United States Environmental Protection Agency
Office of Water
Washington, DC
EPA No. 841-B-24-003

National Coastal Condition Assessment 2025 Laboratory Operations Manual

Version 1.2 June 5, 2025

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:

- National Coastal Condition Assessment: Quality Assurance Project Plan V 1.2 (EPA **841-B-24-001**)
- National Coastal Condition Assessment: Field Operations Manual V1.2 (EPA **841-B-24-002**)
- National Coastal Condition Assessment: Laboratory Methods Manual V1.2 (EPA **841-B-24-003**)
- National Coastal Condition Assessment: Site Evaluation Guidelines 1.2 (EPA **841-B-24-004**)

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 2025. All NCCA cooperator laboratories must follow the guidelines presented in the document.

With the exception of the requirements in Chapters 3 for evaluating microcystins, mention of trade names or commercial products in this document does not constitute a recommendation for use. Chapters 3 requires use of a specific kit and supplemental materials manufactured by a single firm.

More details on specific methods for site evaluation and sampling can be found in the appropriate companion documents (*Site Evaluation Guidelines and Field Operations Manual, respectively)*.

The suggested citation for this document is:

USEPA. 2024. National Coastal Condition Assessment 2025: Laboratory Operations Manual. V.1.2. U.S. Environmental Protection Agency, Washington, DC. EPA 841-B-24-003

VERSION HISTORY

Version	Date	Changes Made
1.0	March 2025	Not Applicable
1.1	March 26, 2025	Updates aligning with updates to FOM and QAPP. (change to Version 1.1, Change to current date.)
1.2	June 5, 2025	• Updates to Section 7.7 through 7.10 (water chemistry and chlorophyll *a* analysis) incorporating updates to ORD-PESD Lab protocols. • Inserted citations to the OST QAPPs for the Human Health Fish Fillet Tissue Indicator (Section 9). • SOP for Research Indicator – Total Alkalinity (Appendix B) updated from SOP ID: J-ACESD-MAB-SOP-3809-0 to SOP ID: J-ACESD-MAB-SOP-3809-1. • Changed to V 1.2, change to current date.

TABLE OF CONTENTS

FIGURES

TABLES

LIST OF ACRONYMS

A	Absorbance
ASTM	American Society for Testing and Materials
Avg	Average
CAS	Chemical Abstracts Service Assigns Unique Identifiers to Chemicals
Chl-*a*	Chlorophyll *a*
Cl	Chloride
CRM	Certified Reference Material
CV	Curriculum Vitae
D	Duplicate Sample
DDT	Dichloro-Diphenyl-Trichloroethane
DI	De-Ionized Water
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DW	Distilled Water
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ETOH	Ethyl Alcohol
FOM	Field Operations Manual
g	Grams
HDPE	High Density Polyethylene
HNO_3	Nitric Acid
HRP	Antibody-Horseradish Peroxidase
$H2SO_4$	Sulphuric Acid
ISBN	International Standard Book Number
ISO	International Organization for Standardization
ITIS	Integrated Taxonomic Information System
KC	Kit Control
kg	Kilograms
L	Liters
LCR	Labeled Compound Recovery
LCS	Laboratory Control Sample
LIMS	Laboratory Information Management System
LOM	Laboratory Operations Manual
LRL	Laboratory Reporting Limit
mg	Milligrams
mg/kg	Milligrams per Kilogram
mL	Milliliters
MDL	Method Detection Limit
MS	Matrix Spike
NABS	North American Benthological Society
NARS	National Aquatic Resource Surveys
NC	Negative Control
ND	Non-Detect
NELAC	National Environmental Laboratory Accreditation Conference
ng	Nanograms
NH_4	Ammonium
NIST	National Institute of Standards
NO_2	Nitrite
NO_3	Nitrate

ORD	EPA's Office of Research and Development
OW	EPA's Office of Water
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyl
PDE	Percent Difference in Enumeration
ppb	Parts per Billion
ppm	Parts per Million
ppt	Parts per Trillion
P	Primary Sample
PSE	Percent Sorting Efficiency
psu	Practical Salinity Units
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
QCF	Quality Control Failure
QMP	Quality Management Plan
RL	Reporting Limit
RO	Reverse-Osmosis
RPD	Relative Percent Difference
RQM	Relative Quantitation Method
RSD	Relative Standard Deviation
RTH	Richest Targeted Habitat
S	Standard Deviation
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
TOCOR	Task Order Contracting Officer's Representative
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
U	Unknown or REGULAR
USGS	United States Geological Survey
WSA	Wadeable Streams Assessment
WQX	Water Quality Exchange

1.0 INTRODUCTION

The U.S. Environmental Protection Agency (EPA), in partnership with states and Tribes, has designed the National Coastal Condition Assessment (NCCA) 2025 to assess the condition of estuarine and Great Lakes nearshore waters in the United States. The NCCA is one in a series of National Aquatic Resource Surveys (NARS) conducted to provide the public with comprehensive assessments of the condition of waters in the U.S.

This manual describes methods for laboratory analyses of the samples to be collected during the National Coastal Condition Assessment. The purposes of this manual are to:

1) document the standardized sample processing and analysis procedures used in the various laboratories for the NCCA 2025; and
2) provide guidance for data quality objectives and, where appropriate, a performance-based method approach to obtain comparable results across all participating laboratories.

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 1st through September 30th. Field crews will collect a variety of measurements and samples from the statistically selected sampling locations identified by geographical coordinates. The complete list of indicators is included in **Table 1-1**. Samples shipped to laboratories for analyses are described below. The indicators are similar to those evaluated in previous NCCA.

Detailed laboratory procedures are described in this document for the following indicators: microcystins, benthic macroinvertebrates, sediment chemistry (including sediment grain size and TOC), sediment toxicity, and ecological fish tissue contaminants.

Specific laboratory analysis procedures for water chemistry and chlorophyll *a* samples are not presented here. A list of parameters to be analyzed are outlined. Additionally, the performance-based methods and pertinent quality assurance/quality control (QA/QC) procedures, as well as filtering, preservation and holding time requirements, are outlined as requirements for laboratories to follow when analyzing water chemistry and chlorophyll *a* samples. Alternative analytical methods for water chemistry are acceptable if they meet all specified performance requirements described in this document. Acceptability is determined by the NCCA project management team. Please contact the NARS Laboratory Coordinator with any questions about method acceptability.

In addition to the indicators above, fish tissue fillets are being analyzed for contaminants by USEPA's Office of Science and Technology (OST). USEPA's Office of Research and Development (ORD) is analyzing water for enterococci and total alkalinity. Analytical methods are provided in separate QAPPs maintained by ORD and OST for these three indicators.

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_2NO_3, NO_3, NH_3, PO_4; Total N and P Chlorophyll *a*	**Nutrient enrichment**
	Silica (Pacific territories only)	**Signature of groundwater seepage/freshwater sources**
	Total Alkalinity (addressed in a separate QAPP)	**Societal value and ecosystem production**
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 • 16 metals • 25 PAHs • 20 PCBs • 14 pesticides • 6 DDT metabolites	**Risk of biological response to sediment contamination**
	Sediment toxicity (10-day bioassay with Leptocheirus or Hyalella)	**Biological response to sediment exposure**
Ecological Fish Tissue Contamination	Whole body fish contaminants • 13 metals (no Sb or Mn) • 20 PCBs • 14 pesticides • 6 DDT metabolites	**Environmentally available contaminant exposure**
Biological Quality	Benthic community structure	**Biological response to site conditions**
	Submerged Aquatic Vegetation	**Societal value and ecosystem production**
Human Health	Microcystins Enterococci (addressed in a separate QAPP) Contaminants in fish tissue filets (addressed in a separate QAPP)	**Societal value**
Other	**Underwater video (Great Lakes only)**	**Invasive species; lake floor characteristics**

2.0 GENERAL LABORATORY GUIDELINES

This section describes the general laboratory guidelines with an overview to the quality assurance / quality control (QA/QC) requirements. Each of the following chapters describes analytical procedures, and the relevant QA/QC requirements, for a different indicator. In addition, the Quality Assurance Project Plan (QAPP) provides comprehensive descriptions of the QA/QC requirements for NCCA 2025.

2.1 Responsibility and Personnel Qualifications

In advance of analyses for NCCA 2025, each laboratory shall train its personnel in in the use of equipment and standard operating procedure(s) (SOP) for which they are responsible. All personnel are responsible for complying with all QA/QC requirements that pertain to the samples to be analyzed. Each laboratory follows its institutional or organizational requirements for instrument maintenance. Additionally, each laboratory should have documentation of experience of working with samples collected in estuarine/marine and/or the Great Lakes environments. **Appendix C**: Laboratory Remote Evaluation Forms identifies the specific documentation that each laboratory must submit to demonstrate its qualifications for performing the analyses.

2.2 Roles and Contact Information

Table 2.1 presents contact information for the key personnel associated with NCCA 2025. The **EPA Headquarters Project Management Team** consists of the Project Manager, EPA Logistics Coordinator, Project Quality Assurance Coordinator, and EPA HQ NCCA 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 EPA team is the final authority on all decisions regarding laboratory analysis.

Table 2-1 NCCA: Contact Information

TITLE[a]	NAME	CONTACT INFORMATION
Project Manager	Hugh Sullivan, EPA OW	sullivan.hugh@epa.gov 202-564-1763
Project QA Coordinator	Sarah Lehmann, EPA OW	Lehmann.sarah@epa.gov 202-566-1379
NARS Laboratory Coordinator	Kendra Forde, EPA OW	forde.kendra@epa.gov 202-564-0417
Logistics Coordinator	Brian Hasty, EPA OW	hasty.brian@epa.gov 202- 564-2236
NARS Team Leader	Sarah Lehmann, EPA OW	lehmann.sarah@epa.gov 202-566-1379

[a] 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 discussions, the laboratories are permitted to contact these people.

TITLE[a]	NAME	CONTACT INFORMATION
NARS Information Management Coordinator (Contractor)	Michelle Gover, GDIT	gover.michelle@epa.gov 541-754-4793
NARS Data Manager	Karen Blocksom, EPA ORD	blocksom.karen@epa.gov 541-754-4470
Contractor Field Logistics Coordinator	Chris Turner, GLEC	cturner@glec.com 715-829-3737

The **Field Logistics Lead**, **Contractor Logistics Coordinator** and the **NARS Information Management (IM) Coordinator** track the location of each NCCA 2025 sample to be processed in the laboratory. The Contractor Logistics Coordinator and the NARS IM Coordinator will be the laboratories' main point of contact regarding sample tracking.

The following personnel will be required for indicators outlined in the chapters below. Additional personnel will be required for individual indicators and will be listed within those chapters.

Laboratory Technician is one who is familiar and qualified to conduct the procedure outlined in the indicator chapters within this NCCA Laboratory Operations Manual and the NCCA Quality Assurance Project Plan.

External QC Coordinator is an EPA staff person who is responsible for selecting and managing the "**QC contractor**."

QC Contractor is a person who must be dedicated to QA/QC functions and must not be a primary laboratory or a field sampling contractor for NCCA. This is done to eliminate inherent bias and maintain objectivity in QA/QC activities. 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.

2.3 Laboratory Capability Review

Capability reviews 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. The EPA may elect to conduct a lab capability review at any time during the period of performance of any lab task order and will provide no fewer than five working days' notice via written technical direction prior to the review. If EPA decides to conduct a capability review, a qualified EPA scientist or contractor will review a checklist based upon the steps described in this section. Lab capability reviews may be conducted virtually or in-person. EPA will develop, review, and approve the checklist and share the checklist with the lab prior to conducting a capability review.

2.4 Sample Tracking

Samples are collected by many different field crews during the index period (June 1st through September 30th). The actual number of sites sampled on a given day will vary widely during this time. Field crews will submit data via the NCCA app or in rare instances, on electronic forms. When sample information is submitted on the app, it will immediately update the NARS IM database. Field crews submit electronic forms when they have shipped samples, and the NARS IM Center uploads the information into the NARS IM database. The NARS IM Center provides laboratories with spreadsheets of samples that the batch lab or field crews (as appropriate) have sent to them. Upon sample receipt, the analysis laboratory must immediately complete and email the sample tracking spreadsheet (containing the sample login and sample condition information) to the IM Center Coordinator for confirmation of sample receipt. Each laboratory will make arrangements with the EPA HQ NCCA Laboratory Review Coordinator for access to the NARS SharePoint site and the NARS IM Center Coordinator, both listed above, to ensure the process of sample check-in has been organized before samples begin to arrive.

When the samples arrive from the field crews or batch laboratory, laboratories will also receive tracking forms in the shipment (refer to the NCCA Field Operations Manual (FOM)). These forms will list the samples included in the shipment. Laboratory personnel must cross check the forms with all samples received to verify that there are not any inconsistencies. If any sample is missing or damaged, laboratory personnel are to contact the NARS IM Coordinator and/or Contractor Logistics Coordinator immediately. For state laboratories conducting analyses in their own laboratories, a state sample tracking spreadsheet is available from EPA.

2.5 Reporting

All labs must provide data analysis information to the HQ Project Management Team by **March 1st, 2026** or as stipulated in contractual or other agreements. These reports must include the data elements specified for each analytical method in this manual including but not limited to the following information and be reported in the data templates available separately from EPA.

- Sample Type (indicator)
- Site ID (ex: NCCA25_AL-10007)
- Sample ID (ex: 999000)
- Pertinent information to the indicator
- Metadata for all fields

The submitted filename must use the following naming convention:

- Indicator name (ex: microcystins)
- Date of files submission to EPA by year, month, and day (ex: 2025_11_01)
- Laboratory name (ex: MyLab)

Combined, the file name would look as follows: Microcystins_2025_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 described below.

The analyst shall review the data to ensure that:

- Sample preparation information is correct and complete;
- Analysis information is correct and complete;
- Results are reported using the requested units;
- The appropriate method and standard operating procedures were followed and identified in the metadata;
- Analytical results are correct and complete;
- Appropriate QA codes are reported when necessary;
- QA Codes/case narratives are clearly defined in the comments and used consistently from batch to batch unless an alternate approach is approved by the Laboratory QC Coordinator or TOCOR;
- Quality control sample results 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 sample results were within established control limits;
- Qualitative and quantitative results are correct and reported using the requested units; 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 2025 Project Manager. 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 NCCA 2025 datafiles on the public website. 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 three (3) 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 1035.
- EPA's project records which under Schedule 1035 are permanent.

See Section 6 of the NCCA 2025 QAPP for additional information on laboratory quality control and competency processes.

3.0 MICROCYSTINS IMMUNOASSAY PROCEDURE

This section describes an immunoassay procedure that measures concentrations of total microcystins in water samples using Gold Standard Diagnostics Microcystins-ADDA ELISA Test Kits ("kits"). Each kit is an enzyme-linked immunosorbent assay (ELISA) for the determination of microcystins and nodularins in water samples.

Field Collection/Sample Summary: Crews collect a microcystins sample in a 500ml PETG bottle. Frozen microcystins samples will be shipped on dry ice from the field crews to the contract batching laboratory (unless the sample is being processed by a state or other non-NCCA national laboratory). The contract batching laboratory will send the batched frozen samples to the analysis laboratory in coolers on ice where they can be held in a freezer until ready for analysis. If a state or other non-NCCA national laboratory is processing the samples, crews may ship or hand deliver the frozen samples to their lab based on internal procedures.

3.1 Summary of the Method

Microcystin analyses laboratories will need to process the samples within the **90-day holding time** and in accordance with timeframes outlined in contractual agreements.

