSUE: No peaks at all

16.7.1.1 *Ensure that the system is powered.*

16.7.2 ISSUE: Low sensitivity

16.7.2.1 *Make sure that you have freshly changed soda lime in the soda lime trap, and that it is from a good source.*

16.7.2.2 *Do not use old calibration standards to calibrate the system.*

16.7.2.3 *Make sure you are running fresh $SnCl_2$ solution.*

16.7.2.4 *Make sure that your stock Hg standard has not expired and is from a reliable source and that it is not compromised.*

16.7.2.5 Check the lamp voltage

16.7.3 ISSUE: High blanks

[redacted]

16.7.3.1 Check reagent (including water) quality

16.7.3.2 Check for system contamination

16.7.4 ISSUE: Nonlinearity of the calibration curve

16.7.4.1 *Check and investigate high blanks.*

16.7.4.2 *Contaminated and expired soda lime. Change soda lime.*

16.7.4.3 *Make sure your calibration standards are fresh and properly prepared.*

17 Calculations:

17.1 Average all instrument blanks (PH_X) using the peak area values from the TekMDS software. Subtract the average (IB) from the peak area for each standard and sample.

17.2 Calculate the calibration factor (CF_x) for mercury in each of the five standards using the mean instrument-blank-subtracted peak area and the following equation:

$$CF_x = PA_x - IB / C_x$$

Where:

17.2.1 PAx=peak area (or peak height) for mercury in standard

17.2.2 IB= mean peak height (or peak area) for mercury in bubbler blank

17.2.3 Cx=mass in standard analyzed (ng/L)

17.2.4 CFx=Calibration Factor of each concentration

17.2.4.1 Average the five calibration factors to establish mean value: CF(Avg) (units/ng/L).

17.3 Sample results are then corrected for the average peak area values of at least three preparation blanks (PBs), unless otherwise requested. This result is shown as the Initial Result on the Excel spreadsheet and in LIMS.

17.4 Total Mercury in Water:

Instrument Value (ng/L) = (Peak Height – BB) / $CF_{(Avg)}$

Final Result (ng/L) = [(Instrument Value x DF) – (BLK)] x (Vf /Vi)

Where:

17.4.1 $CF_{(avg)}$ = average calibration factor for curve (in units/ng/L).

17.4.2 BB = average bubbler blank peak area or peak height (in units)

17.4.3 V_f = Final volume of sample (in mL) from bench sheet.

17.4.4 V_i = initial volume of sample analyzed in mL prior to addition of BrCl.

17.4.5 DF = Dilution Factor - takes into account any instrumental dilution of the sample

17.4.6 BLK = average of the preparation blanks in ng/L.

17.5 A linear regression can be used as alternate calibration. A linear regression will not change values significantly. If linear regression is used, the correlation coefficient (R) must be ≥0.995.

18 Statistical Information/Method Performance:

18.1 The Method Detection Limit (MDL) is determined according to 40 CFR Part 136 Section B. Ten replicates (9 degrees of freedom) spiked 3-10 times the expected MDL are run. The standard deviation (s) is taken from the resulting data and the MDL is calculated as follows: MDL=2.821*s. This value should not be interpreted as the method reporting limit.

18.2 The Practical Quantitation Limit (PQL) is the reporting limit for this method and is included as the lowest calibration point (2003 NELAC regulation 5.5.5.2.2.1.h.3). The PQL is determined by running ten samples with a concentration that will produce a recovery of 70-130 %. The PQL is referred to as the Method Reporting Limit (MRL) in LIMS.

18.3 Using clean handling techniques and reagents tested low for Hg content, the LOD value for Total Hg in water is typically less than 0.2 ng/L, while the PQL is 0.50 ng/L.

18.4 Current LODs, LOQs, MDLs, and PQLs are stored at: Cuprum\General and Admin\Quality Assurance\MDLs & PQLs.

19 Quality Assurance/Quality Control:

19.1 A minimum of three preparation blanks and one LCS/LCSD (preferably NIST 1641d), must be analyzed per preparation batch. The upper control limit for each preparation blank is equal to the PQL.

