Quality Assurance Project Plan for

Sample Preparation and Analysis for the 2015 National Coastal Condition Assessment Great Lakes Human Health Fish Fillet Tissue Study

Revision 4
April 4, 2017

Prepared for:

United States Environmental Protection Agency
Office of Water
Office of Science and Technology
Standards and Health Protection Division

Prepared jointly by:

CSRA
under:
Scientific and Technical Support
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and

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and

Tetra Tech, Inc.
under:
Standards and Health Protection Division
Contract No. EP-C-14-016

EPA-822-B-24-002

Revision History

April 3, 2017 Revision 4

- This revision removes all references to contaminants of emerging concern (CECs). It also changes the way that the National Coastal Condition Assessment is identified (e.g., NCCA 2015 instead of the earlier use of 2015 NCCA). Each individual occurrence is *not* called out in this history.
- This revision includes additions to the approval page in A1 and the distribution list in A3.
- Section A4 was updated to add Yildiz Chambers-Velarde as the CSRA Project Leader under the WSD contract.
- Figure 1, the organization chart, was updated to add Yildiz Chambers-Velarde and to include the dioxin and furan analysis lab contact.
- Table 2 in Section B1 was revised to reflect the actual numbers of samples collected.
- Section B4.6 has been revised to describe the dioxin and furan analysis details.
- Similarly, Section B5.6 has been revised to describe the dioxin and furan quality control details.
- Section B7 was revised to include a description of the instrument calibration procedures for the dioxin and furan analyses.
- Section C1.1 has been revised to include the details for the dioxin and furan analyses.
- Section C1.4 has been revised to describe the readiness review for the dioxin and furan analysis lab.
- Section D3 was revised to describe the assessment of the dioxin and furan results against the associated QC criteria.
- Appendix C was updated to add the details relevant to the dioxin and furan analyses.
- Appendix F was added to the document with the quality control acceptance criteria for the dioxin and furan analyses. Given the length of this material, it was added as a new appendix, rather than attempting to fit it into the flow of the text in Section B.

January 31, 2017 Revision 3

- This revision addresses the name change of Computer Science Government Solutions (CSGov) to CSRA that occurred during the course of the overall study. Each individual occurrence of the name change is *not* called out in this history.
- This revision includes edits to the distribution list in A3, including the addition of a cell for the fatty acid analysis lab.
- Figure 1, the organization chart, was updated to include the fatty acid analysis lab contact.
- Section B4.5 has been revised to describe the fatty acid analysis procedures, and the placeholder for the dioxin/furan analysis details has been moved to Section B4.6 because those analyses will appear in the next revision of the QAPP.
- Similarly, Section B5.5 has been revised to describe the fatty acid quality control procedures, including a new Table 10, and the placeholder for the dioxin/furan quality control details has

been moved to Section B5.6 because those analyses will appear in the next revision of the QAPP.

- Section B7 was revised to include a description of the instrument calibration procedures for the fatty acid analyses.
- Section C1.1 has been revised to include the details for the fatty acid analyses.
- Section C1.4 has been revised to describe the readiness review for the fatty acid analysis lab.
- Section D3 was revised to describe the assessment of the fatty acid results against the associated QC criteria.
- The Reference section has been updated with the citation for the QA manual for the fatty acid analysis lab.
- Appendix C was updated to add the details relevant to the fatty acid analyses of tissue samples.

June 2, 2016 Revision 2

- This revision includes edits to the distribution list in A3, including the addition of cells for the PCB and PFC analysis labs.
- Figure 1, the organization chart, was updated to include the PCB and PFC analysis lab contacts.
- Section A6 was updated to reflect the collection of additional samples in May 2016 and Figure 2 was updated to show all of the sample locations.
- Section A7 was updated to reference the sections of the QAPP where the details on the methods and QC criteria for the mercury, PCB, and PFC analyses are found.
- Section B4.3 was updated to include the method specifics for the PCB analysis of fish tissue and rinsate samples.
- Section B4.4 was updated to include the method specifics for the PFC analysis of fish tissue and rinsate samples.
- Section B5.1.2 was updated with information about how the rinsate results for PFCs and dioxins and furans will be used.
- Section B5.3 was updated to include the QC specifics for the PCB analysis of fish tissue and rinsate samples.
- Section B5.4 was updated to include the QC specifics for the PFC analysis of fish tissue and rinsate samples.
- Section B7 was updated to add the details relevant to the PCB and PFC analyses of tissue samples and rinsates.
- Sections C1.1 and C1.4 were updated to add the details relevant to the PCB and PFC analyses of tissue samples.
- The Reference section was updated with the citations for the QA manuals from the PCB and PFC analysis labs.
- Appendix C was updated with the MDLs and MLs for the PCB and PFC analyses of tissue samples. The final values are based on information from the PCB and PFC analysis labs.

- Appendix D was added to the document with the quality control acceptance criteria for the PCB congener analyses. Given the length of this material, it was added as a new appendix, rather than attempting to fit it into the flow of the text in Section B.
- Appendix E was added to the document with the quality control acceptance criteria for the PFC analyses. Given the length of this material, it was added as a new appendix, rather than attempting to fit it into the flow of the text in Section B.

April 20, 2016 Revision 1

- This revision includes edits to the distribution list in A3, including the addition of a cell for the mercury analysis lab.
- Figure 1, the organization chart, was updated to include the mercury analysis lab, a new placeholder was added for the dioxin/furan analysis lab, and a missing line of communication was added between the two Project Co-Managers.
- The text in Section B4 was divided into new subsections.
    - Section B4.1 includes both the fish sample and rinsate preparation.
    - Section B4.2 includes the analyses of both tissue samples and rinsates for mercury and the details of the mercury tissue analyses were added.
    - Section B4.3 is a placeholder for the PCB analysis of fish tissue and rinsate samples.
    - Section B4.4 is a placeholder for the PFC analysis of fish tissue and rinsate samples.
    - Section B4.5 is a placeholder for the dioxin and furan analysis of fish tissue and rinsate samples.
    - Section B4.6 is a placeholder for the fatty acid analysis of fish tissue samples.
- Section B5.2 was added to address the fish tissue mercury quality control requirements including the addition of Tables 5 and 6.
- Section B5.5 was added as a placeholder for the QC specifications for the dioxin and furan analysis of fish tissue and rinsate samples and the existing B5.5 was renumbered as B5.6.
- B7 was updated to add the details relevant to the analyses of tissue samples and rinsates.
- Sections C1.1 and C1.4 were updated to add the details relevant to the analyses of tissue samples.
- The Reference section was updated with the citation for the QA manual from the mercury analysis lab, plus two references for the mercury preparation and analysis procedures from Methods 1631B and 1631E.
- Appendix B was revised to reflect the most recent version of the fish sample preparation procedures that now explicitly include preparation of tissue aliquots for eventual dioxin/furan analyses.
- Appendix C was added with the MDL and ML for the mercury analyses of tissue samples. The final values are based on information from the mercury analysis lab.

February 24, 2016 - Original QAPP signed

Sample Preparation and Analysis for the 2015 National Coastal Condition Assessment Great Lakes Human Health Fish Fillet Tissue Study

A. PROJECT MANAGEMENT

This Quality Assurance Project Plan (QAPP) presents performance criteria, acceptance criteria, and objectives for the preparation and analysis of fish fillet tissue composite samples for the Great Lakes Human Health Fish Fillet Tissue Study (GLHHFFTS) under the 2015 National Coastal Condition Assessment (NCCA 2015). Fish fillet tissue samples prepared under the QAPP will be analyzed for mercury, polychlorinated biphenyls (PCBs), perfluorinated compounds (PFCs), dioxins and furans (polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, or PCDDs/PCDFs), and fatty acids. This QAPP also describes the methods and procedures that will be followed during the 2015 GLHHFFTS to ensure that the criteria and objectives are met. The scope of the initial QAPP was limited to fish sample preparation. Details for the chemical analyses for mercury, PCBs, PFCs, and fatty acids were added to the QAPP in the first three revisions. Details for the analyses of the dioxins and furans have been added to this fourth revision of the QAPP.

This QAPP was prepared in accordance with the most recent version of EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* (USEPA 2001a), 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 OST Project Co-Manager for the 2015 GLHHFFTS and the EPA Standards and Health Protection Division (SHPD) Quality Assurance Coordinator 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 information and description is provided in Sections A5 and A6, respectively.

