Quality Assurance Project Plan for

Fish Sample Preparation and Analysis of Mercury, Perfluorinated Compounds (PFCs), Polybrominated Diphenyl Ethers (PBDEs), Polychlorinated Biphenyls (PCBs), and Fatty Acids in Fish Tissue from the Great Lakes Human Health Fish Tissue Study

Revision 2

April 18, 2012

Prepared for:

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Standards and Health Protection Division

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Revision History

June 21, 2011 - Original QAPP signed

October 20, 2011 - Revision 1 prepared

- This revision added summary level information on PBDE analysis to Sections A4, A6, A7, B1, and B3 and details on the analysis of PBDEs in tissue samples by a commercial laboratory to Sections B4, including the decision that 20-gram aliquots will be analyzed for PBDEs.
- It added the associated QC discussion and acceptance limits for PBDEs as a new Section B5.4, including an update to the 2-tiered PBDE method blank acceptance criteria that lowered the threshold for the second tier from 5 times the minimum level (ML) to 2 times the ML.
- It deleted all references to future PBDE analyses by NERL-Cincinnati.
- It corrected the list of omega-3 fatty acid target analytes in Table 2 to reflect the final list for which the laboratory can obtain authentic standards.
- It added the list of PBDE target analytes as Table 3, which resulted in renumbering all subsequent tables in the document.
- It added new references associated with the PBDE analyses.
- It revised Table 1 in Appendix B to reflect changes to the target mass to be collected for each sample aliquot and added instructions to archive all remaining tissue mass available after production of the second archive jar.
- The revision number and the date in the header and on the title page were changed to reflect the fact that the QAPP has been revised.
- The title was updated to include addition of PBDE analysis to the GLHHFTS.
- NERL-Cincinnati staff were removed from the Distribution List.
- Appendix C was added to provide method detection limits (MDLs) and minimum level (ML) summaries for all GLHHFTS target analytes.
- References were added to Sections A4 and A6 to indicate the need for a second revision to the QAPP to add PCB analysis to the GLHHFTS.

April 18, 2012 - Revision 2 prepared

- This revision added summary level information on PCB analysis to Sections A4, A6, A7, B1, and B3 and details on the analysis of PCBs in tissue samples by a commercial laboratory to Section B4.
- It added the associated QC discussion and acceptance limits for PCBs as a new Section B5.5.
- It added the list of PCB target analytes to Appendix C (avoiding inserting a very long table in the middle of the document).
- It added new references associated with the PCB analyses.
- The revision number and the date in the header and on the title page were changed to reflect the fact that the QAPP has been revised.
- The title was updated to include addition of PCB analysis to the GLHHFTS.
- Appendix C was revised to include PCB method detection limits (MDLs) and minimum levels (MLs).

Fish Sample Preparation and Analysis of Mercury, Perfluorinated Compounds (PFCs), Polybrominated Diphenyl Ethers (PBDEs), Polychlorinated Biphenyls (PCBs), and Fatty Acids in Fish Tissue from the Great Lakes Human Health Fish Tissue Study

A. PROJECT MANAGEMENT

This Quality Assurance Project Plan (QAPP) presents performance criteria, acceptance criteria, and objectives for the analysis of mercury, perfluorinated compounds (PFCs), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and omega-3 fatty acids in fish composites collected for the Great Lakes Human Health Fish Tissue Study under the National Coastal Condition Assessment (NCCA). This QAPP also describes the methods and procedures that will be followed during the Great Lakes Human Health Fish Tissue Study (GLHHFTS) to ensure that the criteria and objectives are met. This document addresses mercury, PFCs, PBDEs, PCBs, and omega-3 fatty acid analytical activities only.

This QAPP was prepared in accordance with the most recent version of EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* (USEPA 2001), that was reissued in 2006. In accordance with EPA QA/R-5, this QAPP is a dynamic document that is subject to change as analytical activities progress. Changes to procedures in this QAPP must be reviewed by the EPA Project Manager and the EPA Standards and Health Protection Division (SHPD) Quality Assurance Coordinator for the GLHHFTS to determine whether the changes will impact the technical and quality objectives of the project. If so, the QAPP will be revised accordingly, circulated for approval, and forwarded to all project participants listed in the QAPP distribution list (Section A3). Key project personnel and their roles and responsibilities are discussed in the QAPP section to follow (Section A4), and project background perspective and description is provided in Sections A5 and A6, respectively.

A1. Approvals

Leanne Stahl — 04/18/12
Leanne Stahl, OST Project Manager, EPA — Date

Denise F. Hawkins — April 18, 2012
Denise Hawkins, Chief, FSBOB, EPA — Date

4/18/12
Robert Shippen, SHPD QA Coordinator, EPA — Date

4/18/12
Marion Kelly, OST QA Officer, EPA — Date

APRIL 18, 2012
Blaine Snyder, Tetra Tech Project Leader — Date

Susan Lanberg — 4/18/12
Susan Lanberg, Tetra Tech QA Officer — Date

4/18/12
Harry McCarty, CSC Project Leader — Date

Marguerite E. Jones — 4/19/2012
Marguerite Jones, CSC QA Officer — Date

Nancy — 4/23/12
Project Officer, EP-C-10-060

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List of Acronyms and Abbreviations

AOAC	Association of Official Analytical Chemists
CAS	Chemical Abstract Service
CEC	Contaminant of emerging concern
DDT	Dichlorodiphenyltrichloroethane
ECO	Ecological
EPA	Environmental Protection Agency
FSBOB	Fish, Shellfish, Beach, and Outreach Branch
GC/FID	Gas chromatography/flame ionization detector
GLHHFTS	Great Lakes Human Health Fish Tissue Study
GLNPO	Great Lakes National Program Office
HDPE	High density polyethylene
HPLC–MS/MS	High performance liquid chromatography–tandem mass spectrometry
ID	Identification
LCS	Laboratory control sample
MDL	Method detection limit
MS/MSD	Matrix spike/matrix spike duplicate
NCCA	National Coastal Condition Assessment
NERL	National Exposure Research Laboratory
NHEERL	National Health and Environmental Effects Research Laboratory
ORD	Office of Research and Development
OST	Office of Science and Technology
OW	Office of Water
OWOW	Office of Wetlands, Oceans, and Watersheds
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PFC	Perfluorinated compound
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PTFE	Polytetrafluoroethylene
QA	Quality assurance
QAPP	Quality Assurance Project Plan
QC	Quality control
QSA	Quality system audit

RF	Response factor
RPD	Relative percent difference
SHPD	Standards and Health Protection Division
SOP	Standard operating procedure
SOW	Statement of work
SPE	Solid-phase extraction
SWRI	Southwest Research Institute
USEPA	U.S. Environmental Protection Agency
VCSB	Voluntary consensus standard body
WED	Western Ecology Division

A3. Distribution List

Michael Arbaugh
Microbac Laboratories
2101 Van Deman Street
Baltimore, MD 21224
(Contact Harry McCarty at CSC)

Louis Blume
USEPA/GLNPO (G-17J)
77 West Jackson Boulevard
Chicago, IL 60604
312/353-2317
blume.louis@epa.gov

Gregory Colianni
USEPA/OWOW (4503T)
1200 Pennsylvania Ave., N.W.
Washington, DC 20460
202/566-1249
colianni.gregory@epa.gov

Jacqueline Fisher
USEPA/GLNPO (G-17J)
77 West Jackson Boulevard
Chicago, IL 60604
312/353-1481
fisher.jacqueline@epa.gov

Michael Flournoy
TestAmerica Laboratories
880 Riverside Parkway
West Sacramento, CA 95605
(Contact Harry McCarty at CSC)

Denise Hawkins
USEPA/OST (4305T)
1200 Pennsylvania Ave., N.W.
Washington, DC 20460
202/566-1384
hawkins.denise@epa.gov

Marguerite Jones
CSC
6101 Stevenson Avenue
Alexandria, VA 22304
703/461-2247
mjones214@csc.com

Marion Kelly
USEPA/OST (4303T)
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
202/566-1045
kelly.marion@epa.gov

Susan Lanberg
Tetra Tech, Inc.
10306 Easton Place, Suite 340
Fairfax, VA 22030
703/385-6000
susan.lanberg@tetratech.com

Sarah Lehmann
USEPA/OWOW (4503T)
1200 Pennsylvania Avenue, N. W.
Washington, DC 20460
202/566-1379
lehmann.sarah@epa.gov

Harry McCarty
CSC
6101 Stevenson Avenue
Alexandria, VA 22304
703/461-2392
hmccarty@csc.com

Rod McLeod
ALS Canada Ltd.
5420 Mainway Drive
Burlington ON, Canada L7L 6A4
(Contact Harry McCarty at CSC)

Elizabeth Murphy
USEPA/GLNPO (G-17J)
77 West Jackson Boulevard
Chicago, IL 60604
312/353-4227
murphy.elizabeth@epa.gov

Mari Nord
USEPA Region 5 (WQ-16J)
77 West Jackson Boulevard
Chicago, IL 60604
312/886-3017
nord.mari@epa.gov

John O'Donnell
Tetra Tech, Inc.
10306 Easton Place, Suite 340
Fairfax, VA 22030
703/385-6000
john.odonnell@tetratech.com

Tony Olsen
USEPA/ORD/NHEERL/WED
200 S.W. 35th Street
Corvallis, OR 97333
541/754-4790
olsen.tony@epa.gov

Lorraine Scheller
Southwest Research Institute
6220 Culebra Road
San Antonio, TX 78238
(Contact Harry McCarty at CSC)

Robert Shippen
USEPA/OST (4305T)
1200 Pennsylvania Ave., N.W.
Washington, DC 20460
202/566-0391
shippen.robert@epa.gov

Edwin Smith
USEPA/GLNPO (G-17J)
77 West Jackson Boulevard
Chicago, IL 60604
312/353-6571
smith.edwin@epa.gov

Blaine Snyder
Tetra Tech, Inc.
400 Red Brook Blvd., Suite 200
Owings Mills, MD 21117
410/356-8993
blaine.snyder@tetratech.com

Leanne Stahl
USEPA/OST (4305T)
1200 Pennsylvania Ave., N.W.
Washington, DC 20460
202/566-0404
stahl.leanne@epa.gov

Cynthia Tomey
AXYS Analytical Services, Ltd.
2045 Mills Road W.
Sidney, BC, Canada V8L 5X2
(Contact Harry McCarty at CSC)

Lynn Walters
CSC
6101 Stevenson Avenue
Alexandria, VA 22304
703/461-2060
lwalters3@csc.com

John Wathen
USEPA/OST (4305T)
1200 Pennsylvania Ave., N.W.
Washington, DC 20460
202/566-0367
wathen.john@epa.gov

Tom Yoder
TestAmerica Laboratories
5815 Middlebrook Pike
Knoxville, TN 37921
(Contact Harry McCarty at CSC)

A4. Project/Task Organization

EPA's National Coastal Condition Assessment (NCCA) is a probability-based survey designed to assess the condition of coastal waters of the United States. It includes collection and analysis of physical, chemical, and biological indicator data that will allow a statistically-valid characterization of the condition of the Nation's coastal waters. EPA used an unequal probability design to select 682 marine sites along the coasts of the contiguous United States and 225 freshwater sites from nearshore areas throughout the Great Lakes. The Office of Wetlands, Oceans, and Watersheds (OWOW) within the Office of Water (OW) is responsible for the overall planning and implementation of the NCCA.

One national and three regional fish contamination studies are being conducted under the NCCA. The national assessment is using fish collected from all sampling sites in outer coastal waters as indicators of ecological (ECO) contamination, based on whole body contaminant concentrations. The ECO fish samples will be analyzed for 12 metals (including mercury), 21 polychlorinated biphenyl (PCB) congeners, and 14 pesticides (including DDT and its metabolites). Results from these analyses of whole body tissue will be used in conjunction with data from other indicators (e.g., water chemistry) to determine the ecological integrity of all U.S. coastal resources. The three other fish tissue surveys are regional studies of the Great Lakes, which involve two ecological assessments and one assessment of fish contamination relevant to human health. The first ecological assessment is the Great Lakes Embayment Enhancement study. It includes 150 randomly selected sites in embayments across all five Great Lakes to improve the ability to assess the lake-wide condition of bays and harbors, and it focuses on whole-body analyses of fish for evaluation of ecological condition.

The second ecological study and the human health study involve collection of fish from Great Lakes nearshore sites (depths up to 30 m or distances up to 5 km from shore). Fish for the second ECO fish study were collected from the full complement of 225 randomly selected nearshore sites (45 sites per lake) and will be analyzed for the same group of chemicals identified above for the national assessment. The Great Lakes Human Health Fish Tissue Study (GLHHFTS) sample collection effort targeted game fish from a statistically representative subset of about 150 nearshore sites (about 30 sites per lake). Field crews collected fish composite samples for the GLHHFTS during a June through November 2010 sampling season. Routine composite samples for this study consist of five similarly-sized adult fish of a single species commonly consumed by humans. All of the samples collected for the GLHHFTS were shipped as whole fish to a central storage facility at Microbac Laboratories in Baltimore, Maryland, and staff at this laboratory will be preparing the fish samples for analysis (i.e., filleting the fish samples and homogenizing the fillet tissue). The fillet tissue from these fish samples will be analyzed for mercury, perfluorinated compounds (PFCs), omega-3 fatty acids (hereafter simply referred to as fatty acids), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs). Other contaminants are under consideration for future analysis (e.g., pharmaceutical compounds).

EPA's Office of Science and Technology (OST) within OW is collaborating with the Great Lakes National Program Office (GLNPO) in Chicago, Illinois and with the Office of Research and Development (ORD) Western Ecology Division in Corvallis, Oregon to conduct the GLHHFTS under the NCCA. OST is responsible for management of the GLHHFTS under the NCCA with financial and technical support from GLNPO. ORD's Western Ecology Division in Corvallis, Oregon developed the study design and selected all the sampling locations for the

NCCA, including the 157 GLHHFTS sites. Statisticians in the Western Ecology Division will also be analyzing the fish tissue concentration data.

In 2010, OWOW developed the NCCA Quality Assurance Project Plan (QAPP) (USEPA 2010a) that describes the procedures and associated quality assurance/quality control (QA/QC) activities for collecting and shipping NCCA fish tissue samples. It includes the human health fish collection and shipping procedures that OST developed for the GLHHFTS based on the protocols used for the National Lake Fish Tissue Study. In June 2011, OST developed the first version of this QAPP that covers laboratory activities associated with GLHHFTS fish sample preparation and analysis of fillet tissue for mercury, PFCs, and fatty acids. The first revision of the OST QAPP added PBDE analyses and addresses other minor changes to the original QAPP (see the revision history at the front). The current document represents the second revision to the QAPP that adds PCB analyses to the GLHHFTS.

The GLHHFTS project team currently consists of managers, scientists, statisticians, and QA personnel in OST, the ORD Western Ecology Division, and GLNPO, along with contractors providing scientific and technical support to OST from CSC and Tetra Tech, Inc. (Figure 1). Project team members from GLNPO are providing support for developing and reviewing technical and program information related to all aspects of the study, including training materials, standard operating procedures, QAPPs, analytical QA reports, briefings and reports on study results, and outreach materials. Responsibilities for other key members of the project team are described below.

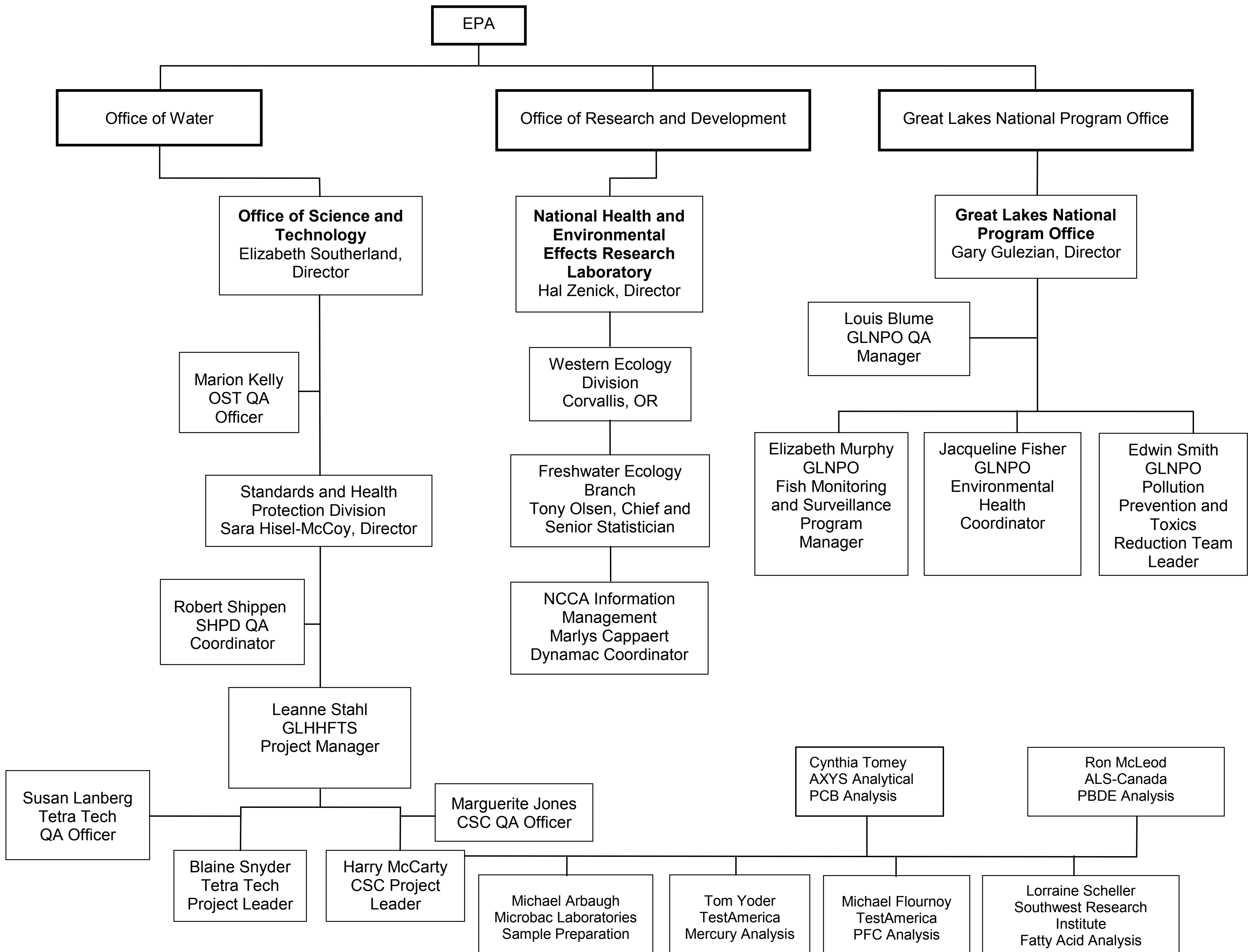

Figure 1. GLHHFTS project team organization

Leanne Stahl of OST is the **GLHHFTS Project Manager** who is providing overall direction for planning and implementation of this regional Great Lakes study being conducted under the NCCA. This role involves the following responsibilities related to the GLHHFTS:

- developing technical information for GLHHFTS fish sample collection that includes preparation of the sampling SOP and coordination with the NCCA Project Leaders in OWOW to integrate field sampling technical information for the GLHHFTS into NCCA documents and training materials
- providing technical support to conduct training on the GLHHFTS field sampling requirements in coordination with the NCCA Project Leaders in OWOW
- developing the fish preparation SOP, implementing training for laboratory processing of NCCA fish samples, and providing technical direction for and oversight of fish preparation activities, including technical support for review of fish preparation QA data
- managing analysis of fish samples for target chemicals, including obtaining technical support for chemical analysis of fish tissue, directing development of this QAPP, providing for QA review of the analytical results, developing the data files for statistical analysis of the data, reviewing and approving the final analytical QA report, and providing oversight for development of the database to store GLHHFTS fish tissue results
- facilitating communication among GLHHFTS project team members and coordinating with all of these individuals to ensure technical quality and adherence to QA/QC requirements
- developing and managing work assignments under OST contracts to provide technical support for the GLHHFTS, providing oversight of all OST contractor activities, and reviewing and approving study deliverables for each work assignment
- scheduling and leading meetings and conference calls with project team members for planning study activities, reporting progress on study tasks, and discussing and resolving technical issues related to the study
- working with QA staff to identify corrective actions necessary to ensure that study quality objectives are met
- managing the development of and/or reviewing and approving all major work products associated with the GLHHFTS
- collaborating with the GLHHFTS project team for reporting the study results in technical journal articles and federal technical reports