19.2 Matrix Spikes: One Matrix Spike/Matrix Spike Duplicate (MS/MSD) must be performed for every 10 samples. The recovery of the MS/MSD must be between 71%-125% recovery, and the Relative Percent Difference (RPD) below 24%. If an MS/MSD is out of control, the analyst should investigate to identify the source of the failure. The MS and MSD may be used as duplicates. Some failures may be qualified using QA Qualification Flow Charts (Appendix A).

19.2.1 For aqueous samples, the MS/MSD is spiked at 1 to 5 times the ambient concentration, with 0.25 ng, in the bubbler, being the minimum spiking level. Sample aliquots for the MS/MSD should be the same as the ambient sample aliquot, if sufficient sample volume exists. Spikes are added to the split aliquots for volumes of 10mL or greater. For less than 10mL aliquots, spikes are added directly to the bubbler. NEVER ADD SPIKE DIRECTLY TO THE ORIGINAL SAMPLE VESSEL UNLESS OTHERWISE STATED.

19.3 Matrix Duplicates – One Matrix Duplicate (MD) may be analyzed for every batch of 20 samples. Upon request, a Matrix Triplicate (MT) may be performed. The MSD may serve as the MD if necessary. The Relative Percent Difference (RPD) and the Relative Standard Deviation (RSD) of duplicate samples must be less than 24%. Some failures may be qualified using QA Qualification Flow Charts.

19.3.1 For aqueous samples, analyze the parent, duplicate and triplicate at the same dilution.

19.4 Laboratory Control Standard (LCS) or Quality Control Sample (QCS): For every batch of samples, at least one LCS is processed and analyzed. The recovery of the LCS must be within 80-120% for the aqueous NIST 1641d. An LCS Duplicate (LCSD) should accompany the LCS.

19.4.1 A Certified Reference Material (CRM) is the preferred LCS, but a Blank Spike may serve as an LCS if an appropriate CRM does not exist. The spiking level is based on client request, historical data, or a default of mid-curve. A duplicate blank spike must also be prepared as an LCSD.

19.5 Ongoing Precision and Recovery (OPR): An OPR must be analyzed at the beginning and end of each analytical batch, or at the end of each 12-hour shift. The recovery of the OPR must be within 77-123% to be considered in control.

19.6 All calibration standards must be traceable to the original standard source. The calibration curve must be established at the beginning of the analytical run. It must include at least five different concentrations, with the lowest concentration equal to the PQL. The average response factor of each calibration standard is used to calculate the sample values. The RSD of the response factors must be less than 15% of the mean or the calibration fails.

19.7 ICV and CCV control limit is 77-123%. The CCV is analyzed every 10 analyses, and at the end of an analytical run. CCBs are always analyzed after the CCVs.

19.8 Field Blanks: To be compliant with EPA 1631, clients must submit a field blank for each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples).

19.8.1 If no field blanks are submitted by the client, their data will be flagged with "FB-1631." "Required equipment/field/filter blank not submitted by the client. The sample has been analyzed according to 1631E, but does not meet 1631E criteria."

19.9 Method or Preparation Blanks (BLK): Method blanks are used to demonstrate that the analytical system is free from contamination that could otherwise compromise sample results. Method blanks are prepared and analyzed using sample containers, labware, reagents, and analytical procedures identical to those used to prepare and analyze the samples.

19.9.1 A minimum of three 1 % BrCl method blanks per analytical batch are required. Any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

19.9.2 If the result for any 1 % BrCl method blank is found to contain ≥0.50 ng/L Hg (0.25 ng/L for DOD), the system is out of control. Mercury in the analytical system must be reduced until a method blank is free of contamination at the 0.50 ng/L level.

19.9.3 For method blanks containing more than 1% BrCl, the control limit is equal to 0.50 ng/L multiplied by the final preservation percentage of BrCl. For example, for a method blank preserved to 2 % BrCl, the control limit for the blank is 0.50

ng/L * (102/101), or 0.50 ng/L. For 3% BrCl the control limit is (103/101)*0.50ng/L, or 0.51ng/L.

19.10 Instrument Blanks (IBL): A minimum of three instrument blanks must be analyzed with each analytical batch. To analyze an instrument blank, attach a clean gold trap to the bubbler. Purge and analyze as previously described and determine the amount of Hg remaining in the system.