The procedure is an adaptation of the instructions provided by Gold Standard Diagnostic for determining total microcystins concentrations using its ELISA-ADDA kits.[2] For samples with salinity less than 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 low as 0.10 µg/L (i.e., the method detection limit is 0.10 µg/L in samples with undiluted salinity of less than 3.5 ppt and the reporting limit to use is 0.15 µg/L. If the lab determines a different MDL or RL is appropriate these should be included in the appropriate field of the data reporting template with an explanation). The procedure also provides additional sample preparation steps for samples with salinities greater than or equal to 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. A seawater clean-up kit is also required for use when the salinity greater than or equal to 3.5 ppt. The MDL is 0.175 µg/L in samples that do not need to be diluted as a result of higher concentrations of microcystins and the RL is 0.263 µg/L. If the lab determines a different MDL or RL is appropriate these should be included in the appropriate field of the data reporting template with an explanation. Note that the reporting limit for diluted samples is the product of the result multiplied by the dilution factor.

[2] Gold Standard Diagnostics, "Microcystins-ADDA ELISA" Retrieved on August 5, 2024 from https://www.goldstandarddiagnostics.com/pub/media/productattachments/files/m/i/microcystins_nodularins-adda-elisa-user-guide-520011.pdf

3.2 Health and Safety Warnings

The laboratory must require its staff to abide by appropriate health and safety precautions, because the microcystins kit contains potential hazardous chemicals. The substrate solution kit 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 potentially 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 (Laboratories and Equipment)

This section provides definitions, competency and quality assurance documents, and equipment and materials required for laboratories to complete the analyses in this section.

3.3.1 Definitions

The procedure uses the following terms:

Absorbance (A) is a measure of the amount of light absorbed by a sample. 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 Gold Standard Diagnostics microcystins test procedure, are defined as samples with salinity greater than or equal to 3.5 parts per thousand (ppt). 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 less than 3.5 ppt range from the reporting limit of 0.15 µg/L to a maximum value of 5.0 µg/L. Please note, NARS IM cannot accommodate character values within numeric fields.

Given the salinity is less than 3.5 ppt, if the result value is:

- Less than 0.10 µg/L, then the laboratory reports the result as is and flags the sample as being a non-detect (DATA_FLAG=ND).
- Between 0.10 µg/L and the reporting limit of 0.15 µg/L (i.e., >=0.10 µg/L and <0.15 µg/L), the laboratory should record the value, but assign a QC code to the value (i.e., DATA_FLAG=J).
- 5.0 µg/L or greater, the laboratory must flag the samples as HI, leave the CONC column blank, dilute and reanalyze the sample (DATA_FLAG= HI).

Given the salinity greater than or equal to 3.5 ppt, the technician must follow the seawater cleanup procedures in **Section 3.5.2** and then analyze the sample. The results are then adjusted by a factor of 1.75 for a reporting range of 0.263 µg/L to 8.75 µg/L. If the adjusted result value is:

- Less than 0.175 µg/L, then the laboratory reports the result as is and flag the sample as a non-detect (DATA_FLAG=ND).
- Between 0.175 µg/L and the reporting limit of 0.263 µg/L (i.e., >0.175 µg/L and <0.263 µg/L), the laboratory should record the value, but assign a QC code to the value (i.e., DATA_FLAG=J).
- Greater than 8.75 µg/L, the laboratory must flag the data as "HI" in the Data Flag, leave the CONC column blank, dilute and reanalyze the sample (DATA_FLAG= HI).

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.

Duplicate samples (D): are defined as the second aliquot of an individual sample within a well plate. Each sample including the standards are run in pairs and both results for the primary and duplicate aliquot are reported in the result column of the lab deliverable.

Method Detection Limit: 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 method 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." The MDL for freshwater samples (salinity <3.5ppt) is 0.10 µg/L and for saline samples (>3.5ppt) is 0.175 µg/L.

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

NARS Information Management (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 estuarine/marine samples will be collected during the field stage of NCCA.

Primary samples (P): are defined as the first aliquot of a sample within a well plate. Each sample is analyzed in pairs. The results of both this aliquot and the secondary, duplicate aliquot are reported in the result column of the lab deliverable.

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. For undiluted samples with a salinity <3.5 ppt, the reporting limit is 0.15 µg/L, for undiluted samples with a salinity ≥3.5 ppt, the reporting limit is 0.263 µg/L. The reporting limit for diluted samples is the product of the result multiplied by the dilution factor.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method detection limit (salinity <3.5 ppt = < 0.10 µg/L or salinity ≥3.5 ppt = < 0.175 µg/L). For diluted samples, the sample-specific detection limit will be the product of the method 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.

Standard Deviation (S) shows variation from the average.

3.3.2 General Requirements for Laboratories

Competency

To demonstrate its competency/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.
- Results from an algal toxin proficiency test program showing acceptable results and that uses standards that cover the range of likely concentrations that will be encountered.

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 assurance for the laboratory shall sign the NCCA QAPP Certification Page.

3.3.3 Equipment/Materials

The procedures require the following equipment and information:

- Gold Standard Diagnostics ADDA Test Kit, Product #520011[3] (see **Section 3.5.3**)
- Adhesive Sealing Film (Parafilm) for Micro Plates (such as Rainin, non-sterile, Cat. No. 96-SP-100): Used to cover plates during incubation.
- Sample Template – **Figure 3.1.**
- Distilled or Deionized Water: For diluting samples when necessary.
- ELISA evaluation software
- 2 Glass scintillation vials (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).

[3]Or Gold Standard Diagnostics, Microcystins-(ADDA)- SAES ELISA (Microtiter Plate): Product No. 520011SAES, which may be used in saline waters up to 38 psu without a cleanup step. If using this kit, the method quality objectives as documented in literature provided with the SAES kit will apply.

- 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 (Gold Standard Diagnostics 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 will keep the samples frozen upon receipt. Periodically, the batching laboratory ships samples to the microcystins laboratory. Samples will arrive in the analytical laboratory frozen where they can be held in a freezer for several months (up to 90 days) after collection date.

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 **Section 2.2** 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. *NF:* Sample is not frozen

c. If any sample is damaged or missing, contact the EPA HQ NCCA Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in **Section 2.2**).

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_NAME	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_NO	numeric	Sequential visits to site (1 or 2)	
SAMPLE_ID	numeric	Sample ID as used on field sheet (on sample label)	
DATE_COL	MMDDYY	Date sample was collected	
CONDITION_CODE	text	Condition codes describing the condition of the sample upon arrival at the laboratory.	
		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
		NF	Sample is not frozen
		Q	Other quality issue to which the above flags are not applicable.
COND_COMMENT	text	Comments about the condition of the sample Required for "Q". Optional for others.	

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 another similar alternative are acceptable. Use one new syringe and filter per sample.

3.5.2 Additional Prep 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 Gold Standard Diagnostic (formerly Abraxis).[4] 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 greater than 3.5 ppt the technician should follow the instructions:

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

[4] Gold Standard Diagnostics “Microcystins in Brackish Water or Seawater Sample Preparation” Retrieved on August 5, 2024 from https://www.goldstandarddiagnostics.com/pub/media/productattachments/files/m/i/microcystin-adda-estuary-sample-application-note-520011.pdf. This cleanup step is required for samples with a salinity of greater than 3.5 ppt only if Gold Standard Diagnostics ADDA Test Kit, Product #520011 is being used. If Microcystins-ADDA SAES ELISA kit, Product # 520011 SAES is being used, the cleanup step is not necessary.

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[5]. 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. Clean 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.
    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.

[5] Additional correspondence between EPA and Abraxis (now Gold Standard) notes that this step leaves the resin in 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 Gold Standard Diagnostics Microcystins-ADDA ELISA Kit beginning with the next section (**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 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 Gold Standard Diagnostics 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 3.1**) 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 NCCA Laboratory Review Coordinator for approval prior to first use. (See **Section 2.2** of the manual for contact information.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S4	P1	P5	P9	P13	P17	P21	P25	P29	P33	P37
B	S0	S4	D1	D5	D9	D13	D17	D21	D25	D29	D33	D37
C	S1	S5	P2	P6	P10	P14	P18	P22	P26	P30	P34	P38
D	S1	S5	D2	D6	D10	D14	D18	D22	D26	D30	D34	D38
E	S2	KC	P3	P7	P11	P15	P19	P23	P27	P31	P35	P39
F	S2	KC	D3	D7	D11	D15	D19	D23	D27	D31	D35	D39
G	S3	NC	P4	P8	P12	P16	P20	P24	P28	P32	P36	P40
H	S3	NC	D4	D8	D12	D16	D20	D24	D28	D32	D36	D40

Figure 3.1 Microcystin: sample template
Key: S0-S5 = Standards; KC = Control supplied with Kit (i.e., Kit Control);
NC = Negative Control (Laboratory Reagent Blank); P = Primary aliquot for each unknown sample collected by field crew; D= "DUPLICATE" aliquot for each matching unknown Primary sample

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 3.1**. Verify that the software displays the same template or make any necessary corrections. Laboratories with access to an auto-pipetter may use said machinery after proper documentation of set up, training and calibration has been provided and approved by EPA HQ NCCA Laboratory Review Coordinator prior to first use. (See **Section 2.2** of the manual for contact information).

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, consisting of the original analysis and, if necessary, one reanalysis):
        i. The concentration value registers as HIGH (exceeds the calibration range). 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 sample-specific 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 "P" or "D" for unknown sample.
    b. **CONC** contains the numeric concentration value. Two special cases:
        i. Non-detected concentrations: If the sample is non-detected, provide the result within CONC column, record the data as 'ND' in the DATA FLAG column and provide the sample-specific detection limit (0.1 µg/L it the sample is undiluted with salinity less than 3.5 ppt) in the method detection limit column (MDL). See step 24 for reporting values for samples with salinity greater than or equal to 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 "HIGH," 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.[6]

    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 less than 3.5 ppt; see step 24 for samples with salinity greater than or equal to 3.5 ppt);
        iii. **HI** if the concentration value registers as HIGH (exceeds the calibration range).
        iv. **QCF** if there is a QC failure per step 23 above. The QCF code must be used for all failures to facilitate data analysis.
        v. **H** if the sample did not meet the holding time and was not analyzed within 90 days of collection.
        vi. **Q** for any other quality issue (describe in **COMMENTS**)
    d. DILUTION FACTOR is only required if the sample was diluted.
    e. **DUP AVG** and **DUP CV** are required for duplicate samples and control samples (use all three values if the controls are used in triplicate).

3.5.5 Dilutions (if needed)

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

[6] EPA compares the microcystin data values to 8 µg/L, which is the magnitude of the EPA criteria for recreational waterbodies in *Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. 2019. EPA 822-R-19-001*. Retrieved June 5, 2019. https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf

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 Eurofins' method detection limit by 10 to obtain the sample-specific detection limit. For example, for a sample with salinity less than 3.5 ppt, Eurofins' 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 Gold Standard Diagnostics' method detection limit by 100 to obtain the sample-specific detection limit. For example, for a sample with salinity less than 3.5 ppt, Gold Standard Diagnostics' method detection limit is 0.1 µg/L and the sample-specific detection limit 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.

Table 3-2 Microcystins: Data Elements for Each Sample

FIELD	FORMAT	DESCRIPTION	
LAB_ID	Character	Name or abbreviation for 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_NO	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_COL	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)

FIELD	FORMAT	DESCRIPTION	
		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 frozen
COND_ COMMENT	Character	Comments about the condition of the sample. If the condition code='W' then provide the temperature	
BATCH_ID	Numeric	Batch identification code, assigned by lab	
TECHNICIAN	Character	Name or initials of technician performing the procedure	
DATE_ANALYZED	MMDDYY	Date when samples are 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.	
SAM_CODE	Character	Type of solution being tested in the well	
		Flag	Definition
		KC	Kit control
		NC	Negative control
		S0, S1, S2, S3, S4, S5	Standard
		QC	Quality control sample
		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)	
PRIM_DUP	Text	Regular samples are listed as "P" for Primary/first aliquot or "D" for second aliquot (see **Figure 3.1**)	
SALINITY	Numeric	If the sample vial has the salinity marked on the vial, record the value in units of parts per thousand (ppt) or practical salinity units (psu). The two units are interchangeable. 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. (Sample-specific detection limit is 0.175 µg/L for samples with salinity ≥3.5 ppt using the ADDA ELISA test kit).	

FIELD	FORMAT	DESCRIPTION
UNITS	Text	The units of the concentration of the CONC column.
MDL	Numeric	Minimum detection limit in same units as the CONC column
RL	Numeric	Reporting Limit in same unit as the CONC column
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 S. 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.
QA_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
		H: Sample did not meet the holding time and was not analyzed within 90 days.
		HI: Result indicated a high concentration (i.e., outside calibration range)
		J: Concentration above detection but below reporting limit
		QCF: QC failure
		Q: Other quality concerns not identified above
LAB_COMMENT	Character	Explanation for QA_FLAG(s) (if needed) or other comments.

3.6 Data Reporting Requirements

Table 3-3 provides the data reporting criteria.

Table 3-3 Microcystins: Data Reporting Criteria

MEASUREMENT	UNITS	NO. SIGNIFICANT FIGURES	MAXIMUM NO. DECIMAL PLACES
Microcystins	ug/L	3	3

3.7 Quality Measures

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

3.7.1 QC Samples

The External QC Coordinator may 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 if QC samples are being sent.

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.

3.7.2 Summary of QA/QC Requirements

Table 3-5 outlines the data quality objectives for microcystins and **Table 3-6** provides a summary of the quality control requirements described in **Sections 3.4** and **3.5**.

Table 3-4 Microcystin: Sample Receipt and Processing Quality Control

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTIONS
Sample Log-in	Upon receipt of a sample shipment, record receipt of samples in the NARS IM system (within 24 clock hours) and the laboratory's Information Management System (LIMS).	Discrepancies, damaged, or missing samples are reported to the EPA HQs Laboratory QA Coordinator
Sample condition upon receipt	Sample issues such as cracked container; missing label; temperature (frozen); adherence to holding time requirements; sufficient volume for test.	Qualify samples
Sample Storage	Store sample frozen	Qualify samples
Holding time	Frozen samples can be stored for several months.	Qualify samples

Table 3-5 Microcystins: Data Quality Objectives

PARAMETER	UNITS	METHOD DETECTION LIMIT OBJECTIVE	REPORTING LIMIT OBJECTIVE
Microcystins, undiluted samples with salinities <3.5 part per thousand (ppt)	µg/L	0.1	0.15
Microcystins, undiluted samples with salinity greater than or equal to 3.5 ppt	µg/L	0.175	0.263
Microcystins, diluted samples with salinities <3.5 ppt	µg/L	0.1 times the dilution factor	Will vary
Microcystins, diluted samples with salinity greater than or equal to 3.5 ppt	µg/L	1.75 times the dilution factor	Will vary

Table 3-6 Microcystins: Sample Analysis Quality Control Activities and Objectives

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTION
Kit – Shelf Life	Shelf life must be 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.

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTION
Kit -Contents	All required contents must be present and in acceptable condition. This is important because Gold Standard Diagnostics 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 average must be between 0.565 µg/L and 0.935 µg/L.	If either requirement fails results from the analytical run are not reported. • 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%).

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTION
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.** If samples are re-run, do not enter concentration information of the first run.
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 Coordinator will determine an appropriate course of action, including no action, flagging the data, or excluding some or all of the laboratory's data.

3.8 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 data files. 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 may follow its own internal protocols for disposal.