A1. Approvals

Leanne Stahl, OST Project Manager, EPA — Date: 04/05/17

Shari Barash, Chief, National Branch, EPA — Date: 4/6/17

Bill Kramer, SHPD QA Coordinator, EPA — Date: 4-6-17

Marion Kelly, OST QA Officer, EPA — Date: 4/5/17

Blaine Snyder, Tetra Tech Project Leader — Date: 4/12/2017

Susan Lanberg, Tetra Tech QA Officer — Date: 4/12/17

Harry McCarty, CSRA Project Leader (STS) — Date: 4/5/2017

Yildiz Chambers-Velarde, CSRA Project Leader (WSD) — Date: 4/5/2017

Marguerite Jones, CSRA QA Officer — Date: 4/10/2017

Nancy Parrotta, WSD Project Officer — Date: 4/13/17

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List of Acronyms and Abbreviations

EPA	Environmental Protection Agency (also known as USEPA)
GLHHFFTS	Great Lakes Human Health Fish Fillet Tissue Study
GLNPO	Great Lakes National Program Office
ID	Identification
MDL	Method Detection Limit
ML	Minimum Level
m/z	Mass-to-charge ratio of a specific ion monitored during high resolution mass spectrometric analyses, where m is the mass and z is the charge
NCCA	National Coastal Condition Assessment
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
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin, also generically known as dioxin
PCDF	Polychlorinated dibenzofuran, also generically known as furan
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
SHPD	Standards and Health Protection Division
SOP	Standard operating procedure
SOW	Statement of work
SPE	Solid-phase extraction

A3. Distribution List

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A4. Project/Task Organization

EPA's 2015 National Coastal Condition Assessment (NCCA) is a probability-based survey designed to assess the condition of coastal waters of the United States. Building on EPA's experience from the 2010 NCCA, 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 684 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.

Multiple fish contamination studies are being conducted under the NCCA. The national assessment is using fish collected from all sampling sites in outer coastal waters and the Great Lakes assessment is using fish collected from all Great Lakes sampling sites as indicators of ecological (ECO) contamination, based on whole body contaminant concentrations. The ECO fish samples will be analyzed for various contaminants. 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. Another fish tissue survey is this regional study of the Great Lakes that involves an assessment of fish fillet contamination relevant to human health. It is referred to as the 2015 Great Lakes Human Health Fish Fillet Tissue Study (2015 GLHHFFTS).

The 2015 GLHHFFTS sampling effort resulted in collection of game fish from a statistically representative subset of 152 nearshore sites (about 30 sites per lake), which exceeded the minimum target of 150 samples. Field crews collected 147 valid fish composite samples for the 2015 GLHHFFTS during a June through October 2015 sampling season. An additional five fish samples were collected in Lake Michigan during May 2016.

Note: Following the completion of the sampling effort, OWOW determined that one of the samples collected in 2015 was not from a valid site, so OST did not use any results from that site. This version of the QAPP corrects the number of samples collected in 2015.

Routine composite samples for this study consist of five similarly sized adult fish of a single species commonly consumed by humans. The samples collected for the 2015 GLHHFFTS were shipped as whole fish to an interim storage facility at Microbac Laboratories in Baltimore, Maryland. Through its support contractor CSRA, EPA contracted the Tetra Tech, Inc. Center for Ecological Sciences to prepare the fish samples for analysis (i.e., filleting the fish samples and homogenizing the fillet tissue). The fillet tissue from these fish samples are being analyzed for mercury, polychlorinated biphenyls (PCBs), perfluorinated compounds (PFCs), dioxins and furans (PCDDs/PCDFs), and fatty acids. As noted earlier, details for each type of chemical analysis have been added to revisions of the QAPP as the information became available.

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 2015 GLHHFFTS under the NCCA 2015. OST and GLNPO are responsible for co-management of the 2015 GLHHFFTS under this NCCA. ORD's Western Ecology Division in Corvallis, Oregon developed the study design and selected all the sampling locations for the NCCA 2015, including

the 150 sites for the 2015 GLHHFFTS. Statisticians in the Western Ecology Division will also be analyzing the fish tissue concentration data.

In 2015, OWOW developed the NCCA Quality Assurance Project Plan (USEPA 2015a) 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 2015 GLHHFFTS based on the protocols used for the National Lake Fish Tissue Study. The initial analytical activities QAPP covered laboratory activities associated with 2015 GLHHFFTS fish fillet tissue sample preparation. As OST and GLNPO have added specific analyses to the project, the analytical activities QAPP has been revised to include the relevant details (see the revision history at the front of the document).

The 2015 GLHHFFTS project team currently consists of managers, scientists, statisticians, and QA personnel in OST, GLNPO, and the ORD Western Ecology Division, along with contractors CSRA and Tetra Tech, Inc. providing scientific and technical support to OST and GLNPO (Figure 1). Responsibilities for key members of the project team are described below.

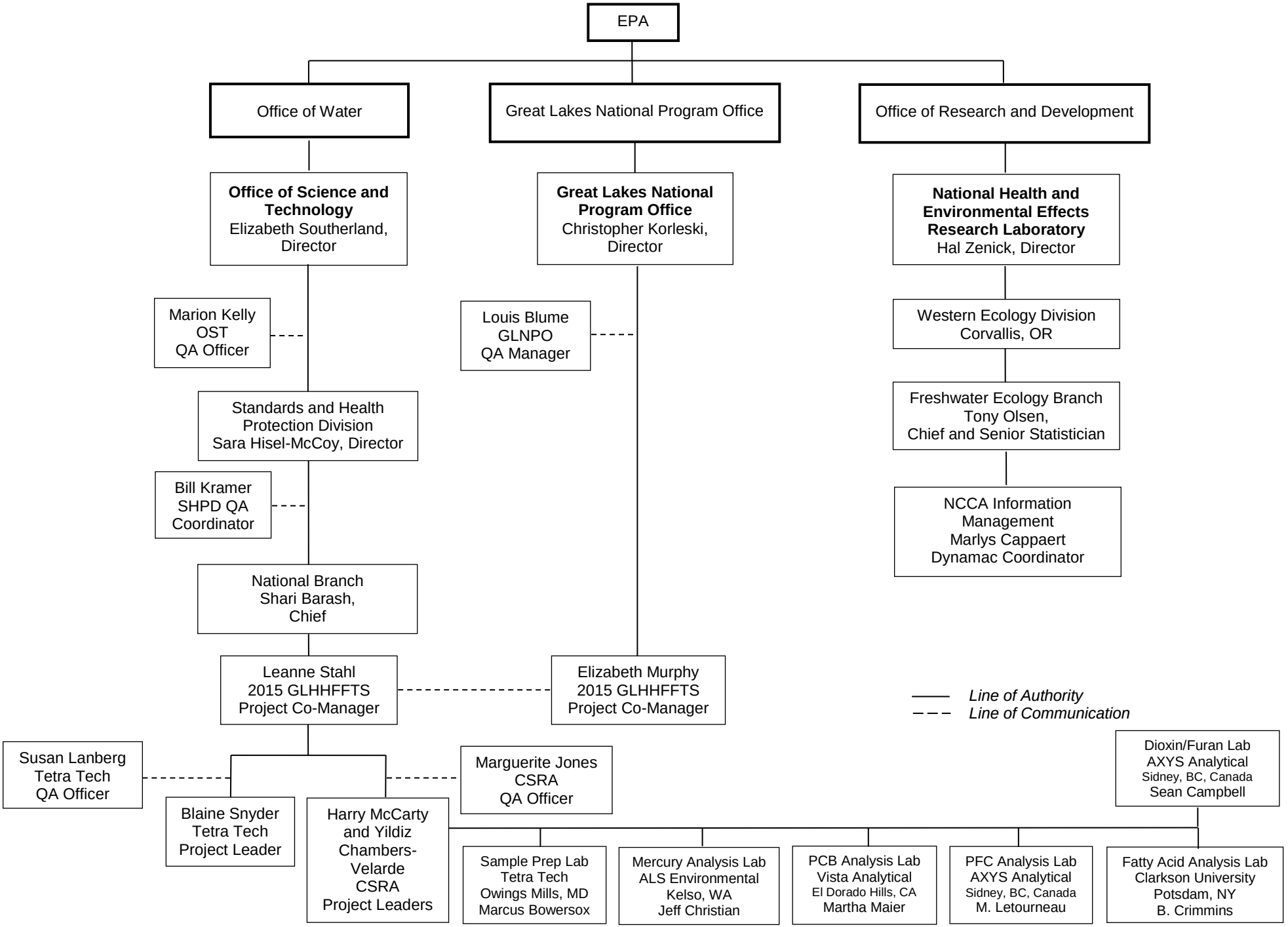

Figure 1. GLHHFFTS project team organization

Leanne Stahl of OST is a **2015 GLHHFFTS Project Co-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 2015 GLHHFFTS:

- developing technical information for 2015 GLHHFFTS fish sample collection that includes preparation of the sampling standard operating procedure (SOP) and coordination with the NCCA Project Leaders in OWOW to integrate field sampling technical information for the 2015 GLHHFFTS into NCCA documents and training materials
- providing technical support to conduct training on the 2015 GLHHFFTS 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 tissue 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 2015 GLHHFFTS fish tissue results
- facilitating communication among 2015 GLHHFFTS 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 2015 GLHHFFTS, 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 2015 GLHHFFTS
- collaborating with the 2015 GLHHFFTS project team for reporting the study results in technical journal articles and federal technical reports
- preparing fish tissue study presentations and presenting them in various forums (e.g., scientific conferences, government meetings, and webinars)

Elizabeth Murphy of GLNPO is a **2015 GLHHFFTS Project Co-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 2015 GLHHFFTS:

- reviewing and concurring on technical information developed for 2015 GLHHFFTS fish sample collection
- providing support to conduct training on the 2015 GLHHFFTS field sampling requirements in coordination with OST
- arranging additional support for 2015 GLHHFFTS fish sample collection through GLNPO fisheries contacts
- reviewing and concurring on the fish sample preparation SOP
- participating in the development, review, and approval of the analytical activities QAPP for the 2015 GLHHFFTS
- managing analysis of fish tissue samples for target chemicals, particularly for the fatty acids
- facilitating communication among 2015 GLHHFFTS project team members to ensure technical quality and adherence to QA/QC requirements
- 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
- managing the development of and/or reviewing and approving all major work products associated with the 2015 GLHHFFTS
- collaborating with the 2015 GLHHFFTS project team for reporting the study results in technical journal articles and federal technical reports
- preparing and/or reviewing fish tissue study presentations and presenting them in various forums (e.g., scientific conferences, government meetings, and webinars)

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. Louis Blume is the **GLNPO Quality Assurance Manager** who is responsible for reviewing and approving all QAPPs that involve scientific work being conducted by GLNPO. Bill Kramer 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, the GLNPO QA Manager, 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 2015 GLHHFFTS activities and procedures
- conducting external performance and system audits of the procedures applied for all 2015 GLHHFFTS 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 2015 GLHHFFTS. His specific responsibilities include the following:

- providing direct technical support for the following 2015 GLHHFFTS activities or providing leadership and oversight for Tetra Tech staff supporting these activities:
  - developing SOPs for field sampling and fish preparation
  - preparing 2015 GLHHFFTS documents (including this QAPP) or project information to incorporate into NCCA documents
  - providing field sampling and fish preparation training
  - planning and implementing 2015 GLHHFFTS 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 fillet 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**. Her role is independent from project line management, and she reports QA issues directly to the Tetra Tech Fairfax Center's Principal-in-Charge, Mr. George Townsend. Her 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 and Yildiz Chambers-Velarde are the **CSRA Project Leaders** who are responsible for managing all aspects of the technical support being provided by CSRA staff for the 2015 GLHHFFTS under two separate EPA contracts. Their specific responsibilities include the following:

- providing direct technical support for the following 2015 GLHHFFTS activities or providing leadership and oversight for CSRA staff supporting these activities:
  - preparing information related to technical and quality assurance requirements for preparation and chemical analysis of fish fillet tissue samples for target chemicals, verification and validation of analytical data, and database development to support project planning and development of 2015 GLHHFFTS documents (including this QAPP) or characterization of the 2015 GLHHFFTS in NCCA 2015 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 fish fillet tissue samples for mercury, PCBs, PFCs, and other target chemicals as required, 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 2015 GLHHFFTS 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 CSRA staff participating in this study to ensure that they are following all QA procedures described in this QAPP that are related to CSRA 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 **CSRA QA Officer**. She is in the independent CSRA Quality Management Office, separate from project line management, and reports through the Quality Management Office to the CSRA Chief of Staff. Her primary responsibilities include the following:

- assisting CSRA's Project Leader with the development and review of this QAPP
- approving this QAPP
- providing oversight for the implementation of QA procedures related to CSRA tasks that are described in this QAPP
- reporting deviations from this QAPP to the CSRA 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 2015 GLHHFFTS by providing technical expertise for study planning and implementation and by assuming responsibility for the following activities:

- study design development for the NCCA 2015, including statistically representative national and regional studies being conducted under the NCCA, such as the 2015 GLHHFFTS
- site selection and tracking for final statistical classification of sites
- statistical analysis of analytical data for 2015 GLHHFFTS fish fillet tissue samples, including trends analysis
- development of cumulative distribution 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, PCBs, and other chemicals of concern is a priority area of interest for EPA. Since 1998, OW has collaborated with ORD to conduct the first national-scale assessments of mercury, PCBs, and selected other target chemicals in fish fillet tissue through statistically based studies of U.S. lakes and rivers. These studies are referred to as the National Lake Fish Tissue Study, the 2008-09 National Rivers and Streams Assessment, the 2010 Great Lakes Human Health Fish Tissue Study (GLHHFTS), and the 2013-14 National Rivers and Streams Assessment. 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 2010 NCCA offered the opportunity to conduct the 2010 Great Lakes Human Health Fish Tissue Study, which was the first statistically representative study of chemical residues in fish relevant to human health for this region. The 2015 GLHHFFTS will provide additional lake-wide data on the occurrence and distribution of contaminants in the Great Lakes and, through comparison with the 2010 results, allow EPA to evaluate temporal trends of these contaminants in the Great Lakes. Collecting statistically representative data for other contaminants not measured in 2010 (e.g., dioxins and furans) is an additional goal of this study.

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 2015 GLHHFFTS within the framework of the NCCA 2015. A total of 152 valid fish samples were collected for the study at a statistical subset of NCCA Great Lakes sites distributed throughout the five Great Lakes (Figure 2). The majority of fish samples (147) were collected from June through October 2015 and an additional five samples were collected in Lake Michigan during May 2016.

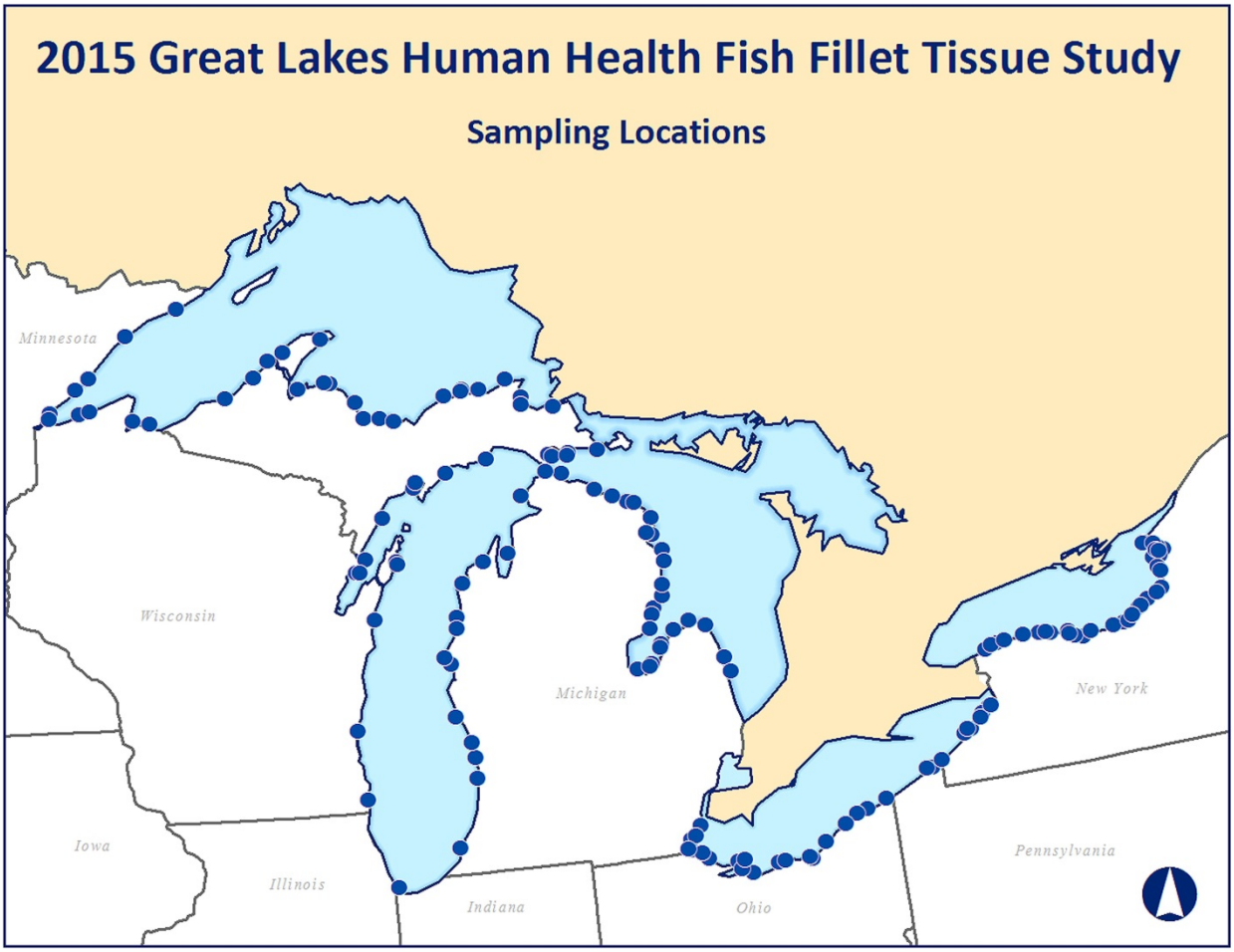

Figure 2. Sampling locations of the 152 valid fish samples collected for the 2015 GLHHFFTS