19.10.1 An instrument blank must be performed on all bubblers used during the analytical run (normally four, but three at a minimum).

19.10.2 If the instrument blank is found to contain more than 0.50ng/L, the system is out of control. The problem must be investigated and remedied and the samples run on that bubbler must be reanalyzed. If the blanks from other bubblers contain less than 0.50 ng/L, the data associated with those bubblers remain valid, provided that all other QC criteria are met.

19.10.2.1.1 The mean result for all instrument blanks must be <0.25ng/L with a standard deviation of 0.10 ng/L.

19.11 The analytical day must close with a CCV/OPR/CCB.

19.12 Because the method is done in real-time, it is EFGS' position that a single non-compliant QC sample result does not automatically invalidate a data set. All data points that can be explained and rerun with a passing result can be qualified. If the source of error cannot be corrected for a QC standard that day, none of the data can be validated. In the event that the system becomes out of control during the analysis day, all results bracketed between valid QC data points shall still be considered valid (CCV, OPR, CCB, etc).

19.13 The Control Limits are established from EPA 1631E.

20 Corrective Action

20.1 The data is reviewed as in the QC section (or matrix specific QC section) for all parameters that pass specific requirements. If the data does not meet QC requirements it is qualified or submitted for reruns. Data may be qualified (based on scientific peer review) by the Group Supervisor, Project Manager, Lab Manager, or QA Officer.

20.2 Control Chart data is generated through LIMS to monitor the performance of the CCV, LCS, MS, and MSD. This is done by the QA department.

20.3 Due to the real-time nature of the CVAFS method, failures must be investigated as they happen. If the source of the problem can be identified, and corrected, the samples may be rerun. If source of problem cannot be isolated, see the Senior Analyst, Group Supervisor, or Laboratory Manager for instructions.

20.4 The Senior Analyst, Group Supervisor, Laboratory Manager, or QA Officer must be informed if QC fails. It is also advisable to always alert the Project Managers.

21 List of Attachments

Table 1: QC Requirements for Total Mercury

Appendix A: Example - Standard Operating Procedure Training Record

[redacted]

Table 1: QC Requirements for Total Mercury

QC Parameter	Acceptance Criteria
Initial Calibration Verification (ICV)	77-123% Recovery
Continuing Calibration Verification (CCV)	77-123% Recovery
Ongoing Precision and Recovery (OPR)	77-123% Recovery
Initial Calibration Blank (ICB)/ Continuing Calibration Blank (CCB)	Individually, IBL and CCB ≤0.50ng/L, but the mean of all the IBLs shall be <0.25ng/L with a standard deviation of 0.10ng/L.
Laboratory Control Standard (LCS) or Quality Control Standard (QCS)	80-120% Recovery for NIST1641d and 75-125% for all other CRMs. RSD<24%
Calibration Curve RSD (Referred to as "Corr. RSD CF" in Excel spreadsheet).	RSD of Calibration Response Factor ≤15%
Lowest Calibration Point	75-125%
1% BrCl Method Blank (BLK)	Less than 0.50ng/L (0.25ng/L for DOD projects) (individually)
Matrix Duplicate (MD) and Analytical Duplicate (AD)	< 24% RPD
Matrix Spike and Matrix Spike Duplicate (MS/MSD) ; Analytical Spike (AS) and Analytical Spike Duplicate (ASD)	71-125% Recovery < 24% RPD

Appendix A: Example - Standard Operating Procedure Training Record

By signing this document, I the employee, certifies to have read, understood and agreed to follow the test method and quality procedure as described in this procedure.

Reading of SOP FGS-137.02:
Mercury in Water by Oxidation, Purge & Trap and CV-AFS (EPA Method 1631, Rev E.