Marion Kelly is the **OST Quality Assurance Officer** who is responsible for reviewing and approving all Quality Assurance Project Plans (QAPPs) that involve scientific work being conducted by OST. Robert Shippen is the **Standards and Health Protection Division QA Coordinator** who is responsible for reviewing and recommending approval of all QAPPs that include scientific work being conducted by the Standards and Health Protection Division (SHPD) within OST. The OST QA Officer and SHPD QA Coordinator are also responsible for the following QA/QC activities:

- reviewing and approving this QAPP

- reviewing and evaluating the QA/QC requirements and data for all the GLHHFTS activities and procedures
- conducting external performance and system audits of the procedures applied for all GLHHFTS activities
- participating in Agency QA reviews of the study

Blaine Snyder is the **Tetra Tech Project Leader** who is responsible for managing all aspects of the technical support being provided by Tetra Tech staff for the GLHHFTS. His specific responsibilities include the following:

- providing direct technical support for the following GLHHFTS activities or providing leadership and oversight for Tetra Tech staff supporting these activities:
    - developing standard operating procedures for field sampling and fish preparation
    - preparing GLHHFTS documents (including this QAPP) or project information to incorporate into NCCA documents
    - providing field sampling and fish preparation training
    - planning and implementing GLHHFTS logistics
    - conducting field sampling at Great Lake sites designated by the OST Project Manager
    - obtaining and performing QA reviews of Great Lakes human health field sampling data
    - preparing fish preparation instructions for human health fish samples collected from Great Lakes nearshore sites
    - evaluating weekly fish processing reports for adherence to the technical and quality requirements in the fish preparation SOP
    - preparing summary project information and graphics for development of project fact sheets, presentations, and other EPA meeting and outreach materials
    - developing technical journal articles and final project reports
- monitoring the performance of Tetra Tech staff participating in this study to ensure that they are following all QA procedures described in this QAPP that are related to Tetra Tech tasks being performed to support this study (see list above)
- ensuring completion of high-quality deliverables within established budgets and time schedules
- participating in meetings and conference calls with project team members for planning study activities, reporting progress on study tasks, and discussing and resolving technical issues related to the study

Susan Lanberg is the **Tetra Tech QA Officer** whose primary responsibilities include the following:

- assisting Tetra Tech's Project Leader with the development and review of this QAPP
- approving this QAPP

- providing oversight for the implementation of QA procedures related to Tetra Tech tasks that are described in this QAPP
- reporting deviations from this QAPP to the Tetra Tech Project Leader and assisting in implementing corrective actions to resolve these deviations

Harry McCarty is the **CSC Project Leader** who is responsible for managing all aspects of the technical support being provided by CSC staff for the GLHHFTS. His specific responsibilities include the following:

- providing direct technical support for the following GLHHFTS activities or providing leadership and oversight for CSC staff supporting these activities:
  - preparing information related to technical and quality assurance requirements for preparation and chemical analysis of fish tissue samples for target chemicals, validation of analytical data, and database development to support project planning and development of GLHHFTS documents (including this QAPP) or characterization of the GLHHFTS in NCCA documents
  - conducting reviews of fish preparation QA/QC data associated with each batch of up to 20 fish samples and preparing a report about the results of each batch review for distribution to the OST Project Manager and the fish preparation laboratory
  - obtaining subcontractor laboratory services to analyze urban river water and fish tissue samples for mercury, PFCs, fatty acids, PBDEs, and PCBs, and providing technical and QA oversight of laboratory operations
  - completing analytical data review for all target chemicals and developing the analytical data QA report
  - formatting the analytical data files for statistical analysis and preparing raw (unweighted) data files for public release
  - developing and maintaining a project database for storing GLHHFTS field and analytical data and initiating queries of the database to respond to data requests from Agency and external data users
  - obtaining freezer space that meets the requirements for long-term storage of archived fish tissue samples, organizing the archived fish tissue samples by project to facilitate retrieval of the samples, and developing and maintaining an inventory of the archived samples
  - preparing summary project information and graphics for development of project fact sheets, presentations, and other EPA meeting and outreach materials
  - supporting development of technical journal articles and final project reports
- monitoring the performance of CSC staff participating in this study to ensure that they are following all QA procedures described in this QAPP that are related to CSC tasks being performed to support this study (see list above)
- ensuring completion of high-quality deliverables within established budgets and time schedules

- participating in meetings and conference calls with project team members for planning study activities, reporting progress on study tasks, and discussing and resolving technical issues related to the study

Marguerite Jones is the **CSC QA Officer** whose primary responsibilities include the following:

- assisting CSC's Project Leader with the development and review of this QAPP
- approving this QAPP
- providing oversight for the implementation of QA procedures related to CSC tasks that are described in this QAPP
- reporting deviations from this QAPP to the CSC Project Leader and recommending corrective actions to resolve these deviations

Tony Olsen is the **Senior Statistician** at the ORD Western Ecology Division in Corvallis, Oregon who is supporting the GLHHFTS by providing technical expertise for study planning and implementation and by assuming responsibility for the following activities:

- study design development for the NCCA, including statistically representative national and regional studies being conducted under the NCCA, such as the GLHHFTS
- site selection and tracking for final statistical classification of sites
- statistical analysis of analytical data for GLHHFTS fish tissue samples
- development of cumulative density functions for analytical data sets with sufficient data points
- participation in development of technical journal articles and final reports for publication

A5. Problem Definition/Background

Obtaining statistically representative environmental data on mercury and chemicals of emerging concern (CECs) is a priority area of interest for EPA. Since 1998, OW has collaborated with ORD to conduct the first national-scale assessments of mercury in fish tissue through statistically-based studies of U.S. lakes and rivers. These studies are referred to as the National Lake Fish Tissue Study and the National Rivers and Streams Assessment, respectively. The Great Lakes were excluded from the National Lake Fish Tissue Study because assessment of a freshwater system of that magnitude required a separate sampling design. The probability-based Great Lakes sampling design developed for the NCCA offered the opportunity to conduct the GLHHFTS, which is the first representative study of chemical residues in fish relevant to human health for this region. The GLHHFTS will also provide the first lake-wide data on the occurrence and distribution of CECs (e.g., PFCs) in the Great Lakes. In addition, the GLHHFTS will generate species-specific data on fatty acids to address an existing data gap and to identify fish with higher omega-3 levels and potentially greater health benefits.

A6. Project/Task Description

OST is collaborating with the Great Lakes National Program Office and with ORD's Western Ecology Division in Corvallis, Oregon, to conduct the GLHHFTS within the framework of the

NCCA. Fish composite samples were collected from June through November 2010 at a statistical subset of NCCA Great Lakes sites, which consisted of over 150 randomly selected nearshore sites distributed throughout the five Great Lakes (Figure 2).

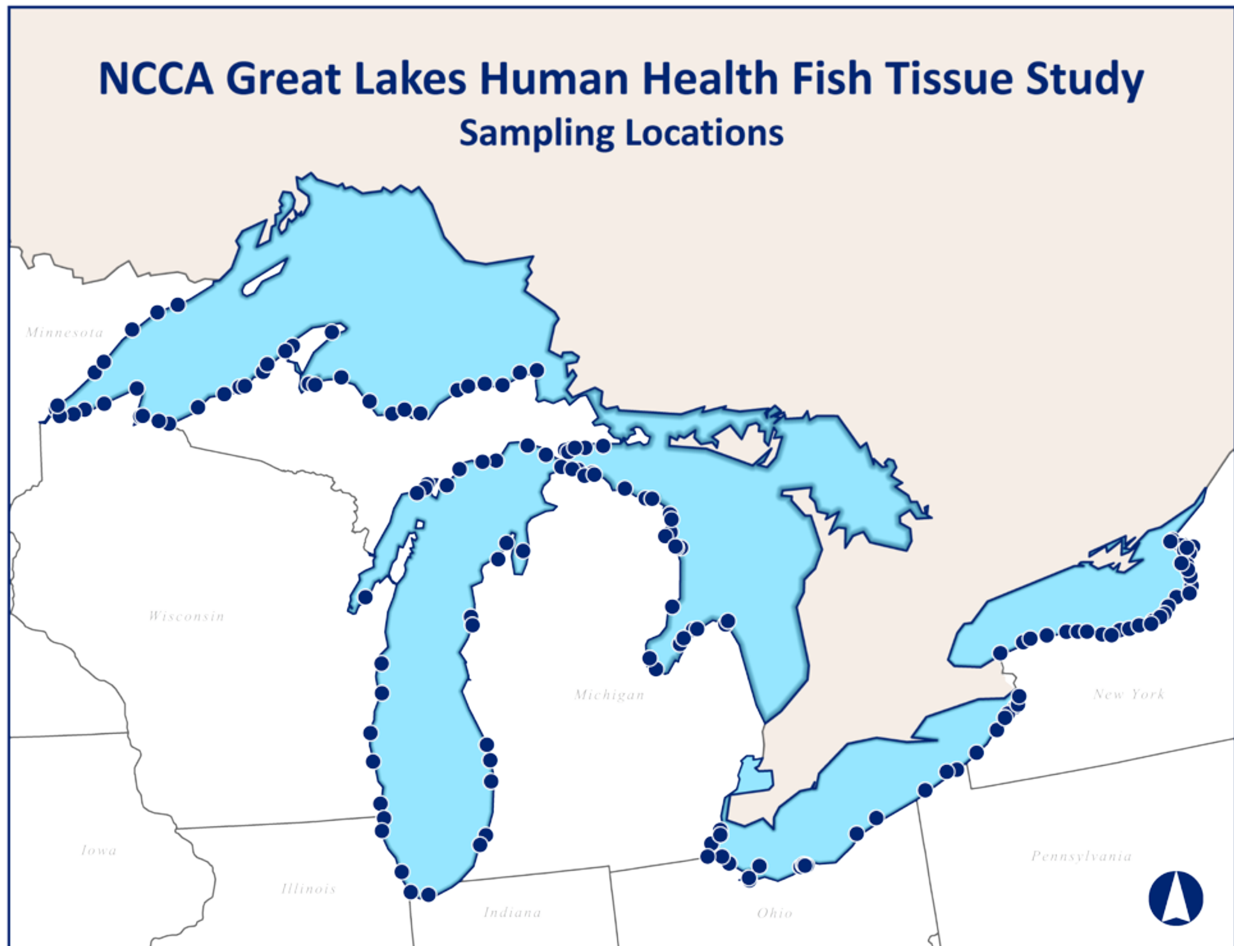

Figure 2. Location of the 157 randomly selected nearshore Great Lakes sampling locations, a statistical subset of NCCA Great Lake sites

Following are the key design components for the GLHHFTS:

- sampling over 150 randomly selected sites (about 30 sites per lake) in the nearshore regions (depths up to 30 m or distances up to 5 km from shore) during 2010 (Appendix A).

- collecting one fish composite sample for human health applications (i.e., five similarly sized adult fish of the same species that are commonly consumed by humans) from each site.

- shipping whole fish samples to a commercial laboratory for storage and fish sample preparation, which includes filleting the fish, homogenizing the fillet tissue composites, and preparing fillet tissue aliquots for analysis of specific contaminants, along with a series of archive samples that may be used for future analyses of other contaminants.

- analyzing the fillet tissue samples for mercury (total), 13 perfluorinated compounds (including PFOA and PFOS), 5 fatty acids, 52 PBDE congeners (and 2 other brominated compounds), and 209 PCB congeners.

Microbac Laboratories in Baltimore, Maryland, is storing the GLHHFTS fish samples and preparing the fish tissue samples for analysis as outlined in the third bullet above. As shown in Figure 1, commercial laboratories under subcontract to CSC will be analyzing the GLHHFTS fish tissue samples for mercury, PFCs, fatty acids, PBDEs, and PCBs. For each sample, Microbac staff are also preparing and holding multiple aliquots of archived fillet tissue in a freezer at their facility to allow for further analyses of GLHHFTS samples if resources can be identified in the future to support these analyses.

Note: The sample aliquots that will be analyzed for PCBs are those originally prepared for the analysis of pharmaceuticals and personal care products (PPCPs).

A7. Quality Objectives and Criteria

The overall quality objective for the analysis of the GLHHFTS fish tissue samples for mercury, PFCs, fatty acids, PBDEs, and PCBs is to obtain a complete set of data for each chemical or chemical group and to produce data of known and documented quality. Completeness is defined as the percentage of samples collected in the study for which usable analytical results were produced. The goal for completeness is 95% and it is calculated at the sample-analyte level, such that an issue with the quality of one analyte out of many does not invalidate the entire sample.

Commercial laboratories proposed analytical methods and quality control acceptance criteria for analyses of GLHHFTS fish tissue samples for mercury, PFCs, fatty acids, and PBDEs. The information describing the proposed methods has been added to Section B4 of this QAPP. Data usability for each analysis will be assessed using QC criteria established by the respective laboratories and summarized in Section B.5. For the PCB analyses, EPA specified the use of Method 1668C and the QC acceptance criteria from that method are summarized in Section B.5.5.

A8. Special Training/Certification

Fish Tissue Sample Preparation

All laboratory staff involved in the preparation of fish tissue samples must be proficient in the associated tasks, as required by the NCCA GLHHFTS Tissue Preparation, Homogenization, and Distribution Procedures (Appendix B).

Specialized training was provided for laboratory technicians who will be preparing fish tissue fillets and homogenates for this project. This training was conducted at Microbac in Baltimore, Maryland, for all laboratory staff involved with GLHHFTS fish tissue sample preparation to accomplish the following objectives:

- present GLHHFTS fish tissue preparation, homogenization and distribution procedures described in Appendix B,

- demonstrate filleting and homogenizing techniques with fish from invalid GLHHFTS samples, and

- provide hands-on opportunities for fish preparation laboratory staff to develop proficiency with filleting and homogenizing fish samples.

Analysis of Fish Tissue Samples

All laboratory staff involved in the analysis of fish tissue samples must be proficient in the associated tasks, as required by each analytical laboratory's existing quality system. All contractor staff involved in analytical data review and assessment will be proficient in data review, and no specialized training is required for data reviewers for this project.

A9. Documents and Records

The Statements of Work (SOWs) for the analytical subcontracts provide the specific requirements for laboratory deliverables. The major points are summarized below:

- The laboratory must provide reports of all results required from analyses of environmental and QC samples.
- Summary level data must be submitted in electronic format and must include the following information: EPA sample number, analyte name and CAS number, laboratory sample ID, measured amount, reporting units, sample preparation date, and analytical batch ID (if applicable).
- The laboratory shall provide raw data in the form of direct instrument readouts with each data package. Raw data include:
  - Copy of traffic report, chain-of-custody records, or other shipping information
  - Instrument readouts and quantitation reports for analysis of each sample, blank, standard and QC sample, and all manual worksheets pertaining to sample or QC data or the calculations thereof
  - Copies of bench notes, including preparation of standards and instrumental analyses

The laboratories will maintain records and documentation associated with these analyses for a minimum of five years after completion of the study. Additional copies will be maintained by CSC for at least five years and will be transferred to EPA on request.

B. DATA GENERATION AND ACQUISITION

B1. Sampling Process Design (Experimental Design)

The objective of the GLHHFTS is to investigate the occurrence of mercury, PFCs, fatty acids, PBDEs, and PCBs in the edible tissue (fillets) of harvestable-sized adult freshwater fish that are typically consumed by humans. The study will provide:

- statistically representative data on the levels of mercury, PFCs, PBDEs, and PCBs in Great Lakes fish commonly consumed by humans,

- information on the potential for the target PFCs to bioaccumulate in fish tissue,
- data to answer questions concerning the occurrence of PFCs in fish and the potential for human exposure through fish consumption, and
- species-specific information on fatty acid content of Great Lakes fish that are commonly targeted by fishermen and consumed by humans.

The details of the sampling process design, sampling methods, and sample handling and custody procedures are described in EPA's *National Coastal Condition Assessment Quality Assurance Project Plan* prepared by OWOW (USEPA 2010a). However, to provide some context for the readers of this QAPP, those aspects of the NCCA are summarized below.

The NCCA target population included nearshore areas of U.S. waters in the Great Lakes. The Great Lakes survey design was stratified by lake and country (U.S. and Canada) with unequal probability of selection based on state (or province) shoreline length within each stratum. The nearshore zone was defined as the region from the shoreline to a depth of 30 m or to a distance of 5 km from the shoreline in shallower waters of the Great Lakes (e.g., Lake Erie). NCCA sites were randomly selected in the five Great Lakes (Lakes Superior, Michigan, Huron, Erie, and Ontario) bordered by eight Great Lakes states (Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, and Wisconsin). The sample frame for the NCCA was derived by ORD's Western Ecology Division in Corvallis, Oregon. The target population for the GLHHFTS consists of a statistically representative subset of 157 NCCA sites distributed throughout the U.S. nearshore zone of the five Great Lake (about 30 sites per lake). Sampling at the GLHHFTS locations included collection of fish for analysis of mercury, PFCs, fatty acids, PBDEs, and PCBs in the fillets.

To meet the study objective, one fish sample was collected from each site. A routine fish composite sample consists of five fish of adequate size to provide a minimum of 300 grams of edible tissue for analysis. Fish are selected for each composite applying the following criteria:

- all are of the same species
- all satisfy legal requirements of harvestable size (or weight) for the sampled lake, or at least be of consumable size if no legal harvest requirements are in effect,
- all are of similar size, so that the smallest individual in a composite is no less than 75% of the total length of the largest individual, and
- all are collected at the same time, i.e., collected as close to the same time as possible, but no more than one week apart. (Note: Individual fish may have to be frozen until all fish to be included in the composite are available for delivery to the designated laboratory).

Accurate taxonomic identification is essential in preventing the mixing of closely related target species. Under no circumstances are individuals from different species used in a composite sample.

Initially, OWOW designated June through September 2010 as the sampling period for the NCCA, including the Great Lakes region. Field crews in the Great Lakes scheduled fish collection at the majority of the nearshore sites during June and July, which turned out to be a period when many of the target species for the GLHHFTS are difficult to find in shallow waters.

Consequently, OST arranged re-sampling at over 50 nearshore sites after July and extended the sampling period through mid-November to complete the goal of collecting fish for the GLHHFTS from at least 150 nearshore sites.

B2. Sampling Methods

Sampling method procedures and requirements for collection of human health fish samples are detailed in EPA's *National Coastal Condition Assessment Quality Assurance Project Plan* (USEPA 2010a) and *National Coastal Condition Assessment Field Operations Manual* (USEPA 2010b). These sampling procedures and requirements are summarized below.

The field objective was for sampling teams to obtain one representative fish composite sample from each sampling site. Collecting fish composite samples is a cost-effective means of estimating average chemical concentrations in the tissue of target species, and compositing fish ensures adequate sample mass for analysis of multiple chemicals. The sampling procedures specified that each composite should consist of five similarly sized adult fish of the same species. OST developed a recommended fish species list with GLNPO concurrence that contained 26 priority target fish species and 18 alternative fish species. Fish teams used this list as the basis for selecting appropriate fish species for the GLHHFTS samples. The method applied for fish collection was at the discretion of the field team, but it typically involved angling or gillnetting and occasionally trawling.

In preparing fish samples for shipping, field teams recorded sample number, species name, specimen length, sampling location and sampling data and time on a fish collection form. Each fish was wrapped in solvent-rinsed, oven-baked aluminum foil, with the dull side in using foil sheets provided by EPA. Individual foil-wrapped specimens were placed into a length of food-grade polyethylene tubing, each end of the tubing was sealed with a plastic cable tie, and a fish specimen label was affixed to the outside of the food-grade tubing with clear tape. All of the wrapped fish in the sample from each site were placed in a large plastic bag and sealed with another cable tie, then placed immediately on dry ice for shipment to Microbac in Baltimore, Maryland. Field crews were directed to pack fish samples on dry ice in sufficient quantities to keep samples frozen for up to 48 hours (50 pounds were recommended), and to ship them via priority overnight delivery service (e.g., Federal Express), so that they could arrive at Microbac in less than 24 hours from the time of sample collection. CSC was responsible for receiving and examining the fish samples at Microbac before they were stored in a walk-in freezer at the laboratory.

B3. Sample Handling and Custody

This section describes the sample handling and custody procedures that apply once the homogenized fish tissue samples are shipped from Microbac to each of the analytical laboratories selected for analysis of GLHHFTS fish tissue samples for mercury, PFCs, fatty acids, and PBDEs. All sample handling and custody procedures prior to those described here are discussed in the QAPP prepared by OWOW (USEPA 2010a) for other portions of the study and are not repeated here.

CSC will ship the fish tissue homogenates from Microbac to the GLHHFTS analytical laboratories. Samples will be packaged in sturdy coolers for shipping and wrapped with bubble wrap or other suitable packaging to protect the samples in transit. Samples will be shipped

frozen with sufficient dry ice in the coolers to ensure that the samples remain frozen for at least 48 hours. Step 29 of the fish preparation procedures (Appendix B) provides specific information about dry ice requirements for shipping fish tissue samples. CSC will prepare sample tracking paperwork and include it in each shipment.