Following are the key design components for the 2015 GLHHFFTS:

- sampling at least 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) (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 an interim frozen storage facility
- transferring the whole fish samples to a laboratory for fish sample preparation, which includes filleting the fish, homogenizing the fillet tissue composites, and preparing fillet

tissue aliquots for analysis of specific chemicals, 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), PCB congeners, PFCs, dioxins and furans, and fatty acids

EPA stored the 2015 GLHHFFTS fish samples in freezers at Microbac Laboratories in Baltimore, Maryland, under contract to CSRA, prior to shipping them to the sample preparation laboratory. Tetra Tech's Center for Ecological Sciences worked under a subcontract to CSRA as the sample preparation laboratory to prepare the fish fillet tissue samples for analysis as outlined in the fourth bullet above. The sample preparation laboratory prepared aliquots of fillet tissue for mercury, PCBs, PFCs, dioxins and furans, fatty acids, and additional archive aliquots to allow for future analyses of 2015 GLHHFFTS samples. Those additional archive samples will be transferred to Microbac Laboratories after completion of all of the analyses listed above and stored along with archived fish tissue samples from earlier studies. Analytical laboratories will be analyzing the 2015 GLHHFFTS fish fillet tissue samples for mercury, PCBs, PFCs, dioxins and furans, and fatty acids under project-specific purchase orders issued by CSRA. Procedures for handling and shipping homogenized fish tissue samples to Microbac and the analysis laboratories are described in Appendix B.

A7. Quality Objectives and Criteria

The overall quality objective for the analysis of the 2015 GLHHFFTS fish fillet tissue samples for mercury, PCBs, PFCs, dioxins and furans, and fatty acids 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.

The methods and quality control acceptance criteria employed by the laboratories under contract for analyses of 2015 GLHHFFTS fish fillet tissue samples for mercury, PCBs, PFCs, fatty acids, and dioxins and furans are described in Sections B4 and B5 of this QAPP. Data usability for each analysis will be assessed using processes described in Section C and the QC criteria summarized in Section B5 of this QAPP.

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 Revised 2015 GLHHFFTS Tissue Preparation, Homogenization, and Distribution Procedures (Appendix B).

Specialized training will be provided for laboratory technicians who will be preparing fish tissue fillets and homogenates for this project. This training will be conducted at the sample preparation laboratory for all laboratory staff involved with 2015 GLHHFFTS fish tissue sample preparation to accomplish the following objectives:

- present 2015 GLHHFFTS fish tissue preparation, homogenization and distribution procedures described in Appendix B
- demonstrate filleting and homogenizing techniques with fish from invalid 2015 GLHHFFTS samples or from other sources of fish samples
- 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 will 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 CSRA 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 primary objective of the 2015 GLHHFFTS is to investigate the occurrence of mercury, PCBs, PFCs, dioxins and furans, and fatty acids 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 concentrations of mercury, PCBs, PFCs, and dioxins and furans in Great Lakes fish commonly consumed by humans
- species-specific information on fatty acid content of Great Lakes fish that are commonly targeted by fishermen and consumed by humans

Fish tissue data from this study will also provide EPA with the first opportunity to analyze trends in the levels of Great Lakes fish contamination by comparing 2015 GLHHFFTS fillet tissue results to the fillet tissue data generated during the 2010 GLHHFTS.

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 2015a). However, to provide some context for the readers of this QAPP, those aspects of the NCCA are summarized below.

The NCCA 2015 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). The NCCA 2015 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 Great Lakes portion of the NCCA 2015 study design consists of two independent survey designs. One design resampled sites visited during the 2010 NCCA Great Lakes assessment. The other design selected new sites using the same survey design applied for the 2010 NCCA. Both designs used a Generalized Random Tessellation Stratified (GRTS) survey design for an area resource. Application of the two Great Lakes survey designs resulted in selection of 225 U.S. nearshore sites (45 sites per lake): 110 (49%) were sites previously sampled during the 2010 NCCA Great Lakes assessment and 115 (51%) were new sites selected for the NCCA 2015 Great Lakes assessment. The site selection results are summarized in Table 1.

Table 1. NCCA 2015 Sampling Sites by State

State	Previously Sampled 2010 Sites	New 2015 Sampling Sites	Total
IL	0	0	0
IN	1	2	3
MI	56	55	111
MN	3	3	6
NY	28	28	56
OH	12	14	26
PA	1	2	3
WI	9	11	20
Total	110	115	225

The target population for the 2015 GLHHFFTS consists of a statistically representative subset of 150 NCCA sites distributed throughout the U.S. nearshore zone of the five Great Lakes (about 30 sites per lake). Half of the sites (75 sites or approximately 15 sites per lake) are ones that were previously sampled during the 2010 NCCA Great Lakes assessment. The other 75 sites (15

sites per lake) are new sites selected for the NCCA 2015 Great Lakes assessment. Including sites that were previously sampled in 2010 enhanced EPA's capability for estimating change in fish contamination levels between the 2010 and 2015 studies. Table 2 provides more specific information about the 2015 GLHHFFTS target population and a summary of the sites actually sampled for the study.

Table 2. 2015 GLHHFFTS Sampling Sites by Lake and State

Lake	State	Proposed Previously Sampled 2010 Sites	Proposed New 2015 Sampling Sites	Total Proposed Sites	Actual Previous 2010 Sites Sampled	Actual New 2015 Sites Sampled	Total Actual Sites Sampled
Superior	MI	10	10	20	10	10	20
	MN	3	3	6	3	3	6
	WI	2	2	4	2	2	4
Michigan	IL	0	0	0	0	0	0
	IN	0	1	1	0	1	1
	MI	11	10	21	10	9	19
	WI	4	4	8	4	4	8
Huron	MI	15	15	30	14	16	30
Erie	MI	2	2	4	2	2	4
	NY	4	4	8	4	4	8
	OH	8	8	16	8	9	17
	PA	1	1	2	1	1	2
Ontario	NY	15	15	30	16	17	33
Total		75	75	150	74	78	152

Sampling at the 2015 GLHHFFTS sites involves collection of whole fish samples for analysis of fillet tissue samples for mercury, PCBs, PFCs, fatty acids, and dioxins and furans. To meet the study objectives, one fish sample is collected from each site. Ideally, each fish sample is a routine fish composite sample that consists of five fish of adequate size to provide a minimum of 485 grams of edible tissue for analysis. Fish are selected for each composite sample by 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
- 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.

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 2015a) and *National Coastal Condition Assessment Field Operations Manual* (USEPA 2015b). These sampling procedures and requirements are summarized below.

The field objective is 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 specify 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 contains 25 priority target fish species and 18 alternative fish species. Fish teams use this list as the basis for selecting appropriate fish species for the 2015 GLHHFFTS samples. The method applied for fish collection is left to the discretion of the field team, but it typically involves angling or gillnetting and occasionally trawling.

In preparing fish samples for shipping, field teams record sample number, species name, specimen length, sampling location and sampling date on a fish collection form. Each fish is wrapped in solvent-rinsed, oven-baked aluminum foil, with the dull side in using foil sheets provided by EPA. Individual foil-wrapped specimens are placed into a length of food-grade polyethylene tubing, each end of the tubing is sealed with a plastic cable tie, and a fish specimen label is affixed to the outside of the food-grade tubing with clear tape. All of the wrapped fish in the sample from each site are 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 are directed to pack fish samples on dry ice in sufficient quantities to keep samples frozen for up to 48 hours (50 pounds are recommended), and to ship them via priority overnight delivery service (e.g., Federal Express), so that they can arrive at Microbac in less than 24 hours from the time of sample collection. Alternatively, field crews have the option to transport whole fish samples on wet or dry ice (depending on the distance) to an interim facility where the fish samples are frozen and stored for up to two weeks before shipping them overnight express delivery to Microbac on dry ice as described above.