3.9 References

Gold Standard Diagnostics, "Microcystins-ADDA SAES ELISA (Microtiter Plate): Product No. 520011SAES" Retrieved on August 5, 2024 from https://www.goldstandarddiagnostics.com/microcystins-nodularins-adda-saes-elisa-96-tests.html

Gold Standard Diagnostics, "Microcystins in Brackish Water or Seawater Sample Preparation" Undated. Retrieved on March 12, 2020 from https://www.goldstandarddiagnostics.com/pub/media/productattachments/files/m/i/microcystin-adda-estuary-sample-application-note-520011.pdf

Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. 2019. EPA 822-R-19-001. Retrieved June 5, 2019. https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf

4.0 BENTHIC MACROINVERTEBRATES

This section describes the steps for identifying benthic macroinvertebrate organisms in samples collected in estuarine coastal waters and the nearshore Great Lakes during the 2025 NCCA.

Field Collection/Sample Summary: Field crews collect samples in one or more one-liter HDPE bottles depending on the amount of material. The samples are preserved in the field with buffered formalin stained with rose bengal and shipped from the field crews to the contract batching laboratory (unless the sample is being processed by a state or other non-NCCA national laboratory). The contract batching laboratory will send batched samples to the analysis laboratory in coolers. If a state or other non-NCCA national laboratory is processing the samples, crews may ship or deliver the samples to their lab based on internal procedures.

This procedure requires the laboratory to fully sort and identify all organisms in the sample. Subsampling procedures are not authorized for any samples collected for the NCCA program.

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 (e.g., organic detritus, random body parts that are not identifiable, empty shells, etc.). For each species or lowest practical taxonomic level, the taxonomist includes at least one representative organism in the laboratory's reference collection for NCCA 2025.

As part of the quality control measures, a second taxonomist at each lab 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 to minimize problems during data analysis.

Upon completion of the taxonomy at each taxonomy lab, an external QC contractor will randomly select 10% of samples for independent taxonomic verification.

4.2 Health and Safety Warnings

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

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

2. When working with potentially hazardous chemicals (e.g. rose bengal) or biological agents (benthic organisms and sediments), avoid inhalation (e.g., use a fume hood or other appropriate ventilation when necessary), skin contact, eye contact, or ingestion. Treat any potential exposures appropriately and according to internal laboratory protocols.

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

This section provides definitions, competency and quality assurance documents, and equipment and materials required for laboratories to complete the analyses in this section.

4.3.1 Definitions

The procedure uses the following throughout the document:

AphiaID: A stable and globally unique identifier that the World Register of Marine Species (WoRMS) couples with each scientific name to serve as the "common denominator" for accessing information. AphiaIDs are preferred for marine samples, but Taxonomic Serial Number (TSN) may be used for low salinity species lacking AphiaIDs. When either of these are unavailable, secondary sources are acceptable.

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.

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

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:
    - NCCA2025
    - 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.
    - Append "e" to the filename if the photograph was edited (e.g., cropped).

For example, on September 8, 2025, 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: NCCA2025_ABC_1234_ capitella capitata_40x_20250908.gif and NCCA2025_ABC_1234_ capitella capitata_40x_20250908e.gif.

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 used for Great Lakes taxa and low salinity estuarine taxa.

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 NARS 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 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 for Great Lakes taxa and low salinity estuarine taxa without AphiaIDs, but when they are not available, secondary sources are acceptable.

World Register of Marine Species (WoRMS): a database with standardized and reliable information on species nomenclature and their hierarchical taxonomic information used for estuarine taxa.

4.3.1 General Requirements for Laboratories

Competency

The procedure may be used by any laboratory that demonstrates documented competency in completing taxonomic analytical work, adhering to quality procedures and reporting standardized data:

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 successful 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 QMPs, QAPPs, and applicable Standard Operating Procedures (SOPs).
    b. To demonstrate its ongoing commitment, the person in charge of quality assurance for the laboratory shall sign the NCCA 2025 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.

Personnel

The procedure may be used by laboratory personnel who have 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, and make available to EPA upon request, documentation of the qualifications for the Internal Taxonomy QC Officer. The Internal Taxonomy QC Officer is an experienced taxonomist who meets the following requirements:

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 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. 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. Taxonomists identifying marine taxa should have experience with marine and estuarine fauna, while those identifying Great Lakes fauna should be familiar with those fauna. 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 *CV* 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 greater than or equal to 90% sorting efficiency in five consecutive samples.

4.3.2 Equipment/Materials

Examples of equipment and materials for sample preparation, sorting, and taxonomic identifications (not all-inclusive).

Sample Preparation 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)

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 (Protocol 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 **Section 2.2** for contact information).

2. Inspect each jar THE SAME DAY THEY ARE RECEIVED:
   a. Add 70-80% EtOH 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. As soon as possible, notify the NCCA Project Lead or NARS Laboratory Coordinator (see contact information in **Section 2.2**) if any jars were broken and/or there are discrepancies between the custody form and jars (e.g., three jars expected but only two were received; Sample ID and SiteID numbers don't coincide with those that are expected; etc.).

3. Store the sample containers at room temperature and in a dark location 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. See quality control requirements in **Table 4-3.**

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 4-1 Benthic 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_NO	Numeric	Sequential visits to site (1 or 2, if specified on label)	
SAMPLE_ID	Numeric	NCCA sample number as used on field sheet (on sample label)	
DATE_COL	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_COMMENT)
COND_COMMENT	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. Verify that the internal label matches the external one.

2. Carefully decant the formalin from the sample container by pouring the fluid through a sieve (U.S. 35) into a separate container in a well-ventilated area (i.e. fume hood). 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 (i.e., do not include in counts):
    - Empty snail or bivalve shells
    - Organisms of water surface-dwelling or strict water column[2] arthropod taxa,
    - 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. 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

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

- 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.

7. Remove the remaining material left on the sorting pan (i.e., material such as sticks and 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 number and sample identification 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_COMMENT 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. Complete the top portion of a Taxonomic Bench Sheet (for an example, see **Attachment 5.1**), using the information from the internal sample 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 apply a coverslip. All organisms must be visible, which generally means a maximum of 10-20 organisms per slide. Label the slides with the sample identification number and lab tracking number (per internal SOPs).
        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. In other words, EPA would *not* consider a request for combined locations of a prime contract laboratory and its subcontract laboratories. Instead, a separate waiver request must be submitted for each of the individual prime and subcontractor laboratories, and EPA would evaluate and grant (or deny) a waiver individually, based upon each individual laboratory's qualifications.
            2) The request for a waiver must identify and describe a minimum of three studies, for each of which, the external QC evaluation demonstrated 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 (organized by group) 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, which are not counted in the NCCA 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 Manager for guidance. Add any necessary data qualifiers (see list with Required Data Elements in **Table 4-2**).
    a. Enter the AphiaID for estuarine taxa (when available) or the Taxonomic Serial Number (TSN) for Great Lakes and low salinity estuarine taxa 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. For example: If some organisms from a sample are identified to *Macoma*, but other organisms in the sample could only be identified to Tellinidae (Family) and/or Veneroida (Order), 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.
        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 an 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.

Record the identifications. For example, using the taxonomic bench sheet in **Protocol 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.

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. Examples of problematic taxa name entries include:
    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)
    j. Invalid name (e.g., taxonomic change, synonym; Sphaeriidae vs. Pisiidae)

6. Check whether taxa names are "reasonable" (i.e., likely to occur in the region from where the sample was collected).

7. 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.

8. Provide the data to the Internal Taxonomic QA 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.

9. 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.

10. 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 Taxonomy QC Officer, who will periodically review the reference collection to ensure that it is complete.

11. Verify that the reference collection contains at least one organism that represents each genus (or lowest taxonomic level) identified from all samples. 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: one for organisms removed for the reference collection; one for the non-reference organisms that remain in the sample container.
    c. Obtain a good quality representative digital photograph of the specimen (see instructions in **Section 4.3.1**).

12. 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 each 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 original taxonomist fails to maintain a ≥ 90% identification as determined by QC checks, previous samples will be re-counted and identified. (If the Internal Taxonomy QC Officer performs the QC check more frequently, then all QC data must be submitted.)

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

14. 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**.

15. 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: Data Elements for Each Sample

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_NO	Numeric	Sequential visits to site (1 or 2, if specified on label)
SAMPLE_ID	Numeric	NCCA sample number as used on field sheet (on sample label)
DATE_COL	MMDDYY	Date sample was taken
SALINITY	Numeric	Salinity in ppt or psu of the water from which the sample was collected (from label)
CONDITION_CODE	Character	Condition of sample upon arrival in lab, from **Table 4-1** in LOM
COND_COMMENT	Character	Explanation of condition code (if needed). “Q” condition code always requires a comment.
DATE_TAXON	MMDDYY	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
PHYLUM	Character	Taxonomic phylum
CLASS	Character	Taxonomic class
ORDER	Character	Taxonomic order
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
WORMS_APHIA_ID	Numeric	World Register of Marine Resources Database ID
ITIS_TSN	Numeric	Integrated Taxonomic Information System Taxonomic Serial Number (for Great Lakes samples and low salinity marine samples)
LAB_TIN (OPTIONAL)	Character	Lab taxa ID number
TAXA_NAME	Character	Unique taxon name in the taxa list provided by EPA

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

4.7 Data Entry

Table 4-1 and **Table 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.

4.8 Sample and Record Retention

The laboratory shall retain:

1. The sample materials, including vials, slides, and sorting residuals, for a minimum of three years from the date the EPA makes the data public. 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.

a. Sample collection permits (i.e., for some samples collected in waters under the jurisdiction of the National Park Service) may require samples to be returned to the permitting entity. In this case, the EPA Headquarters Project Management Team will contact the laboratory to arrange for the samples to be returned after QC reconciliation has been completed.

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 may follow its own 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 among taxonomists within a lab, the purpose of the external QC evaluation is to ensure taxonomic consistency among different laboratories and taxonomists. To achieve this objective, EPA compares the primary laboratory results to those from a secondary 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 regularly 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 primary 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 primary 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 their 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 required 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. Although rare, 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, if needed.
    a. In preparation for the teleconferences:
        i. The QC contractor instructs the secondary laboratory to photograph representative specimens for each taxon requiring a reconciliation 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 QC contractor may also consider and provide information on whether the taxa names are "reasonable" (i.e., likely to occur in the

region from where the sample was collected) although this is not required.

iv. The primary and secondary laboratories provide participants with the relevant reference (or citation) and photograph for each taxonomic identification for the discussion.

v. The QC contractor emails a meeting announcement for a convenient time for all participants. The email provides instructions for accessing the External QC Coordinator's teleconference line or video conference platform.

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); and

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[7]:

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

[7] Depending on the outcome of reconciliation calls, reevaluations, changes, and incorporation of common nomenclature may be required of primary or secondary taxonomy laboratories, or both.

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/or 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.

4.10 Summary of Benthic Macroinvertebrates QA/QC Requirements

Table 4-3 provides information on the sample storage quality control activities. Equations for percent sorting efficiency (PSE), percent disagreement in enumeration (PDE) and percent taxonomic disagreement (PTD) are listed in **Section 4.3.1**.

Table 4-4 and **Table 4-5** outline the measurement quality objectives and describe the laboratory quality control measures for benthic macroinvertebrates.

Table 4-3 Benthic Macroinvertebrates Sample: Sample Storage Quality Control

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTION
Sample Storage	Store benthic samples in a cool, dark place.	Qualify sample as suspect for all analyses
Preservation	Transfer storage to 70% ethanol for long term storage	Qualify samples
Holding time	Preserved samples can be stored indefinitely; periodically check jars and change the ethanol if sample material appears to be degrading.	Qualify samples

Table 4-4 Benthic Macroinvertebrates: Measurement Quality Objectives

VARIABLE OR MEASUREMENT	PRECISION	ACCURACY
Sort and Pick	90% [a]	90% [a]
Identification	85% [b]	95% [c]

[a] As measured by PSE; [b] As measured by (100%-PTD); [c] As measured by (100%-PDE)

Table 4-5 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 (minimum of 1) completed per sorter	PSE ≥ 90%	If < 90%, examine all residuals of samples by that sorter and retrain 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.

CHECK OR SAMPLE DESCRIPTION	FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
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	Checks whether taxa are known to occur in estuarine or nearshore Great Lakes water from which the sample was collected.	Check against known ranges (can occur during internal or external QC; also conducted by EPA data reviewers as part of assessment process.)

Protocol 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

Alpha ID (Use # in Unique Identifier from taxa list provided by EPA)	TSN (Use # in Unique Identifier 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		
Add additional rows as necessary									

Comments:

Use back side or additional pages as needed.

5.0 WHOLE BODY FISH PROCESSING AND CONTAMINANT ANALYSIS

This section 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 2025 NCCA and related studies.

Field Collection/Sample Summary: At each sampling site, the FOM instructs the crews to collect five fish of the same species and similar size for each sample. The samples are frozen and shipped from the field crews to the contract batching laboratory on dry ice (unless the sample is being processed by a state or other non-NCCA national laboratory). The contract batching laboratory will send batched, frozen samples to the analysis laboratory in coolers. If a state is using a lab other than the NCCA national laboratory to process fish samples, crews may ship or deliver the frozen samples to their lab per internal protocols.

The laboratory shall perform analysis to determine the lipid content, moisture content, concentrations of metals, pesticides, and PCBs found in fish within estuarine waters and nearshore Great Lakes.

5.1 Summary of the Procedure

This section describes the processing and contaminant analysis of whole fish samples collected for EPA's 2025 NCCA. To ensure consistent preparation across all fish samples and to avoid sample contamination, it is important that all NCCA participating laboratories adhere to the fish 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, alternate methods must still meet the measurement quality objectives required in the QAPP and associated QA documents).

5.2 Health and Safety Warnings

In addition to understanding the laboratory's hazard communication, safety and disposal requirements, persons using this procedure must abide by 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 potentially 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 (Laboratories and Equipment)

This section provides definitions, competency and quality assurance documents, and equipment and materials required for laboratories to complete the analyses in this section.

5.3.1 Definitions

The procedure uses the following terms:

Method 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 NARS IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. Freshwater and marine 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. EPA will provide instructions for the use, if necessary, of the non-routine samples. Non-routine includes most species not listed in **Appendix A**: Target Fish Species For Whole Fish Analyses with the exception of species listed in the Endangered Species Act, cartilaginous fishes, and invertebrates of any type except sea urchins. These instructions may

also 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.

Percent Recovery: Recovery is measured by comparing the concentrations of a sample split into two aliquots; and one aliquot is spiked with a known concentration value. C_s is the concentration measured in the spiked aliquot; *C* is the concentration measured in the un-spiked aliquot; and *s* is the known concentration amount for the spike. The following equation is used to calculate the percent recovery ($\%Rs$):

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

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. The laboratory homogenizes the fish to prepare one composite sample. The species must be one of the target species identified in **Appendix A**: Target Fish Species For Whole Fish Analyses of this LOM.

Sample-Specific Detection Limit: Most samples will have a sample-specific detection equal to the method detection limit. For diluted samples, the sample-specific detection limit will be the product of the method 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.

Quality Control Check Sample (QCCS) is a sample prepared from an independent standard at a concentration within the calibration range. A QCCS is intended as an independent check of technique, methodology, and standards and should be run with every batch.

5.3.2 General Requirements for Laboratories

Competency

To demonstrate its competency, the laboratory shall provide analyte and matrix specific information to EPA. In addition to documentation of achieving the method detection limits, accuracy, and precision targets (as specified by the NCCA QAPP) for the required analytes in fish samples, 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.

Quality assurance and quality control requirements

The organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include QMPs, QAPPs, and applicable Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment to maintaining data quality, the person in charge of quality assurance for the laboratory shall sign the NCCA QAPP Certification Page.