When received at the respective analytical laboratories, the samples are inspected for damage, logged into the laboratory, and immediately placed into freezers. Because the samples are shipped frozen, typical temperature blanks consisting of a bottle of water are not practical (they may break due to expansion), nor required. The laboratories measure and record the temperature of the coolers containing the samples on receipt using an infrared temperature sensor or other suitable device. CSC is notified of the receipt of samples by email. CSC will advise EPA of tissue sample receipt. Any questions from the laboratories regarding sample paperwork or condition will be sent to CSC, routed to OST or Tetra Tech as appropriate, and CSC will send the answers back to the appropriate laboratory.

Fish tissue samples will be stored frozen at $\leq$ -20°C until analyzed. There are no formal holding time studies or requirements that apply to these analytes, except mercury and PCBs. EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1* (USEPA 2000) recommended a 28-day holding time for fish tissue mercury analysis, i.e., from receipt of the fish at the sample processing laboratory to analysis. Recently, Peterson et al. (2007) conducted a holding time study focused specifically on mercury. They reported that results for frozen tissue homogenates retained at -20°C between their original analysis in 2002 and a subsequent analysis in 2006 revealed no statistical differences in mercury concentrations over time. They concluded that wet fish tissue homogenates can be held frozen for at least four years without affecting analytical results for mercury. Considering those findings, a 1-year administrative holding time (from homogenization to analysis) will be applied for GLHHFTS mercury analyses in order to adhere to the study schedule and ensure sufficient time for data compilation, review, and statistical analysis. For PCBs, EPA Method 1668C specifies a 1-year holding time for solid samples, including tissues. For this study, that holding time will begin at the completion of the homogenization of the composite sample.

EPA will note any results for either mercury or PCBs generated outside of these 1-year holding times, but will not preclude use of such results for the purposes of this project.

B4. Analytical Methods

Fish Sample Preparation

Microbac was selected as the fish sample preparation laboratory (prep lab) for the GLHHFTS. In this role, Microbac is responsible for filleting each valid fish sample, homogenizing the fillet tissue, preparing the required number of fish tissue aliquots for analysis and archive, shipping the fish tissue aliquots for each analysis to the designated analytical laboratory, and storing archived fish tissue samples in a freezer at their facility. The specific procedures for all GLHHFTS fish sample preparation activities are described in Appendix B.

Fish are filleted by qualified prep lab technicians using thoroughly clean utensils and cutting boards (cleaning procedures are detailed in Appendix B). Each fish is weighed to the nearest gram wet weight, rinsed with deionized water, and filleted on a glass cutting board. For the

GLHHFTS, fillets from both sides of each fish are prepared with scales removed, skin on, and belly flap (ventral muscle and skin) attached. Fillets are composited using the "batch" method, in which all of the individual specimens that comprise the sample are homogenized together, regardless of each individual specimen's proportion to one another (as opposed to the "individual" method, in which equal weights of each specimen are added together).

An electric meat grinder is used to prepare homogenate samples. Entire fillets (with skin and belly flap) from both sides of each fish are homogenized, and the entire homogenized volume of all fillets from the fish sample is used to prepare the tissue sample. Tissues are mixed thoroughly until they are completely homogenized as evidenced by a fillet homogenate that consists of a fine paste of uniform color and texture. The collective weight of the homogenized tissue from each sample is recorded to the nearest gram (wet weight) after processing. Microbac prepares fillet tissue aliquots according to the specifications listed in Step 15 of the fish sample preparation procedures in Appendix B.

Fish Tissue Analysis

Mercury

Fish tissue samples will be analyzed by TestAmerica - Knoxville using a microwave-assisted strong acid digestion, followed by cold-vapor atomic absorption detection of mercury (CAS Number 7439-97-6). The digestion and analysis procedures are based on SW-846 Method 3051A and Method 7470A, respectively. Approximately 0.5 g of tissue is used for the analysis. Because the microwave-assisted digestion procedure dissolves all of the tissue, the digestate can be analyzed as a liquid sample.

The rinsate samples for mercury are being analyzed by Microbac Laboratories, during the course of the homogenization of the fish tissue samples. Microbac is analyzing these aqueous samples using EPA Method 245.1, a cold-vapor atomic absorption procedure applicable to water samples.

Tissue sample results are reported based on the wet weight of the tissue sample, in micrograms per kilogram (µg/kg). Mercury method detection limits (MDLs) and minimum levels (MLs) are listed in Appendix C.

Rinsate results are reported based on the volume of the rinsate sample, in micrograms per liter (µg/L).

PFCs

There are no formal analytical methods from EPA or any voluntary consensus standard bodies (VCSBs) for the PFC analyses. Therefore, fish tissue samples will be analyzed by the TestAmerica - West Sacramento using procedures developed, tested, and documented in that laboratory. The SOPs for those procedures are considered proprietary by the laboratory and therefore are not attached to this QAPP. However, the SOPs have been reviewed by CSC and the analytical procedures are briefly described below.

The 13 target PFC analytes are shown in Table 1 below.

Table 1. PFC Target Analytes and Identifiers

Name	Abbreviation	Formula	CAS Number
Perfluorobutyric acid	PFBA	C_3F_7COOH	375-22-4
Perfluoropentanoic acid	PFPeA	C_4F_9COOH	2706-90-3
Perfluorohexanoic acid	PFHxA	$C_5F_{11}COOH$	307-24-4
Perfluoroheptanoic acid	PFHpA	$C_6F_{13}COOH$	375-85-9
Perfluorooctanoic acid	PFOA	$C_7F_{15}COOH$	335-67-1
Perfluorononanoic acid	PFNA	$C_8F_{17}COOH$	375-95-1
Perfluorodecanoic acid	PFDA	$C_9F_{19}COOH$	375-76-2
Perfluoroundecanoic acid	PFUnA	$C_{10}F_{21}COOH$	2058-94-8
Perfluorododecanoic acid	PFDoA	$C_{11}F_{23}COOH$	307-55-1
Perfluorobutanesulfonic acid	PFBS	$C_4F_9SO_3H$	375-73-5
Perfluorohexanesulfonic acid	PFHxS	$C_6F_{13}SO_3H$	355-46-4
Perfluorooctanesulfonic acid	PFOS	$C_8F_{17}SO_3H$	1763-23-1
Perfluorooctanesulfonamide	PFOSA	$C_8F_{17}SO_2NH_2$	754-91-6

The concentration of each PFC is determined using the responses from the $^{13}C_{12}$-labeled standards added prior to sample extraction, applying the technique known as isotope dilution. As a result, all of the target analyte concentrations are corrected for the recovery of the labeled standards, thus accounting for extraction efficiencies and losses during cleanup.

Approximately 1 to 5 g of fish tissue are required for analysis. The sample is spiked with twelve isotopically labeled standards and extracted by shaking the tissue in a caustic solution of methanol, water, and sodium hydroxide. The hydroxide solution breaks down the tissue and allows the PFCs to be extracted into the methanol/water.

After extraction, the solution is centrifuged to remove the solids and the supernatant liquid is diluted with dilute hydrochloric acid (HCl) to a pH < 2. That diluted extract is processed by solid-phase extraction (SPE). The PFCs are eluted from the SPE cartridge and the eluant is spiked with additional labeled recovery standards and analyzed by HPLC-MS/MS.

A 250-mL aliquot of an aqueous rinsate sample is spiked with the labeled standards and acidified with dilute hydrochloric acid (HCl) to a pH < 2. That diluted extract is processed by solid-phase extraction (SPE), in a similar manner as the tissue samples. The PFCs are eluted from the SPE cartridge and the eluant is spiked with additional labeled recovery standards and analyzed by HPLC-MS/MS.

Tissue sample results are reported based on the wet weight of the tissue sample, in nanograms per kilogram (ng/kg). MDLs and MLs for PFCs are listed in Appendix C. Aqueous rinsate results are reported based on the volume of the rinsate sample, in nanograms per liter (ng/L).

Fatty Acids

As with the PFCs, there are no formal analytical methods from EPA for the fatty acids, largely because they are natural products and not environmental contaminants. However, there are procedures for analysis of fats and oils available from some VCSBs, including the Association of Official Analytical Chemists (AOAC). The fatty acid samples will be analyzed by Southwest Research Institute (SwRI) using a combination of an extraction procedure from the literature and an AOAC analytical method, as described below. The SOPs for those procedures are considered

proprietary by the laboratory and therefore are not attached to this QAPP. However, the analytical procedures are briefly described below.

The 5 target fatty acid analytes are shown in Table 2 below.

Table 2. Target Omega-3 Fatty Acids and Identifiers

Common name	Abbreviation	Structural Shorthand	CAS Number
alpha-Linolenic acid	ALA	18:3 (n-3)	463-40-1
Eicosatrienoic acid	ETE	20:3 (n-3)	2091-27-2
Eicosapentaenoic acid	EPA	20:5 (n-3)	10417-94-4
Docosapentaenoic acid	DPA	22:5 (n-3)	24880-45-3
Docosahexaenoic acid	DHA	22:6 (n-3)	6217-54-5

EPA initially planned to include four other fatty acids as target analytes, but neither the laboratory nor CSC could locate authentic standards for those four fatty acids, so they have been dropped from the list in Table 2.

The method used to extract the fatty acids from the fish tissue samples is based on the procedure described by Sathivel *et al.* (2002). Briefly, a 1-g aliquot of homogenized fish tissue is placed in a centrifuge tube and spiked with a surrogate solution containing triheneicosanoin (a C_{21}-triglyceride). The sample is extracted with 25 mL of a 1:4:4 solution of distilled water, chloroform, and methanol and vortexed for 1 minute. The sample is placed on a mechanical shaker for 15 minutes. After shaking, the mixture is filtered through Whatman No. 1 filter paper to remove the solids, and the filtrate is collected in a separatory funnel, where it separates into two layers. If needed, additional water is added to the separatory funnel to ensure phase separation. The chloroform layer is drawn off from the bottom of the separatory funnel and passed through anhydrous sodium sulfate to remove any remaining water. The extract is reduced to dryness using nitrogen evaporation.

An internal standard is added to the extract and the fatty acids are derivatized to their methyl esters by adding 1.5 mL of 0.5 N methanolic sodium hydroxide solution. The sample is blanketed with either nitrogen or argon to prevent oxidation and heated to 100 ºC for 30 min. The sample is cooled to about 40 °C and 2 mL of isooctane are added. The sample is vortexed for 30 sec. and 5 mL of saturated NaCl solution is added to the isooctane, followed by another 1 min of vortexing, after which the layers are allowed to separate. The isooctane layer is transferred to a clean vial and the process is repeated once. The isooctane aliquots are combined and the volume is adjusted to 1 mL and analyzed by GC/FID, using a DB-23 GC column.

For the rinsate samples, an aliquot of the rinsate will be evaporated to dryness with nitrogen and the fatty acids will be derivatized following AOAC Method 991.39. That method uses boron trifluoride (BF_3) to derivatize the fatty acids to their methyl esters. As with the tissue samples, the methyl esters are extracted with isooctane, concentrated, and analyzed by GC/FID.

Tissue results will be reported as the fractional percentage of each fatty acid methyl ester, based on the wet weight of the sample. MDLs and MLs for fatty acids are listed in Appendix C. Rinsate results will be reported as the fractional percentage of each fatty acid methyl ester, based on the volume of the hexane rinsate sample.

PBDEs

The PBDE samples will be prepared and analyzed by ALS - Canada in general accordance with EPA Method 1614A (USEPA 2010c) and as detailed in the laboratory's proprietary SOP. The ALS SOP deviates from the published EPA method in several aspects, including:

- The use of more ^{13}C-labeled extraction standards than called for in the method
- Approximately 20 g of fish tissue is used for the analysis
- GC performance criteria are monitored for every 12-hour run sequence instead of requiring that the absolute retention time for decabromodiphenyl be at least 48 minutes
- The concentrations of labeled and native spiking solutions differs from those listed in Method 1614A
- The labeled clean-up standard hexabromo-BDE-139L has been replaced with hexabromo-BDE-138L
- The list of injection standards has been enhanced to include four ^{13}C-labeled BDEs (BDE-79L, -139L, -180L, and -206L), rather than two labeled PCBs
- The initial calibration range has been narrowed from 1 to 2500 ng/mL to 1 to 500 ng/mL, with the CS4 standard at 150 and CS5 at 500 ng/mL

These changes fall within the method's established allowance for flexibility, and EPA has accepted these deviations from Method 1614A for the purposes of this study.

The target analytes are listed in Table 3 and include 52 PBDE congeners and two other brominated analytes. Of the 47 PBDE congeners, 41 are determined as individual congeners and 6 are determined as coeluting pairs that cannot be separated chromatographically. MDLs and MLs for target PBDEs are listed in Appendix C.

Table 3. PBDE Target Analyte List

Full name	Abbreviation	CAS Number*
2,4-Dibromodiphenyl ether	BDE-7	171977-44-9
2,4'-Dibromodiphenyl ether coeluting with 3,3'-Dibromodiphenyl ether	BDE-8/11	147217-71-8/ 6903-63-5
2,6-Dibromodiphenyl ether	BDE-10	51930-04-2
3,4-Dibromodiphenyl ether coeluting with 3,4'-Dibromodiphenyl ether	BDE-12/13	189084-59-1/ 83694-71-7
4,4'-Dibromodiphenyl ether	BDE-15	2050-47-7
2,2',4-Tribromodiphenyl ether coeluting with 2,3',4-Tribromodiphenyl ether	BDE-17/25	147217-75-2/ 147217-77-4
2,4,4'-Tribromodiphenyl ether coeluting with 2',3,4-Tribromodiphenyl ether	BDE-28/33	41318-75-6/ 147217-78-5
2,4,6-Tribromodiphenyl ether	BDE-30	155999-95-4
2,4',6-Tribromodiphenyl ether	BDE-32	189084-60-4
3,3',4-Tribromodiphenyl ether	BDE-35	147217-80-9
3,4,4'-Tribromodiphenyl ether	BDE-37	147217-81-0
2,2',4,4'-Tetrabromodiphenyl ether	BDE-47	5436-43-1
2,2',4,5'-Tetrabromodiphenyl ether	BDE-49	243982-82-3
2,2',4,6'-Tetrabromodiphenyl ether	BDE-51	189084-57-9
2,3',4,4'-Tetrabromodiphenyl ether	BDE-66	189084-61-5
2,3',4',6-Tetrabromodiphenyl ether	BDE-71	189084-62-6

Full name	Abbreviation	CAS Number*
2,4,4',6-Tetrabromodiphenyl ether	BDE-75	189084-63-7
3,3',4,4'-Tetrabromodiphenyl ether	BDE-77	93703-48-1
3,3',4,5'-Tetrabromodiphenyl ether	BDE-79	446254-48-4
2,2',3,4,4'-Pentabromodiphenyl ether	BDE-85	182346-21-0
2,2',4,4',5-Pentabromodiphenyl ether	BDE-99	60348-60-9
2,2',4,4',6-Pentabromodiphenyl ether	BDE-100	189084-64-8
2,3,3',4,4'-Pentabromodiphenyl ether	BDE-105	373594-78-6
2,3,4,5,6-Pentabromodiphenyl ether	BDE-116	189084-65-9
2,3',4,4',5-Pentabromodiphenyl ether	BDE-118	446254-80-4
2,3',4,4',6-Pentabromodiphenyl ether coeluting with 2,3',4,5,5'-Pentabromodiphenyl ether	BDE-119/120	189084-66-0/ 417727-71-0
3,3',4,4',5-Pentabromodiphenyl ether	BDE-126	366791-32-4
2,2',3,3',4,4'-Hexabromodiphenyl ether	BDE-128	NA
2,2',3,4,4',5'-Hexabromodiphenyl ether coeluting with 2,3,4,4',5,6-Hexabromodiphenyl ether	BDE-138/166	182677-30-1/ 189084-58-0
2,2',3,4,4',6'-Hexabromodiphenyl ether	BDE-140	243982-83-4
2,2',4,4',5,5'-Hexabromodiphenyl ether	BDE-153	68631-49-2
2,2',4,4',5',6-Hexabromodiphenyl ether	BDE-154	207122-15-4
2,2',4,4',6,6'-Hexabromodiphenyl ether	BDE-155	35854-94-5
2,3,3’,4,4’,5-Hexabromodiphenyl ether	BDE-156	NA
2,2',3,4,4',5,6-Heptabromodiphenyl ether	BDE-181	189084-67-1
2,2',3,4,4',5',6-Heptabromodiphenyl ether	BDE-183	207122-16-5
2,2’,3,4,4’,6,6’-Heptabromodiphenyl ether	BDE-184	117948-63-7
2,3,3',4,4',5,6-Heptabromodiphenyl ether	BDE-190	189084-68-2
2,3,3’,4,4’,5’,6-Heptabromodiphenyl ether	BDE-191	NA
2,2’,3,3’,4,4’,5’,6-Octabromodiphenyl ether	BDE-196	NA
2,2’,3,3’,4,4’,6,6’-Octabromodiphenyl ether	BDE-197	NA
2,2',3,4,4',5,5',6-Octabromodiphenyl ether	BDE-203	337513-72-1
2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether	BDE-206	63387-28-0
2,2',3,3',4,4',5,6,6'-Nonabromodiphenyl ether	BDE-207	437701-79-6
2,2',3,3',4,5,5',6,6'-Nonabromodiphenyl ether	BDE-208	NA
2,2',3,3',4,4’,5,5',6,6'-Decabromodiphenyl ether	BDE-209	1163-19-5
Pentabromoethylbenzene	PBEB	85-22-3
Hexabromobenzene	HBB	87-82-1

* CAS numbers for coeluting congeners are shown for information only and will not be used in the project database
NA = Not available - no CAS number found for this congener

PCBs

The PCB samples will be prepared and analyzed by AXYS Analytical Services, in general accordance with EPA Method 1668C (USEPA 2010d). The samples will be analyzed for all 209 PCB congeners, and reported as either individual congeners or coeluting groups of congeners. The following method modifications have been reviewed, found to be within the allowance for flexibility in Section 9.1.2 of Method 1668C, supported by performance data that are maintained on file at the laboratory, and have been approved for use in this study:

Section	Modification	Original Method Approach
Table 2	The use of 34 ^{13}C-labeled extraction standards	32 ^{13}C-labeled extraction standards
4.2.1, 4.2.2	The protocol for washing reusable glassware includes a detergent wash, water rinse and baking at a minimum of 300 °C for 8 h. Immediately prior to use, glassware is solvent rinsed with toluene and hexane.	Glassware should be rinsed with solvent and washed with a detergent solution. After detergent washing, glassware should be rinsed immediately, first with methanol, then with hot tap water. The tap water rinse is followed by another methanol rinse, then acetone, and then methylene chloride.
4.7	The first cleanup column for tissue extracts is a gravity gel permeation column (SX-3 Biobeads). An anthropogenic isolation column 7.5.3 is not used.	Lipids must be removed by the anthropogenic isolation column procedure in Section 13.6, followed by the gel permeation chromatography procedure.
6.5.1	Glass wool is cleaned by rinsing twice with toluene and twice with hexane.	Glass wool is solvent-extracted using a Soxhlet or SDS extractor for 3 h minimum.
7.2.1	Sodium sulfate is baked at a minimum of 300 °C for 8 h.	Sodium sulfate, reagent grade, granular, anhydrous, rinsed with methylene chloride (20 mL/g), baked at 400 ºC for 1 h minimum.
7.5.1	Silica is activated by baking at 450 °C in a muffle oven for at least 8 h.	Activated silica gel, rinsed with methylene chloride, baked at 180 ºC for a minimum of 1 h.
7.5.4.1.1	Florisil is baked at 450 °C in a muffle oven for at least 8 h, then deactivated with water to 2.1% deactivation.	Place in an oven at 130-150 ºC for a minimum of three days to activate the Florisil.
7.12, 7.13, 9.0, 11.0	The concentration of the labeled toxics/level of chlorination and the cleanup standard spiking solutions is 100 ng/mL and the sample spiking volume is 20 µL. The resulting final concentrations in the extracts are as specified in the method.	The concentration of the labeled toxics/level of chlorination and the cleanup standard spiking solutions is 2 ng/mL and the sample spiking volume is 1 mL.
7.14	Concentration of the labeled injection internal standard spiking solution is modified so that a volume of 5 µL is added. The resulting amount of standard added to the final extract is the same as specified in the method. The solution is spiked into a 15 µL extract volume for a final extract volume of 20 µL.	Concentration of the injection internal standards is 1000 ng/mL. When 2 µL of this solution is spiked into a 20 µL extract, the concentration of each injection internal standard will be nominally 100 ng/mL.
10.3.3, 15.3.3	S:N ratio of 3:1 for di-PCBs and nona-PCBs in CS0.2 calibration solution is acceptable. *(Note, this standard is 5 times lower than the standard in the method, hence the lesser S:N requirement)*	The peaks representing the CBs and labeled compounds in the CS-1 calibration standard must have signal-to-noise ratios (S/N) ≥ 10.
11.5, 11.5.2, 11.5.5, 12.3	Solid samples are dried by mixing with anhydrous sodium sulfate. The dried solid is extracted using a Soxhlet extraction apparatus. The surrogate spike is incorporated after the drying step. Equilibration time for the surrogate is 30 minutes. The extracting solvent for solids is dichloromethane. *(Note, the method warns that the use of toluene and SDS may result in loss of some of the mono- through trichlorinated analytes)*	Weigh a well-mixed aliquot of each sample sufficient to provide 10 g of dry solids into a clean beaker or glass jar. Spike with labeled compounds and stir or tumble and equilibrate the aliquots for 1 to 2 h. Extract the sample using the SDS procedure using toluene.
11.8, 12.4	The surrogate spike is incorporated into the sample after the drying step to eliminate the possibility of disproportional loss of volatile labeled and target compounds.	Spike the labeled compounds, then mix in the sodium sulfate drying agent.
12.4.2	The precleaning of the Soxhlet apparatus is carried out using toluene instead of dichloromethane, for 2 h.	Pre-extract the Soxhlet apparatus using methylene chloride (dichloromethane).
12.6.1.1	Rotary evaporation is done at 30 °C. Daily cleaning of the rotary evaporators include dismantling and rinsing/soaking with solvent. Proofs are run periodically but are not archived daily.	Rotary evaporation – Concentrate the extracts in a water bath at 45 ºC. Pre-clean the rotary evaporator daily by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary.
12.7.4	Before Florisil or alumina cleanup procedures, a solvent exchange is done by reducing under nitrogen to 300 µL and bulking up to 1 mL in hexane. If toluene is present, the extract is reduced to 50 µL under nitrogen and bulked up to 1 mL.	When the volume of the liquid is approximately 100 µL, add 2 to 3 mL of the hexane and continue concentration to approximately 100 µL. Repeat the addition of solvent and concentrate once more. Bring the final volume to 1.0 mL with hexane.