Microbac is serving as EPA's interim storage facility for 2015 GLHHFFTS whole fish samples that are designated for fillet tissue analysis. Microbac staff are responsible for receiving and examining the fish samples at Microbac before they are stored in a walk-in freezer at the laboratory. The specific procedures that the laboratory staff follow upon receipt of whole fish samples are described in Appendix B (2015 GLHHFFTS Fish Tissue Preparation, Homogenization, and Distribution Procedures). Microbac staff are also responsible, for forwarding electronic copies of the sample records to CSRA, who in turn forwards them to OST and other fish study team members.

B3. Sample Handling and Custody

This section describes the sample handling and custody procedures that apply to the shipment of the whole fish samples to the sample preparation laboratory and that also will apply once the homogenized fish tissue samples are shipped from the sample preparation laboratory to each of the analytical laboratories selected for analysis of 2015 GLHHFFTS fish fillet tissue samples for

mercury, PCBs, PFCs, dioxins and furans, and fatty acids. All sample handling and custody procedures prior to those described here are discussed in the Field Operations Manual prepared by OWOW (USEPA 2015b) or in Appendix B as referenced in Section B2 above.

Transfer of Whole Fish Samples to the Sample Preparation Laboratory

Tetra Tech will transport the whole fish samples from the sample repository at Microbac Laboratories in batches of 20 samples determined by EPA. As samples arrive, the sample custodian at the sample preparation laboratory:

- Checks that each shipping container has arrived undamaged and verifies that samples are still frozen and in good condition
- Checks the temperature of one of the samples in the cooler using a thermometer that reads to -20 degrees Celsius (°C) or less, or an infra-red (IR) temperature "gun" and records the reading on the sample tracking form
- Verifies that all associated paperwork is complete, legible, and accurate
- Compares the information on the label on each individual fish specimen to the sample tracking form for each composite and verifies that each specimen was included in the shipment and is properly wrapped and labeled
- Notifies CSRA of the fact that samples were received and of any discrepancies in the paperwork identified above
- Transfers the samples to the freezer for long-term storage

The sample preparation laboratory notifies CSRA immediately about any problems encountered upon receipt of samples. Problems involving sample integrity, conformity, or inconsistencies for whole fish samples are required to be reported to CSRA in writing (e.g., by email) as soon as possible following sample receipt and inspection.

Following sample processing, the sample preparation laboratory must store sample aliquots frozen to less than or equal -20 °C until they are distributed to the laboratories performing analyses under separate CSRA purchase orders or other EPA funding mechanisms.

Shipment of Fillet Tissue Samples to the Analytical Labs

CSRA will be responsible for oversight of shipping the fillet tissue samples from the sample preparation laboratory to the 2015 GLHHFFTS analytical laboratories. The tissue samples will be packaged in sturdy coolers for shipping and wrapped with bubble wrap or other suitable packaging to protect the samples in transit. Fillet tissue samples will be shipped frozen with sufficient dry ice in the coolers to ensure that the samples remain frozen for at least 48 hours. The fish preparation procedures (Appendix B) provide specific information about dry ice requirements for shipping the fillet tissue samples. CSRA will prepare sample tracking paperwork and include it in each shipment.

When received at the respective analytical laboratories, the fillet tissue 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), so they are not 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. CSRA is notified of the receipt of the fillet tissue samples by email. CSRA will advise OST of tissue sample receipt. Any questions from the laboratories regarding sample paperwork or condition will be sent to CSRA, routed to OST or Tetra Tech as appropriate, and CSRA will send the answers back to the appropriate laboratory.

Fillet tissue samples will be stored frozen at less than or equal to -20 ^{o}C until analyzed. There are formal holding time studies or requirements that apply to mercury and PCBs, but not to PFCs. 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 2015 GLHHFFTS 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 fillet tissue sample. For PFCs, EPA will use a 1-year holding time for the fish tissue samples, because there is no evidence that indicates that there are practical limitations regarding the loss of PFCs from tissues in that time frame. EPA will note any results for mercury, PCBs, or PFCs generated outside of these 1-year holding times, but this will not preclude use of these results for the purposes of this project.

B4. Methods

B4.1 Fish Sample and Rinsate Preparation

Fish Sample Preparation

The services of Tetra Tech's Center for Ecological Services, the fish sample preparation laboratory for the 2015 GLHHFFTS, were procured by CSRA via a competitive solicitation. The fish sample preparation laboratory is responsible for filleting each valid fish sample, homogenizing the fillet tissue, preparing the required number of fillet tissue aliquots for analysis and archive, providing support for shipping the fillet tissue aliquots for each analysis to the designated analytical laboratory, and storing archived fillet tissue samples in a freezer at their facility until CSRA arranges for transfer of the archived fillet tissue samples to a facility for long-term storage of these samples. The specific procedures for all 2015 GLHHFFTS fish sample preparation activities are described in Appendix B.

Fish are filleted by qualified 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, scaled, rinsed with deionized water, and filleted on a glass cutting board. For the 2015 GLHHFFTS, 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 fillets from 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 fillets from 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. Homogeneity is confirmed by conducting triplicate analyses of the lipid content in one of every twenty samples. The collective weight of the homogenized tissue from each sample is recorded to the nearest gram (wet weight) after processing. The sample preparation laboratory prepares fillet tissue aliquots according to the specifications listed in Step 20 of the fish sample preparation procedures in Appendix B.

Rinsate Preparation

As part of the fish sample preparation process, the sample preparation laboratory will create rinsates of the equipment used to homogenize fillet tissue samples, as described in Appendix B. Two of these rinsates will be analyzed for mercury and selected PCB congeners by a laboratory under subcontract to the sample preparation laboratory, and two other rinsates will be analyzed for PFCs and PCDDs/PCDFs by laboratories under project-specific purchase orders issued by CSRA. The results of the mercury and PCB rinsates will be used to assess the ongoing effectiveness of the laboratory's equipment cleaning procedures.

B4.2 Mercury Analysis of Fillet Tissue and Rinsate Samples

Fish tissue samples are being prepared and analyzed by ALS-Environmental (Kelso, WA), using Procedure I from "Appendix to Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation" from Revision B of Method 1631 (1631B) for sample preparation (USEPA 2001b), and Revision E of Method 1631 (1631E) for the analysis of mercury in fish tissue samples (USEPA 2002). This method requires approximately 1 g of tissue for the analysis. The sample is digested with a combination of nitric and sulfuric acids. The mercury in the sample is oxidized with bromine monochloride (BrCl) and analyzed by cold-vapor atomic fluorescence spectrometry.

Tissue sample results are reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g). The mercury method detection limit (MDL) and minimum level (ML) are listed in Appendix C.

The rinsate samples for mercury are being analyzed by ALS-Pennsylvania, under subcontract to the sample preparation laboratory, Tetra Tech, during the course of the homogenization of the fish tissue samples. ALS-Pennsylvania is analyzing these aqueous samples using EPA Method 245.1, a cold-vapor atomic absorption procedure applicable to water samples.

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

B4.3 PCB Analysis of Fillet Tissue and Rinsate Samples

Fish tissue samples are being prepared and analyzed by Vista Analytical Laboratory (El Dorado Hills, CA), in general accordance with Revision C of EPA Method 1668 (1668C), Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS (USEPA 2010). The samples are being analyzed for all 209 PCB congeners, and reported as either individual congeners or coeluting groups of congeners.

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, along with their MDLs and MLs.

Tissue sample results are reported based on the wet weight of the tissue sample in units of nanograms per gram (ng/g).

The following method modifications have been reviewed by CSRA during the solicitation, 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 by EPA for use in this study:

- Section 7.6.4: Vista uses sodium sulfate as the reference matrix for QC samples associated with tissue analyses rather than vegetable oil because they have not found a source of vegetable oil that did not have traces of PCBs in it.

- Section 12.5: Vista uses sodium hydroxide to adjust the pH of the solution in the back-extraction procedure, rather than potassium hydroxide.

- Sections 7.10.1 and 15.4.2.1: Vista uses a CS-3 (mid-level calibration) standard that contains all 209 of the PCB congeners, rather than the subset of congeners listed in the method. Therefore, they do not run a separate standard containing all 209 congeners during the calibration verification process in Section 15.4.2.1.

- Table 3: Vista adds 44 ^{13}C-labeled compounds to each sample, five more than the 39 labeled compounds specified in the method, and monitors the recoveries of all of these standards in each sample.

The rinsate samples for PCBs are being analyzed by Pace Analytical Services, under subcontract to Tetra Tech, during the course of the homogenization of the fish tissue samples. Pace is analyzing these samples using SW-846 Method 8082A (USEPA, 2007) and results are reported in units of nanograms per liter (ng/L).