SOP name and Revision number

Employee name (print)

Employee name (sign) Date:

Supervisor name (sign) Date:

Initial SOP Training (leave blank if not applicable)

Initial reading of method and training	Initials	Date	Supervisor
1. Read method			
2. Observe the method			
3. Detailed review of method and associated literature			
4. Supervised practice of method with trainer			
5. Unsupervised practice of the method with trainer			
6. Review of work with trainer and/or peer-review			
7. IDOC to determine precision and accuracy			
8. Determination of blanks			

[redacted]

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Table of Contents

Approvals:

Prepared by: _____ Date: 5/20/13

Approved by: _____ Date: 5/16/13

Approved by: _____ Date: 5/20/13

1 Revision Log:

Revision: 05	Effective Date: This version	
Section	**Justification**	**Changes**
Cover	Required change	Changed company name from Frontier Global Sciences to Eurofins Frontier Global Sciences.
All	Formatting requirement per LOM SOP-LAB-201	Reformatted document to new corporate specifications.
8.9	Required	Updated spiking levels for the matrix spike
14.3, 14.4	Required	Updated max contamination levels of reagent acids
17.3	Required	Replaced MDL with LOD
18.2 – 18.5	Required	Updated QC limits
18.3	Required	Incorporated QA MOC 2011-007

2 Reference:

2.1 Chemical Hygiene Plan, Eurofins Frontier Global Sciences, current version.

2.2 EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, 2002.

2.3 National Environmental Laboratory Accreditation Conference, NELAC Standard September 8, 2009.

2.4 Department of Defense Quality Systems Manual for Environmental Laboratories, prepared by DoD Environmental Quality Workgroup, Final Version 4.2, October 2010

3 Cross Reference:

Document	Document Title
SOP FGS-003	Pipette Verification, Calibration and Maintenance
SOP FGS-008	Ultra Clean Aqueous Sample Collection
SOP FGS-038	Data Review and Validation
SOP FGS-094, App F	Standard Operating Procedure Training Record
SOP FGS-099	Waste Disposal Procedure for Client Sample Waste
SOP FGS-121	Determination of Total Mercury in Various Matrices by Flow Injection Atomic Fluorescence Spectrometry (EPA Method 1631E)
SOP FGS-155	Calibration of Volumetric Dispensers

4 Purpose:

4.1 The purpose of this Standard Operating Procedure (SOP) is to describe the method for digesting biological tissue samples prior to analysis by CV-AFS for total mercury.

5 Scope:

5.1 This method is for the preparation of biological tissue samples for the determination of total mercury at concentrations less than 1 ng/g. Through the analysis of smaller digestate aliquots, contaminated tissues of up to 10,000 ng/g can be directly measured. Using clean handling techniques and low-level reagents, the typical detection limit for samples prepared by this method is less than 1 ng/g.

5.2 Total mercury, as defined by this method, is all HNO3/H2SO4/BrCl-oxidizable mercury forms and species found in tissue matrices. This includes, but is not limited to, Hg(II), Hg(O), HgS, strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg,

and several covalently bound organo-mercurials (i.e., CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$).

6 Basic Principles:

6.1 Samples are collected using clean sample handling protocols into commercially available clean glass containers with Teflon-lined caps (i.e., I-Chem glass jars) or 125 mL or 250 mL HDPE jars. Freezing (< -15°C) preserves tissue samples until sample preparation is performed.

6.2 A subsample of homogenized sample is digested with 10 mL of 70:30 HNO_3/H_2SO_4.

6.3 The digested sample is diluted up to 40 mL with 10% (v/v) BrCl.

7 Reference Modifications:

7.1 No significant modifications were made to this method.

8 Definitions:

8.1 Batch – no more than 20 client samples grouped for preparation. 3 Preparation Blanks, *1 CRM* or 1 LCS/LCSD (or BS/BSD) set and 1 MD are prepared per every 20 samples; 1 MS/MSD set is prepared for *every 10 samples*.

8.2 Celsius (C), conversion of Celsius to Fahrenheit: (C * 1.8) + 32.

8.3 Fahrenheit (F), conversion of Fahrenheit to Celsius: (F - 32) * 5/9.

8.4 Method Detection Limit (MDL) – the limit derived from an exercise as described in 40 CFR, Part 136, Appendix B. The exercise produces a defined value that is the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero from a given matrix.

8.5 Certified Reference Material (CRM) – a standard of known composition that is certified by a recognized authority and representing a sample matrix. It is used to verify the accuracy of a method.

8.6 Laboratory Control Sample (LCS) and Laboratory Control Sample Duplicate (LCSD), is a sample containing known concentrations of the analytes of interest that is taken through the entire preparation and analysis process in the same manner as the samples to monitor complete method performance. A Certified Reference Material (CRM) is preferred as the LCS, but a blank spiked sample also meets the requirement.