Section	Modification	Original Method Approach
12.7.7	Toluene (1 mL) is added to the eluate from the final column prior to rotary evaporation and nitrogen concentration steps. Transfer the extract to the autosampler vial, rinsing with hexane and adding the rinse to the vial. Add 15 µL of nonane to the vial, and evaporate the solvent to the level of the nonane. Add 5 µL of labeled injection internal standard spiking solution, as described in 7.14 above.	Quantitatively transfer the extract to a 0.3-mL conical vial for final concentration, rinsing the larger vial with hexane and adding the rinse to the conical vial. Add 20 µL of nonane to the vial, and evaporate the solvent to the level of the nonane.
13.3.1	Routine layered silica column is as follows: 0.5 g neutral silica, 2 g 28% basic silica, 0.5 g neutral silica, 4 g 44% acidic silica, 4 g 22% acidic silica, 1 g neutral silica. *(Note, these masses are half of those in the method, but in the same relative proportions)*	Pack the column with 1 g silica gel, 4 g basic silica gel, 1 g silica gel, 8 g acid silica gel, 2 g silica gel.
13.3.4	The sample is loaded onto the column, followed by 2-3 rinses of a least 1 mL, and eluted with 100 mL of hexane.	Rinse the receiver twice with 1-mL portions of hexane, and apply separately to the column. Elute the PCBs with 25 mL of hexane and collect the eluate.
14.2	The volume of labeled injection internal standard added to the extract is 5 µL, for a final extract volume of 20 µL. Hexane, rather than nonane, is used as the solvent to bring extract back to volume for re-analysis or to dilute extracts.	Add 2 µL of the labeled injection internal standard spiking solution to the 20 µL sample extract immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. If an extract is to be reanalyzed and evaporation has occurred, do not add more labeled injection internal standard spiking solution. Rather, bring the extract back to its previous volume with pure nonane.
15.3	The calibration solution containing all 209 PCB congeners is used as the CAL/VER solution.	Table 4 of the method includes only 27 PCB congeners.
17.0	The concentrations of target analytes, and the labeled compound concentrations and recoveries, are calculated using slight variations of the equations described in the method. The modified procedures are equivalent to those described in the method, but are more direct.	*Note, neither set of equations is reproduced in this QAPP, given their length, but they are on file at CSC.*
17.5	Extracts are diluted with hexane. The concentration of the labeled injection internal standard is not re-adjusted to 100 pg/µL when dilutions are performed.	Dilute the sample extract by the factor necessary to bring the concentration within the calibration range, adjust the concentration of the labeled injection internal standard to 100 pg/µL in the extract.

Note: Given the large number of target analytes involved, the final list of PCB congeners and coelutions is provided in Appendix C of this QAPP.

B5. Quality Control

The analytical procedures being applied by the laboratories designated for analysis of GLHHFTS fish tissue samples include many of the traditional EPA analytical quality control activities. For example, all samples are analyzed in batches and each batch includes:

- up to 20 samples, including both field samples and QC samples
- blanks – 5% of the samples within a batch are method blanks

Other common quality control activities vary by the analysis type, as described in the subsections below.

B5.1 Mercury

Quality control samples associated with each batch of tissue samples analyzed for mercury are summarized in Table 4 below.

The cold-vapor atomic absorption instrument is calibrated daily, as described in SW-846 Method 7470A and the laboratory's SOP. At least five calibration standards and a blank are used for calibration, and the resulting calibration curve must have a correlation coefficient of at least 0.995. The calibration is verified after every 10 samples by the analysis of a mid-range standard. The results for the verification standard must fall within 20% of the true value.

The rinsate samples are prepared and analyzed individually, not in batches of up to 20, in order to provide timely feedback of the cleanliness of the homogenization equipment. Therefore, the quality control samples associated with the rinsate samples analyzed for mercury are usually analyzed with each rinsate sample, and are summarized in Table 5 below.

Table 4. QC Samples and Acceptance Criteria for Mercury Analysis of Fish Tissue

Quality Control Sample	Frequency	Acceptance Criteria
Method blank	One per sample batch	As noted elsewhere, all results, including blanks, are reported down to the MDL. - If the method blank result is above the MDL, but below the laboratory's nominal quantitation limit, the laboratory will flag all associated field sample results as having a detectable method blank for mercury. (Subsequent validation of the results by EPA or its contractors will evaluate the potential contribution of the blank to such field sample results.) - If the method blank result is above the quantitation limit, the laboratory will reanalyze the method blank. • If the reanalysis result is below the quantitation limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the reanalysis result is still above the quantitation limit, then the laboratory will redigest and reanalyze all field samples with original results above the MDL.
Laboratory control sample	One per sample batch	80 - 120% recovery of mercury. Otherwise, correct instrumental problems, and redigest and reanalyze the batch of field samples and QC samples.
Matrix spike sample	One per sample batch	75 - 125% recovery of mercury. Otherwise, compare spiking level to background concentration in unspiked sample. If spiked too low, adjust spiking level for future batches. If spiked at least 5x background, and spike recovery criteria is not met, flag all associated sample results and contact CSC to discuss options for future batches.
Duplicate sample	One per sample batch	RPD ≤ 20%. Flag results outside the limit.

Table 5. QC Samples and Acceptance Criteria for Mercury Analysis of Rinsates

Quality Control Sample	Frequency	Acceptance Criteria
Instrument blank	With each rinsate sample	Result must be less than the MDL. Otherwise redigest and reanalyze the rinsate sample.
Laboratory control sample	With each rinsate sample	80 - 120% recovery of mercury. Otherwise, correct instrumental problems, and redigest and reanalyze the rinsate sample.

Because the rinsates are prepared in reagent water, there is little chance of a "matrix effect" and the laboratory control sample, which is also prepared in reagent water, provides sufficient information on the performance of the method and the laboratory in reagent water.

The rinsate samples for mercury are being analyzed by Microbac Laboratories, during the course of the homogenization of the fish tissue samples. Microbac is analyzing these aqueous samples using EPA Method 245.1, a cold-vapor atomic absorption procedure applicable to water samples. As is the case for the tissue sample analyses, the instrument is calibrated daily as described in Method 245.1

B5.2 PFCs

Quality control samples associated with each batch of tissue samples analyzed for PFCs are summarized in Table 6 below.

Table 6. QC Samples and Acceptance Criteria for PFC Analysis of Fish Tissue

Quality Control Sample	Frequency	Acceptance Criteria
Method blank	One per sample batch	As noted elsewhere, all results, including blanks, are reported down to the MDL. - If the method blank result for any PFC is above the MDL, but below the laboratory's nominal quantitation limit, the laboratory will flag all associated field sample results as having a detectable method blank for that analyte. (Subsequent validation of the results by EPA or its contractors will evaluate the potential contribution of the blank to such field sample results.) - If the method blank result is above the quantitation limit, the laboratory will reanalyze the method blank. • If the method blank reanalysis result is below the quantitation limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the method blank reanalysis result is still above the quantitation limit, then the laboratory will re-extract and reanalyze all field samples with original results above the MDL.
Laboratory control sample	One per sample batch	60 - 140% recovery of each analyte quantified by isotope dilution, and 50 -150% for each analyte quantified by internal standard. Otherwise, correct instrumental problems, and re-extract and reanalyze the batch of field samples and QC samples.
Matrix spike and matrix spike duplicate samples	One pair per sample batch	60 - 140% recovery of each analyte quantified by isotope dilution, and 50 - 150% for each analyte quantified by internal standard. Otherwise, compare spiking level to background concentration in unspiked sample. If spiked too low, adjust spiking level for future batches. If spiked at least 5x background, then correct instrumental problems, and re-extract and reanalyze the batch of field samples and QC samples. If spike recovery criteria still cannot be met, flag all associated sample results. RPD of MS/MSD pair ≤ 30%. Otherwise, flag results outside the limit.
Labeled compound recovery	Every field and QC sample	25 - 150% recovery of each labeled compound. For recoveries <25%, examine results for native analyte and assess impact. Contact CSC for direction, as needed.

The rinsate samples are prepared and analyzed individually, not in batches of up to 20, in order to provide timely feedback of the cleanliness of the homogenization equipment. Therefore, the quality control samples associated with the rinsate samples analyzed for PFCs are summarized in Table 7 below.

Table 7. QC Samples and Acceptance Criteria for PFC Analysis of Rinsates

Quality Control Sample	Frequency	Acceptance Criteria
Method blank	With each rinsate sample	As noted elsewhere, all results, including blanks, are reported down to the MDL. - If the method blank result for any PFC is above the MDL, but below the laboratory's nominal quantitation limit, the laboratory will flag all associated field sample results as having a detectable method blank for that analyte. (Subsequent validation of the results by EPA or its contractors will evaluate the potential contribution of the blank to such field sample results.) - If the method blank result is above the quantitation limit, the laboratory will reanalyze the method blank. • If the method blank reanalysis result is below the quantitation limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the method blank reanalysis result is still above the quantitation limit, then the laboratory will re-extract and reanalyze the rinsate sample if the original rinsate sample results were above the MDL.
Laboratory control sample	With each rinsate sample	60 - 140% recovery of each analyte quantified by isotope dilution, and 50 - 150% for each analyte quantified by internal standard. Otherwise, correct instrumental problems, and re-extract and reanalyze the rinsate sample.
Labeled compound recovery	Every rinsate sample	25 - 150% recovery of each labeled compound. For recoveries <25%, examine results for native analyte and assess impact. Contact CSC for direction, as needed.

Because the rinsates are prepared in reagent water, there is little chance of a "matrix effect" and the laboratory control sample, which is also prepared in reagent water, provides sufficient information on the performance of the laboratory in reagent water.

The HPLC-MS/MS instrument is calibrated using nine calibration standards and modeling the instrument response with either a linear regression through the origin that is based on calculating the response factor (RF) for each standard, or a linear regression not through the origin. For a calibration employing response factors, the relative standard deviation of the nine response factors must be < 35%. If a linear regression that does not pass through the origin is employed, the correlation coefficient must be > 0.995.

The calibration is verified at the beginning of each analytical shift and after every 10 samples, using a single standard that must meet ± 40% for each analyte quantified by isotope dilution, and ± 50% for each analyte quantified by internal standard.

B5.3 Fatty Acids

Quality control samples associated with each batch of tissue samples analyzed for fatty acids are summarized in Table 8 below.

Table 8. QC Samples and Acceptance Criteria for Fatty Acid Analysis of Fish Tissue

Quality Control Sample	Frequency	Acceptance Criteria
Method blank	One per sample batch	As noted elsewhere, all results, including blanks, are reported down to the MDL. - If the method blank result for any fatty acid is above the MDL, but below the laboratory's nominal quantitation limit, the laboratory will flag all associated field sample results as having a detectable method blank for that analyte. (Subsequent validation of the results by EPA or its contractors will evaluate the potential contribution of the blank to such field sample results.) - If the method blank result is above the quantitation limit, the laboratory will reanalyze the method blank. • If the reanalysis result is below the quantitation limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the reanalysis result is still above the quantitation limit, then the laboratory will re-extract and reanalyze all field samples with original results above the MDL.
Surrogate	Every field and QC sample	70 - 130% recovery in each sample. Otherwise re-extract and reanalyze the sample.
Laboratory control sample	One per sample batch	70 - 130% recovery of each target fatty acid. Otherwise, correct instrumental problems, and reanalyze the batch of samples.
Reference material (NIST SRM 1946 Lake Superior fish tissue)	One per sample batch	Within ± 30% of the certified value for each analyte. Otherwise, correct instrumental problems, and re-extract and reanalyze the batch of samples.
Duplicate sample	One per sample batch	RPD ≤ 20%. Flag results outside the limit.

The rinsate samples are prepared and analyzed individually, not in batches of up to 20, in order to provide timely feedback of the cleanliness of the homogenization equipment. Therefore, the quality control samples associated with the rinsate samples analyzed for fatty acids are summarized in Table 9 below.

Table 9. QC Samples and Acceptance Criteria for Fatty Acid Analysis of Rinsates

Quality Control Sample	Frequency	Acceptance Criteria
Method blank	With each rinsate sample	Result must be less than the MDL. Otherwise reanalyze the associated rinsate sample.
Surrogate	Every field and QC sample	70 - 130% recovery in each sample. Otherwise reanalyze the sample.
Laboratory control sample	With each rinsate sample	70 - 130% recovery of each analyte Otherwise, correct instrumental problems, and reanalyze the rinsate sample.

Because the rinsates and blanks are prepared from hexane and no sample extraction is required, "matrix effects" are highly unlikely. Therefore matrix spike and duplicate samples are **not** required for these hexane samples. Analysis of a LCS prepared in hexane with each set of rinsate samples will provide sufficient evidence of the performance of the method in hexane.

The GC/FID is calibrated using a series of six standards of the target analytes that are carried through the esterification process along with the samples. The calibration must attain a correlation coefficient of at least 0.995. The calibration is verified at the beginning and end of each analytical sequence and after every 15 injections using a mid-level calibration standard that must meet ± 20% for each analyte.

B5.4 PBDEs

Quality control samples associated with each batch of tissue samples analyzed for PBDEs are summarized in Table 10 below.

Table 10. QC Samples and Acceptance Criteria for PBDE Analysis of Fish Tissue

Quality Control Sample	Frequency	Acceptance Criteria
Method blank	One per sample batch	As noted elsewhere, all results, including blanks, are reported down to the MDL. Given the ubiquitous nature of PBDEs, it can be difficult to produce method blanks that are completely free of these analytes. The following scheme will be used by the laboratory to evaluate method blank results: • If any PBDE congener other than 28, 47, 99, 100, and 209 is found in the method blank above the concentration equivalent to the low point of the initial calibration (e.g., EPA's Minimum Level in Method 1614A), analysis will be halted until the source of the contamination can be identified and corrected. Samples associated with a contaminated method blank will be re-extracted and reanalyzed. • For the frequently occurring congeners 28, 47, 99, 100, and 209, similar corrective action will be taken if the results are greater than 2 times the low point of the initial calibration. **Note:** CSC will evaluate the sample results based on the relation between the reported *detection limit* for each congener in the method blank and the results in the field sample, and qualify sample results accordingly.
Laboratory duplicate sample	One per sample batch	The acceptance criterion for the relative percent difference (RPD) is 50% for results that are above the low point of the calibration range. Results below that level will be compared, but may not meet the acceptance criterion in every case.
Laboratory control sample	One per sample batch	See acceptance criteria in Table 11
Labeled compounds	Spiked into every field sample	See acceptance criteria in Table 12

Table 11. Laboratory Control Sample Limits (%) for PBDE Analysis

Compound	Lower Limit	Upper Limit	Source
Native Analytes			
BDE-7	20	150	In-house
BDE-8/11	20	150	In-house
BDE-10	20	150	In-house
BDE-12/13	20	150	In-house
BDE-15	50	150	In-house
BDE-17/25	50	150	In-house
BDE-28/33	50	150	1614A
BDE-30	20	150	In-house
BDE-32	50	150	In-house
BDE-35	50	150	In-house
BDE-37	50	150	In-house
BDE-47	50	150	1614A
BDE-49	50	150	In-house
BDE-51	50	150	In-house
BDE-66	50	150	In-house

Compound	Lower Limit	Upper Limit	Source
BDE-71	50	150	In-house
BDE-75	50	150	In-house
BDE-77	50	150	In-house
BDE-79	50	150	In-house
BDE-85	50	150	In-house
BDE-99	50	150	1614A
BDE-100	50	150	1614A
BDE-105	50	150	In-house
BDE-116	50	150	In-house
BDE-118	50	150	In-house
BDE-119/120	50	150	In-house
BDE-126	50	150	In-house
BDE-128	50	150	In-house
BDE-138/166	50	150	In-house
BDE-140	50	150	In-house
BDE-153	50	150	1614A
BDE-154	50	150	1614A
BDE-155	50	150	In-house
BDE-156	50	150	In-house
BDE-181	50	150	In-house
BDE-183	50	150	1614A
BDE-184	50	150	In-house
BDE-190	50	150	In-house
BDE-191	50	150	In-house
BDE-196	50	150	In-house
BDE-197	50	150	In-house
BDE-203	50	150	In-house
BDE-206	50	200	In-house
BDE-207	50	200	In-house
BDE-208	50	200	In-house
BDE-209	50	200	1614A
PBEB	50	150	In-house
HBB	50	150	In-house
Extraction Standards			
$^{13}C_{12}$-BDE-3	10	140	In-house
$^{13}C_{12}$-BDE-15	20	140	In-house
$^{13}C_{12}$-BDE-28	30	140	1614A
$^{13}C_{6}$-HBB	30	140	In-house
$^{13}C_{12}$-BDE-47	30	140	1614A
$^{13}C_{12}$-BDE-77	30	140	In-house
$^{13}C_{12}$-BDE-99	30	140	1614A
$^{13}C_{12}$-BDE-100	30	140	1614A
$^{13}C_{12}$-BDE-126	30	140	In-house
$^{13}C_{12}$-BDE-153	30	140	1614A
$^{13}C_{12}$-BDE-154	30	140	1614A
$^{13}C_{12}$-BDE-183	30	140	1614A
$^{13}C_{12}$-BDE-197	30	140	In-house
$^{13}C_{12}$-BDE-207	25	200	In-house
$^{13}C_{12}$-BDE-209	25	200	1614A
Cleanup Standard			
$^{13}C_{12}$-BDE-138	40	125	1614A

Table 12. Labeled Compound Recovery Limits (%) for PBDEs in Samples

Compound	Lower Limit	Upper Limit	Source
Extraction Standards			
$^{13}C_{12}$-BDE-3	10	150	In-house
$^{13}C_{12}$-BDE-15	20	150	In-house
$^{13}C_{12}$-BDE-28	25	150	1614A
$^{13}C_{6}$-HBB	25	150	In-house
$^{13}C_{12}$-BDE-47	25	150	1614A
$^{13}C_{12}$-BDE-77	25	150	In-house
$^{13}C_{12}$-BDE-99	25	150	1614A
$^{13}C_{12}$-BDE-100	25	150	1614A
$^{13}C_{12}$-BDE-126	25	150	In-house
$^{13}C_{12}$-BDE-153	25	150	1614A
$^{13}C_{12}$-BDE-154	25	150	1614A
$^{13}C_{12}$-BDE-183	25	150	1614A
$^{13}C_{12}$-BDE-197	25	150	In-house
$^{13}C_{12}$-BDE-207	20	200	In-house
$^{13}C_{12}$-BDE-209	20	200	1614A
Cleanup Standard			
$^{13}C_{12}$-BDE-138	40	125	1614A

B5.5 PCBs

Quality control samples associated with each batch of tissue samples analyzed for PCBs are summarized in Table 13, below, and are based on the QC requirements of Method 1668C, with the project-specific addition of one laboratory duplicate sample per batch.