B4.4 PFC Analysis of Fillet Tissue and Rinsate Samples

There are no formal analytical methods from EPA or any voluntary consensus standards bodies for the PFC analyses of tissue samples. Therefore, fish tissue samples will be analyzed by AXYS Analytical Services, Ltd. (Sidney, BC, Canada), using procedures developed, tested, and documented in that laboratory. The SOP was reviewed by CSRA during the solicitation process, along with the supporting materials from all of the bidders. A copy of the AXYS SOP will be maintained on file at CSRA and will be made available to EPA for review on request.

The analytical procedures are briefly described below, based on information in the SOP. The 13 target PFC analytes are shown in Appendix C.

The concentration of each PFC is determined using the responses from one of the ^{13}C- or ^{18}O-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. (Because a labeled standard for perfluorobutanesulfonic acid is not commercially available, this target analyte is quantified using the response for ^{18}O-labeled perfluorohexanesulfonic acid, a closely related compound.)

Approximately 2 g of fish tissue are required for analysis. (If matrix-related analytical problems are identified during the analysis of a given fish tissue sample, a sample aliquot of 1 g may be used to minimize those problems.) The sample is spiked with eight isotopically labeled standards and extracted by shaking the tissue in a caustic solution of methanol, water, and potassium 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 reagent water and 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 high performance liquid chromatography with tandem mass spectrometry.

The aqueous rinsate samples will be analyzed using a procedure based on EPA Method 537 from the Office of Groundwater and Drinking Water (USEPA 2009). The 250-mL aqueous rinsate sample is spiked with the labeled standards and processed by SPE, in a similar manner as is used for the tissue samples. The PFCs are eluted from the SPE cartridge and the eluant is spiked with additional labeled recovery standards and analyzed by high performance liquid chromatography with tandem mass spectrometry.

Tissue sample results are reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g). Method detection limits and Minimum Levels 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).

B4.5 Fatty Acid Analysis of Fillet Tissue

Because they are not environmental contaminants, there are no formal EPA methods for the analysis of fatty acids in any matrix. Therefore, fish tissue samples will be analyzed by Clarkson University (Potsdam, NY), using procedures developed, tested, and documented in that laboratory and currently employed under GLNPO Grant No. GL 00E01505. The laboratory's SOPs were reviewed by CSRA during the solicitation process, along with the supporting materials. Copies of the Clarkson SOPs will be maintained on file at CSRA and will be made available to EPA for review on request.

The analytical procedures are briefly described below. The 38 target fatty acid analytes are shown in Appendix C.

Approximately 2 g of homogenized fish tissue is spiked with a surrogate solution (nonadecacanoic acid, C19:0), mixed with cross-linked polyacrylic acid, and extracted with methylene chloride using pressurized fluid extraction. The extract is dried with sodium sulfate, concentrated to approximately 20 mL. A 10-µL aliquot of the extract is transferred to a clean autosampler vial, purged for 30 seconds with nitrogen, capped, and then placed on the instrument for derivatization and injection.

The automated instrument adds 100 µL of deuterated C18:0 (as an internal standard) and 250 µL of 12% boron trifluoride (BF_3) in methanol. The solution is mixed and heated to 70 °C for 50 minutes. After heating, 25 µL of water is added to quench the derivatization reaction and the derivatized extract is mixed, followed by the addition of 0.65 mL of hexane and further mixing to separate the fatty acid methyl esters (FAMEs) from the aqueous solution.

An aliquot of the hexane extract is analyzed by gas chromatography, with flame ionization detection (GC/FID), using a 100 m x 250 µm x 0.2 µm HP-88 column. The concentration of each fatty acid is calculated based on a multi-point calibration curve and reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g). Method detection limits and quantitation limits for the fatty acids are listed in Appendix C.

B4.6 Dioxin and Furan Analysis of Fillet Tissue and Rinsate Samples

Fish tissue samples and rinsate samples are being prepared and analyzed by AXYS Analytical Services, Ltd. (Sidney, BC, Canada), using Revision B of EPA Method 1613 (1613B), Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS (1994A). The samples are being analyzed for the 17 2,3,7,8-substituted PCDD/PCDF congeners listed in Appendix C.

In order to achieve tissue MDLs and MLs similar to those used in the National Lake Fish Tissue Study, the dioxin analysis laboratory has proposed method modifications as follows:

- 25 grams of fish tissue will be extracted, instead of the method-specified 10 grams, which increases method sensitivity by a factor of 2.5.

- A 6-point instrument calibration will be performed using an additional low-level standard, (CS0.2) at 0.1-0.5 ng/mL, which lowers the method-specified initial calibration range by a factor of 5.

- Because of the low detection limits required, the use of the cleanup standard ($^{37}Cl_4$-2,3,7,8-TCDD) is not required. The presence of the cleanup standard causes a low-level interference in the quantification of 2,3,7,8-TCDD. Specifically, the $^{37}Cl_4$-2,3,7,8-TCDD has a small response at mass-to-charge ratio (m/z) 322, one of the two ions used to quantify the unlabeled 2,3,7,8-TCDD. The method-specified concentration of the cleanup standard is 8,000 pg in the sample extract, which could result in a contribution to the 2,3,7,8-TCDD response of approximately 0.8 pg. Given the low detection limits required for this project, that 0.8 pg potential contribution is enough to distort the ion ratios and create a high bias in low-level results. (The cleanup standard was included in the original method as a diagnostic tool to assist the laboratory in assessing the recoveries of the other labeled standards used for quantification of the target analytes. The elimination of this standard from the fish tissue

analyses has no direct effect on the sample results, assuming that the other labeled compounds meet the recovery specifications in Appendix F.)

The rinsate samples for dioxins and furans will not require method modifications, other than the fact that the rinsates were prepared in solvent and therefore will not require extraction prior to analysis.

B5. Fish Sample Preparation and Analytical Quality Control

The fish fillet tissue sample preparation procedures being applied for the 2015 GLHHFFTS are specific to the project, but are based on the procedures specified by OST for previous fish tissue studies. The associated quality control activities are described in Subsection B5.1.

The analytical procedures being applied by the laboratories designated for analysis of 2015 GLHHFFTS fillet tissue samples will 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 field samples and the associated QC samples
- blanks – 5% of the samples within a batch are method blanks

Other common quality control activities vary by the analysis type, and they will be described in the subsections below as the method-specific information becomes available. The QC activities associated with the mercury analyses are described in Subsection B5.2. The QC activities associated with the PCB analyses are described in Subsection B5.3. The QC activities associated with the PFC analyses are described in Subsection B5.4. QC information for other analytes will be added to subsequent revisions of this QAPP that address those additional analytes.

B5.1 Fish Sample Preparation

The project-specific QC procedures for fish sample preparation 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 training workshop with EPA, and (2) during normal operations (i.e., 2015 GLHHFFTS fish sample preparation procedures).

B5.1.1 Initial demonstration of capabilities

After the kickoff meeting and training workshop, the fish preparation laboratory staff will prepare three test fish samples provided by CSRA (with support from Tetra Tech). Each test sample will consist of a single large fish that 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. In between processing each individual fish sample, fish preparation laboratory staff will clean all of the sample preparation equipment as described in Step 24 of Appendix B. After each cleaning, fish preparation laboratory staff will prepare the entire series of equipment rinsates and solvent blanks described in Step 27 of Appendix B.

The fish preparation laboratory 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 31 of Appendix B. The fish preparation laboratory will have the rinsate samples analyzed for mercury and PCB congeners by a subcontract laboratory under their control, using the procedures described in Table 3, as shown below. Due to the lack of standardized analytical methods for the PFCs, the rinsates will not be analyzed by the fish preparation laboratory for PFCs, but will be analyzed later by AXYS Analytical Services, Ltd., (Sidney, BC, Canada) at the same time as the PFC analyses of the fish tissue samples. Analyses of the rinsates for dioxins and furans also will be performed later by AXYS at the same time as the dioxin and furan analyses of the fish tissue samples.

Table 3. Methods for Determination of Lipids and Analyses of Rinsate Samples

Parameter	Method	Laboratory
Lipids	SW-846 Method 3541 (automated Soxhlet extraction) (USEPA, 1994) followed by gravimetric determination, using a procedure from NOAA that was originally derived from Bligh and Dyer (1959)	ALS, Pennsylvania
Mercury	EPA 245.1 (USEPA 1983)	ALS, Pennsylvania
PCBs	SW-846 Method 8082A (USEPA, 2007)	Pace Analytical Services, New York

The results of the analyses of the rinsates and the homogeneity testing (three sets each) will be submitted to CSRA for review. The fish preparation laboratory will not begin 2015 GLHHFFTS sample preparation until CSRA and EPA determine that the sample preparation laboratory has successfully demonstrated proficiency in meeting QC requirements for equipment cleaning and tissue homogenization.