The national EPA task order award incorporated data quality objectives proposed by the winning offeror for the whole-body fish tissue analytical laboratory if they were less stringent than those originally requested by the EPA. In addition, EPA will exclude parameters marked with an asterisk (*) in **Table 5-6** in the LOM from its evaluation of the laboratory's ability to meet EPA's targets for ≥80% of the parameters.

5.3.3 Equipment/Materials

The procedures require the following equipment and information:

- Scale (Electronic balance)
- Powder-free nitrile gloves
- Tape measure, ruler or fish measuring board
- 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 many samples, the laboratory may email a spreadsheet with the sample login and sample condition information to NARSIM (see **Section 2.0** 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 °C (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 number. For example, if the sample number is "149333," then the crew should label specimen ".01" as "149333.01." 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 300 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. As soon as possible following sample receipt and inspection, notify the EPA about any problems involving sample integrity, conformity, or other inconsistencies.

Table 5-1 Whole Body Fish Login: Required Data Elements

FIELD	TYPE	DESCRIPTION	
SITE_ID	Character	NCCA site identification code	
SAMPLE_ID	Numeric	NCCA sample number, typically six digits	
DATE_COL	MMDDYY	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)	
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 (**Section 2.2** 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 definitions provided in **Section 5.3.1**. If instructions are unclear for the handling and/or processing of ***any*** composite fish sample (e.g. samples collected from incorrect sampling location, unnecessary duplicate sample, inappropriate fish species, etc.), the laboratory shall contact EPA for clarification before proceeding with further activities involving the sample. The laboratory is expected to maintain the integrity of the sample (e.g., frozen) until EPA determines next activities involving the fish sample in question.

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, ".01", ".02"…)
    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 A**: Target Fish Species For Whole Fish Analyses 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 **Section 2.0** for contact information).

11. Verify that all required data elements, per **Table 5-2** and **Table 5-3**, have been recorded. Enter any missing elements into the database.

12. Rinse each fish with deionized water and remove any adhering slime as a precautionary measure to mitigate 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

FIELD	TYPE	DESCRIPTION
SITE_ID	Character	Site identification code
SAMPLE_ID	Character	Sample number
SPECIMEN_ID	Character	Identification code assigned to a single fish
SPECIES	Character	Genus and species
FISH_WT	Numeric	Weight of fish
WT_UNIT	Character	Units of fish weight (g, kg wet weight)
FISH_LEN	Numeric	Length of fish
LEN_UNIT	Character	Units of fish length (mm, cm)
COMMENT	Character	Comment about condition of fish or other observations

Table 5-3 Whole Body Fish: Data Elements from Examination of Each Sample

FIELD	TYPE	DESCRIPTION	
SITE_ID	Character	NCCA site identification code	
SAMPLE_ID	Character	NCCA 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 specimen's lengths are within 75% of longest fish
		NS	Specimen number is fewer than minimum of 5 or greater than 20 maxima
		WT	Mass does not meet minimum of 300 grams
		LL	Longest fish exceeds 400 mm maximum length
		LS	Shortest fish below 100 mm minimum length
		Q	Other quality concerns, not identified above

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), and pesticides 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. 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.

4. 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.

5. 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.

6. 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.

7. 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 a 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 300 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:

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

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

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

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 storing 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.

8. 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

ANALYSIS	TARGET MASS	SAMPLE JAR REQUIREMENTS
Pesticides	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

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. With the exception of sea urchins, NCCA does not provide support for analyses of any other invertebrates such as crustaceans (e.g., lobster, crabs).

EPA intends to compare the 2025 data to 2010, 2015 and 2020 data sets. Therefore, EPA is requiring the whole fish contamination laboratories to use the same extraction and analysis methods from these earlier surveys. After preparing the fish composites as described in **Section 5.5**, laboratories use the analysis methods detailed in **Table 5-5**, that measures contaminants to the levels of the method detection limits identified in **Table 5-6**. In addition, the laboratories must meet the target precision of 30% and the target accuracy as follows:

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

The laboratory must store the fish samples frozen at a maximum of -20° C and complete the analyses within one year.[8]

Table 5-5 Whole Body Fish: Analytical Methods

ANALYSIS	EXTRACTION	REQUIRED METHODS
Metals (except Mercury)	microwave assisted digestion[9]	EPA Method 6020B[10]
Mercury	microwave assisted digestion[8]	EPA Method 245.7[11]

[8] 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 May 22, 2019 from https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf

[9] "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils," retrieved May 22, 2019 from https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf

[10] Method 6020B "Inductively Coupled Plasma-Mass Spectrometry" retrieved February 29, 2024 from https://www.epa.gov/esam/sam-2022-chemical-determinative-method-summary?methodNumber=ChemicalMethod28.

[11] Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved March 12, 2020 from https://nepis.epa.gov/Exe/ZyPDF.cgi/P1008IY8.PDF?Dockey=P1008IY8.PDF

ANALYSIS	EXTRACTION	REQUIRED METHODS
PCBs and Pesticides	EPA Method 3540C[12]	EPA Method 8270[13]
Percent Lipids	Any method using hexane	EPA Method 9071B[14]

Table 5-6 Whole Body Fish: Lipids, Moisture and Required Contaminants

TYPE	UNITS	PARAMETERS	CAS NUMBER	PCB NUMBER	MAX CONC (2010 - 2020)	MDL TARGET**	Target ACCURACY	TARGET PRECISION
LIPID	% Wet Weight	% LIPID				0.01		
MOISTURE	% Moisture	% MOISTURE			46.1			
METAL	µg/wet g	Aluminum	7429-90-5		1100	10	20	30
	µg/wet g	Iron	7439-89-6		1730	50	20	30
	µg/wet g	Lead	7439-92-1		707	0.1	20	30
	µg/wet g	Mercury	7439-97-6		1190	0.01	20	30
	µg/wet g	Nickel	7440-02-0		22.798	0.5	20	30
	µg/wet g	Silver	7440-22-4		11.5	0.3	20	30
	µg/wet g	Tin	7440-31-5		184	0.05	20	30
	µg/wet g	Arsenic	7440-38-2		63.6	0.025	20	30
	µg/wet g	Cadmium	7440-43-9		22.377	0.2	20	30
	µg/wet g	Chromium	7440-47-3		28.495	0.1	20	30
	µg/wet g	Copper	7440-50-8		212	5	20	30
	µg/wet g	Vanadium	7440-62-2		24.923	1	20	30
	µg/wet g	Zinc	7440-66-6		694.607	50	20	30
	µg/wet g	Selenium	7782-49-2		24.774	0.025	20	30
PCB	ng/wet g	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	2051-24-3	209	76	2	35	30
	ng/wet g	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118	458.7	2	35	30
	ng/wet g	2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66	207.3	2	35	30
	ng/wet g	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	77	95.2	2	35	30
	ng/wet g	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105	121.4	2	35	30
	ng/wet g	2,4'-Dichlorobiphenyl	34883-43-7	8	60.3	2	35	30
	ng/wet g	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153	621.6	2	35	30
	ng/wet g	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138	402.3	2	35	30

[12] Method 3540C "Soxhlet Extraction" retrieved March 12, 2020 from https://www.epa.gov/sites/production/files/2015-12/documents/3540c.pdf

[13] Method 8270D "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)" retrieved March 12, 2020 from https://19january2017snapshot.epa.gov/sites/production/files/2015-07/documents/epa-8270d.pdf

[14] Method 9071B "n-Hexane Extractable Material (HEM) for Sludge, Sediment, And Solid Samples," retrieved May 22, 2019 from https://www.epa.gov/sites/production/files/2015-12/documents/9071b.pdf

TYPE	UNITS	PARAMETERS	CAS NUMBER	PCB NUMBER	MAX CONC (2010 - 2020)	MDL TARGET**	Target ACCURACY	TARGET PRECISION
	ng/wet g	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	180	362.1	2	35	30
	ng/wet g	2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170	154	2	35	30
	ng/wet g	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52	471	2	35	30
	ng/wet g	2,2',5-Trichlorobiphenyl	37680-65-2	18	113.1	2	35	30
	ng/wet g	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101	393.5	2	35	30
	ng/wet g	2,3,3',4,6'-Pentachlorobiphenyl	38380-03-9	110	291.4	2	35	30
	ng/wet g	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	128	86.2	2	35	30
	ng/wet g	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206	84.5	2	35	30
	ng/wet g	2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44	104.2	2	35	30
	ng/wet g	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187	243	2	35	30
	ng/wet g	2,2',3,3',4,4',5,6-Octachlorobiphenyl	52663-78-2	195	83.8	2	35	30
	ng/wet g	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	126	87.2	2	35	30
	ng/wet g	2,4,4'-Trichlorobiphenyl	7012-37-5	28	339.3	2	35	30
PEST	ng/wet g	Heptachlor Epoxide	1024-57-3		636.9	2	35	30
	ng/wet g	Endosulfan Sulfate	1031-07-8		503	2	35	30
	ng/wet g	Hexachlorobenzene	118-74-1		401.7	2	35	30
	ng/wet g	Mirex	2385-85-5		357.6	2	35	30
	ng/wet g	Oxychlordane	26880-48-8		478.3	2	35	30
	ng/wet g	Aldrin	309-00-2		431.8	2	35	30
	ng/wet g	Alpha-BHC	319-84-6		566.6	2	35	30
	ng/wet g	Beta-BHC	319-85-7		800.9	2	35	30
	ng/wet g	Delta-BHC	319-86-8		435.9	2	35	30
	ng/wet g	Endosulfan II	33213-65-9		462.1	2	35	30
	ng/wet g	2,4'-DDE	3424-82-6		522.6	2	35	30
	ng/wet g	Trans-Nonachlor	39765-80-5		523.9	2	35	30
	ng/wet g	4,4'-DDT	50-29-3		754.8	2	35	30
	ng/wet g	Alpha-Chlordane	5103-71-9		566.3	2	35	30
	ng/wet g	Cis-Nonachlor	5103-73-1		384.8	2	35	30
	ng/wet g	2,4'-DDD	53-19-0		668.3	2	35	30
	ng/wet g	Endrin Ketone	53494-70-5		644.9	2	35	30
	ng/wet g	Gamma-Chlordane	5566-34-7		593.7	2	35	30
	ng/wet g	Lindane	58-89-9		559	2	35	30
	ng/wet g	Dieldrin	60-57-1		557.3	2	35	30
	ng/wet g	Endrin	72-20-8		1735.2	2	35	30
	ng/wet g	4,4'-DDD	72-54-8		666.9	2	35	30
	ng/wet g	4,4'-DDE	72-55-9		1080	2	35	30
	ng/wet g	Endrin Aldehyde	7421-93-4		406.8	2	35	30
	ng/wet g	Heptachlor	76-44-8		799.2	2	35	30
	ng/wet g	2,4'-DDT	789-02-6		670	2	35	30
	ng/wet g	Endosulfan I	959-98-8		11943.4	2	35	30

** In the event that the MDL is elevated because sample dilution is necessary to overcome the matrix effect, please notify EPA Laboratory Coordinator. The EPA is not requiring laboratories to meet pre-specified laboratory reporting limits. Prior to

analyzing samples, laboratories shall provide historical Laboratory Reporting Limits to the EPA. Every effort should be made to achieve the historical reporting limit for each sample. If the laboratory reporting limit increases by more than 10% over the historical reporting limit (after accounting for dilution factors), the laboratory shall contact the NCCA Lead or QA Coordinator to discuss corrective actions.

5.7 Data Entry

Table 5-1, **Table 5-2**, **Table 5-3**, and **Table 5-7** 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

FIELD	TYPE	DESCRIPTION	
SITE_ID	Character	NCCA site identification code or type of QC sample (e.g., LAB BLANK)	
SAMPLE_ID	Numeric/ Character	6-digit NCCA sample number, LCS, BLANK, MS, or Rinsate	
REPEAT	Numeric	Duplicate or Triplicate (otherwise blank)	
DATE_COL	MMDDYY	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	
PER_MOIST	Numeric	Moisture percentage of fish	
PER_LIPID	Numeric	Lipid percentage based on lab proposed data quality objectives using standard operating procedures.	
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 FISH_CODE (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

FIELD	TYPE	DESCRIPTION	
		LE	Not all specimen's lengths are within 75% of longest fish
		NS	Specimen number is fewer than minimum of 5 or greater than 20 maxima
		WT	Mass does not meet minimum of 300 grams
		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	
LAB	Character	Name of Laboratory processing sample (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	MMDDYY	Date that the sample homogenization started	
DATE_ANALYSIS	MMDDYY	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	Measured concentration value	
REASON	Character	Reason for qualification in RESULT_QUAL (usually blank)	
RESULT_QUAL	Character	Data qualifier (usually blank)	

FIELD	TYPE	DESCRIPTION
UNIT	Character	Unit of measurement for RESULT, MDL, and RL
QC_CODE	Character	Apply laboratory defined QC codes and describe in the comments field. Define laboratory QC codes within the case narrative.
COMMENT	Character	Explain situation that created QC code, or any unusual aspects of the analysis

* In the event that the MDL is elevated because sample dilution is necessary to overcome the matrix effect, please notify EPA Laboratory Coordinator.

5.8 Date Reporting

Data reporting units and significant figures are given in **Table 5-8.**

Table 5-8 Whole Body Fish: Data Reporting Criteria

MEASUREMENT	UNITS	EXPRESSED TO THE NEAREST
Pesticides and PCBs	ng/ wet g	0.01
Metals	µg/wet g	0.01
Hg	µg/wet g	0.001
Lipid content	% lipid	.01
Moisture content	% moisture	.01

5.9 Summary of Whole Body Fish QA/QC Requirements

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 5-9** provides a summary of the quality control requirements.

Table 5-9 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.
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.

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTION
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 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.

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTION
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 5-6** (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 5-6**) 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.

QUALITY CONTROL ACTIVITY	DESCRIPTION AND REQUIREMENTS	CORRECTIVE ACTION
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 5-6**), 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/ wet g. PCBs and pesticides in ng/ wet g	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 units in the comments field.
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.

***Section 2.2** 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 EPA HQ NCCA Laboratory Review Coordinator.

5.10 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 may follow its own internal protocols for disposal.

5.11 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. Third edition. EPA/823/B-00/007. https://www.epa.gov/sites/default/files/2015-06/documents/volume1.pdf

Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved March 12, 2020 from https://nepis.epa.gov/Exe/ZyPDF.cgi/P1008IY8.PDF?Dockey=P1008IY8.PDF.

Method 3051A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils," retrieved March 12, 2020 from https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf.

Method 6020A "Inductively Coupled Plasma-Mass Spectrometry" retrieved February 29, 2024 from https://www.epa.gov/esam/sam-2022-chemical-determinative-method-summary?methodNumber=ChemicalMethod28.

Method 8270D "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS) retrieved March 12, 2020 from https://19january2017snapshot.epa.gov/sites/production/files/2015-12/documents/8270d.pdf.

Method 9071B "n-Hexane Extractable Material (HEM) for Sludge, Sediment, And Solid Samples," retrieved from https://www.epa.gov/sites/production/files/2015-12/documents/9071b.pdf.

6.0 SEDIMENT CONTAMINANT, GRAIN SIZE, AND TOC ANALYSES

This section 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 2025 NCCA and related studies.