Table 13. QC Samples and Acceptance Criteria for PCB Analysis of Fish Tissue

Quality Control Sample	Frequency	Acceptance Criteria
Method blank	One per sample batch	5 times the MDL for each congener (As noted elsewhere, all results, including blanks, are reported down to the MDL.) If the method blank result is above 5 times the MDL, the laboratory will reanalyze the method blank extract. • If the reanalysis result is below 5 times the MDL, then the laboratory will reanalyze all of the associated field and QC samples. • If the reanalysis result is still above 5 times the MDL, then the laboratory will re-extract and reanalyze all field samples with original results above the MDL.
Laboratory duplicate sample	One per sample batch	The RPD of the duplicate measurements must be: • < 50% for sample concentrations greater than or equal to 5 times the MDL, and • <100% for sample concentrations less than 5 times the MDL. (When comparing the sample concentration to the MDL, use the lower of the two concentrations in the paired samples.) If the RPD exceeds the acceptance limit, the laboratory will reanalyze the laboratory duplicate extract. • If the reanalysis result does not exceed the RPD limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the reanalysis result still exceeds the RPD limit, then the laboratory will re-extract and reanalyze all field samples with original results above the MDL.

Quality Control Sample	Frequency	Acceptance Criteria
Laboratory control sample	One per sample batch	Per Table 14 below
Labeled compounds	Spiked into every field sample	Per Table 14 below
Calibration verification (VER)	At the beginning of every 12-h analytical shift	Per Table 14 below

Table 14. Calibration Verification Limits (%), Laboratory Control Sample Recovery Limits (%), and Labeled Compound Recovery Limits (%) for PCB Analyses[1]

Compound	CAL VER	LCS Recovery	Labeled Compound Recovery in Samples
PCB-1	75 - 125	60 - 135	Limits for each labeled compound are shown below
PCB-3	75 - 125	60 - 135	
PCB-4	75 - 125	60 - 135	
PCB-15	75 - 125	60 - 135	
PCB-19	75 - 125	60 - 135	
PCB-37	75 - 125	60 - 135	
PCB-54	75 - 125	60 - 135	
PCB-77	75 - 125	60 - 135	
PCB-81	75 - 125	60 - 135	
PCB-104	75 - 125	60 - 135	
PCB-105	75 - 125	60 - 135	
PCB-114	75 - 125	60 - 135	
PCB-118	75 - 125	60 - 135	
PCB-123	75 - 125	60 - 135	
PCB-126	75 - 125	60 - 135	
PCB-155	75 - 125	60 - 135	
PCB-156	75 - 125	60 - 135	
PCB-157	75 - 125	60 - 135	
PCB-167	75 - 125	60 - 135	
PCB-169	75 - 125	60 - 135	
PCB-188	75 - 125	60 - 135	
PCB-189	75 - 125	60 - 135	
PCB-202	75 - 125	60 - 135	
PCB-205	75 - 125	60 - 135	
PCB-206	75 - 125	60 - 135	
PCB-208	75 - 125	60 - 135	
PCB-209	75 - 125	60 - 135	
^{13}C-Labeled Compounds			
$^{13}C_{12}$-PCB-1	50 - 145	15 - 145	5 - 145
$^{13}C_{12}$-PCB-3	50 - 145	15 - 145	5 - 145
$^{13}C_{12}$-PCB-4	50 - 145	15 - 145	5 - 145
$^{13}C_{12}$-PCB-15	50 - 145	15 - 145	5 - 145
$^{13}C_{12}$-PCB-19	50 - 145	15 - 145	5 - 145
$^{13}C_{12}$-PCB-37	50 - 145	15 - 145	5 - 145
$^{13}C_{12}$-PCB-54	50 - 145	15 - 145	5 - 145
$^{13}C_{12}$-PCB-77	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-81	50 - 145	40 - 145	10 - 145

Compound	CAL VER	LCS Recovery	Labeled Compound Recovery in Samples
$^{13}C_{12}$-PCB-104	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-105	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-114	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-118	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-123	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-126	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-155	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-156	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-157	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-167	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-169	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-170	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-180	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-188	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-189	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-202	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-205	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-206	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-208	50 - 145	40 - 145	10 - 145
$^{13}C_{12}$-PCB-209	50 - 145	40 - 145	10 - 145
Cleanup Standards			
$^{13}C_{12}$-PCB-28	65 - 135	15 - 145	5 - 145
$^{13}C_{12}$-PCB-111	75 - 125	40 - 145	10 - 145
$^{13}C_{12}$-PCB-178	75 - 125	40 - 145	10 - 145

[1] Adapted from Table 6 of Method 1668C

B6. Instrument/Equipment Testing, Inspection, and Maintenance

The inspection, testing, and maintenance of all laboratory equipment and instrumentation is addressed in the individual laboratory operating procedures to be used, or in each laboratory's existing overall quality system documentation (TestAmerica 2010, TestAmerica 2011, Southwest Research Institute 2010, ALS 2009, and AXYS 2012). There are no additional requirements specific to this project, and therefore, none are described here.

B7. Instrument/Equipment Calibration and Frequency

Each laboratory's instrument calibration procedures and frequency are included in Section B5 of this QAPP and in the laboratory's existing overall quality system documentation (TestAmerica 2010, TestAmerica 2011, Southwest Research Institute 2010, ALS 2009, and AXYS 2012). No additional discussion is required.

B8. Inspection/Acceptance of Supplies and Consumables

The inspection and acceptance of any laboratory supplies and consumables are addressed in the individual laboratory operating procedures to be used, and/or in the laboratory's existing overall quality system documentation (TestAmerica 2010, TestAmerica 2011, Southwest Research Institute 2010, ALS 2009, and AXYS 2012). There are no additional requirements specific to this project, and therefore, none are described here.

B9. Non-direct Measurements

Non-direct measurements are not required for this project.

B10. Data Management

Data management practices employed in this study will be based on standard data management practices used for EPA's National Lake Fish Tissue Study. The data management (i.e., sample tracking, data tracking, data inspection, data quality assessment, database development) procedures have been regularly applied to other technical studies. These procedures are being employed because they are effective, efficient, and have successfully withstood repeated internal and external audits, including internal review by EPA Quality Staff, public review and comment, judicial challenge, and the Government Accountability Office audit. These procedures, as implemented for the GLHHFTS, are summarized below.

Laboratory Data Management

Laboratory data management procedures include the following:

- Each laboratory is required to maintain all records and documentation associated with the preparation and analysis of study samples for a minimum period of five years after completion of the study.
- To facilitate data tracking, each laboratory is required to use EPA-assigned sample numbers when reporting results.
- All results of field sample analyses and QC sample analyses must be reported on electronic media.
- All required reports and documentation, including raw data, must be sequentially paginated and clearly labeled with the laboratory name, contract number, episode number, and associated EPA sample numbers. Any electronic media submitted must be similarly labeled.
- Each laboratory will adhere to a comprehensive data management plan that is consistent with the principles set forth in Good Automated Laboratory Practices, EPA Office of Administration and Resources Management, October 10, 1995 (USEPA 1995). Those data management plans are incorporated in their overall quality system documentation (e.g., their quality management plan, TestAmerica 2010, TestAmerica 2011, Southwest Research Institute 2010, ALS 2009, and AXYS 2012).

CSC Data Management

Data management procedures employed by CSC include the use of 1) standardized data review guidelines to promote consistency in data quality audits (data reviews) across reviewers and over time, 2) a multi-stage data review process designed to maximize the amount of useable data generated in each study, and 3) a standardized database development process that facilitates rapid development of a database with at least 99.9% accuracy.

Standardized data review guidelines will be used in this study to facilitate rapid, consistent, accurate, and thorough data quality audits. The data review guidelines are those that were employed for the National Lake Fish Tissue Study and are in use for a variety of analyses performed for EPA programs. These guidelines detail method-specific data review procedures for commonly used methods and more general procedures that can be applied to less frequently used methods. Where appropriate, CSC will modify existing data review guidelines as necessary to reflect the methods, method modifications, and data quality objectives for the GLHHFTS. Descriptions of any modifications will be retained in CSC's project records.

Although each guideline is written for a specific method, technique, or group of analytes, all guidelines specify a general review process that ensure data are in proper format, are complete, are contractually compliant, and are usable. CSC data review chemists use this multi-stage process to verify the quality of each laboratory submission under the GLHHFTS. If an error is detected in any stage of the review, the CSC data review chemist and the CSC Project Leader will initiate corrective action procedures to obtain the maximum amount of usable data from the study. These actions may serve to obtain missing data, correct typographical or transcription errors on data reporting forms, or initiate reanalysis of field or QC samples that do not meet the performance criteria for this study. Any such actions will be documented in CSC's project records.

Concurrent with the performance of data quality audits, CSC will begin developing a MS Access database of combined field and analytical results for tissue samples. At a minimum, this database will be formatted in a manner that is consistent with the National Lake Fish Tissue Study. At a minimum, each record should include fields containing the following information for each tissue sample:

- the site identification number assigned by EPA;
- the EPA sample number;
- sample matrix (tissue);
- sample type (indicates the type of sample, whether it was a primary composite, duplicate composite, matrix spike, etc);
- fish species (scientific and common names);
- fish specimen number;
- length of fish specimen;
- weight of fish specimen;
- retention of fish specimen for homogenization;
- method of collection;
- sample collection date;
- Great Lake from which samples were collected;
- state where site is located;
- latitude/longitude where site is located;
- year samples were collected;
- ecological group for fish samples (predator or bottom dweller);
- sample analysis date;
- measured value for each target analyte; and
- fish tissue lipid content measurements.

The MS Access database will contain the field and analytical results from all study samples until the complete data set is transmitted to EPA. The database also will contain data for the QC samples described in Section B, associated with the field sample results, as well as applicable surrogates and labeled compounds. The structure of the database will allow CSC to segregate these QC results from those in the field samples.

Results for the rinsate samples will not be included in the same database, but will be used to assess the homogenization and equipment cleaning procedures employed during sample preparation activities before the analyses take place.

As with the data quality audits, a multi-stage process of inspections and corrective actions are used to facilitate timely, efficient construction of databases that are least 99.9% accurate. The database development process will begin with a completeness check to verify the laboratory has submitted data on an electronic medium that contains all data in an appropriate format. If deficiencies are found, appropriate corrective action measures will be initiated.

The CSC data review chemist responsible for performing the data quality audit will verify that the electronic data accurately reflect the hard copy submission. Accuracy will be confirmed by spot checking at least 10% of all results that were downloaded directly from an analytical instrument in the laboratory and by performing a 100% QC check of data that were manually entered by the laboratory or CSC. Corrective actions will be taken as needed to resolve deficiencies. Following completion of the data quality audit, the CSC data review chemist and the CSC database administrator will modify the database to reflect data usability determinations. A report, generated to reflect the modified database, will then be reviewed by the CSC data review chemist to verify database accuracy before submission to EPA. These reports are maintained in CSC's project files.

C. ASSESSMENT AND OVERSIGHT

C1. Assessments and Response Actions

The laboratory contracts prepared to support this study stipulate that each laboratory has a comprehensive QA program in place and operating at all times during the performance of their contract, and that in performing laboratory work for this study, each laboratory shall adhere to the requirements of that QA program (TestAmerica 2010, TestAmerica 2011, Southwest Research Institute 2010, ALS 2009, and AXYS 2012).

Sections C1.1 through C1.6 describe other types of assessment activities and corresponding response actions identified to ensure that data gathering activities in the GLHHFTS are conducted as prescribed and that the performance criteria defined for the study are met.

C1.1 Surveillance

The CSC Project Leader will schedule and track all analytical work performed by laboratories for mercury, PFC, fatty acid, and PBDE analyses. The Project Leader will coordinate with staff at Microbac regarding fish tissue sample shipments.

When samples are shipped to an analytical laboratory, the Project Leader will contact designated laboratory staff by email to notify them of the forthcoming shipment(s) and request that they

contact CSC if the shipments do not arrive intact as scheduled. Within 24 hours of scheduled sample receipt, CSC will contact the laboratory to verify that the samples arrived in good condition, and if problems are noted, will work with the laboratory and EPA to resolve the problem as quickly as possible to minimize data integrity problems.

CSC also will communicate periodically with laboratory staff by telephone or email to monitor the progress of analytical sample preparation, sample analysis, and data reporting. If technical problems are encountered during sample preparation and analysis, CSC will identify a technical expert within CSC to assist in resolving the problem, and work with EPA to identify and implement a solution to the problem. If the laboratory fails to deliver data on time, or if the laboratory notifies CSC of anticipated reporting delays, CSC will notify the EPA Project Manager of the situation. To the extent possible, CSC will adjust schedules and shift resources within CSC as necessary to minimize the impact of laboratory delays on EPA schedules. CSC also will immediately notify the Project Manager of any laboratory delays that are anticipated to impact EPA schedules.

Finally, the CSC Project Leader will monitor the progress of the data quality audits (data reviews) and database development to ensure that each laboratory data submission is reviewed in a timely manner. In the event that dedicated staff are not able to meet EPA schedules, CSC will identify additional staff who are qualified and capable of reviewing the data in a timely manner. If such resources cannot be identified, and if training new employees is not feasible, CSC will meet with the EPA Project Manager to discuss an appropriate solution.

C1.2 Product Review

Product reviews for validated analytical data packages will be performed within CSC to verify that the CSC data reviews are being performed consistently over time and across data reviewers, that the review findings are technically correct, and that the reviews are being performed in accordance with this QAPP. Product reviewers will be charged with evaluating the completeness of the original CSC data review, the technical accuracy of the reviewer's findings, and the technical accuracy of the analytical database developed to store results associated with the data package. The CSC data reviewers will be responsible for identifying and assigning qualified product reviewers and for selecting packages to be product reviewed. Qualified product reviewers will include any staff members that have been trained in CSC data review procedures, that are experienced in reviewing data similar to those being reviewed, and are familiar with the requirements of this QAPP. To ensure the findings of each data review are documented in a consistent and technically accurate manner, CSC staff will review 100% of the data qualifier flags entered into the project database.

The EPA Project Manager and SHPD QA Coordinator will review the analytical QA report developed by CSC, and the EPA Project Manager will approve the final analytical QA report. The GLHHFTS data files prepared by CSC for statistical analysis of the data will be reviewed internally by CSC staff and independently by the EPA Project Manager with support from Tetra Tech before being forwarded to Tony Olsen at NHEERL-Corvallis, who will complete statistical analysis of the GLHHFTS data and deliver the results to the EPA Project Manager.

C1.3 Quality Systems Audit

A quality system audit (QSA) is used to verify, by examination and evaluations of objective evidence, that applicable elements of the quality system are appropriate and have been developed, documented, and effectively implemented in accordance and in conjunction with specified requirements. The focus of these assessments is on the quality system processes – not on evaluating the quality of specific products or judging the quality of environmental data or the performance of personnel or programs.

The SHPD QA Coordinator may perform a QSA of the GLHHFTS mercury, PFC, fatty acid, and PBDE analyses portion of the NCCA.

C1.4 Readiness Review

A readiness review of each laboratory's capability to produce precise and accurate results with the methods specified in this study will be performed before the laboratory is allowed to analyze field samples collected during the study. Because there are no formal EPA methods for most of these analytes in tissue samples, there are no independent acceptance criteria by which to judge laboratory performance. As part of the laboratory contracting process, CSC has requested information from each laboratory regarding their demonstrated detection and quantitation limits in tissue samples, as well as their in-house QC acceptance criteria for all QC operations associated with the methods that each laboratory proposes to use for this project. CSC has also requested information regarding each laboratory's capacity for the analyses (e.g., how many samples per month), any relevant proficiency testing results in tissue samples, and any accreditations relevant to tissue analyses. CSC will examine all information provided by each laboratory to determine if the laboratory is capable of supporting the project.

Readiness reviews will be performed by CSC data reviewers. If problems are identified during these reviews, CSC will work with the laboratory, to the extent possible, to resolve the problem. If the problem cannot be resolved within the time frame required by EPA or within the scope of the laboratory's existing contract, CSC will notify the EPA Project Manager immediately. Records of these reviews and any corrective actions are maintained by CSC separate from the analytical results for the field samples. CSC staff will document their findings and recommendations concerning the readiness review as part of a written analytical QA report to EPA.

C1.5 Technical Systems Audit

Each laboratory contract will require that the laboratory be prepared for and willing to undergo an on-site, or technical systems, audit of its facilities, equipment, staff, and sample analysis, training, record keeping, data validation, data management, and data reporting procedures. An audit will be conducted only if the results of the readiness reviews, data quality audits, and surveillance suggest serious or chronic laboratory problems that warrant on-site examinations and discussion with laboratory personnel.

If such an audit is determined to be necessary, a standardized audit checklist may be used to facilitate an audit walkthrough and document audit findings. Audit participants may include the EPA Project Manager or the SHPD QA Coordinator (or a qualified EPA staff member designated by the OST QA Officer) and a CSC staff member experienced in conducting

laboratory audits. One audit team member will be responsible for leading the audit and conducting a post-audit debriefing to convey significant findings to laboratory staff at the conclusion of the audit. The other audit team member will be responsible for gathering pre-audit documentation of problems that necessitated the audit, customizing the audit checklist as necessary to ensure that those problems are addressed during the audit, documenting audit findings on the audit checklist during the audit, and drafting a formal report of audit findings for review by EPA.

C1.6 Data Quality Assessment

Upon completion of data verification and validation procedures (see Section D1), CSC will create an analytical database that contains all field sample results from the GLHHFTS (see Section B10).

At selected intervals and upon completion of the study, CSC's database development staff will perform statistical analyses to verify the accuracy of the database. The statistical procedures will be directed at evaluating the overall quality of the database against data quality objectives established for the study, and in identifying trends in field and QC results obtained during the study. CSC staff will document their findings and recommendations concerning this data quality assessment as part of a written analytical QA report to EPA.

C2. Reports to Management

Following data verification and validation of all project data, CSC will apply standardized data qualifier flags to the results in the project database that describe data quality limitations and recommendations concerning data use. The data qualifier flags are based on those developed for the National Lake Fish Tissue Survey and the complete list of qualifier flags and their implications for data use will be summarized in a report to EPA at or near the end of the data assessment process.

On request, CSC also will provide a report that describes the status of all current analysis and data review activities, and periodic database status reports that provide up-to-date information concerning database revisions that occurred since distribution of previous reports.

D. DATA VALIDATION AND USABILITY

This QAPP addresses the generation of data for mercury, PFCs, fatty acids, PBDEs, and PCBs in fish tissue samples. Sections D1, D2, and D3 of this QAPP apply to all of the analytical data generation for the GLHHFTS.

D1. Data Review, Verification, and Validation

D1.1 Data Review

All laboratory results and calculations will be reviewed by the Laboratory Manager prior to data submission. Any errors identified during this peer review will be returned to the analyst for correction prior to submission of the data package. Following correction of the errors, the Laboratory Manager will verify that the final package is complete and compliant with the

contract, and will sign each data submission to certify that the package was reviewed and determined to be in compliance with the terms and conditions of the contract.

D1.2 Data Verification

The basic goal of data verification is to ensure that project participants know what data were produced, if they are complete, if they are contractually compliant, and the extent to which they meet the objectives of the study.

Every laboratory data package submitted under this study will be subjected to data verification by qualified CSC staff who have been trained in procedures for verifying data and who are familiar with the laboratory methods used to analyze the samples. This includes all of the mercury, PFC, fatty acid, PBDE, and PCB analysis results generated under this QAPP. The verification process is designed to identify and correct data deficiencies as early as possible in order to maximize the amount of usable data generated during this study.

CSC staff will also conduct reviews of the QC sample results for homogenized fish tissue samples prepared by Microbac. This will involve review of data for percent lipid measurements that serve as a surrogate for homogeneity testing and review of the results from reagent water rinsates, methanol rinsates, and hexane rinsates of the sample processing equipment, which are analyzed by Microbac and the other contract laboratories. The CSC Project Leader will verify the summary level results for these QC samples, determine if they meet the project objectives in this QAPP, and report the verification findings to OST.

D1.3 Data Validation

Data validation is the process of evaluating the quality of the results relative to their intended use. Data need not be "perfect" to be usable for a particular project, and the validation process is designed to identify data quality issues uncovered during the verification process that may affect the intended use. One goal of validation is to answer the "So what?" question with regard to any data quality issues.