From the lipid results, the fish preparation laboratory will 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.

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

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

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

If the RSD of the triplicate results is less than or equal to 15%, then CSRA and EPA will judge the homogenization effort to be sufficient for all samples in that preparation batch.

If the results for the rinsate samples are below the limits in Table 4 for mercury and PCBs, then CSRA and EPA will judge the equipment cleaning effort to be sufficient for all samples in that preparation batch.

Table 4. Acceptance Limits for Rinsate Samples

Parameter	Acceptance Limit	Basis for Limit
Mercury	0.2 µg/L for total mercury	Method detection limit for an aqueous sample
PCBs	0.5 ng/mL per congener	Instrument detection limit for a 0.5-mL final volume of solvent concentrated from the original 50-mL rinsate sample

B5.1.2 Normal Operations

During normal sample preparation efforts, the fish preparation laboratory 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 31 and 32 of Appendix B. The batch-specific rinsate and homogeneity results will be reviewed by CSRA and EPA against the same QC specifications used for the initial demonstration of capabilities in Table 4. The fish sample preparation laboratory may continue to process up to two additional batches of 20 samples (based on sample preparation instructions provided by CSRA) during that review process. However, the fish sample preparation laboratory may not continue beyond that third batch of samples until receiving notification from CSRA that review of the first batch rinsate and homogeneity test results is complete and the results were deemed satisfactory.

As noted in Section B5.1.1, the rinsate samples will be analyzed for PFCs and dioxins and furans separately (and later) than the analyses for mercury and PCBs. Therefore, the results for those contaminants in the rinsates will not be used to assess the initial or routine cleaning procedures used by the sample preparation laboratory in the manner described for the mercury and PCB results. Rather, the PFC and dioxin/furan results for the rinsates will be compared to the final results for those contaminants in the fish tissue samples to determine if there was any potential influence on the tissue sample results.

B5.2 Mercury Analysis Quality Control

Quality control samples associated with each batch of tissue samples analyzed for mercury are summarized in Table 5 below.

The cold-vapor atomic fluorescence instrument is calibrated daily, as described in Method 1631E and the laboratory's SOP. At least five calibration standards and a blank are used for calibration, and the variability in the calibration factors for the five standards must have a relative standard deviation (RSD) less than or equal to 15%. The calibration is verified after every 20 samples by the analysis of the ongoing precision and recovery (OPR) standard, or the laboratory control sample (LCS). The results for the OPR/LCS standard must fall within the limits in Table 5.

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

QC Operation	Frequency	Acceptance Limit	Corrective Action
Bubbler blank	3 blanks run during calibration and with each analytical batch of up to 20 field samples	50 picograms (pg) of mercury	If the bubbler is above 50 pg, take corrective action to reduce the blank level to below 50 pg, and reanalyze any samples in the affected batch.

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

QC Operation	Frequency	Acceptance Limit	Corrective Action
Method blank	3 method blanks per batch of up to 20 field samples, with analyses interspersed among the samples in the analysis batch	0.4 nanograms (ng) (400 pg) of mercury, *or* Less than one tenth the concentration of an associated sample	If any of the three method blank results is above 0.4 nanograms, • take corrective action to reduce the blank level to below 0.4 ng, • reanalyze any samples in the affected batch with results less than 10 times the observed results for any of the three blanks, and • flag sample results greater than 10 times the observed blank level to advise the data user of the potential contamination.
OPR/LCS	Prepared once per batch of up to 20 field samples, analyzed *once prior to* the analysis of any field samples, *and again at* the end of each analytical batch, spiked at 4.0 ng	70 - 130% recovery (5.6 –10.4 ng/g)	If the OPR recovery is not within the QC acceptance limits, • take corrective action and repeat the OPR analysis, beginning with a fresh aliquot, • reanalyze all samples in the affected analytical batch.
QC sample	Once per batch of up to 20 field samples	Per the provider of the QCS *or* 75 - 125% recovery if no criteria provided by the supplier	If the QCS results are not within the provider's acceptance limits, • take corrective action and repeat the QCS analysis, beginning with a fresh aliquot, • reanalyze all samples in the affected analytical batch.
MS/MSD	Once per every 10 field samples (e.g., twice per 20 samples in a preparation batch) See note below table.	70 - 130% recovery and Relative Percent Difference (RPD) ≤ 30%	If either the MS or MSD recovery is not within the QC acceptance limits, • take corrective action and repeat the MS/MSD analysis, beginning with fresh aliquots, • reanalyze all samples in the affected analytical batch. If the RPD exceeds the acceptance limit, the laboratory will reanalyze the MS/MSD samples: • If the reanalysis results meet the RPD limit, then the laboratory will reanalyze all of the associated field and QC samples.

Note: Provision of useful MS/MSD data is highly dependent on selection of an appropriate spiking level relative to the background concentration of mercury in the unspiked sample. After the first batch of samples, the MS/MSD sample may be prepared from excess volume of tissue from a sample in the previous batch, such that the background level is known. Spiking should be performed at approximately 3 to 5 times the background concentration.

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 6 below.

Table 6. QC Samples and Acceptance Criteria for Mercury Analysis of Rinsates

QC 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.

B5.3 PCB Analysis Quality Control

The high resolution gas chromatograph/high resolution mass spectrometer used for fish tissue analysis is calibrated periodically as described in Method 1668C and the laboratory's SOP. At least five calibration standards are used for calibration, and the variability in the response factors for the five standards must have a RSD less than or equal to 20%. The calibration is verified every 12 hours by the analysis of the calibration verification standard. The results for the calibration verification must meet the requirements in Appendix D of this QAPP.

Quality control samples associated with each batch of tissue samples analyzed for PCBs are summarized in Table 7 below, and are based on the QC requirements of Method 1668C, with the project-specific addition of one laboratory duplicate sample per batch.

Table 7. QC Samples and Acceptance Criteria for PCB Analysis of Fish Tissue

QC Operation	Frequency	Acceptance Limit	Corrective Action
Labeled compounds	Spiked into every sample	Per Appendix D of this QAPP	Per Method 1668C
Calibration verification (VER)	At the beginning of every 12-h analytical shift	Per Appendix D of this QAPP	Per Method 1668C
Laboratory duplicate	Once per batch of up to 20 field samples	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 meets the RPD limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the reanalysis result still does not meet the RPD limit, then the laboratory will re-extract and reanalyze all field samples with original results above the MDL.
OPR/LCS	One per sample batch	Per Appendix D of this QAPP	If the OPR recovery is not within the QC acceptance limits: take corrective action and repeat the OPR analysis, beginning with a fresh aliquot, reanalyze all samples in the affected analytical batch.

Table 7. QC Samples and Acceptance Criteria for PCB Analysis of Fish Tissue

QC Operation	Frequency	Acceptance Limit	Corrective Action
Method blank	Once per batch of up to 20 field samples	5x MDL for each congener (As noted elsewhere, all results, including blanks, are reported down to the MDL.)	If the method blank result is above 5x MDL, the laboratory will reanalyze the method blank extract to confirm the presence of the blank contaminants. If the reanalysis result is still above 5x MDL, then the laboratory will compare the results in the method blank to the results in all of the associated field samples in the batch and take corrective action as follows: 1. If the result for a congener (or group of coeluting congeners) that is present in the method blank at 5x MDL or higher is *not present* in the field sample, then the result for that field sample may be reported without corrective actions. The result must be flagged with a "B" flag that indicates the presence of the analyte in the associated blank and the data package narrative must discuss the comparison of the blank and sample results for that sample. 2. If the result for the congener in the field sample is more than 10 times the level found in the method blank, then the result for that field sample also may be reported without corrective actions. The result must be flagged with a "B" flag that indicates the presence of the analyte in the associated blank and the data package narrative must discuss the comparison of the blank and sample results for that sample. 3. If the result for the congener in the field sample is less than or equal to 10 times the level found in the method blank, then re-extraction and reanalysis of the affected sample is **required** (but not samples that meet the conditions in #1 and #2 above) in conjunction with a new method blank and all other method-specified QC samples. CSRA will work with the laboratory to schedule any required reanalyses in a manner that does not delay analyses of subsequent batches of field samples. 4. If the results of the re-extraction and reanalysis of the field sample do not resolve the problem, i.e., the background levels in the method blank are still a concern, CSRA will require that the laboratory provide information on historical levels of blank contaminants for similar matrices. CSRA and EPA will evaluate those historical results and the reanalysis results on a case-by-case basis to determine if there is a pattern of blank contamination that is indicative of a broader problem and if any further corrective actions are required by the laboratory.