Field Collection/Sample Summary: At each sampling site, the FOM instructs the crews to collect surficial sediment samples. Samples for sediment grain size (SEDG) collected in one-quart plastic bag. Crews collect sediment in a single 250 mL glass jar for both sediment contaminant (SEDO) and total organic carbon (SEDC) analysis. The SEDG samples are shipped on wet ice while the SEDO/SEDC samples are frozen and shipped from the field crews to the contract batching laboratory on dry ice (unless the sample is being processed by a state or other non-NCCA national laboratory). The contract batching laboratory will send batched, frozen samples (SEDO/SEDC) to the analysis laboratory in coolers and batched SEDG samples on wet ice to the analysis lab in coolers. If a state or other non-NCCA national laboratory is processing the samples, crews may ship or deliver the samples to their lab.

The laboratory shall perform analysis to determine the moisture content, concentrations of metals, mercury, pesticides, PAHs, and PCBs found in sediments in coastal waters and Great Lakes.

6.1 Summary of the Procedure

This section describes the contaminant, grain size, and TOC determination of sediment samples collected for EPA's 2025 NCCA. As described in **Section 6.5**, unless contractually bound by other requirements, the laboratory may choose to use any method that meets EPA's specifications for contaminant and grain size analyses.

6.2 Health and Safety Warnings

In addition to understanding the laboratory's hazard communication, safety and disposal requirements, persons using this procedure must abide by 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 potentially 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 (Laboratories and Equipment)

This section provides definitions, competency and quality assurance documents, and equipment and materials required for laboratories to complete the analyses in this section.

6.3.1 Definitions

The procedure uses the following terms:

Certified Reference Materials (CRM) are materials of various matrices for which analytical information has been determined and certified by a recognized authority to provide a quantitative assessment of the accuracy of an analytical method. CRMs provide evidence that the laboratory preparation and analysis produces results that are comparable to those obtained by an independent organization.

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.

Grain Size Classifications are broken down into three categories:

- Sand (0.0625 mm < 2.0 mm)
- Silt (0.0039 mm < 0.0625 mm)
- Clay (< 0.0039 mm)

Method Detection Limit the lowest level of analyte that can be distinguished from zero with 99 percent confidence based on a single measurement (Glaser et al., 1981) 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.”

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 NARS IM system is used to track the samples from field collection to the laboratory.

NCCA: National Coastal Condition Assessment. Freshwater (Great Lakes) and estuarine 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 aliquots; and one aliquot is spiked with a known concentration value. C_s is the concentration measured in the spiked aliquot; C is the concentration measured in the unspiked aliquot; and s is the known concentration amount for the spike. The following equation is used to calculate the percent recovery (Rs):

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

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 detection limit. For diluted samples, the sample-specific detection limit will be the product of the method 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 NARS in the past five years, showing competency for both estuarine and Great Lake samples.
- Documentation detailing the competency of the organization, including professional certifications for sediment-related analyses, membership in professional societies, and experience with analyses for estuarine and Great Lakes sediments that are the same or similar to the requirements of this method.
- Demonstration of competency with sediment samples from estuarine and freshwater environments in achieving the method detection limits, accuracy, and precision targets.

Quality assurance and quality control requirements

The organization shall provide EPA with copies of the quality-related documents relevant to the procedure. Examples include QMPs, QAPPs, and applicable Standard Operating Procedures (SOPs).

To demonstrate its ongoing commitment to maintaining data quality, the person in charge of quality assurance for the laboratory shall sign the NCCA QAPP Certification Page.

The national EPA task order award incorporated data quality objectives proposed by the winning offeror for the sediment contaminants, TOC and grain size analytical laboratory if they were less stringent than those originally requested by the EPA. In addition, EPA will exclude parameters marked with an asterisk (*) in **Table 6-3** in the LOM from its evaluation of the laboratory's ability to meet EPA's targets for ≥80% of the parameters.

6.3.3 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 as samples arrive:

1. Log the samples into NARS IM within 24 clock hours of receiving the samples. 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 **Section 2.2** 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 °C (i.e., room temperature) down to -20 °C 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 organic contaminants [or chemical] (SEDO) and TOC (SEDC) samples and ships with dry ice. Record the condition and temperature of the samples 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 chemical and TOC 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

FIELD	TYPE	DESCRIPTION	
SITE_ID	Character	Site identification code	
SAMPLE_ID	Character	Sample number	
DATE_COL	MMDDYY	Date that the field crew collected the sample	
ANALYSIS_TYPE	Character	Contaminant (SEDO), TOC (SEDC), or Grain Size (SEDG)	
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
		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.

EPA intends to compare the 2025 data to 2010, 2015 and 2020 data sets. Therefore, EPA is requiring the sediment contamination laboratories to use the same extraction and analysis methods from these earlier surveys. Laboratories shall use the analysis methods detailed in **Table 6-2** to measure contaminants 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 listed in **Table 6-3.** For each batch of contaminant samples, precision is assessed using the 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: Storage Requirements and Analytical Methods

STORAGE REQUIREMENTS	TYPE	METHODS THAT MEET THE QA/QC REQUIREMENTS*
Freeze samples at a temperature ≤ -20° C	Metals (except Mercury)	Extraction: EPA Method 3051A[15] Analysis: EPA Method 6020B[16]
	Mercury	EPA Method 245.7[17]
	PCBs, Pesticides, PAHs	Extraction: EPA Method 3540C[18] Analysis: EPA Method 8270D[19]
	TOC	USEPA Method 9060[20]
Refrigerate at 4 °C (do not freeze).	Sediment Grain Size	Any method that reports the determination as percent silt, clay, and sand, and meets the QA/QC requirements.

*Any laboratory variation that meets the QA/QC requirements are acceptable

[15] Method 3051A "Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, And Oils" retrieved November 13, 2018 from https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf ; and
[16]Method 6020B "Inductively Coupled Plasma-Mass Spectrometry" retrieved February 29, 2024 from https://www.epa.gov/esam/sam-2022-chemical-determinative-method-summary?methodNumber=ChemicalMethod28
[17] Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved March 12, 2020 from https://nepis.epa.gov/Exe/ZyPDF.cgi/P1008IY8.PDF?Dockey=P1008IY8.PDF
[17] Method 245.7 "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, Revision 2.0" (EPA-821-R-05-001, February 2005), retrieved March 12, 2020 from https://nepis.epa.gov/Exe/ZyPDF.cgi/P1008IY8.PDF?Dockey=P1008IY8.PDF
[18] Method 3540C "Soxhlet Extraction" retrieved March 12, 2020 from https://www.epa.gov/sites/production/files/2015-12/documents/3540c.pdf
[19]Method 8270D "Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)" retrieved March 12, 2020 from https://19january2017snapshot.epa.gov/sites/production/files/2015-07/documents/epa-8270d.pdf
[20] Method 9060a "Total Organic Carbon" retrieved March 5, 2024 from https://www.epa.gov/sites/default/files/2015-12/documents/9060a.pdf

Table 6-3 Sediment Chemistry, Grain Size, and TOC: Required Parameters

TYPE	UNITS	CAS NUMBER	PARAMETER	PCB NUMBER (WHERE APPLICABLE)	MAX CONC BASED UPON 2010 Through 2020 DATA	MDL TARGET**	TARGET ACCURACY	TARGET PRECISION
METAL	µg/dry g	7429-90-5	Aluminum		162000	1500	20	30
METAL		7440-36-0	Antimony		38.1	0.2	20	30
METAL		7440-38-2	Arsenic		237	1.5	20	30
METAL		7440-43-9	Cadmium		9.9	0.05	20	30
METAL		7440-47-3	Chromium		1078.78	5	20	30
METAL		7440-50-8	Copper		2290	5	20	30
METAL		7439-89-6	Iron		169000	500	20	30
METAL		7439-92-1	Lead		461	1	20	30
METAL		7439-96-5	Manganese		45700	1	20	30
METAL		7439-97-6	Mercury		3.84	0.01	20	30
METAL		7440-02-0	Nickel		360.17	1	20	30
METAL		7782-49-2	Selenium		121.019	0.1	20	30
METAL		7440-22-4	Silver		35.34	0.3	20	30
METAL		7440-31-5	Tin		258	0.1	20	30
METAL		7440-62-2	Vanadium		4734	1	20	30
METAL		7440-66-6	Zinc		1750	2	20	30
PAH	ng/dry g	83-32-9	Acenaphthene		32200	1	23	30
PAH		208-96-8	Acenaphthylene		1990	1	22	30
PAH		120-12-7	Anthracene		51400	1	20	30
PAH		56-55-3	Benz(a)anthracene		8940	1	20	30
PAH		205-99-2	Benzo(b)fluoranthene		11125.6	1	20	30
PAH		207-08-9	Benzo(k)fluoranthene		8530.9	1	20	30
PAH		191-24-2	Benzo(g,h,i)perylene		4650	1	20	30
PAH		50-32-8	Benzo(a)pyrene		10158.6	1	20	30
PAH		192-97-2	Benzo(e)pyrene		5510	1	20	30
PAH		92-52-4	Biphenyl		11200	1	23	30
PAH		218-01-9	Chrysene		13600	1	20	30

TYPE	UNITS	CAS NUMBER	PARAMETER	PCB NUMBER (WHERE APPLICABLE)	MAX CONC BASED UPON 2010 Through 2020 DATA	MDL TARGET**	TARGET ACCURACY	TARGET PRECISION
PAH		53-70-3	Dibenz(a,h)anthracene		1513.5	1	20	30
PAH		132-65-0	Dibenzothiophene		1000	1	20	30
PAH		581-42-0	2,6-Dimethylnaphthalene		1570	1	22	30
PAH		206-44-0	Fluoranthene		38970	1	20	30
PAH		86-73-7	Fluorene		17100	1	20	30
PAH		193-39-5	Indeno(1,2,3-c,d)pyrene		6615.5	1	20	30
PAH		90-12-0	1-Methylnaphthalene		9700	1	27	30
PAH		91-57-6	2-Methylnaphthalene		11500	1	23	30
PAH		832-69-9	1-Methylphenanthrene		2870	1	20	30
PAH		91-20-3	Naphthalene		33400	1	35	30
PAH		198-55-0	Perylene		2441.68	1	20	30
PAH		85-01-8	Phenanthrene		44200	1	20	30
PAH		129-00-0	Pyrene		30400	1	20	30
PAH		2245-38-7	2,3,5-Trimethylnaphthalene		411	1	20	30
PCB	ng/dry g	2051-24-3	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	209	22.4	1	20	30
PCB		35065-30-6	2,2',3,3',4,4',5-Heptachlorobiphenyl	170	115.4	1	20	30
PCB	ng/dry g	52663-68-0	2,2',3,4',5,5',6-Heptachlorobiphenyl	187	56.8	1	20	30

TYPE	UNITS	CAS NUMBER	PARAMETER	PCB NUMBER (WHERE APPLICABLE)	MAX CONC BASED UPON 2010 Through 2020 DATA	MDL TARGET**	TARGET ACCURACY	TARGET PRECISION
PCB		35065-29-3	2,2',3,4,4',5,5'-Heptachlorobiphenyl	180	249.4	1	20	30
PCB		38380-07-3	2,2',3,3',4,4'-Hexachlorobiphenyl	128	61.3	1	20	30
PCB		35065-28-2	2,2',3,4,4',5'-Hexachlorobiphenyl	138	362	1	20	30
PCB		35065-27-1	2,2',4,4',5,5'-Hexachlorobiphenyl	153	168.7	1	20	30
PCB		40186-72-9	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	206	75.5	1	20	30
PCB		52663-78-2	2,2',3,3',4,4',5,6-Octachlorobiphenyl	195	40	1	20	30
PCB		32598-14-4	2,3,3',4,4'-Pentachlorobiphenyl	105	78.2	1	20	30
PCB		37680-73-2	2,2',4,5,5'-Pentachlorobiphenyl	101	256	1	20	30
PCB		31508-00-6	2,3',4,4',5-Pentachlorobiphenyl	118	201	1	20	30
PCB		38380-03-9	2,3,3',4,6'-Pentachlorobiphenyl	110	249	1	20	30
PCB		57465-28-8	3,3',4,4',5-Pentachlorobiphenyl	126	3.5	1	20	30
PCB		41464-39-5	2,2',3,5'-Tetrachlorobiphenyl	44	54.3	1	20	30
PCB		32598-13-3	3,3',4,4'-Tetrachlorobiphenyl	77	8.8	1	20	30

TYPE	UNITS	CAS NUMBER	PARAMETER	PCB NUMBER (WHERE APPLICABLE)	MAX CONC BASED UPON 2010 Through 2020 DATA	MDL TARGET**	TARGET ACCURACY	TARGET PRECISION
PCB		35693-99-3	2,2',5,5'-Tetrachlorobiphenyl	52	123	1	20	30
PCB		32598-10-0	2,3',4,4'-Tetrachlorobiphenyl	66	37.9	1	20	30
PCB		37680-65-2	2,2',5-Trichlorobiphenyl	18	18.4	1	20	30
PCB		7012-37-5	2,4,4'-Trichlorobiphenyl	28	64.3	1	20	30
PEST	ng/dry g,	53-19-0	2,4'-DDD		92.5	1	20	30
PEST		3424-82-6	2,4'-DDE		59.5	1	20	30
PEST		789-02-6	2,4'-DDT		114.1	1	20	30
PEST		72-54-8	4,4'-DDD		157	1	20	30
PEST		72-55-9	4,4'-DDE		159	1	20	30
PEST		50-29-3	4,4'-DDT		194	1	20	30
PEST		309-00-2	Aldrin		13.3	1	21	30
PEST		319-84-6	Alpha-BHC*		0.047591	1	20	30
PEST		319-85-7	Beta-BHC*		510.4	1	20	30
PEST		319-86-8	Delta-BHC*		7.2	1	20	30
PEST		5103-71-9	Alpha-Chlordane		10.9	1	20	30
PEST		5566-34-7	Gamma-Chlordane		12.1	1	20	30
PEST		60-57-1	Dieldrin		2.3	1	20	30
PEST		959-98-8	Endosulfan I		#N/A	1	24	30
PEST		33213-65-9	Endosulfan II		21.2	1	21	30
PEST		1031-07-8	Endosulfan Sulfate		8.1	1	20	30
PEST		72-20-8	Endrin		13.2	1	20	30
PEST		7421-93-4	Endrin Aldehyde*		#N/A	1	58	30

TYPE	UNITS	CAS NUMBER	PARAMETER	PCB NUMBER (WHERE APPLICABLE)	MAX CONC BASED UPON 2010 Through 2020 DATA	MDL TARGET**	TARGET ACCURACY	TARGET PRECISION
PEST		53494-70-5	Endrin Ketone*		#N/A	1	20	30
PEST		76-44-8	Heptachlor		5.3	1	20	30
PEST		1024-57-3	Heptachlor Epoxide		12.7	1	20	30
PEST		118-74-1	Hexachlorobenzene		173.7	1	20	30
PEST		58-89-9	Lindane		163.3	1	20	30
PEST		2385-85-5	Mirex		9.1	1	20	30
PEST		5103-73-1	Cis-Nonachlor*		5.57	1	20	30
PEST		26880-48-8	Oxychlordane*		45.1	1	20	30
PEST		39765-80-5	Trans-Nonachlor		8.17	1	20	30
	% silt, % sand, % clay		Grain Size			0.05 %		10% (LCS)
TOC	mg/kg or %		Total Organic Carbon		54.5	0.01 %	10%	10%

* EPA will exclude these parameters in its evaluation of the laboratory's ability to meet EPA's targets for ≥80% of the parameters.

** In the event that MDLs are elevated because sample dilution is necessary to overcome the matrix effect, please notify EPA Laboratory Coordinator.