CSC data review chemists will validate all of the mercury, PFC, fatty acid, PBDE, and PCB analysis results to be generated under this QAPP.

D2. Verification and Validation Methods

D2.1 Verification Methods

In the first stage of the data verification process, CSC data review chemists will perform a "Data Completeness Check" in which all elements in each laboratory submission will be evaluated to verify that results for all specified samples are provided, that data are reported in the correct format, and that all relevant information, such as preparation and analysis logs, are included in the data package. Corrective action procedures will be initiated if deficiencies are noted.

The second stage of the verification process will focus on an "Instrument Performance Check" in which the CSC data review chemists will verify that calibrations, calibration verifications, standards, and calibration blanks were analyzed at the appropriate frequency and met method or

study performance specifications. If errors are noted at this stage, corrective action procedures will be initiated immediately.

Stage three of the verification process will focus on a "Laboratory Performance Check" in which CSC data review chemists will verify that the laboratory correctly performed the required analytical procedures and was able to demonstrate a high level of precision and accuracy. This stage includes evaluation of QC elements such as the laboratory control samples, method blanks, matrix spike samples and/or reference samples, where applicable. Corrective action procedures will be initiated with the laboratories to resolve any deficiencies identified.

In stage four of the verification process, the CSC data review chemist will perform a "Method/Matrix Performance Check" to discern whether any QC failures are a result of laboratory performance or difficulties with the method or sample matrix. Data evaluated in this stage may include matrix spike, matrix spike duplicate, duplicate sample, reference sample, labeled compound, and surrogate spike results. The CSC data review chemist also will verify that proper sample dilutions were performed and that necessary sample cleanup steps were taken. If problems are encountered, the CSC data review chemist will immediately implement corrective actions.

D2.2 Validation Methods

CSC data review chemists will perform a data quality and usability assessment in which the overall quality of data is evaluated against the performance criteria (see Section B5 for a description of performance criteria). This assessment will strive to maximize use of data gathered in this study based on performance criteria established for this study. This will be accomplished by evaluating the overall quality of a particular data set rather than focusing on individual QC failures. Results of this assessment will be documented in a project QA report developed after all of the results have been evaluated, and before they are used in any final decision making.

D3. Reconciliation with User Requirements

As data qualifier flags are applied to the project results to identify any results that did not meet the method- or project-specific requirements, CSC data review chemists still may also apply additional qualifiers that indicate an assessment of the impact of the problem. For example, individual sample results are often qualified based on the presence of the analyte in a method blank associated with samples prepared together (e.g., extracted or digested in the same batch). While it is important to identify any result associated with the presence of the analyte in the blank, the relative significance of the potential for sample contamination will be assessed using commonly accepted "rules." In instances where the amount of the analyte found in the method blank has very limited potential to affect the field sample result, an additional data qualifier will be applied to that field sample result to indicate that the result was not affected by the observed blank contamination. Similar assessments made for other data quality concerns may result in the application of additional flags that reconcile the observed data quality concerns with the user requirements and warn the end user of any limitations to the results (i.e., potential low or high bias, qualitative uncertainty, etc.). All of the data qualifiers will be included in the database along with summary level comments that explain the implication in relatively plain English.

Where data quality concerns suggest that no valid result was produced for a given analyte, the result for that analyte will be excluded from the database, and the comments will provide the rationale for the exclusion. As noted earlier, the overall verification and validation process is designed to maximize the amount of usable data for the project, and excluding results from the final database is intended as a last resort.

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Appendix A
List of Great Lakes Human Health Fish Tissue Study Sampling Locations

List of Great Lakes Human Health Fish Tissue Study Sampling Locations				
Site ID Number	**Lake**	**State**	**Latitude**	**Longitude**
NCCAGL10-1001	Lake Superior	Minnesota	47.14114	-91.45036
NCCAGL10-1002	Lake Superior	Minnesota	47.55628	-90.86774
NCCAGL10-1003	Lake Superior	Michigan	47.38864	-87.92476
NCCAGL10-1005	Lake Superior	Wisconsin	46.77051	-91.62224
NCCAGL10-1006	Lake Superior	Michigan	46.88719	-88.32472
NCCAGL10-1007	Lake Superior	Michigan	47.28380	-88.51741
NCCAGL10-1008	Lake Superior	Michigan	46.68530	-86.16970
NCCAGL10-1009	Lake Superior	Wisconsin	46.95817	-90.84602
NCCAGL10-1010	Lake Superior	Michigan	46.92451	-87.84378
NCCAGL10-1011	Lake Superior	Minnesota	46.79049	-92.04478
NCCAGL10-1012	Lake Superior	Michigan	46.79342	-85.23359
NCCAGL10-1013	Lake Superior	Wisconsin	46.67280	-90.81696
NCCAGL10-1014	Lake Superior	Michigan	47.04289	-88.98127
NCCAGL10-1015	Lake Superior	Michigan	46.51201	-87.14860
NCCAGL10-1018	Lake Superior	Michigan	46.48751	-86.74091
NCCAGL10-1019	Lake Superior	Wisconsin	46.72925	-91.78798
NCCAGL10-1021	Lake Superior	Michigan	46.72029	-85.76284
NCCAGL10-1022	Lake Superior	Michigan	46.73077	-89.96820
NCCAGL10-1023	Lake Superior	Michigan	46.84623	-89.57309
NCCAGL10-1024	Lake Superior	Michigan	46.68694	-85.50666
NCCAGL10-1025	Lake Superior	Michigan	46.58207	-90.40632
NCCAGL10-1026	Lake Superior	Michigan	47.11605	-88.91043
NCCAGL10-1028	Lake Superior	Wisconsin	46.81943	-91.33555
NCCAGL10-1029	Lake Superior	Minnesota	47.71590	-90.47964
NCCAGL10-1030	Lake Superior	Michigan	46.71267	-86.00895
NCCAGL10-1031	Lake Superior	Minnesota	47.24485	-91.31001
NCCAGL10-1032	Lake Superior	Michigan	46.87086	-88.23574
NCCAGL10-1033	Lake Superior	Wisconsin	46.67815	-90.78103
NCCAGL10-1034	Lake Superior	Michigan	46.90615	-89.34169
NCCAGL10-1035	Lake Superior	Michigan	46.54149	-86.96612
NCCAGL10-1036	Lake Superior	Michigan	46.91310	-89.26228
NCCAGL10-1037	Lake Superior	Michigan	46.65890	-87.46141
NCCAGL10-1039	Lake Superior	Minnesota	47.78138	-90.16989
NCCAGL10-1040	Lake Superior	Michigan	47.23721	-88.63144
NCCAGL10-1041	Lake Superior	Wisconsin	46.71701	-91.99021
NCCAGL10-1043	Lake Superior	Michigan	46.79607	-84.98365
NCCAGL10-1044	Lake Superior	Wisconsin	46.61594	-90.55170
NCCAGL10-1046	Lake Michigan	Michigan	45.93795	-84.99405
NCCAGL10-1048	Lake Michigan	Wisconsin	42.61470	-87.81058
NCCAGL10-1051	Lake Michigan	Michigan	45.76909	-86.74218
NCCAGL10-1054	Lake Michigan	Michigan	45.00071	-85.47705
NCCAGL10-1055	Lake Michigan	Michigan	44.94403	-85.84072
NCCAGL10-1056	Lake Michigan	Michigan	44.39603	-86.30882
NCCAGL10-QLM-10-01	Lake Michigan	Illinois	42.46726	-87.77979
NCCAGL10-1058	Lake Michigan	Michigan	45.88809	-86.25744
NCCAGL10-1059	Lake Michigan	Wisconsin	43.32892	-87.86407
NCCAGL10-QLM-10-05	Lake Michigan	Illinois	42.34262	-87.82165
NCCAGL10-1062	Lake Michigan	Michigan	42.94420	-86.24677
NCCAGL10-1064	Lake Michigan	Michigan	45.79750	-84.79227
NCCAGL10-1065	Lake Michigan	Michigan	43.10240	-86.27177
NCCAGL10-1067	Lake Michigan	Michigan	45.93451	-85.71997
NCCAGL10-1068	Lake Michigan	Michigan	45.09776	-85.69919
NCCAGL10-1069	Lake Michigan	Indiana	41.66361	-87.26672

List of Great Lakes Human Health Fish Tissue Study Sampling Locations				
Site ID Number	**Lake**	**State**	**Latitude**	**Longitude**
NCCAGL10-1071	Lake Michigan	Wisconsin	43.71907	-87.65705
NCCAGL10-1072	Lake Michigan	Michigan	44.31255	-86.29955
NCCAGL10-1073	Lake Michigan	Michigan	46.05134	-85.24134
NCCAGL10-1074	Lake Michigan	Michigan	45.73460	-86.77751
NCCAGL10-1075	Lake Michigan	Wisconsin	44.69307	-87.76815
NCCAGL10-1077	Lake Michigan	Michigan	45.69042	-86.90180
NCCAGL10-1078	Lake Michigan	Michigan	45.93913	-85.91562
NCCAGL10-1081	Lake Michigan	Wisconsin	43.04134	-87.86151
NCCAGL10-1082	Lake Michigan	Wisconsin	44.01482	-87.62677
NCCAGL10-1083	Lake Michigan	Michigan	42.20083	-86.41114
NCCAGL10-1084	Lake Michigan	Michigan	45.73852	-86.46434
NCCAGL10-1085	Lake Michigan	Michigan	42.73211	-86.26326
NCCAGL10-QLM-10-20	Lake Michigan	Illinois	41.91638	-87.60207
NCCAGL10-1088	Lake Michigan	Indiana	41.70618	-87.50341
NCCAGL10-1090	Lake Michigan	Michigan	42.11036	-86.50359
NCCAGL10-1091	Lake Huron	Michigan	43.66117	-83.81375
NCCAGL10-1093	Lake Huron	Michigan	45.75036	-84.56395
NCCAGL10-1094	Lake Huron	Michigan	44.83935	-83.24250
NCCAGL10-1096	Lake Huron	Michigan	45.96307	-84.71430
NCCAGL10-1097	Lake Huron	Michigan	45.37810	-83.64797
NCCAGL10-1099	Lake Huron	Michigan	44.00505	-83.22758
NCCAGL10-1100	Lake Huron	Michigan	45.93965	-84.66939
NCCAGL10-1101	Lake Huron	Michigan	45.00660	-83.35906
NCCAGL10-1102	Lake Huron	Michigan	43.87991	-83.43664
NCCAGL10-1103	Lake Huron	Michigan	44.01350	-82.76739
NCCAGL10-1104	Lake Huron	Michigan	45.96071	-84.41915
NCCAGL10-1105	Lake Huron	Michigan	45.18651	-83.33389
NCCAGL10-1106	Lake Huron	Michigan	44.26273	-83.47572
NCCAGL10-1107	Lake Huron	Michigan	45.36534	-83.55898
NCCAGL10-1108	Lake Huron	Michigan	43.93339	-83.37510
NCCAGL10-1110	Lake Huron	Michigan	44.97528	-83.44224
NCCAGL10-1113	Lake Huron	Michigan	45.70090	-84.35747
NCCAGL10-1115	Lake Huron	Michigan	44.04340	-82.72642
NCCAGL10-1116	Lake Huron	Michigan	45.97315	-84.56772
NCCAGL10-1120	Lake Huron	Michigan	43.77893	-83.88343
NCCAGL10-1121	Lake Huron	Michigan	44.00634	-83.16876
NCCAGL10-1122	Lake Huron	Michigan	45.76363	-84.64443
NCCAGL10-1124	Lake Huron	Michigan	45.68168	-84.48027
NCCAGL10-1125	Lake Huron	Michigan	45.13464	-83.32074
NCCAGL10-1126	Lake Huron	Michigan	45.95788	-84.16044
NCCAGL10-1127	Lake Huron	Michigan	44.84472	-83.30373
NCCAGL10-1130	Lake Huron	Michigan	45.50311	-83.92721
NCCAGL10-1131	Lake Huron	Michigan	44.85847	-83.31801
NCCAGL10-1136	Lake Erie	Ohio	41.74625	-83.37917
NCCAGL10-1137	Lake Erie	Ohio	41.51048	-82.13912
NCCAGL10-1138	Lake Erie	New York	42.73212	-78.97097
NCCAGL10-1139	Lake Erie	New York	42.53829	-79.27534
NCCAGL10-1140	Lake Erie	Michigan	41.85549	-83.37181
NCCAGL10-1141	Lake Erie	Ohio	41.50063	-82.21454
NCCAGL10-1142	Lake Erie	New York	42.68146	-79.08613
NCCAGL10-1144	Lake Erie	Ohio	41.63394	-83.16825
NCCAGL10-1146	Lake Erie	Pennsylvania	42.21606	-79.90829
NCCAGL10-1148	Lake Erie	Michigan	41.97839	-83.22607

List of Great Lakes Human Health Fish Tissue Study Sampling Locations				
Site ID Number	**Lake**	**State**	**Latitude**	**Longitude**
NCCAGL10-1149	Lake Erie	Ohio	41.56669	-82.76520
NCCAGL10-1152	Lake Erie	New York	42.75304	-78.92968
NCCAGL10-1155	Lake Erie	New York	42.64527	-79.13889
NCCAGL10-1157	Lake Erie	Ohio	41.71204	-83.24907
NCCAGL10-1161	Lake Erie	Ohio	41.43843	-82.92615
NCCAGL10-1163	Lake Erie	Ohio	41.73072	-83.44522
NCCAGL10-1165	Lake Erie	Ohio	41.86898	-81.10047
NCCAGL10-1168	Lake Erie	Michigan	41.92854	-83.23335
NCCAGL10-1169	Lake Erie	Ohio	41.46351	-82.92912
NCCAGL10-1170	Lake Erie	New York	42.35038	-79.60465
NCCAGL10-1173	Lake Erie	Pennsylvania	42.06438	-80.38134
NCCAGL10-1174	Lake Erie	Ohio	41.50192	-82.13009
NCCAGL10-1175	Lake Erie	New York	42.83341	-78.89074
NCCAGL10-1176	Lake Erie	Pennsylvania	42.21322	-80.05048
NCCAGL10-1178	Lake Erie	Ohio	41.74465	-81.39602
NCCAGL10-1179	Lake Erie	Ohio	41.51697	-82.17261
NCCAGL10-1181	Lake Ontario	New York	43.96827	-76.1154
NCCAGL10-1182	Lake Ontario	New York	43.91360	-76.18341
NCCAGL10-1183	Lake Ontario	New York	43.35820	-78.70273
NCCAGL10-1185	Lake Ontario	New York	43.50622	-76.48772
NCCAGL10-1188	Lake Ontario	New York	43.25480	-77.48873
NCCAGL10-1189	Lake Ontario	New York	43.58759	-76.25065
NCCAGL10-1190	Lake Ontario	New York	44.07588	-76.37700
NCCAGL10-1191	Lake Ontario	New York	43.38128	-78.08532
NCCAGL10-1192	Lake Ontario	New York	43.43145	-76.62718
NCCAGL10-1193	Lake Ontario	New York	43.80338	-76.25182
NCCAGL10-1195	Lake Ontario	New York	43.36138	-77.93097
NCCAGL10-1196	Lake Ontario	New York	43.31913	-76.87901
NCCAGL10-1200	Lake Ontario	New York	43.38012	-78.59547
NCCAGL10-1201	Lake Ontario	New York	43.29485	-77.35129
NCCAGL10-1202	Lake Ontario	New York	43.68424	-76.23963
NCCAGL10-1204	Lake Ontario	New York	43.36850	-76.69966
NCCAGL10-1205	Lake Ontario	New York	43.75382	-76.25459
NCCAGL10-1206	Lake Ontario	New York	44.00692	-76.28367
NCCAGL10-1207	Lake Ontario	New York	43.28966	-77.21855
NCCAGL10-1209	Lake Ontario	New York	43.95098	-76.24824
NCCAGL10-1210	Lake Ontario	New York	43.28736	-77.60575
NCCAGL10-1211	Lake Ontario	New York	43.51761	-76.29959
NCCAGL10-1213	Lake Ontario	New York	43.82965	-76.32422
NCCAGL10-1214	Lake Ontario	New York	44.06539	-76.41119
NCCAGL10-1216	Lake Ontario	New York	43.29208	-79.04195
NCCAGL10-1217	Lake Ontario	New York	43.34313	-77.80294
NCCAGL10-1218	Lake Ontario	New York	43.30384	-77.07563
NCCAGL10-1221	Lake Ontario	New York	43.38191	-78.36226
NCCAGL10-1222	Lake Ontario	New York	43.26149	-77.47764
NCCAGL10-1223	Lake Ontario	New York	43.34353	-76.76442
NCCAGL10-1224	Lake Ontario	New York	43.96971	-76.20600
NCCAGL10-1225	Lake Ontario	New York	43.29486	-76.90896
NCCAGL10-2005	Lake Superior	Minnesota	46.82415	-92.01783
NCCAGL10-2093	Lake Huron	Michigan	45.68080	-84.33828
NCCAGL10-2140	Lake Erie	Ohio	41.50642	-82.15552

Appendix B
GLHHFTS Fish Tissue Preparation, Homogenization, and Distribution Procedures

Note: The discussion of the tissue preparation, homogenization, and distribution procedures in this appendix represents the approach that was implemented at the time that the samples were prepared. EPA subsequently decided to use the sample aliquots prepared for the pharmaceuticals and personal care products (PPCPs) for the analysis of the PCBs. Because the procedures described in this appendix were already complete when that decision was made, the text of this appendix has not been modified other than to add a brief note below the table of sample aliquots.

National Coastal Condition Assessment
Great Lakes Human Health Fish Tissue Study
Tissue Preparation, Homogenization, and Distribution Procedures

I. PURPOSE

This document describes the procedures that Microbac Laboratories will follow when preparing fish tissue samples for EPA's National Coastal Condition Assessment (NCCA) Great Lakes Human Health Fish Tissue Study (GLHHFTS) under contract to CSC. Adherence to these procedures will ensure that fish tissue preparation activities are performed consistently across all study samples and in a manner consistent with previous EPA fish tissue studies. The effort is divided into four components:

- A kickoff meeting and workshop involving all study participants, including Microbac staff, EPA, CSC, and Tetra Tech
- An initial demonstration of capabilities, also referred to as the QA study
- Normal fish tissue processing and distribution procedures, including quality control steps
- Analyses of rinsate samples and blanks for mercury and polybrominated diphenyl ethers (PBDEs)

Each of these components is described in detail below.

II. KICKOFF MEETING AND WORKSHOP

EPA held a kickoff meeting and workshop at Microbac on February 23, 2011. Staff from all study participants, including Microbac, EPA, CSC, and Tetra Tech, met at Microbac to review the overall GLHHFTS project goals, the roles of each participant, the fish sample preparation procedures, and the communication strategies necessary to ensure successful completion of the project. In conjunction with that meeting, CSC provided whole fish samples that will be used during a hands-on workshop on the specific procedures for fish sample preparation. All Microbac staff involved in the preparation of fish samples attended the kickoff meeting and workshop.

III. INITIAL DEMONSTRATION OF CAPABILITIES

A routine aspect of any procedure for sample preparation or analysis is an initial demonstration of capabilities, or QA study. For the GLHHFTS project, Microbac will receive three whole large fish provided by Tetra Tech. Each of these fish will be treated as a separate project sample and will be prepared using the procedures detailed in Section IV (i.e., Steps 1 to 20). In between each fish, Microbac will prepare the entire series of equipment rinsate samples and blanks described in Section IV, Steps 22 to 23, but analyze only the rinsates and blanks for mercury and PBDEs (Steps 24, 25, and Section V). Microbac will perform triplicate determinations of lipids on each test sample, as described in Step 21. The results of the QA study will be reported to CSC.

Note: Microbac will not be authorized to process actual project samples until CSC determines that the QA study results meet the project objectives, including the adequacy of Microbac's equipment cleaning and homogenization procedures.

The sample aliquots prepared from these samples will be stored frozen at Microbac for possible future use by EPA. Each of the samples prepared for the QA study will be billable under the CSC subcontract at the cost for a normal project sample.

IV. NORMAL FISH TISSUE PROCESSING AND DISTRIBUTION PROCEDURES

The procedures for processing and distributing GLHHFTS composite fish tissue samples are described below. The process description is organized into the following eight components, including the quality control (QC) procedures:

A. Sample Handling
B. Filleting and Homogenization Procedures
C. Aliquoting and Distribution Procedures
D. Equipment Cleaning between Composite Samples
E. Lipid Determination on Every Homogenized Composite Sample
F. Quality Control (QC) Procedures
G. Reporting Requirements
H. Shipping Samples

The individual steps in the overall process are presented as a series of numbered steps across the eight components listed above.