8.7 Preparation Blank (BLK) – Method blanks consist of the same reagents used to digest the samples, in the same volume or proportion, and are carried through the complete sample preparation and analytical procedure. Teflon boiling chips are added to the preparation blanks.

8.8 Matrix Duplicate (MD) – a representative sample is selected and digested in the same manner. This QC sample will indicate sample homogeneity on the analytes of interest

8.9 Matrix Spike (MS) and Matrix Spike Duplicate (MSD) – a representative sample is selected and spiked with a secondary source at *two* to five times the ambient

concentration or at *two* to five times the MRL, whichever is greater. These QC samples will indicate sample matrix effects on the analytes of interest.

8.10 May: This action, activity or procedure is optional.

8.11 May Not: This action, activity or procedure is prohibited.

8.12 Shall: This action, activity or procedure is required.

8.13 Should: This action, activity or procedure is suggested, but is not required.

9 Interferences:

9.1 Due to the high levels of halogens (i.e., iodine) typically found in tissue digestates, it is recommended that aliquots of no more than 5.0 mL of the digestate be analyzed. Otherwise, soda-lime traps may be overloaded and the gold traps may lose the ability to amalgamate and retain mercury.

9.2 The high acidity and halogen levels that are found in tissue digestates necessitate the changing of the bubbler water after every 10 mL of digestate analyzed. Failure to do so can lead to low recoveries that would be reflected in the analysis of QC samples.

10 Safety Precautions, Pollution Prevention and Waste Handling:

10.1 Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan. This includes, but is not limited to, laboratory coat, safety goggles, and nitrile gloves under clean gloves.

10.2 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the MSDS (Material Safety Data Sheets) for each chemical they are working with.

10.2.1 Note: Use particular caution when preparing and using BrCl, as it releases extremely irritating, corrosive fumes similar in effect to free chlorine. Always handle this reagent in an approved fume hood.

10.2.2 Note: Use particular caution when preparing and using the Nitric/Sulfuric Mixture. Always handle this reagent in an approved fume hood.

10.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease-causative agents. Eurofins Frontier will reimburse the expense of Hepatitis A and B immunizations for any laboratory staff member who desires this protection.

10.4 Nitric acid (HNO_3): Corrosive. Strong oxidizer. Contact with other material may cause a fire. Causes eye and skin burns. May cause severe respiratory tract irritation with possible burns. May cause severe digestive tract irritation with possible burns. For more information see MSDS.

10.5 Sulfuric acid (H_2SO_4): Corrosive. Causes eye and skin burns. May cause severe eye irritation with possible burns. May cause severe respiratory tract irritation with possible burns. May cause severe digestive tract irritation with possible burns. Cancer hazard.

Animal studies suggest this acid may cause fetal effects. May cause kidney damage. May cause lung damage. May be fatal if inhaled. Hygroscopic. Strong oxidizer. Contact with other material may cause a fire. For more information see MSDS.

10.5.1 Eyes: Get medical aid immediately. Do NOT allow victim to rub or keep eyes closed. Extensive irrigation with water is required (at least 30 minutes).

10.5.2 Skin: Get medical aid immediately. Flush skin with soap and water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Destroy contaminated shoes.

10.6 See Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP) for general information regarding employee safety, waste management, and pollution prevention.

10.7 Pollution prevention information can be found in the current Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP), which details and tracks various waste streams and disposal procedures.

10.8 All laboratory waste is accumulated, managed, and disposed of in accordance with all federal, state, and local laws and regulations. Any waste generated by this procedure should be disposed of according to SOP FGS-099 "Waste Disposal Procedure for Client Sample Waste," which provides instruction on dealing with laboratory and client waste.

11 Personnel Training and Qualifications:

11.1 An analyst must perform an initial demonstration of capability (IDOC) that includes four replicates of a secondary source before being qualified to analyze samples without supervision. Continuing DOC will be maintained and monitored via performance on CRMs and other QC samples, as well as obtaining acceptable results on proficiency testing exercises.