Note: Aluminum and iron historical values, derived from the analysis of matrix spike samples, are not available. This is because the concentrations of these two elements in sediments are high, and the sample digestates must be diluted to bring the concentration within the range of the calibration curve, which renders the spike concentration undetectable. Physis will provide RPD data to demonstrate that laboratory precision of duplicate sample analyses meets EPA target values. However, no useful accuracy data can be gleaned from the analysis of matrix spike samples.

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 must define the codes. **Table 6-5** provides the data reporting criteria.

Table 6-4 Sediment Chemistry, Grain Size, and TOC: Data Elements for Each Sample

FIELD	TYPE	DESCRIPTION	
SITE_ID	Character	NCCA site identification code or type of QC sample (e.g., LAB BLANK)	
SAMPLE_ID	Character	NCCA sample identification code	
VISIT_NO	Numeric	Sequential Visits to site (1 or 2)	
SAM_CODE	Character	REGULAR, DUP LCS, Blank, MS or CRM	
DATE_COL	MMDDYY	Date that the field crew collected the sample	
SAMPLE_TYPE	Character	Metals (except Mercury); Mercury; PCBs, Pesticides, PAHs; TOC; GRAIN SIZE	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory	
REPEAT	Numeric	Identifies a duplicate run of a sample: 1 or 2. Do not enter a number if no duplicate of sample is analyzed.	
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	
LAB_NAME	Character	Laboratory name (abbreviation)	
METHOD	Character	Laboratory method used	
ANALYST	Character	Last name or initials of person who performed the analysis	

FIELD	TYPE	DESCRIPTION
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
HOLDING_TIME	Y/N	Analysis performed within holding time
DATE_ANALYZED	MMDDYY	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 (LCS), matrix spike (MS), laboratory duplicate, method blank (BLANK), and CRM samples.
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)
RL	Numeric	Actual Reporting limit (based on the unique matrix of sediment for each batch of samples)
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
RESULT_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 codes as part of the case narrative
COMMENT	Character	Explain situation that created QC code, or any unusual aspects of the analysis

* In the event that MDLs are elevated because sample dilution is necessary to overcome the matrix effect, please notify EPA Laboratory Coordinator

Table 6-5 Sediment Contaminants, TOC and Grain Size indicators: Data Reporting Criteria

MEASUREMENT	UNITS	EXPRESSED TO THE NEAREST
Sediment		
Pesticides and PCBs	ng/g; ppb (sediment: dry wt)	0.01
Metals	ug/g; ppm (sediment: dry wt)	0.01
Hg	ug/g; ppm (sediment: dry wt)	0.001
PAHs	ng/g; ppb (dry wt)	0.01
TOC	%	0.01
Grain Size	%	0.01

6.7 Summary of Sediment Contaminant, Grain Size and TOC QA/QC Requirements

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, matrix spike duplicate 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 6-6** provides previsions and accuracy objectives and **Table 6-7** provides a summary of the quality control requirements.

Table 6-6 Sediment Contaminants, Grain Size and TOC: Precision and Accuracy Objectives

PARAMETER	PRECISION OBJECTIVE (MEASURED BY)	ACCURACY OBJECTIVE (MEASURED BY)
All Contaminants	30% (RPD between MS and MSD)	20% (average %Rs between MS and MSD)
TOC	10% (RPD between duplicates)	10% (CRM)
Grain Size	10% (LCS)	Not Applicable

* RPD=Relative Percent Difference; %Rs=%Recovery; MS=Matrix Spike; MSD=Matrix Spike Duplicate; CRM=Certified Reference Material; LCS=Lab Control Sample.

Table 6-7 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. They 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 control by analyzing three

ACTIVITY	EVALUATION	CORRECTIVE ACTION
		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 **Table 6-3**.	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 6-3**) 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 (not required for TOC and grain size).	Evaluate performance after the first 3 batches; and then every subsequent batch. Ideally, control limits for recovery will not exceed the target accuracy goal, but this may not be realistic	If both the original and duplicate 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 first 3 batches, within 2 working days, contact the EPA HQ NCCA

ACTIVITY	EVALUATION	CORRECTIVE ACTION
	for all parameters with this matrix.	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 the EPA HQ NCCA Laboratory Review Coordinator immediately if issues affect laboratory's ability to meet completeness objective.

* **Section 2.0** 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 EPA HQ NCCA 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 and TOC analyses and refrigerate those used for grain size. 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 may follow its own 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 March 12, 2020 from https://nepis.epa.gov/Exe/ZyPDF.cgi/P1008IY8.PDF?Dockey=P1008IY8.PDF.

Method 3051A “Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, And Oils” retrieved November 13, 2018 from https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf

Method 6020B “Inductively Coupled Plasma-Mass Spectrometry” retrieved February 29, 2024 from https://www.epa.gov/esam/sam-2022-chemical-determinative-method-summary?methodNumber=ChemicalMethod28.

Method 8270D “Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)” retrieved March 12, 2020 from https://19january2017snapshot.epa.gov/sites/production/files/2015-07/documents/epa-8270d.pdf

SW-846 Method 3540C “Soxhlet Extraction” retrieved November 8, 2018 from https://www.epa.gov/sites/production/files/2015-12/documents/3540c.pdf

7.0 WATER CHEMISTRY AND CHLOROPHYLL *A*

This section describes the analysis requirements for water quality samples.

Field Collection/Sample Summary: At each sampling site, the FOM instructs the crews to collect water samples. Samples for total nitrogen and total phosphorus analyses (CHEM) are collected in 250 mL amber HDPE bottles. Crews collect water in a 2 L amber HDPE bottle to filter for chlorophyll *a* [WCHL]; and dissolved ammonia, nitrites, nitrates, and phosphorus [NUTS]. Using water from the 2 L amber bottle. the crews filter water from for chlorophyll *a* sediment through a Whatman GF/F 47 mm 0.7 micron filter. Crews place the filter in a 50 mL tube, wrap the tube in a foil square and then place the tube in a zip-top plastic bag. The crews collect 250 mL of filtrate produced from processing the chlorophyll *a* sample and place this filtrate into a 250 mL clear HDPE bottle. This filtrate is used by laboratories to analyze for dissolved nutrients samples. The CHEM, WCHL and NUTS samples are shipped on wet ice directly to the analysis laboratory in coolers. If a state or other non-NCCA national laboratory is processing the samples, crews may ship or deliver the samples to their lab.

The purpose is to determine concentrations of water quality parameters and chlorophyll *a* in water quality samples collected in the NCCA 2025 and related studies. The laboratory shall perform analysis to determine levels of ammonia (NH_3), nitrite (NO_2) (optional), nitrate plus nitrite (NO_3 plus NO_2), total nitrogen (TN), total phosphorus (TP) and ortho-phosphate (PO_4) (also called soluble reactive phosphorus (SRP) or dissolved inorganic phosphorus (DIP)), pH, conductivity (required for Great Lakes, optional for estuaries) 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, and measure salinity in estuarine samples (optional). For Pacific Island territories (reef flats), silica (SIO_2) will also be measured.

7.1 Summary of the Procedure

This section describes the water chemistry and chlorophyll *a* procedures. As described in **Section 7.6**, unless contractually bound by other requirements, the laboratory may choose to use any method that meets EPA's specifications for water chemistry and chlorophyll *a* analyses.

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 potentially 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 (Laboratories and Equipment)

This section provides definitions, competency and quality assurance documents, and equipment and materials required for laboratories to complete the analyses in this section.

7.3.1 Definitions

The procedure uses the following terms:

Cl: Chloride

Method 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 NARS 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_2: Nitrite

NO_3: Nitrate

NO_3 Plus NO_2: Nitrate plus 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):

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

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.

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

SiO_2: Silica

SO_4: Sulfate.

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

SRP: Soluble Reactive Phosphorus (also called orthophosphate or dissolved inorganic phosphate)

TN: Total nitrogen

TP: Total phosphorus

7.3.2 General Requirements for Laboratories

Competency

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 QMPs, Laboratory Quality Assurance Manuals, QAPPs, and applicable Standard Operating Procedures (SOPs).

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

7.3.3 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
- One 250 mL clear bottle labeled 'NUTS' for dissolved nutrient analyses
- A filter in a 50 ml tube for chlorophyll *a* labeled 'WCHL'

The laboratory 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 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 **Section 2.0** 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 °C (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 0-6 °C. 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 stored at 0-6 ° C.
    b. Chlorophyll *a* filters are stored at ≤ -10 °C.
    c. Dissolved nutrient samples are stored at ≤ -10 °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

FIELD	TYPE	DESCRIPTION	
SITE_ID	Character	NCCA site identification code	
SAMPLE_ID	Character	NCCA sample number	
DATE_COL	MMDDYY	Date that the field crew collected the sample	
ANALYSIS_TYPE	Character	Water Chemistry or Chlorophyll *a* or Nutrients	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory (CHEM, WCHL 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:

1. Split the sample into two aliquots as shown in Figure 7.1.
2. Store in darkness at ≤ -10 °C

For the unfiltered, water chemistry (CHEM) sample:

1. Split the sample into two aliquots as shown in Figure 7.1.
2. Add ultra-pure acid (H_2SO_4,) to one aliquot of the unfiltered, CHEM sample and homogenize.
3. Store aliquot in darkness at 0-6 °C.

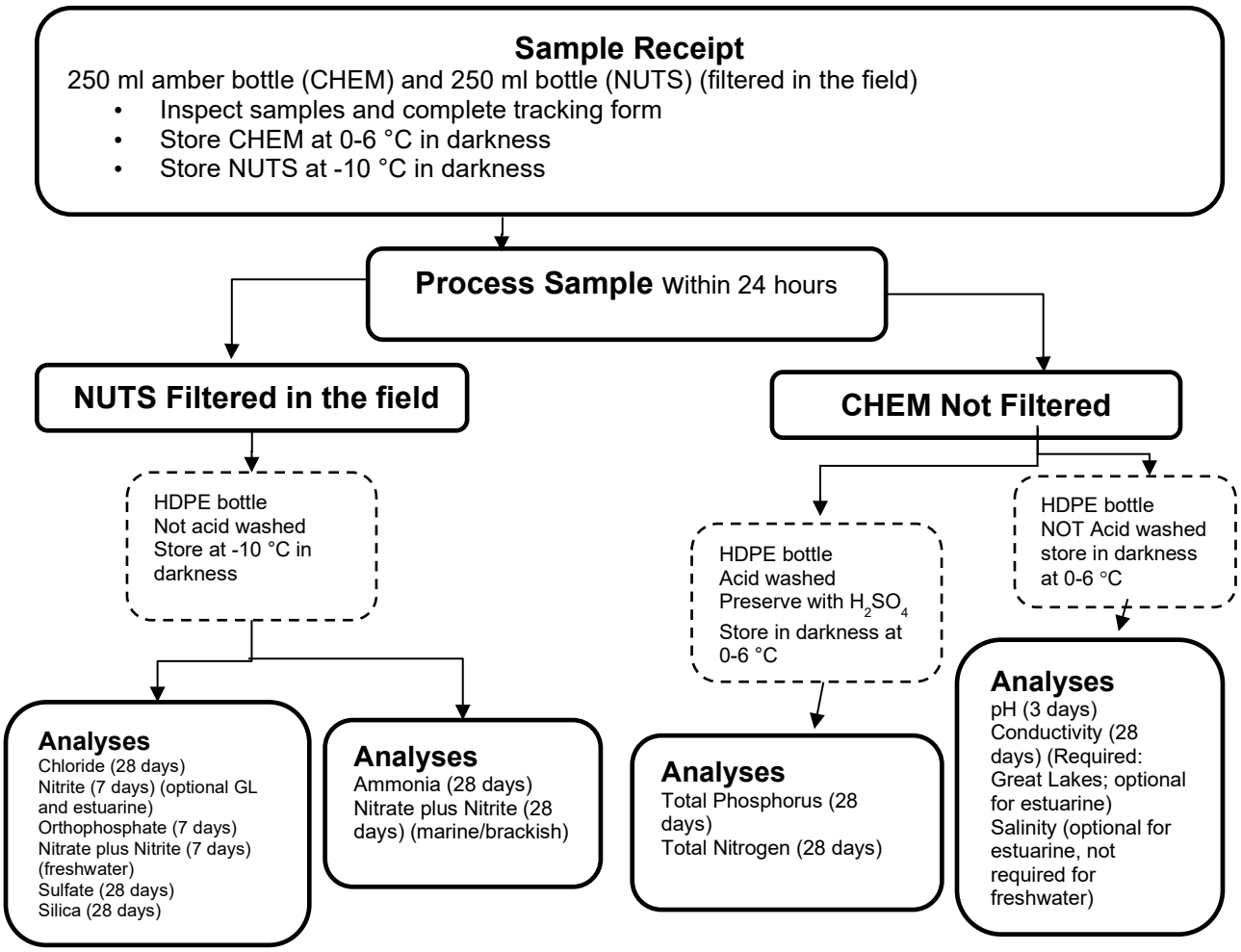

Figure 7.1 Water Chemistry and Dissolved Nutrient Samples: Receipt and Holding Times

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. Rinse vacuum filtration funnel units thoroughly with reagent water five times before each use and in between samples. Place a 0.4 µm polycarbonate filter in the funnel unit, filter ~100 mL reagent water through the filtration unit and discard the rinse water.
2. Filter sample directly into the NUTS bottle. If a new filter is needed, remove the sample bottle, and pre-rinse the new filter with reagent water before continuing.
3. Store filtered NUTS sample in darkness at ≤ -10 °C.

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 plus NO_3

7.6 Water Chemistry and Chlorophyll *a* Analysis: Requirements

The laboratory shall perform the following analyses on all samples:

- ammonia (NH_3)
- nitrate plus nitrite (NO_3 plus NO_2)
- nitrite (NO_2) (optional)
- total nitrogen (TN)
- ortho-phosphate (PO_4)
- total phosphorus (TP)
- chlorophyll *a*
- pH

The laboratory shall perform the following analyses on only the samples indicated:

- conductivity (Great Lakes- required)
- conductivity (estuaries- optional)
- salinity (estuarine samples only, optional)
- chloride (Cl) (Great Lakes samples only)
- sulfate (SO_4) (Great Lakes samples only)
- silica (SiO_2) (Pacific Island Reef flats samples only)

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 method detection 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 reporting limit value for each analyte to double the long-term method detection limit (LT-MDL), following guidance presented in Oblinger Childress et al. (USGS, 1999) and EPA document 821-R-16-006 (EPA, 2016).

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 C** identifies the information that the laboratory should provide to the EPA HQ NCCA Laboratory Review Coordinator to use in determining whether the laboratories meet the necessary requirements.