Note: Microbac may **not** process any fish tissue samples until directed by CSC to proceed. No normal samples may be processed until after the kickoff meeting and workshop and until CSC reviews the results of the initial demonstration of capabilities (QA study) described in Section III above.

Composite Sample Classifications

For the purposes of the GLHHTS, EPA has classified each valid sample as a “routine” composite sample, or a “non-routine” composite sample, based on the following definitions:

- **Routine sample** – A routine composite sample consists of five individual adult fish of a single species that meet EPA’s length requirements (i.e., length of the smallest specimen in the composite is at least 75% of the length of the largest individual). Fillets from both sides of all five fish will be removed (total of 10 fillets) and homogenized to prepare one composite fillet sample.

- **Non-routine sample** – A non-routine sample is any sample that does not meet the definition of a routine sample, including those that do not meet the 75% rule and those with fewer or greater than five fish. When non-routine samples are sent to the prep lab, EPA and CSC will provide instructions for processing the non-routine samples. These instructions may include discarding some of the fish in the composite sample based on size before proceeding with filleting and homogenizing. In cases when fewer or more than five fish were collected, instructions may include processing some or all of those fish in the composite sample.

Each of the five fish in the routine samples must be filleted before homogenization. **For non-routine composites, only the designated specimens (identified by specimen number) will be filleted and homogenized.** For both types of samples, the specimens to be included in each

composite must be scaled (i.e., scales removed) and both fillets from each specimen prepared as skin-on fillets (belly-flap included) to form the fillet composites.

Note: The classifications described above do not include samples that were collected from an incorrect sampling location, were an unnecessary duplicate sample, or contained an inappropriate fish species. EPA does not plan on using these "invalid" samples for the GLHHFTS, so it is imperative that Microbac not process any sample without specific instructions from CSC. Therefore, samples will be retained in frozen storage and processed only upon receipt of CSC-issued instructions. If the status of any composite sample in the instructions is not clear, contact CSC and wait for clarification.

IV.A. Sample Handling

The whole fish collected for the GLHHFTS are being stored frozen (e.g., -20 ºC) at CSC's sample repository in stackable trays. Samples to be processed must be retrieved from the freezer, with their associated paperwork, and allowed to partially thaw before they can be filleted.

1. CSC will send sample processing instructions to the laboratory The instructions consist of an Excel spreadsheet file that details the site and sample identifiers for fish that EPA has determined are routine valid five-fish composites, or non-routine composites to be prepared. At a minimum, the Excel file will list the following fields for each individual fish specimen in a given composite sample:

   - Site ID (NCCAGL10XXXX = National Coastal Condition Assessment Great Lakes, 2010, and a 4-digit site number, except for three samples from Illinois that use a different format)
   - Lake
   - Date (of collection)
   - Sample ID (526###.X, where X usually ranges from 1 to 5 specimens in the composite, but can range up to 10)
   - Species (scientific and common names)
   - Total Length (mm) of each specimen
   - Composite Type (predator or bottom dweller)
   - Composite Classification (Routine, Non-Routine, or Invalid)
   - Deviation (e.g., why it is not routine or not valid)
   - Instructions (sample-specific details about which fish to process)

   CSC will provide the storage tray number for each sample, as part of the instructions, or separately.

2. When retrieving samples from the freezer, the sample custodian must:

   - Verify that all associated hardcopy paperwork stored with the samples is complete, legible, and accurate.
   - Compare the information on the label on each individual fish specimen to the processing instructions and notify CSC of any discrepancies between the sample labels and the Excel file of instructions. Problems involving sample paperwork, sample integrity, or custody inconsistencies for all fish tissue samples should be reported to CSC in writing (e.g., by

email) as soon as possible following sample retrieval and inspection. **Do not proceed with sample processing until discrepancies are resolved.**

Note: The hardcopy paperwork generated by the field samplers and stored with the samples does *not* contain all of the information in the Excel instruction files. Therefore, lack of information on hardcopy field paperwork regarding the composite type, composite classification, or deviation is *not* a discrepancy that must be reported.

IV.B. Filleting and Homogenization Procedures

3. Prior to preparing each composite sample, thoroughly clean utensils and cutting boards using the following series of procedures:

   - Wash with a detergent solution (phosphate- and scent-free) and warm tap water
   - Rinse three times with warm tap water
   - Rinse three times with DI water
   - Rinse with acetone
   - Rinse three times with DI water
   - Rinse with (not soak in) 5% nitric acid
   - Rinse three times with DI water

   To control contamination, separate sets of utensils and cutting boards must be used for scaling fish and for filleting fish.

4. Put on powder-free nitrile gloves before unpacking individual fish specimens for filleting and tissue homogenization. As samples are unpacked and unwrapped, inspect each fish carefully to verify that it has not been damaged during collection or shipment. If damage (e.g., tearing the skin or puncturing the gut) is observed, document it in the laboratory project logsheet and notify CSC.

5. Weigh each fish to the nearest gram (wet weight) prior to any sample processing. Enter weight information for each individual fish into a laboratory project logsheet. Individual specimen weights eventually will be transferred to spreadsheets for submission to CSC.

6. Rinse each fish with deionized water as a precautionary measure to treat for possible contamination from sample handling in the field. Use HDPE wash bottles for rinsing fish and for cleaning homogenization equipment and utensils. Do **NOT** use Teflon® wash bottles for these procedures, because PFCs are among the target analytes for this study.

7. Put on new powder-free nitrile gloves during the scaling and filleting processes. Fish with scales must be scaled (and any adhering slime should be removed) prior to filleting. Scale each fish by laying it flat on a clean glass cutting board and scraping from the tail to the head using a stainless steel scaler or the blade-edge of a clean stainless steel knife. Rinse the cutting board and scaler or knife with deionized water between fish to minimize the risk of cross-contamination. Filleting can proceed after all scales have been removed from the skin and a separate clean cutting board and scaler or fillet knife are prepared or available.

8. Place each fish on a clean glass cutting board in preparation for the filleting process. Note that filleting should be conducted under the supervision of an experienced fisheries biologist,

if possible. Ideally, fish should be filleted while ice crystals are still present in the muscle tissue. Fish should be thawed only to the point where it becomes possible to make an incision into the flesh. Remove both fillets (lateral muscle tissue with skin attached) from each fish specimen using clean, high-quality stainless steel knives. Include the belly flap (ventral muscle and skin) with each fillet. Care must be taken to avoid contaminating fillet tissues with material released from inadvertent puncture of internal organs. In the event that an internal organ is punctured, rinse the fillet with deionized water immediately after filleting and make a note on the laboratory project logsheet that a puncture has occurred. Bones still present in the tissue after filleting should be carefully removed.

9. Samples should be homogenized partially frozen for ease of grinding. Composite fillets using the "batch" method, in which all of the fillets from the individual specimens that comprise the sample are homogenized together, regardless of each individual specimen's proportion to one another (as opposed to the "individual" method, in which equal weights of tissue from each specimen are added together).

10. Process each sample using a size-appropriate homogenization apparatus (e.g., automatic grinder or high-speed blender). Entire fillets (with skin and belly flap) from both sides of each fish must be homogenized, and the entire homogenized volume of all fish fillets from the composite will be used to prepare the composite. Mix the tissues thoroughly until they are completely homogenized as evidenced by a final composite sample that consists of a fine paste of uniform color and texture. Chunks of skin or tissue will hinder extraction and digestion and, therefore, are NOT acceptable. Grinding of tissue may be easier when tissues are partially frozen. Chilling the grinder briefly with a few chips of dry ice may also keep the tissue from sticking to the equipment.

11. Grind the sample a second time, using the same grinding equipment. This second grinding should proceed more quickly. The grinding equipment does not need to be cleaned between the first and second grinding of the sample. The final sample must consist of a fine paste of uniform color and texture. If there are obvious differences in color or texture, grind the entire sample a third time.

12. Measure the collective weight of the homogenized tissue from each composite to the nearest gram (wet weight) after processing and record the total homogenized tissue weight of each composite on a laboratory project log sheet. The collective weight of the homogenized tissue from each sample will be transferred to spreadsheets for submission to CSC. At least 300 g of homogenized tissue will be needed to fill all of the containers in Table 1 below. **If a sample does not yield at least 300 g of homogenized tissue, contact CSC via email immediately and await instructions.** As appropriate, place any less-than-300-g homogenized samples in the freezer while waiting for instructions, which are likely to involve preparing smaller archive aliquots.

13. After the final (second or third) grinding, clean the **grinding equipment and all other sample preparation equipment** using the procedures described in Step 19.

14. Once in every batch of 20 samples, verify the continued absence of equipment contamination and uniformity of homogenization using the procedures described in Steps 22 to 27.

IV.C. Aliquoting and Distribution Procedures

15. The prep lab will prepare one bulk homogenate tissue aliquot per fish composite sample and use it to fill the pre-cleaned sample containers specified for each type of sample listed in Table 1, following the procedures described in Step 16. All containers will be provided by the prep lab. Documentation of their cleanliness provided by the vendor (i.e., certificates of analysis) must be retained by the prep lab and provided to CSC on request.

Table 1. NCCA Initial Tissue Sample Aliquot Requirements

Analysis	Target Mass	Container Type	Destination
Mercury	5 - 10 g	50-mL HDPE straight-sided jar with **foil-lined lid**, or conical HDPE tube with snap top	TestAmerica-Knoxville
Fatty acids	25 - 30 g	125-mL straight-sided amber or clear glass jar with **PTFE-lined lid**	Southwest Research Institute
PFCs	20 - 25 g	50-mL HDPE straight-sided jar with **foil-lined lid**, or conical HDPE tube with snap top. ***PTFE lid liners not allowed.***	TestAmerica-West Sacramento
PBDEs	20 - 25 g	125-mL straight-sided amber or clear glass jar with **PTFE-lined lid**	ALS - Canada
PPCPs	20 - 25 g	125-mL straight-sided amber or clear glass jar with **PTFE-lined lid**	*To be determined*
Lipids	10 - 15 g	Lab's choice, as this aliquot will be used in-house to determine the lipid content of the sample	In-house
Archive 1	100 g	250-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSC Sample Repository
Archive 2	100 g	250-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSC Sample Repository
Total*	300 - 330 g		
Additional Archives	All available tissue is used to fill jars up to 80% full	500-mL straight-sided amber or clear glass jars with **foil-lined lids**	CSC Sample Repository

* In the event that insufficient fish tissue mass exists to prepare the required number of aliquots, contact CSC for instructions, per Step 12.

Note: After the fish preparation procedures were completed, EPA decided to utilize the samples prepared for the PPCPs for the analysis of PCBs. Therefore, this document has not been modified beyond the addition of this note. The procedures were implemented as described, but the PPCP aliquot was sent to the laboratory performing the PCB analyses.

16. Prepare the sample aliquots for **mercury, fatty acids, PFCs, PBDEs, and PPCPs**. Weigh an appropriate clean sample container (Table 1) to the nearest 0.5 g and record the weight. Transfer sufficient aliquots of ground sample to the container to achieve the minimum mass for that container in Table 1, weigh the container again, record the weight, and determine the weight of the aliquot to the nearest 0.5 g by difference. **The prep lab must use foil-lined lids for jars containing the tissue aliquots for PFC analysis and the archived tissue samples, as specified in Table 1.**

    Note: The archive sample jars are not filled until after sufficient volume for lipids determination has been collected, as described in Step 18. For the sample used for

homogeneity testing, the archive jars are not filled until triple the lipid mass is collected (see Step 26).

When filling jars, leave sufficient space at the top of each jar to allow for expansion of the tissue as it freezes. *In no case should jars be filled beyond 80% capacity, as this may result in breakage on freezing.* Wipe off the outside of the jars to remove any tissue residue or moisture. Fill out a label for each container using a waterproof marker. Include the following information (at a minimum) on each label:

- site identification number (e.g., NCCAGL10-1023),
- sample identification number (e.g., 560208),
- analysis type (e.g., mercury, PFCs, PBDEs, etc.),
- aliquot weight (to the nearest 0.5 gram),
- preparation batch ID, and
- and preparation date (e.g., mm/dd/yyyy)

Affix the label to the container with clear wide tape. Place each container 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 directed by CSC to ship them to the analytical laboratories. (CSC will not issue such instructions until equipment rinsate and homogeneity tests described in Steps 22 to 27 have been completed, reported, evaluated, and determined to be acceptable.)

17. After filling all of the containers for the aliquots for mercury, fatty acids, PFCs, PBDEs, and PPCPs, remove 5 to 10 g of homogenized tissue to be used by the prep lab to determine the lipid content of each sample. Place this aliquot in a clean glass or plastic container of suitable size and label it with the site ID and sample number. Transfer the lipid aliquot to the appropriate staff performing the lipid determinations described in Steps 21, 26, and 27.

18. The archive sample jars are not filled until after sufficient volume for lipids determination has been collected. Once the aliquots for mercury, fatty acids, PFCs, PBDEs, PPCPs, and lipids have been collected, the remaining tissue mass is used to create at least two archive samples. Transfer 100 g of tissue to each of the first two archive containers, and seal and label the containers as described in Step 16 for the other aliquots.

Note: Step 12 requires that the laboratory contact CSC whenever a sample does not yield at least 300 g of tissue. CSC will provide direction to the laboratory regarding samples yielding less than 300 g of tissue that must be followed at this point in the procedure.

If additional tissue mass remains after filling the first two archive sample containers, use it to fill successive 500-mL glass jars with foil-lined lids until all of the excess tissue has been archived. Label the containers as described in Step 16 for the other aliquots. ***No tissue will be discarded without written EPA approval.***

IV.D. Equipment Cleaning between Composite Samples

19. All of the homogenization equipment must be thoroughly cleaned between each composite sample. Once all of the fillets from the individual specimens in a given composite sample have been homogenized, 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**. At a minimum:

- Wash with a detergent solution (phosphate- and scent-free) and warm tap water
- Rinse three times with warm tap water
- Rinse three times with deionized (DI) water
- Rinse with acetone
- Rinse three times with DI water
- Rinse with (not soak in) 5% nitric acid
- Rinse three times with DI water
- Allow the components to air dry

20. Reassemble the homogenization equipment and proceed with homogenization of the next sample in the batch (e.g., begin with Step 4 above).

IV.E. Lipid Determination on Every Homogenized Composite Sample

The procedures for determining the lipid content of every fillet composite are described in Step 21 below. (Additional lipid determinations are required for one sample in every preparation batch, as described in Steps 26 and 27.)

21. Use the 5 to 10 g of homogenized tissue collected in Step 17 to determine the lipid content of the sample. Extract the aliquot using the method of the laboratory's choice. (This method was previously pre-approved by CSC and EPA.) Determine the lipid content of that aliquot and record it in units of percent (i.e., grams of lipid per gram of tissue x 100), and provide the results to CSC by email, as described in Section IV.G. These results may be used by the laboratories conducting the other analyses to lipid-normalize their results.

IV.F. Quality Control (QC) Procedures

The project-specific QC procedures include preparation and testing of equipment rinsate samples and homogeneity testing, using lipids as a surrogate. The QC procedures are performed in two distinct phases: (1) as part of an initial demonstration of capabilities after the kickoff meeting and workshop with EPA, and (2) during normal operations.

Initial demonstration of capabilities: After the kickoff meeting and workshop, Microbac staff will prepare three test fish samples provided by Tetra Tech. Each test sample will consist of a single large fish which will be processed separately. Each of these test samples will be carried through the entire sample preparation and aliquoting procedures separately. The resulting sample aliquots will not be distributed to other laboratories at this time, but stored frozen. In between processing each individual fish sample, Microbac staff will clean all of the sample preparation equipment as described in Step 19 above. After each cleaning, Microbac staff will prepare the entire series of equipment rinsates and solvent blanks described in Step 22 below.

Microbac also will collect three lipid aliquots from each sample prepared during the initial demonstration and use them for triplicate determinations of lipids, as described in Step 26 below.

The results of the analyses of the rinsates and the homogeneity testing (three sets each) will be submitted to CSC for review. Microbac may **not** begin Great Lakes Human Health Fish Tissue Study sample preparation until CSC and EPA determine that Microbac has successfully demonstrated proficiency in meeting QC requirements for equipment cleaning and tissue homogenization.

Normal Operations: During normal sample preparation efforts, Microbac will prepare one set of rinsate samples and will conduct one set of triplicate lipid determinations per batch of 20 composite fish samples, as described in Steps 22 to 27, below. The batch-specific rinsate and homogeneity results will be reviewed by CSC and EPA. Microbac may continue to process up to one additional batch of 20 samples (based on sample preparation instructions provided by CSC) during that review process. However, Microbac may **not** continue beyond that next batch of samples until receiving notification from CSC that review of the prior batch rinsate and homogeneity test results is complete and the results were deemed satisfactory.

Thus, continued sample processing is dependent on both the quality of Microbac's efforts and on the timeliness of their delivery of QC results.

Rinsate Sample Production

22 Prior to reassembling the homogenization equipment (Step 20) between each of the samples processed during the initial demonstration of capabilities, and once per batch during normal operations, prepare five rinsate samples, as follows:

- Prepare **two hexane rinsate samples** by pouring two 100-mL portions of pesticide-grade hexane over all parts of homogenization equipment, including the cutting boards and knives, and collect each 100-mL portion in a separate clean glass container. Place two additional 100-mL aliquots of clean hexane in similar glass containers for use as solvent blanks. Allow the solvent to evaporate from the equipment. One set of these rinsates and blanks will be analyzed by Microbac for PBDEs and the other set will be archived for analysis of fatty acids by a laboratory to be determined later. CSC will provide Microbac with the name and shipping information for the fatty acids laboratory as soon as it is available. Label and store the fatty acids rinsate and blank as described in Step 23. Label, store, and analyze the PBDE rinsate and blank as described in Step 24.

- Once the hexane has evaporated off the equipment, prepare the **methanol rinsate** in a similar fashion, using 100-mL of pesticide-grade methanol. Collect that rinsate in a clean glass container and place a second aliquot of methanol in a separate similar clean glass container for use as a solvent blank. This rinsate and blank will be analyzed for PPCPs by a laboratory to be determined later. CSC will provide Microbac with the PPCP laboratory name and shipping information as soon as it is available. Label and store these PPCP rinsate and blanks as described in Step 23.

- Once the methanol has evaporated, prepare the **first 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. Analyze these rinsate and blank samples for mercury as described in Step 25.

- Prepare the **second DI water rinsate** using an additional 250 mL of DI water. Collect this rinsate in a clean glass container **with a non-PTFE lid liner**. Place a second aliquot of DI water in a separate similar clean glass container for use as a blank. This rinsate and blank will be analyzed for PFCs by a laboratory to be determined later, thus the non-PTFE lid liners are essential. CSC will provide Microbac with the PFC laboratory name and shipping information as soon as it is available. Label and store these PFC rinsates and blanks as described in Step 23.

  Note: In order 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, 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.).

23. Label each container as either "rinsate - [insert name of solvent]" or "blank - [insert name of solvent]," and include the date it was prepared (mm/dd/yyyy), the analysis type (Hg, PBDE, PPCP, PFC, or fatty acids), and the preparation batch identifier. Store the rinsates and blanks cold (<6 ºC).

Rinsate Analyses

24. As part of the initial demonstration of capabilities, Microbac will analyze three sets of hexane rinsate and blank samples for PBDEs using a GC/ECD procedure (e.g., one set prepared after each tissue sample prepared during the initial demonstration process). During normal operations, Microbac will analyze one set of the hexane rinsate and blank samples per batch for PBDEs using a GC/ECD procedure. That procedure will require concentration of the hexane to a final volume of 1 mL, and analysis on two dissimilar GC columns, in order to identify the PBDE congeners of interest by retention time. Requirements for the PBDE analyses are provided in Section V.

25. As part of the initial demonstration of capabilities, Microbac will analyze three sets of DI water rinsate and blank samples for mercury using a cold-vapor atomic absorption procedure (e.g., one set prepared after each tissue sample prepared during the initial demonstration process). During normal operations, Microbac will analyze one set of the DI water rinsate and blank samples for mercury using a cold-vapor atomic absorption procedure. Requirements for the mercury analyses are provided in Section V.

Corrective Actions for Rinsates

CSC will evaluate the rinsate results based on the mass of each analyte detected, and assuming that all of the apparent contamination could be transferred to a nominal 300-g mass of homogenized tissue. Results for mercury or any PBDEs above the anticipated reporting limits for these analytes in tissue samples may be cause for corrective actions by Microbac. Such corrective actions may include revisions to Microbac's equipment cleaning procedures, followed by a successful demonstration of the revised cleaning procedures through preparation and analysis of additional rinsate samples.