11.2 The analyst/laboratory technician must have read this SOP and other relevant SOPs and have the training documented on the applicable form(s). The analyst may be questioned on SOP by supervisor(s) and/or trainers.

11.3 Training is documented by the employee and supervisor, and is kept on file in the QA Office. The employee must read, understand, and by signing the training document, agree to perform the procedures as stated in all Standard Operating Procedures (SOPs) related to this method.

11.4 Reading of the SOP must be documented on the correct form such as "Standard Operating Procedure Training Record," Appendix F in FGS-094, the last page of this SOP, Appendix A "Standard Operating Procedure Training Record" or a similar document."

11.5 All employees must also, on a yearly basis, read the Quality Manual (QM), and complete the yearly Ethics training.

11.6 All training documents including IDOCs, CDOCs, SOP reading, Initial QA orientation, and Ethics training are stored by the Quality Assurance Manager in the employees training file for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

11.7 Chemical Safety Training, Compressed Gas Training, Chemical Hygiene Plan documentation, and Shipping of Hazardous goods, are stored by the Health and Safety Officer for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

12 Sample Collection, Preservation, and Handling:

12.1 Samples must be collected in accordance with established ultraclean sampling techniques (see FGS-008 "Ultra Clean Aqueous Sample Collection"). Samples may be in commercially available clean glass containers with Teflon-lined caps (i.e., I-Chem glass jars), or 125 mL or 250 mL HDPE jars.

12.2 Tissue sample preservation - The tissue sample must be frozen in the sampling container at less than -15°C or freeze-dried and stored at room temperature. The holding time for tissue samples is 1 year.

12.3 Just prior to digestion, samples are thawed and if necessary homogenized. The sample is well mixed to ensure the most representative sample possible.

13 Apparatus and Equipment:

13.1 LIMS – Element, version 5.85 or higher; Computer – Windows XP, 7 or 8

13.2 40 mL or 20 mL I-Chem Vials: Borosilicate glass, series 300 vials with Teflon-lined septa in lids. The size used depends on the amount of sample available. The vials are volumetrically accurate to ± 0.5 mL when filled such that the meniscus is just to the bottom of the vial neck. The person performing the preparation should verify this.

13.3 Hot plate: A hot plate with the ability to achieve and maintain a temperature of 75 °C.

13.4 *Pipettors: All-plastic, pneumatic, fixed volume and variable pipettes in the range of 5 µL to 10 mL. Pipettes are to be calibrated weekly according to SOP FGS-003 and FGS-155.*

13.5 Clean hood.

13.6 Analytical Balance: A laboratory analytical balance capable of weighing to ± 1 mg, with documented calibration.

13.7 Calibrated thermometer: Submerged in water in a 20 mL I-Chem vial. This vial is placed on the hotplate during the digestion process. The analysts must record the actual digestion temperature and the serial number of the thermometer used in the digestion logbook.

13.8 Sample Digestion Log.

13.9 Stainless steel tools for homogenization

13.10 Tissue Homogenization Log.

13.11 Disposable spatula.

13.12 Teflon boiling chips.

13.13 Teflon reflux cap to fit the 40 mL and 20 mL I-Chem vials.

14 Reagents and Standards:

14.1 Reagent Water: 18 MΩ ultra-pure deionized water starting from a pre-purified (distilled, R.O., etc.) source. As a final mercury and organic removal step, the activated carbon cartridge on the 18-MΩ system is placed between the final ion exchange bed and the 0.2 µm filter.

14.2 Nitric Acid (HNO_3): Trace metal purified reagent-grade HNO3 is pre-analyzed and lot sequestered. Several brands (Baker, Fisher, Omnitrace) have been found to have lots with acceptably low levels of trace metals. This reagent should be from a lot number that has been previously tested to be low for the analytes of interest. This reagent shall be entered into LIMS and the expiration date is set to the same as the manufacturer's expiration date.

14.3 Sulfuric acid (H_2SO_4) - Trace metal purified reagent-grade H_2SO_4 is pre-analyzed to < 50 ng/L Hg and lot sequestered before purchase. This reagent shall be entered into the LIMS and is considered stable until the expiration date on the bottle (set by the manufacturer.