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	0.020	0.0200	± 0.020 or ±10%	± 0.020 or ±10%
Chloride (Cl) (Great Lakes only)	mg Cl/L	0 to 5,000	0.030	0.600	± 0.030 or ±10%	± 0.30 or ±10%
Conductivity Required for Great Lakes. Optional for estuaries	µS/cm at 25˚C	1-66,000	NA	20.0	±1.0 or ±10%	± 1.0 or ± 5%
Nitrate-Nitrite (NO_3-NO_2)	mg N/L	0 to 360 (as nitrite)	0.0020 marine 0.0100 freshwater	0.0200	± 0.0020 or ±10%	± 0.0020 or ±10%
Salinity Required for estuaries. Optional for Great Lakes.	ppt or psu	0-40				
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	0.010	0.100	± 0.010 or ±10%	± 0.010 or ±10%
Total Phosphorus (TP) and Ortho-Phosphate	mg P/L	0 to 22 (as TP)	0.0020	0.020	± 0.0020 Or ±10%	± 0.0020 Or ±10%
Nitrate (NO_3)*	mg N/L	0. to 360	0.0020 marine 0.03 freshwater	0.10	± 0.0020 or ±5%	± 0.0020 or ±5%
Nitrite (NO_2)	mg N/L		0.01 freshwater	0.10	± 0.01 or ±10%	± 0.01 or ±10%

PARAMETER	UNITS	POTENTIAL RANGE OF SAMPLES[1]	METHOD DETECTION LIMIT OBJECTIVE[2]	TRANSITION VALUE[3]	PRECISION OBJECTIVE[4]	ACCURACY OBJECTIVE[5]
Sulfate (SO_4)	mg/L	0 to 5000	0.090 freshwater	1.80	±0.090 or ±10%	±0.090 or ±10%
Chlorophyll *a*	mg/L in Extract	0.7 to 11,000	0.5	5	± .5 or ±10%	± .5 or ±10%
Silica (SiO_2)	mg SiO_2/L	0 to 20	0.025	0.05	± 0.025 or ± 10%	± 0.025 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 defined as the minimum concentration of an analyte that can be measured and reported with 99% confidence (based on a one-sided 99% confidence interval) that the analyte concentration is greater than zero.

[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.

* Analyte is preferred, but not required.

Table 7-4 Water Chemistry and Chlorophyll *a*: Analytical Methods Used by PESD Analytical Lab

ANALYTE	SUMMARY OF METHOD[21]	REFERENCES[22]	PESD AL SOP[23]
Nitrate+Nitrite, as N **Nitrate as N***	Ion Chromatography (freshwater samples) OR FIA automated colorimetric (cadmium reduction for brackish samples)	SM4500-NO3-N EEPA 353.2 Lachat 10-107-04-1-C	PESD-AL 36B.4 PESD-AL 40A.9
Ammonia, as N	FIA automated colorimetric (salicylate, dichloroisocyanurate)	Lachat 10-107-06-3-D Lachat 31-107-06-1-B	PESD-AL 30B.3 (Brackish and seawater) PESD-AL 30A.7 (Freshwater)
Total nitrogen (TN)	Persulfate Digestion; FIA Automated Colorimetric Analysis (Cadmium Reduction, sulfanilamide)	Digestion: Ameel (1993) Nitrate-N: EPA 353.2M Lachat 10-107-04-1-C & 4-B Orthophosphate-P: EPA 365.1MSM4500-P E Lachat 10-115-01-1-B & 4-B	PESD-AL 34B.1 (Brackish and seawater) PESD-AL 34A.7 (Freshwater)
Total phosphorus (TP)	Persulfate Digestion; FIA Automated Colorimetric Analysis (molybdate, ascorbic acid)	Digestion: Ameel (1993) Nitrate-N: EPA 353.2M Lachat 10-107-04-1-C & 4-B Orthophosphate-P: EPA 365.1MSM4500-P E Lachat 10-115-01-1-B & 4-B	PESD-AL 34B.2 (Brackish and seawater) PESD-AL 34A.8 (Freshwater)
Orthophosphate	FIA automated colorimetric (molybdate, ascorbic acid)	EPA 353.2M SM4500-P E Lachat 10-115-01-1-B	PESD_AL 36B.4
Chloride, Sulfate	Ion Chromatography (Great Lakes samples only)	SW 9056A; EPA 300.1 SM 4110B	PESD-AL 40A.9

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

[22] 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.

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

ANALYTE	SUMMARY OF METHOD[21]	REFERENCES[22]	PESD AL SOP[23]
Chlorophyll *a* (Chl-a)	Extraction 90% acetone analysis by fluorometry	EPA 445.0, EPA 446.0	PESD-AL 71A.8
Silica	Discrete analyzer (Reef Flat samples only)	EPA 370.1 SM4500-SiO2-D	PESD-AL 61A.2
pH (lab)	Automated, using ManSci PC-Titrate w/ Titra-Sip autotitrator and Ross combination pH electrode. Initial pH determination for ANC titration	EPA 150.1 SM4500-H	PESD-AL 16A.5
Specific conductance @ 25°C*	Electrolytic, Man-Tech TitraSip automated analysis OR manual analysis, electrolytic	EPA 120.1 SM2510	PESD-AL 16A.5

*Analyte is preferred, but not required.

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	NCCA site identification code or type of QC sample (e.g., LAB BLANK)	
SAMPLE	Character	NCCA sample number, LCS, QCCS, Blank, MS, or CRM	
ANALYSIS_TYPE	Character	Contaminant	
REPEAT	Numeric	Duplicate	
DATE_COL	MMDDYY	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	

VARIABLE	TYPE	DESCRIPTION
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 LCS, MS, laboratory duplicate, BLANK, and CRM samples.
HOLDING TIME	Y/N	Analysis performed within holding time
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 codes as part of the case narrative
COMMENT	Character	Explain situation that created QC code, or any unusual aspects of the analysis

7.8 Data Reporting Requirements

Table 7-6 presents the data reporting criteria for the water chemistry and chlorophyll *a* parameters.

Table 7-6 Water Chemistry Indicator: Data Reporting Criteria

MEASUREMENT	UNITS	NO. SIGNIFICANT FIGURES	MAXIMUM NO. DECIMAL PLACES
Total phosphorus (TP)	mg P/L	3	4
Ortho-phosphate	mg P/L	3	4
Total nitrogen	mg N/L	3	3
Nitrate-Nitrite	mg N/L	3	4
Ammonia	mg N/L	3	4
Silica	mg SiO_2/L	2	2
Chlorophyll *a*	µg/L	2	2
pH (laboratory)	pH units	3	2

Conductivity (Laboratory)	µS/cm at 25 °C	3	1

7.9 Summary of Water Chemistry and Chlorophyll *a* QA/QC Requirements

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 given a unique sample identification. **Table 7-7** provides a summary of the quality control requirements.

Table 7-7 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 C:** Laboratory Remote Evaluation Forms	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	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.**
Sample Storage	All	Check the temperature of the refrigerator per laboratory's standard	Record temperature of sample upon arrival at the laboratory. Check	Store refrigerated samples at 0-6° C and frozen	If at any time samples are warmer than required, note temperature and duration (either from the continuous temperature log or from

QC SAMPLE TYPE AND DESCRIPTION	INDICATORS	DESCRIPTION	FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
		operating procedures.	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.	samples at ≤ -10° C	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	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.
Laboratory/ Reagent Blank	All		Once per analytical batch prior to sample analysis	No analytes > RL	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.
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	No analytes > RL	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
			are used from that box.		
Determine LT-MDL Limit for Quality Control Check Sample (QCCS)	All analytes requiring MDL studies	Prepared so concentration is four to six times the LT-MDL objective	Once per analytical batch	Within accuracy objective	Confirm achieved LRL by repeated analysis of LT-MDL QCCS. Evaluate affected samples for possible re-analysis.
Initial Calibration Verification (ICV) and Continuous Calibration Verification (CCV)	All		Analyze ICV after calibration. Analyze CCV after every 10 samples and at end of analytical batch.	±10% or method criteria	Perform corrective action and re-analyze all associated samples since last successful CCV. Alternatively, recalibrate and re-analyze all samples since last successful CCV.
Analytical Duplicate Sample	All		One per 1-sample	Within precision objectives	If results are below LRL: Prepare and analyze duplicate from different sample (volume permitting). Review precision of QCCS measurements for batch. Check preparation of duplicate sample. Qualify all samples in batch for possible reanalysis.
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

QC SAMPLE TYPE AND DESCRIPTION	INDICATORS	DESCRIPTION	FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
					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 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 the EPA HQ NCCA Laboratory Review Coordinator immediately if issues affect laboratory's ability to meet completeness objective.
Deliver data using format required by EPA	All	Compare electronic data deliverable format to template	Data Reporting	All fields are correctly filled in, using text, numbers and characters according to the EPA data template	Correct data entry errors prior to sending data deliverable to EPA.

Sample and Record Retention

The laboratory shall retain:

1. The sample materials for a minimum of one year after collection. During this time, the laboratory shall store the materials at -6 ° C and in darkness. The lab shall retain the sample materials from the one-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 may follow its own internal protocols for disposal.

7.11 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 November 4, 2019 from https://nepis.epa.gov/Exe/ZyPDF.cgi/30003K0S.PDF?Dockey=30003K0S.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 https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=309421

USEPA. December 2016. EPA 821-R-16-006 "Definition and Procedure for the Determination of the Method Detection Limit, Revision 2", retrieved March 12, 2020 from https://www.epa.gov/sites/production/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.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 November 4, 2019 from http://pubs.usgs.gov/of/1999/0193/report.pdf.

8.0 SEDIMENT TOXICITY TESTING

This section describes the analysis requirements for sediment toxicity testing. The purpose is to assess the toxicity of sediment samples collected in the NCCA 2025 and related studies.

Field Collection/Sample Summary: At each sampling site, the FOM instructs the crews to collect surficial sediment samples. Estuary crews collect sediment for analysis in 0.6 gallon plastic buckets (to achieve 2 liters of sediment). Great Lake crews collect sediment for analysis in 1 quart plastic buckets (to achieve 1 liter of sediment). The samples are shipped from the field crews to the contract batching laboratory on wet ice (unless the sample is being processed by a state or other non-NCCA national laboratory). The contract batching laboratory will send batched samples on wet ice to the analysis laboratory in coolers. If a state or other non-NCCA national laboratory is processing the samples, crews may ship or deliver the samples to their lab following internal procedures.

8.1 Summary of the Procedure

This section describes toxicity testing of sediment samples collected for EPA's 2025 National Coastal Condition Assessment (NCCA).

8.2 Health and Safety Warnings

In addition to understanding the laboratory's hazard communication, safety and disposal requirements, persons using this procedure must abide by 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 potentially 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 (Laboratories and Equipment)

This section provides definitions, competency and quality assurance documents, and equipment and materials required for laboratories to complete the analyses in this section.

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.

CNTRL_CORR_SURV: Average percentage of organisms that survived in the replicate test chambers divided by the corresponding batch control average percent survival.

MEAN_NSURV: Average number of organisms that survived in the test chambers for each set of replicates.

MEAN_PSURV: Average percentage of organisms that survived in the test chambers for each set of replicates.

NUM_SURVIVAL: The number of organisms that survived in each replicate test chamber.

PER_SURVIVAL: The percentage of organisms that survived in each replicate test chamber.

8.3.2 General Requirements for Laboratories

Competency

To demonstrate its expertise, the laboratory shall provide EPA with performance data demonstrating their proficiencies in analyzing sediment toxicity 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 sediment-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 QMPs, QAPPs, and applicable Standard Operating Procedures (SOPs).

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

Preparation for the work
Prior to the start of any work, the laboratory shall provide documentation that it has complied with the following control analyses.

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. The laboratory shall supply results of grain size, percent moisture and percent TOC analyses for all control sediment sources at the beginning of the project and before any changes to a different control sediment source. To the extent possible control sediment source changes should be kept to a minimum. Please notify the NCCA Project Manager and Quality Assurance Coordinator if a change in control sediment source may be necessary.
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 estuarine 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 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 **Section 2.0** 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 °C (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_NAME	Character	Name or abbreviation for laboratory
TYPE	Character	Control or NCCA Sample
DATE_COL	MMDDYY	Date Sample was collected by the crew in the field (from sample label)
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_NO	Numeric	Sequential visits to site (1 (or blank) or 2); leave blank for control

FIELD	FORMAT	DESCRIPTION	
SAMPLE_ID	Numeric	Sample ID as used on field sheet (on sample label); leave blank for control	
DATE_COL	MMDDYY	Date sample was collected; leave blank for control	
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* (estuarine). 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. Estuarine: Test Method 100.4 in EPA 600/R-94/025[24] or ASTM E1367-03[25]
    b. Freshwater: Test Method 100.1 in EPA 600/R-99/064[26] or ASTM E1706[27]

[24] USEPA. June 1994. Chapter 11 in *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*. Retrieved October 21, 2024 from https://nepis.epa.gov/Exe/ZyPDF.cgi/300032A9.PDF?Dockey=300032A9.PDF

[25] ASTM. 2023. E1367-03 "Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates" West Conshohocken, PA. March 21, 2023, retrieved November 1, 2024 from https://www.astm.org/e1367-03r23.html

[26] USEPA. March 2000. Chapter 11 in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Second Edition. Retrieved November 5, 2024 from NEPIS.

[27] ASTM. 2020. E1706. "Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates." Retrieved November 5, 2024 from https://www.astm.org/e1706-20.html

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

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

4. Use the following organisms for the tests:
    a. Estuarine: *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. The laboratory shall provide information on how the laboratory maintains the quality of the water used for the tests.
    a. For estuarine sediments, 175 mL of sediment and 800 mL of overlying seawater
    b. For freshwater sediments, 100mL of sediment and 175mL of overlying freshwater

7. 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. The laboratory shall supply results of grain size, percent moisture and percent TOC analyses for all control sediment sources at the beginning of the project and before any changes to a different control sediment source. All tests of the control sediment sources are to be done by the lab and results are to be submitted to EPA. To the extent possible control sediment source changes should be kept to a minimum. Please notify the NCCA Project Manager and Quality Assurance Coordinator if a change in control sediment source may be necessary.

8. Implement the following for exposure/feeding:
    a. For estuarine sediments, exposure is static (i.e., water is not renewed), and the animals are not fed over the 10-day 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. Use 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 estuarine 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. Large indigenous organisms and large debris can be removed using forceps.

Additional details are provided in the summary **Table 8-2** and **Table 8-3**.

Table 8-2 Sediment Toxicity: Test Conditions for Conducting 10-d Tests for Estuarine Sediments

PARAMETER	CONDITIONS
1. Test type	Whole sediment toxicity test, static
2. Temperature	25 °C 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 measurements	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 Sediment Toxicity: Test Conditions for Conducting 10-d 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

PARAMETER	CONDITIONS
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)
Overlying water quality measurements	Estuarine Toxicity Tests: Temperature daily; pH, ammonia, conductivity, and DO at test start and end. Freshwater Toxicity Tests: Temperature and dissolved oxygen daily. Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test.
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

Table 8-4 and **Table 8-5** identify the method performance requirements and data elements describing the test conditions and outcomes for each replicate and batch, respectively. Laboratories shall provide the data elements to EPA, preferably in EPA's data template, available separately from EPA. The laboratory shall digitize bench sheets and provide them to the EPA TOCOR. **Table 8-6** identifies the data reporting criteria.