Lipid Determination to Confirm Homogeneity

26. For each of the samples processed during the initial demonstration of capabilities, and for one sample in every batch of 20 composite samples prepared during normal operations, Microbac will conduct triplicate analyses of the lipid content of samples to confirm that the samples are homogeneous.

    As with the collection of rinsate samples, the homogeneity testing must be performed 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). Therefore, unless otherwise directed by CSC for a particular batch of samples, Microbac will select one sample processed on the first day of every batch that will provide well over 300 g of total tissue mass.

    From that sample, remove three 5- to 10-g aliquots of tissue before filling the archive sample containers. Place these three aliquots in clean glass or plastic containers of suitable size and label each with the site ID, sample number, and an aliquot identifier of the lab's choice. Transfer the lipid aliquot to the appropriate staff performing the lipid determination.

27. From the lipid results, calculate the mean lipid content (in percent), the standard deviation (SD), and the relative standard deviation (RSD) using the formulae below, or the corresponding functions in Excel.

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

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

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

    If the RSD of the triplicate results is less than or equal to 15%, then the homogenization effort is judged to be sufficient for all samples in that preparation batch. For this sample analyzed in triplicate, the mean lipid content will be the value reported for that sample, following the requirements described in Step 28.

Corrective Actions for Homogeneity

If the RSD is greater than 15%, then corrective action is required for all samples in that preparation batch. Corrective actions will be determined by CSC in direct consultation with the laboratory and EPA, but the default corrective action consists of regrinding all of the aliquots from each composite sample in the affected batch until the RSD criterion is met.

This may entail retrieving all sample aliquots (see Table 1) from the freezer, allowing them to partially thaw, and homogenizing them again, beginning at Step 10. In these instances, all of the equipment cleaning procedures will be repeated between each composite sample, new lipids results will be determined for each composite, and a new homogenization QC

determination (triplicate lipids on one sample per batch) will be performed. New sample containers will be required for any rehomogenized samples.

IV.G. Reporting Requirements

28. Microbac will prepare a weekly progress report to document the status of fish preparation activities and forward the report electronically to CSC. The format of the weekly progress report will be as an Excel spreadsheet. For each composite processed during that period, include at least the following information in the report:

    - site identification number (e.g., NCCAGL10-1023),
    - sample identification number (e.g., 560208),
    - specimen numbers of the fish homogenized for the composite,
    - species name (both scientific and common names)
    - lengths and weights of individual specimens that were filleted and homogenized
    - total composite sample (i.e., homogenate) weight (to the nearest gram),
    - analysis type (e.g., mercury, PFCs, archive sample, etc.),
    - aliquot weight (to the nearest 0.5 gram),
    - preparation batch ID,
    - preparation date (e.g., mm/dd/yyyy),
    - QC sample identifiers associated with the batch of composite samples,
    - lipid results for each composite sample, and
    - airbill numbers for all sample shipments that week (these may include samples prepared during previous weeks).

    (Much of the sample-specific information above will be provided to Microbac electronically in the sample processing instructions from CSC.)

    The weekly report will be due by COB Monday, or as agreed to in writing by CSC after consultation with the laboratory, and will document sample preparation progress for the previous week.

    In addition, the laboratory must report the results of the rinsate analyses for mercury and PBDEs and the triplicate lipid results associated with the sample batch. Those results **must** be reported to CSC as soon after the analyses as practical to facilitate CSC's timely review and to minimize delays in receiving instructions to process future batches.

    Note: As specified in the QC section of this document, Microbac may **not** continue beyond the next batch of samples until receiving notification from CSC that review of the prior batch rinsate and homogeneity test results is complete and the results were deemed satisfactory.

IV.H. Shipping Samples

29. **No samples may be shipped until CSC and EPA have reviewed the sample homogeneity testing and rinsate results.** CSC will notify Microbac by email when specific samples may be shipped, and to whom.

When shipping batches of pre-frozen tissue aliquots, keep the individual containers bagged in the food-grade plastic freezer bags. Place these bags in a cooler with adequate space for the tissue containers, packing materials, and dry ice. (CSC will provide suitable coolers from existing stocks.) Secure each of the tissue containers with packing materials (e.g., bubble wrap or foam) before adding the dry ice. Place a modest layer of newspaper on top of the containers before adding the dry ice, as this can prevent cracking the lids. A single “section” of the local newspaper will usually suffice.

The amount of dry ice required for shipping will depend on the number of tissue samples in the cooler and the time of year. It should be an adequate supply to keep the tissue samples frozen for 48 hours (i.e., a minimum of 25 pounds of dry ice per cooler for up to 10 pounds of tissue samples).

Record the samples contained in the cooler on a chain-of-custody form provided by CSC and place the form in a plastic bag taped to the inside lid of the cooler. Secure the outside of the cooler with sealing tape, address it to the sample recipient identified by CSC, and attach a dry ice (dangerous goods) label. Ship the cooler via an overnight express carrier on a date that will allow delivery of the cooler to the analytical lab on a normal business day (e.g., **no Saturday deliveries and no deliveries on U.S. Federal holidays without express permission from CSC**). Provide the air bill number for each shipment to CSC via email on the day that the shipment occurs. **CSC will provide the prep lab with a third-party FedEx account to which each shipment will be billed.**

V. ANALYSES OF RINSATES AND BLANKS FOR MERCURY AND PBDEs

This section describes the analyses of rinsate samples and blanks generated during the composite fish sample preparation process. The results of those analyses are important in demonstrating that the sample preparation laboratory’s equipment cleaning procedures are effective at preventing cross-contamination between fish tissue samples.

V.A. EQUIPMENT AND MATERIALS:

- Mercury analyzer suitable for aqueous samples. Cold-vapor atomic absorption (CVAA) instruments compatible with EPA Method 254 are acceptable. Must be capable of achieving an MDL of approximately 1 µg/L.
- Gas chromatograph with an electron-capture detector (GC/ECD) and two dissimilar GC columns suitable for analysis of organohalide compounds such as PBDEs.
- Solvent concentration equipment suitable for reducing hexane rinsates to final volumes of 1 to 10 mL.
- A PBDE standard solution containing at least the following PBDE congeners: 47, 66, 99, 100, 138, 153, 154, and 183, to be used to establish retention times and perform at least a 3-point calibration of the GC/ECD.
- Assorted glassware, syringes, etc.

V.B. RINSATE AND BLANK ANALYSES

During the initial demonstration of capabilities, the laboratory will prepare three sets of rinsate samples, i.e., one set after each fish prepared as part of that demonstration. Each set of rinsate samples will include:

- Two de-ionized water (DI) rinsate samples and two DI water blanks per sample for analysis of mercury and for analysis of PFCs.
- Two hexane rinsate samples and two hexane blanks per sample for analysis of PBDEs and fatty acids.
- One methanol rinsate sample and a methanol blank per sample for analysis of PPCPs.

During normal sample preparation efforts, the laboratory will prepare rinsates at a frequency of one set for each batch of 20 fish tissue samples prepared. Up to 8 sets of rinsates are anticipated.

The laboratory will digest and analyze the mercury rinsates and blanks by CVAA. The laboratory will concentrate the PBDE rinsates and blanks to a final volume of 1 mL and analyze the concentrated samples by GC/ECD. For each analysis, the laboratory will determine the mass of each analyte (mercury or PBDE congener) in the total volume of each rinsate or blank sample, rather than the concentration of each analyte.

The laboratory must be able to achieve an MDL of approximately 1 μg/L. Mercury results will be reported down to the mass equivalent to the mass at the method detection limit (MDL) for aqueous samples.

Because the PBDE rinsates are not aqueous samples that are extracted, a traditional MDL study for aqueous samples does not apply. Therefore, the laboratory must perform an instrument detection limit (IDL) study before beginning any rinsate analyses. The IDL study will consist of analyzing 7 low-level standards containing the PBDEs of interest, determining the standard deviation of results for each PBDE across all 7 analyses, and multiplying the standard deviation times 3.143, the Student's t-value for 7 replicates. The laboratory must achieve an IDL on the order of 0.5 ng/mL, for a 1-mL final volume.

PBDE congeners will be identified based on retention time windows on both GC columns (see EPA Methods 608 or 8000C for examples of procedures for determining retention time windows).

PBDE results in the rinsates and blanks will be reported down to the mass equivalent to the IDL. Any PBDEs detected on one GC column must be confirmed by the analysis of the sample on a second GC column with a different stationary phase. Alternatively, GC/ECD analyses may be conducted on an instrument set up for simultaneous dual-column analyses.

The rinsates for PPCPs, PFCs, and fatty acids will not be analyzed by the laboratory, but will be held by Microbac.

V.C. QUALITY CONTROL

The quality control (QC) procedures required for the rinsate analyses include:

- MDL or IDL studies, as described above
- Instrument calibration (see Method 245.1 and Method 608 for procedures and acceptance criteria)
- Instrument blanks for both mercury and PBDE analyses
- Calibration verification (once per analysis batch) for both mercury and PBDE analyses
- Laboratory control sample (LCS) once per analysis batch, for mercury only.

The MDL and IDL results will be reviewed by CSC as soon as they become available, and the laboratory will not be authorized to prepare additional fish tissue samples until that review is complete and the results are acceptable.

The matrix for the mercury rinsates is reagent water, which should not adversely affect method performance. Therefore, matrix spike samples are not required for mercury.

Because the PBDE rinsates do not involve extraction of an environmental matrix, matrix spike samples are not applicable. Likewise, laboratory control samples are not applicable to PBDEs.

The instrument blanks for mercury and PBDEs take the place of a traditional method blank that would be extracted along with environmental samples.

V.D. DELIVERABLES

Summary data from the rinsate analyses are to be delivered to CSC in an Excel file. That file must contain the following information, at a minimum:

- Batch ID - to be established by the laboratory, but a simple approach would be to number or letter each sample batch (e.g., A to H, or 1 to 8). The batch ID for the rinsates prepared during the initial demonstration results may be reported as “QA study”
- Sample ID - as described in the instructions for preparing the rinsates
- Lab sample ID - unique internal identifier used by the laboratory, in any
- Prep date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was prepared
- Analysis type - either “Mercury” or “PBDE”
- Analysis date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was analyzed
- Analyte name - PBDE congeners may be abbreviated as PBDE-047, etc.
- Mass of analyte found - in micrograms for mercury, and either micrograms or nanograms for the PBDEs, provided that the reporting units for PBDEs are consistent throughout the effort
- Retention time on GC Column 1 - PBDEs only
- Retention time on GC Column 2 - PBDEs only
- Lab qualifiers - as needed to describe any analytical concerns. A complete list of the qualifiers and their meanings must be included with each data submission (e.g., in a separate tab on the Excel file)
- Reporting limit (i.e., MDL) for each analyte - in the same mass units used for the results.
- Instrument calibration data - Submit as a separate tab in the Excel file. Must include results for the initial calibrations for mercury and PBDEs, as well as any relevant calibration verifications associated with the analyses. Include calibration equations (e.g., regressions) and metrics (e.g., correlation coefficient or calibration factor), and for PBDEs, the retention times of the analytes in each calibration standard on both GC columns.

Raw data supporting each analysis (e.g., chromatograms or instrument printouts) must be retained by the laboratory and made available to CSC when requested. If requested, raw data may be submitted in hard copy, or as a PDF file.

Appendix C
Method Detection Limits and Minimum Levels for GLHHFTS Target Analytes

Method Detection Limits (MDLs) and Minimum Levels (MLs) for GLHHFTS Target Analytes

Metals *(based on a 0.5-g sample)*		
Analyte	**MDL**[a] (µg/kg)	**ML** (µg/kg)
Mercury	Under development	Under development

PFCs *(based on a 5-g sample)*		
Analyte	**MDL**[a] (µg/kg)	**ML**[b] (µg/kg)
PFBS	0.10	1
PFBA	0.07	1
PFDA	0.06	1
PFDoA	0.12	1
PFHpA	0.09	1
PFHxS	0.12	1
PFHxA	0.07	1
PFNA	0.08	1
PFOS	0.08	1
PFOS	0.13	1
PFOA	0.10	1
PFPeA	0.13	1
PFUnA	0.11	1

Fatty Acids *(based on a 1-g sample)*		
Analyte	**MDL**[a] (weight/weight %)	**ML**[b] (weight/weight %)
ALA	0.0027	0.0053
ETE	0.0027	0.0053
DHA	0.0027	0.0053
EPA	0.0026	0.0053
DPA	0.0026	0.0053

[a] All MDLs are based on the EPA procedure described at 40 CFR 136, Appendix B.
[b] ML values are based on the concentration of the lowest calibration standard.

PBDEs *(based on a 20-g sample)*		
Analyte	**MDL**[a] (ng/kg)	**ML**[b] (ng/kg)
BDE-7	0.46	5
BDE-10	0.33	5
BDE-8/BDE-11	0.47	5
BDE-12/BDE-13	0.58	5
BDE-15	0.12	5
BDE-17/BDE-25	0.48	10
BDE-28/BDE-33	0.50	2.5
BDE-30	0.57	5
BDE-32	0.25	5
BDE-35	0.15	5
BDE-37	0.21	5
BDE-47	0.96	5
BDE-49	0.29	5
BDE-51	0.28	5
BDE-66	0.32	2.5
BDE-71	0.24	5
BDE-75	0.30	5
BDE-77	0.27	5
BDE-79	0.20	5
BDE-85	0.55	5
BDE-99	2.99	5
BDE-100	0.66	2.5
BDE-105	0.42	5
BDE-116	0.75	5
BDE-118	0.48	5
BDE-119/BDE-120	0.61	5
BDE-126	0.26	5
BDE-128	0.87	5
BDE-138/BDE-166	1.33	5
BDE-140	0.73	5
BDE-153	0.39	2.5
BDE-154	0.69	2.5
BDE-155	0.47	5
BDE-156	0.86	5
BDE-181	0.57	5
BDE-183	0.49	5
BDE-184	0.89	5
BDE-190	0.98	2.5
BDE-191	0.84	5
BDE-196	0.9	10
BDE-197	0.38	10
BDE-203	0.32	10
BDE-206	1.36	25
BDE-207	1.56	25
BDE-208	1.76	25
BDE-209	6.06	100
HBB	0.81	5
PBEB	0.26	5

[a] All MDLs are based on the EPA procedure described at 40 CFR 136, Appendix B.
[b] ML values are based on the concentration of the lowest calibration standard.

PCBs (based on a 10-g sample)		
Analyte	**MDL**[a] (ng/kg)	**ML**[b] (ng/kg
PCB-1	0.13	0.5
PCB-2	0.14	0.5
PCB-3	0.20	0.5
PCB-4	0.27	1
PCB-5	0.24	1
PCB-6	0.22	0.5
PCB-7	0.35	1
PCB-8	0.29	1
PCB-9	0.19	0.5
PCB-10	0.29	1
PCB-11	0.24	1
PCB-12/PCB-13	0.36	1
PCB-14	0.31	1
PCB-15	0.14	0.5
PCB-16	0.45	2
PCB-17	0.29	1
PCB-18/PCB-30	0.66	2
PCB-19	0.27	1
PCB-20/PCB-28	0.45	1
PCB-21/33	0.57	2
PCB-22	0.30	1
PCB-23	0.31	1
PCB-24	0.34	1
PCB-25	0.27	1
PCB-26/PCB-29	0.52	2
PCB-27	0.32	1
PCB-31	0.20	0.5
PCB-32	0.30	1
PCB-34	0.27	1
PCB-35	0.31	1
PCB-36	0.40	1
PCB-37	0.33	1
PCB-38	0.30	1
PCB-39	0.32	1
PCB-40/PCB-41/PCB-71	1.33	5
PCB-42	0.44	1
PCB-43	0.52	2
PCB-44/PCB-47/PCB-65	1.23	5
PCB-45/PCB-51	0.87	2
PCB-46	0.33	1
PCB-48	0.43	1
PCB-49/PCB-69	0.85	2
PCB-50/PCB-53	0.72	2
PCB-52	0.50	2
PCB-54	0.15	0.5
PCB-55	0.42	1
PCB-56	0.54	2
PCB-57	0.37	1
PCB-58	0.26	1
PCB-59/PCB-62/PCB-75	1.23	5

PCBs (based on a 10-g sample)		
Analyte	**MDL**[a] (ng/kg)	**ML**[b] (ng/kg
PCB-60	0.51	2
PCB-61/PCB-70/PCB-74/PCB-76	1.81	5
PCB-63	0.43	1
PCB-64	0.36	1
PCB-66	0.43	1
PCB-67	0.26	1
PCB-68	0.32	1
PCB-72	0.36	1
PCB-73	0.32	1
PCB-77	0.17	0.5
PCB-78	0.39	1
PCB-79	0.33	1
PCB-80	0.44	1
PCB-81	0.20	0.5
PCB-82	0.20	0.5
PCB-83/PCB-99	0.66	2
PCB-84	0.50	2
PCB-85/PCB-116/PCB-117	0.68	2
PCB-86/PCB-87/PCB-97/PCB-109/PCB-119/PCB-125	1.41	5
PCB-88/PCB-91	0.91	2
PCB-89	0.50	2
PCB-90/PCB-101/PCB-113	0.43	1
PCB-92	0.51	2
PCB-93/PCB-95/PCB-98/PCB-100/PCB-102	2.19	5
PCB-94	0.51	2
PCB-96	0.32	1
PCB-103	0.37	1
PCB-104	0.10	0.2
PCB-105	0.17	0.5
PCB-106	0.21	0.5
PCB-107	0.77	2
PCB-108/PCB-124	0.57	2
PCB-110/PCB-115	0.52	2
PCB-111	0.21	0.5
PCB-112	0.32	1
PCB-114	0.21	0.5
PCB-118	0.28	1
PCB-120	0.32	1
PCB-121	0.53	2
PCB-122	0.42	1
PCB-123	0.34	1
PCB-126	0.17	0.5
PCB-127	0.28	1
PCB-128/PCB-166	0.50	2
PCB-129/PCB-138/PCB-160/PCB-163	1.54	5
PCB-130	0.28	1
PCB-131	0.41	1
PCB-132	0.29	1
PCB-133	0.32	1
PCB-134/PCB-143	0.59	2

PCBs (based on a 10-g sample)		
Analyte	**MDL**[a] (ng/kg)	**ML**[b] (ng/kg
PCB-135/PCB-151/PCB-154	1.59	5
PCB-136	0.32	1
PCB-137	0.26	1
PCB-139/PCB-140	1.28	5
PCB-141	0.35	1
PCB-142	0.26	1
PCB-144	0.42	1
PCB-145	0.42	1
PCB-146	0.35	1
PCB-147/PCB-149	0.75	2
PCB-148	0.34	1
PCB-150	0.26	1
PCB-152	0.37	1
PCB-153/PCB-168	0.92	2
PCB-155	0.12	0.5
PCB-156/PCB-157	0.32	1
PCB-158	0.27	1
PCB-159	0.36	1
PCB-161	0.25	1
PCB-162	0.32	1
PCB-164	0.30	1
PCB-165	0.26	1
PCB-167	0.22	0.5
PCB-169	0.15	0.5
PCB-170	0.73	2
PCB-171/PCB-173	0.32	1
PCB-172	0.26	1
PCB-174	0.58	2
PCB-175	0.11	0.5
PCB-176	0.27	1
PCB-177	0.41	1
PCB-178	0.25	1
PCB-179	0.28	1
PCB-180/PCB-193	1.53	5
PCB-181	0.34	1
PCB-182	0.26	1
PCB-183/PCB-185	0.43	1
PCB-184	0.15	0.5
PCB-186	0.25	1
PCB-187	0.43	1
PCB-188	0.12	0.5
PCB-189	0.28	1
PCB-190	0.18	0.5
PCB-191	0.26	1
PCB-192	0.19	0.5
PCB-194	0.38	1
PCB-195	0.26	1
PCB-196	0.35	1
PCB-197/PCB-200	1.34	5
PCB-198/PCB-199	0.45	2

PCBs (based on a 10-g sample)		
Analyte	**MDL**[a] (ng/kg)	**ML**[b] (ng/kg
PCB-201	0.39	1
PCB-202	0.41	1
PCB-203	0.22	0.5
PCB-204	0.17	0.5
PCB-205	0.17	0.5
PCB-206	0.31	1
PCB-207	0.21	0.5
PCB-208	0.38	1
PCB-209	0.31	1

[a] All MDLs are based on the EPA procedure described at 40 CFR 136, Appendix B.

[b] The minimum level (ML) for the NLFTS was calculated by EPA based on a tissue MDL study. The ML values shown above are equivalent to 10 times the standard deviation from that MDL study, rounded to the nearest multiple of 1, 2, or 5, consistent with the approach used in both the NLFTS and during the development of EPA Method 1668C.