14.4 Nitric/Sulfuric Acid Mixture: Carefully add 300 mL of pre-analyzed, low mercury (< 50 ng/L) concentrated sulfuric acid to 700 mL of pre-analyzed, low mercury concentrated nitric acid to a pre-marked Teflon bottle. Stir constantly. This reagent shall be entered into the LIMS with an expiration date of six months. **CAUTION: THIS MIXTURE BECOMES VERY HOT AND EMITS CAUSTIC FUMES**.

14.5 Potassium Bromide (KBr), neat: this reagent is pre-certified by the vendor to be low in mercury and is entered into the LIMS with a five year expiration date.

14.6 Potassium Bromate ($KBrO_3$), neat: this reagent is pre-certified by the vendor to be low in mercury and is entered into the LIMS with a five year expiration date.

14.7 0.2N Bromine Monochloride (BrCl):

14.7.1 37.5 g of KBr is added to a 2.5 L bottle of concentrated HCl (pre-analyzed and below 5 ng/L Hg). The bottle is inverted in a fume hood to mix the acid and KBr. The solution sits overnight, allowing the KBr to dissolve.

14.7.2 27.5 g of KBrO3 (certified to be low in Hg) is slowly added to the acid. As the KBrO3 is added, the solution should go from yellow to red to orange.

CAUTION: This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl) which are released from the bottle. Add the $KBrO_3$ SLOWLY in a well operating fume hood.

14.7.3 Loosely cap the bottle and allow to sit for 30 minutes (in a fume hood) before tightening. Once tightly capped, invert bottle to make sure all of the solids go into solution.

14.7.4 This reagent shall be entered into the LIMS with a six month expiration date.

14.8 10% (v/v) of 0.2N BrCl: 200 mL of 0.2N BrCl is diluted up to 2.0 L with reagent water in a clean, empty HCl bottle. This bottle is fitted with a 10 mL repipettor. The expiration time for this reagent is set by default to six months in the LIMS.

15 Procedure:

15.1 If needed, the sample is dissected and homogenized with acid-washed stainless steel tools.

15.1.1 The process used for homogenization, number of samples, work order number, client name, and initials of the technician are entered into the Tissue Homogenization Log.

15.2 Weigh at least a 0.5 g aliquot (but not more than 0.65 g) for common and unknown samples, and up to 1.0 g ± 0.025 g for low-level or large-grain samples. This aliquot is placed into a 40 mL I-Chem glass vial.

15.2.1 If limited sample is available, use 20 mL glass vials and drop the initial mass of the samples to 0.25g ± 0.025 g.

15.2.2 It is imperative that all biological tissue samples are thoroughly homogenized. The importance of representativeness cannot be understated.

15.2.3 Batch requirements for this digestion limit the number of samples to 20. In each batch, there must be three method blanks (BLKs), a Blank Spike and Blank Spike Duplicate (BS/BSD) that is *preferably a Certified Reference Material (CRM) or a Laboratory Control Spike* (LCS, *prepared at 8 ng/g*), a Matrix Duplicate (MD), and a Matrix Spike and Matrix Spike Duplicate (MS/MSD).

15.3 10.0 mL of 70:30 (v/v) HNO_3/H_2SO_4 solution is pipetted in and the sample is swirled. *Note: 5.0 mL of 70:30 (v/v) HNO_3/H_2SO_4 solution is used for limited samples prepared in 20 mL vials (15.2.1).*

15.4 The vial is placed on a hot plate operating at 75±5°C with a Teflon reflux can in place instead of the vial's lid. An aluminum rack id often used to keep the vials from tipping over while on the hot plate.

15.4.1 A calibrated thermometer submerged in water is placed in a 20 mL I-Chem vial. This I-Chem vial with a calibrated thermometer is placed on the hot plate during the digestion process. The analysts must record the actual digestion temperature and the serial number of the thermometer used in the digestion logbook.

15.5 After the samples start to reflux, the samples are heated at 75±5°C for an additional 2 hours or until all organic matter is dissolved.

15.6 The samples are allowed to cool and are diluted to 40 mL (*or to 20 mLs for limited sample digestions as described in 15.2.1*) with a 10% (v/v) solution of 0.2N BrCl, capped with their respective lids, and are thoroughly shaken. Sample digestates should be allowed to settle prior to an aliquot being taken for analysis.