Table 8-4 Sediment Toxicity Replicates: Laboratory Method Performance Data Deliverable Requirements

FIELD	FORMAT	DESCRIPTION
LAB_NAME	Character	Name or abbreviation for laboratory
SITE_ID	Character	Unique SITE_ID reported on sample label
TYPE	Character	Control or NCCA Sample
SAMPLE_ID	Numeric	Sample id as used on field sheet (on sample label); leave blank for control
VISIT_NUMBER	Numeric	Number of visit (sequential) to site ("1" or "2" are the only legal values)
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

FIELD	FORMAT	DESCRIPTION
ORGANISM	Character	Leptocheirus plumulosus (marine) or Hyalella azteca (freshwater)
NUM_SURVIVAL	Numeric	Number of organisms that survived out of 20 (marine) and 10 (freshwater)
PER_SURVIVAL	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
TEMP	Numeric	Daily replicate temperature
pH	Numeric	For each replicate on day 0 and day 10
NH_3	Numeric	For each replicate on day 0 and day 10
Salinity	Numeric	For each estuary replicate on day 0 and day 10
Conductivity	Numeric	For each Great Lakes replicate on day 0 and day 10
DO	Numeric	For each replicate on day 0 and day 10

Table 8-5 Sediment Toxicity Batch Summaries: Laboratory Method Performance Data Deliverable Requirements

FIELD	FORMAT	DESCRIPTION
LAB_NAME	Character	Name or abbreviation for laboratory
SITE_ID	Character	Unique SITE_ID reported on sample label
TYPE	Character	Control or NCCA Sample
SAMPLE_ID	Numeric	Sample id as used on field sheet (on sample label); leave blank for control
VISIT_NUMBER	Numeric	Number of sequential visit to site (“1” or “2” are the only legal values)
DATE_COL	MMDDYY	Date sample was collected by crew (from sample label)
DATE_RECEIVED	MMDDYY	Date sample was received in lab
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival in lab (in degrees C)
CONDITION_CODE	Character	Code for condition of sample upon arrival at lab (See **Table 8-1** for allowable codes)
CONDITION_COMMENT	Character	Comment explaining condition code (required for code “Q”. Optional for others)
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	Estuarine or Freshwater
ORGANISM	Character	Leptocheirus plumulosus (marine) or Hyalella azteca (freshwater)
CONTROL	Character	Source of control sediment (artificial or name of collected reference 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
MEAN_NSURV	Numeric	Mean number survival for all replicates of the sample (or control) calculated using the NUM_SURVIVAL
MEAN_PSURV	Numeric	Mean percent survival for all replicates of the sample (or control) calculated using the PER_SURVIVAL
CNTRL_CORR_SURV	Numeric	Optional Field: Average percentage of organisms that survived in the replicate test chambers divided by the corresponding average control percent survival

FIELD	FORMAT	DESCRIPTION
BATCH_PASS	P/F	Indicate if the batch passed (P) or failed (F) the QA/QC requirements (e.g., mean control survival 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

Table 8-6 Sediment Toxicity: Data Reporting Criteria

Measurement	Units	Expressed to the Nearest
Sediment toxicity	%	Survival integer

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 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 8-7** provides a summary of the quality control requirements.

Table 8-7 Quality Control Activities for Sediment Toxicity Samples

ACTIVITY	EVALUATION	CORRECTIVE ACTION
Laboratory demonstrates competency for conducting sediment toxicity analyses in Section 8.5	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 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**.

ACTIVITY	EVALUATION	CORRECTIVE ACTION
Sample storage	All samples: 4°C upon 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. The laboratory shall describe 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 should be shipped in well oxygenated water in insulated containers to maintain temperature during shipment); and upon arrival at the laboratory.	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 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	Data template includes a field to record if a test passed or failed the control requirements. If a test fails,

ACTIVITY	EVALUATION	CORRECTIVE ACTION
	Leptocheirus treatments must remain ≥80% and ≥90%, respectively.	retest all samples in the batch. EPA prefers that results from failing test be deprecated from the spreadsheet and completely detailed in that narrative progress reports, while passing retest results be included in the spreadsheet. If this is not possible, the lab shall clearly differentiate between the original (failing) test results and the retest results within the spreadsheet. 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.

***Section 2.0** 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 EPA HQ NCCA Laboratory Review Coordinator.

8.8 Sample and Record Retention

The laboratory shall retain:

1. The lab shall retain the samples until the NCCA 2025 report and data are published or the lab is notified in writing by the EPA that samples may be disposed of sooner. 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 may follow its own internal protocols for disposal.

8.9 References

American Society for Testing and Materials (ASTM). 2023. E1367-03 "Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates" West Conshohocken, PA. March 21, 2023, retrieved November 1, 2024 from https://www.astm.org/e1367-03r23.html

ASTM. 2020. E1706. "Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates." West Conshohocken, PA. Retrieved November 5, 2024 from https://www.astm.org/e1706-20.html

USEPA. June 1994. Chapter 11 in *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*. Retrieved October 21, 2024 from https://nepis.epa.gov/Exe/ZyPDF.cgi/300032A9.PDF?Dockey=300032A9.PDF

USEPA. March 2000. Chapter 11 in *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Second Edition. Retrieved November 5, 2024 from NEPIS.

9.0 Human Health Fish Tissue Indicator

Procedures for preparing and analyzing fillet tissue samples are specified in QAPPs and EPA Methods documents listed below:

- U.S. Environmental Protection Agency. 2025. Quality Assurance Project Plan for National Coastal Condition Assessment (NCCA) 2025 Human Health Fish Tissue Sample Preparation. Office of Water. Washington, DC, June 2025.
- U.S. Environmental Protection Agency. 2025. Quality Assurance Project Plan for Analysis of the 2025 National Coastal Condition Assessment Fish Fillet Samples for Mercury, Per- and Polyfluoroalkyl Substances, and Polychlorinated Biphenyl Congeners. Office of Water. Washington, DC, June 2025.
- EPA Method 1631E, Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, EPA 821-R-02-019, August 2002.
- EPA Method 1633A, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. EPA-820-R-24-007. December 2024.
- EPA Method 1668C, Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS), EPA 820-R-10-005, April 2010.

10.0 FECAL INDICATOR: ENTEROCOCCI

All lab work for this supplemental indicator is expected to be done by EPA Office of Research and Development labs. Manuals/QAPP maintained by EPA ORD are listed below.

- EPA Method 1609.1: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) with Internal Amplification Control (IAC) Assay. EPA-820-R-15-099. April 15, 2015
- SOP ID: J-WECD-BMB-SOP-5042-0. Estimation of the Enterococci and Escherichia coli Fecal Indicator Bacteria Densities in Water Sample Filter Retentates from National Aquatic Resource Surveys by Quantitative Polymerase Chain Reaction: 2022 National Lakes Assessment. Effective Date 01/10/2023
- QAPP J-WECD-0033862-QP-1-1. Quantification of Fecal Indicator Bacteria in 2023-2024 National Rivers and Streams Assessment (NRSA) Survey Samples. Updated QAPP for project J-WECD-0033862: Estimation of the Enterococci and Escherichia coli Fecal Indicator Bacteria Densities in Water Sample Filter Retentates from National Aquatic Resource Surveys by Quantitative Polymerase Chain Reaction. Updated 01/19/2024.

APPENDIX A: TARGET FISH SPECIES FOR WHOLE FISH ANALYSES

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

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

Table A.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**
Ariidae	*Ariopsis felis*	Hardhead sea catfish
	Bagre marinus	Gafftopsail sea catfish
Paralichthyidae	*Paralichthys albigutta*	Gulf flounder
	Paralichthys dentatus	Summer flounder
	Paralichthys lethostigma	Southern flounder
Sciaenidae	*Cynoscion arenarius*	Sand weakfish (or seatrout)
	Cynoscion nebulosus	Speckled trout
	Cynoscion regalis	Gray weakfish
	Leiostomus xanthurus	Spot croaker
Sparidae	*Lagodon rhomboides*	Pinfish
SOUTHEAST REGION SECONDARY ECOFISH TARGET SPECIES		
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**

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 A.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**
Ariidae	*Ariopsis felis*	Hardhead sea catfish
	Bagre marinus	Gafftopsail sea catfish
Paralichthyidae	*Paralichthys albigutta*	Gulf flounder
	Paralichthys dentatus	Summer flounder
	Paralichthys lethostigma	Southern flounder
Sciaenidae	*Cynoscion arenarius*	Sand weakfish (or seatrout)
	Cynoscion nebulosus	Speckled trout
	Cynoscion regalis	Gray weakfish
	Leiostomus xanthurus	Spot croaker
	Micropogonias undulatus	Atlantic croaker
	Sciaenops ocellatus	Red drum
Sparidae	*Lagodon rhomboides*	Pinfish
GULF REGION SECONDARY ECOFISH TARGET SPECIES		
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**
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 A.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**
Atherinopsidae	*Atherinops affinis*	Topsmelt silverside
Cottidae	*Leptocottus armatus*	Pacific staghorn sculpin
	Oligocottus rimensis	Saddleback sculpin
Cynoglossidae	*Symphurus atricaudus*	California tonguefish
Embiotocidae	*Cymatogaster aggregata*	Shiner perch

	Embiotoca lateralis	Striped seaperch
Gasterosteidae	*Gasterosteus aculeatus*	Three-spined stickleback
Paralichthyidae	*Paralichthys californicus*	California flounder
	Citharichthys sordidus	Pacific sanddab
	Citharichthys stigmaeus	Speckled sanddab
Pleuronectidae	*Isopsetta isolepis*	Butter sole
	Parophrys vetulus	English sole
	Psettichthys melanostictus	Pacific sand sole
	Platichthys stellatus	Starry flounder
Sciaenidae	*Genyonemus lineatus*	White croaker
Serranidae	*Paralabrax nebulifer*	Barred sand bass
	Paralabrax maculatofasciatus	Spotted sand bass
WESTERN REGION SECONDARY ECOFISH TARGET SPECIES		
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**
Echinodermata/ Toxopneustidae	*Tripneustes gratilla* (Hawaii ONLY)	Collector urchin
Batrachoididae	*Porichthys notatus*	Plainfin midshipman
	Porichthys myriaster	Specklefin midshipman
Embiotocidae	*Amphistichus argenteus*	Barred surfperch
Paralichthyidae	*Xystreurys liolepis*	Fantail sole
Pleuronectidae	*Pleuronichthys guttulatus*	Diamond turbot
	Microstomus pacificus	Dover sole
	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 A.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**
Catostomidae	*Moxostoma macrolepidotum*	Shorthead redhorse
Centrarchidae	*Ambloplites rupestris*	Rock bass
	Lepomis gibbosus	Pumpkinseed
	Lepomis macrochirus	Bluegill
	Micropterus dolomieu	Smallmouth bass
	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
	Pimephales notatus	Bluntnose minnow
Esocidae	*Esox lucius*	Northern pike
	Esox masquinongy	Muskellunge
Gasterosteidae	*Gasterosteus aculeatus*	Three-spined stickleback
Gobiidae	*Neogobius melanostomus*	Round goby
	Proterorhinus marmoratus	Tubenose goby
Ictaluridae	*Ameiurus nebulosus*	Brown bullhead
	Ictalurus punctatus	Channel catfish
	Noturus flavus	Stonecat
Gadidae	*Lota lota*	Burbot

Moronidae	*Morone americana*	White perch
	Morone chrysops	White bass
Osmeridae	*Osmerus mordax*	American/ rainbow smelt
Percidae	*Gymnocephalus cernuus*	Ruffe
	Perca flavescens	Yellow perch
	Percina caprodes	Logperch
	Sander canadensis	Sauger
	Sander vitreus	Walleye
Percopsidae	*Percopsis omiscomaycus*	Trout-perch
Salmonidae	*Coregonus artedi*	Cisco/ lake herring
	Coregonus clupeaformis	Lake whitefish
	Oncorhynchus gorbuscha	Pink salmon
	Oncorhynchus kisutch	Coho salmon
	Oncorhynchus mykiss	Rainbow trout
	Oncorhynchus tshawytscha	Chinook salmon
	Salvelinus namaycush	Lake trout
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum
GREAT LAKES SECONDARY ECOFISH TARGET SPECIES		
FAMILY	**SCIENTIFIC NAME**	**COMMON NAME**
Catostomidae	*Catostomus catostomus*	Longnose sucker
	Catostomus commersonii	White sucker
	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
	Salvelinus fontinalis	Brook trout
	Salvelinus fontinalis x	Splake

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

APPENDIX B: RESEARCH INDICATOR - TOTAL ALKALINITY

All lab work for this supplemental indicator is expected to be done by EPA Office of Research and Development labs. Manuals/QAPP maintained by EPA ORD.

- Project QAPP ID: L-PESD-0032676-QP-1-3. Nutrient Enhanced Coastal Acidification Indicator for National Coastal Condition Assessment Survey Quality Assurance Project Plan (QAPP). Updated March 25, 2025.
- SOP ID: J-ACESD-MAB-SOP-3809-1

APPENDIX C: 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 2025 DOCUMENT REQUEST FORM

In 2025, EPA and its state and Tribal partners will conduct the next 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 NCCA 2025, 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 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 NCCA 2025.

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 and submit the following checklist and appropriate signature form, along with accompanying documentation, for the specific indicator(s) for which your laboratory will be conducting analysis for the NCCA 2025.

Which samples are you analyzing?

- ☐ Water chemistry analytes identified in the LOM and QAPP including chlorophyll *a*
- ☐ Microcystins
- ☐ Whole fish tissue contaminant analyses
- ☐ Sediment contaminants, total organic carbon and grain size
- ☐ Sediment toxicity
- ☐ Benthic macroinvertebrate taxonomy

If your lab has been previously approved for the work under NARS for the specific indicator(s) listed above within the last 5 years:

- ☐ A *signature* on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for labs conducting analyses for the 2025 NCCA.
- ☐ A signature on the Quality Assurance Project Plan (QAPP) indicates that you will follow both the QAPP and the LOM.

For previously approved laboratories, please submit the following to the EPA HQ NCCA Laboratory Review Coordinator if available:

- ☐ 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 the specific indicator(s) listed above** for the same parameters/methods.

If you have not been approved within the last 5 years through the laboratory verification process for the indicator(s) or you do not have the documentation listed above, we request that you submit the following documents (if available) for review so EPA can determine your ability to participate as a laboratory in the NCCA (if none of these are available, please contact Kendra Forde, the EPA HQ NCCA Laboratory Review Coordinator to discuss alternative documentation).

- ☐ 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 NCCA 2025 LOM).
- ☐ Documentation attesting to experience running all analytes for the NCCA 2025 indicators identified above (e.g., water chemistry for NCCA includes a number of parameters, including chlorophyll *a)*.
- ☐ Documentation of NABS (or other) *certification* for the *taxonomists* performing analyses (if applicable).

Laboratory Signature Form – Chemistry and Related Analyses Laboratories

I _____certify that the laboratory_____located in
will abide by the following standards in performing the following data analysis and reporting for the National Coastal Condition Assessment (NCCA) 2025.
This applies to the _____ chemistry indicator(s).

1.) Use procedures identified in the NCCA 2025 Laboratory Operations Manual (LOM) (or equivalent). If using equivalent procedures, please provide the procedures and obtain approval from EPA.
2.) Read and abide by the NCCA Quality Assurance Project Plan 2025 and related Standard Operating Procedures.
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 SharePoint (contact the EPA HQ NCCA Laboratory Review Coordinator for a copy or for questions).
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 March 1, 2026 or as otherwise negotiated with EPA.
7.) 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 _____

Laboratory Signature Form – Biology Laboratories

I _____certify that the laboratory_____ located in _____, will abide by the following standards in performing biological data analysis and reporting for the National Coastal Condition Assessment (NCCA) 2025.
This applies to the _____ biological indicator(s).

1). Use procedures identified in the NCCA Lab Operations Manual (LOM) 2025 (or equivalent). If using equivalent procedures, please provide the procedures and obtain approval from EPA.
2). Read and abide by the NCCA Quality Assurance Project Plan (QAPP) 2025 and related Standard Operating Procedures.
3). Have an organized IT tracking system in place for recording sample tracking and analysis data.
4). Use taxonomic standards outlined in the NCCA 2025 LOM.
5). Participate in taxonomic reconciliation exercises during the field and data analysis season, which include conference calls and other laboratory reviews.
6). Provide Quality Control (QC) data for internal QC checks, including for sorting, on a monthly basis.
7). Provide data using the template provided on the NARS SharePoint.
8). 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 March 1, 2026 or as otherwise negotiated with EPA.
9). 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.
11). Agree to utilize taxonomic nomenclature and hierarchical established for NCCA 2025.

Signature _____ Date _____