15.7 Analysis for total mercury is according to Eurofins Frontier *SOP FGS-121.*

16 Calculations:

16.1 This preparation procedure does not involve calculations.

17 Statistical Information/Method Performance:

17.1 Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) studies are based on 40 CFR 136, Appendix B. The MDL and PQL must be performed for each analyte/matrix/preparation combination.

17.2 The Practical Quantitation Limit (PQL) is the reporting limit for this method and is included as the lowest calibration point (2003 NELAC regulation 5.5.5.2.2.1.h.3). The PQL is determined by running ten replicate samples with a concentration that will produce a recovery of 70-130% for most analytes, but the recovery requirements are analyte dependent. The PQL is referred to as the Method Reporting Limit (MRL) in LIMS.

17.3 The current LOD value for Total Hg in tissue prepared by the Nitric and Sulfuric Acids (70:30) Digestion is 0.16 ng/g, while the PQL is 0.8 ng/g.

17.4 Current LODs and PQLs are stored at: \General and Admin\Quality Assurance\MDLs & PQLs.

18 Quality Assurance/Quality Control:

18.1 Maximum Sample Batch Size: 20 samples.

18.2 Preparation Blanks: Minimum of three per batch. Each preparation blank must be less than *one-half* the PQL for the method.

18.2.1 The preparation blanks are prepared with a similar mass of Teflon boiling chips as the samples, with the same reagents, and put through the same preparation process as the samples.

18.3 *Certified Reference Material (CRM, representing the sample matrix when commercially available); a Laboratory Control Spike (LCS) and Laboratory Control Spike Duplicate (LCSD) prepared at 8 ng/g is used when a suitable CRM is not available*: One per batch in duplicate. The control limits are *77-123%* recovery.

18.4 Matrix Duplicate (MD) Sample: One per batch. The control limit for the RPD is ≤ *24%*.

18.5 Matrix Spike/Matrix Spike Duplicate (MS/MSD) Samples: One set per *10* samples. The control limits are *71-125%* recoveries and an RPD of ≤ *24%*.

18.6 Follow the flow charts in SOP FGS-038 "Data Review and Validation" to determine if any QC falling outside the established control limits can be qualified.

18.7 All of the quality control limits for the analysis method are included on the "Data Review Checklist.

18.7.1 The data review checklists are located at: \\cuprum\General and Admin\Quality Assurance\Data Review\Current Data Review Checklists.

19 Corrective Action:

19.1 Limiting the source of contamination/error in the preparatory stage can decrease QC problems during analysis. Limiting such contamination/error sources may include: cleaning all digestion tools in a 10% HCl solution, ensuring all samples are thoroughly homogenized, changing gloves whenever appropriate, flushing repipettors at least

three times before dispensing into vials and, in general, following ultra-clean procedures.

19.2 A failing QC point does not necessary fail the entire dataset. If upon analysis a QC sample is out of control, some investigation must be performed to assess if the difficulties are related to matrix effects. The cause and method of determining the set's failure must be documented on the checklist and in the MMO notes, and the Group Supervisor shall be informed. See SOP FGS-038 "Data Review and Validation" for flow charts regarding analytical issues.

19.3 Additional corrective actions are listed in the SOP for total mercury analysis (Eurofins Frontier SOP FGS-121).

20 List of Attachments

Appendix A: Example - Standard Operating Procedure Training Record

Appendix A: Example - Standard Operating Procedure Training Record

By signing this document, I the employee, certifies to have read, understood and agreed to follow the test method and quality procedure as described in this procedure.

Reading of SOP EFGS-011.05:
Digestion of Tissues for Total Mercury Analysis Using Nitric and Sulfuric Acids (70:30).

SOP name and Revision number

Employee name (print)

Employee name (sign) Date:

Supervisor name (sign) Date:

Initial SOP Training (leave blank if not applicable)

Initial reading of method and training	Initials	Date	Supervisor
1. Read method			
2. Observe the method			
3. Detailed review of method and associated literature			
4. Supervised practice of method with trainer			
5. Unsupervised practice of the method with trainer			
6. Review of work with trainer and/or peer-review			
7. IDOC to determine precision and accuracy			
8. Determination of blanks			