As with the mercury analyses, 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 PCBs are usually analyzed with each rinsate sample, and are summarized in Table 8 below.

Table 8. QC Samples and Acceptance Criteria for PCB Analysis of Rinsates

QC Sample	Frequency	Acceptance Criteria
Instrument blank	With each rinsate sample	Result must be less than the MDL. Otherwise, reanalyze the rinsate sample.
Laboratory Control Sample	With each rinsate sample	70 - 130% recovery. Otherwise, correct instrumental problems, and reanalyze the rinsate sample.
Surrogate	Added to each rinsate sample	59 - 138% recovery. Otherwise, correct instrumental problems, and reanalyze the rinsate sample.

B5.4 PFC Analysis Quality Control

The high performance liquid chromatograph/tandem mass spectrometer is calibrated daily as described in the laboratory's SOP. At least six calibration standards are used for calibration, using a weighted linear regression. The correlative coefficient for the regression must be ≥ 0.95. The calibration is verified every 12 hours through the analysis of the calibration verification standard. The results for the calibration verification must meet the requirements in Appendix E of this QAPP.

Quality control samples associated with each batch of tissue samples or rinsate samples analyzed for PFCs are summarized in Table 9 below.

Table 9. QC Samples and Acceptance Criteria for PFC Analysis of Tissues and Rinsates

QC Operation	Frequency	Acceptance Limit	Corrective Action
Labeled compounds	Spiked into every sample before extraction	Per Appendix E of this QAPP	Evaluate failure and impact on samples. If sample results are non-detects for analytes which have a high labeled compound recovery, report non-detect results with case narrative comment. For detected analytes with low labeled compound recovery, extract and analyze a smaller sample aliquot.
Calibration Verification	Every 12 hours, before sample analysis.	Per Appendix E of this QAPP	Evaluate failure and impact on samples. If sample results are non-detects for analytes which have a high bias, report non-detect results with case narrative comment. *or* Immediately analyze two additional consecutive verification standards. If both pass, samples may be reported without reanalysis. If either fails, take corrective action(s) and re-calibrate; then reanalyze all affected samples since the last acceptable verification standard.
Lab Control Sample (LCS)	Once per batch of up to 20 field samples	Per Appendix E of this QAPP	Reanalyze LCS once. If acceptable, report. Evaluate samples for detections, and LCS for high bias. If LCS has high bias, and sample results are non-detects, report with case narrative comment. If LCS has low bias, or if there are detected analytes with failures, evaluate and reprepare and reanalyze the LCS and all samples in the associated prep batch for failed analytes.

Table 9. QC Samples and Acceptance Criteria for PFC Analysis of Tissues and Rinsates

QC Operation	Frequency	Acceptance Limit	Corrective Action
Method blank	Once per batch of up to 20 field samples	Less than or equal to the MDLs in Appendix C of this QAPP	As noted elsewhere, all results, including blanks, are reported down to the method detection limit (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 tissue sample and rinsate 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 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 tissue or rinsate samples and QC samples. - If the method blank reanalysis result is still above the quantitation limit, then the laboratory will re-extract and reanalyze all tissue or rinsate samples with original results above the MDL.
Laboratory duplicate	Once per batch of up to 20 field samples	The relative percent difference (RPD) of the duplicate measure-ments must be < 40%	Evaluate the data, and re-extract and reanalyze the original sample and duplicate: • If the reanalysis results meet the RPD limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the reanalysis result still does not meet the RPD limit, then the laboratory will re-extract and reanalyze all field samples with original results above the MDL.

B5.5 Fatty Acid Analysis Quality Control

The GC/FID is calibrated as described in the laboratory's SOP. Five calibration standards are used for calibration, using a weighted linear regression. The correlative coefficient for the regression must be ≥ 0.95. The calibration is verified every 10 samples through the analysis of the calibration verification standard. The results for the calibration verification must be within 70 - 130% of the stated concentration for the solution.

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

Note: The fatty acid analysis laboratory prepares and analyzes samples in batches of 10, not 20 as is typically specified in EPA methods.

Table 10. QC Samples and Acceptance Criteria for Fatty Acid Analysis of Tissues

QC Operation	Frequency	Acceptance Limit	Corrective Action
Surrogate (C19:0)	Added to every field and QC sample	50 to 150% recovery	If the recovery of the surrogate is not within the acceptance criteria, reanalyze the affected samples.
Calibration Verification	Every 10 samples	70 - 130% of stated concentration	Evaluate failure and impact on samples. If sample results are non-detects for analytes which have a high bias, report non-detect results with case narrative comment. *or* Immediately analyze two additional consecutive verification standards. If both pass, samples may be reported without reanalysis. If either fails, take corrective action(s) and re-calibrate; then reanalyze all affected samples since the last acceptable verification standard.
Method blank	Once per analysis batch (10 field samples, plus QC samples)	Method detection limit (MDL)	If any of the analytes are present in the method blank above the MDL, • take corrective action to reduce the blank level to below the MDL, • reanalyze any samples in the affected batch with results less than 10 times the observed results for the blank, and • flag sample results greater than 10 times the observed blank level to advise the data user of the potential contamination.
Reference sample	Analyze 1 aliquot of the GLNPO Lake Superior reference tissue sample per analysis batch	60 to 140% of the certified value	If the results are not within the acceptance limits, • take corrective action and repeat the reference sample analysis, beginning with a fresh aliquot, • reanalyze all samples in the affected analytical batch.
Laboratory duplicate sample	Once per every two analysis batches (10 field samples, plus QC samples)	RPD ≤ 50%	If the RPD exceeds the acceptance limit, the laboratory will reanalyze the duplicate sample: • If the reanalysis results meets the RPD limit, then the laboratory will reanalyze all of the associated field and QC samples.

B5.6 Dioxin and Furan (PCDD/PCDF) Analysis Quality Control

The high resolution gas chromatograph/high resolution mass spectrometer used for fish tissue analysis is calibrated periodically as described in Method 1613B and the laboratory's SOP. As implemented by the laboratory, six standards are used for calibration (one more than required by the method), and the variability in the response factors for the six standards must have a RSD less than or equal to 20%. The calibration is verified every 12 hours by the analysis of the calibration verification standard. The results for the calibration verification must meet the requirements in Appendix F of this QAPP.

Quality control samples associated with each batch of tissue samples analyzed for dioxins and furans are summarized in Table 11 below, and are based on the QC requirements of Method 1613B, with the project-specific addition of one laboratory duplicate sample per batch.

The QC acceptance criteria for laboratory duplicate analyses and method blanks are provided below. Control of background levels of dioxins and furans in the laboratory is critical.

However, the low method detection limits required for this effort increase the risk that method blanks will exhibit signs of contamination. Therefore, the corrective actions described here are designed to assess method blank results relative to the concentrations of dioxins and furans in fish tissue samples.

Table 11. QC Samples and Acceptance Criteria for Dioxin and Furan Analysis of Tissues and Rinsates

QC Operation	Frequency	Acceptance Limit	Corrective Action
OPR/LCS	One per sample batch	Per Appendix F of this QAPP	If the OPR recovery is not within the QC acceptance limits: • take corrective action and repeat the OPR analysis beginning with a fresh aliquot, reanalyze all samples in the affected analytical batch.
Labeled compounds	Spiked into every sample	Per Appendix F of this QAPP	Per Method 1613B
Calibration verification (VER)	At the beginning of every 12-h analytical shift	Per Appendix F of this QAPP	Per Method 1613B
Laboratory duplicate	Once per batch of up to 20 field samples	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 meets the RPD limit, then the laboratory will reanalyze all of the associated field and QC samples. • If the reanalysis result still does not meet the RPD limit, then the laboratory will re-extract and reanalyze all field samples with original results above the MDL.
Method blank	Once per batch of up to 20 field samples	5x MDL for each analyte (As noted elsewhere, all results, including blanks, are reported down to the MDL.)	If the method blank result is above 5x MDL, the laboratory will reanalyze the method blank extract to confirm the presence of the blank contaminants. If the reanalysis result is still above 5x MDL, then the laboratory will compare the results in the method blank to the results in all of the associated field samples in the batch and take corrective action as follows: 1. If the result for an analyte that is present in the method blank at 5x MDL or higher is *not present* in the field sample, then the result for that field sample may be reported without corrective actions. The result must be flagged with a "B" flag that indicates the presence of the analyte in the associated blank and the data package narrative must discuss the comparison of the blank and sample results for that sample.

Table 11. QC Samples and Acceptance Criteria for Dioxin and Furan Analysis of Tissues and Rinsates

QC Operation	Frequency	Acceptance Limit	Corrective Action
			2. If the result for the analyte in the field sample is more than 10 times the level found in the method blank, then the result for that field sample also may be reported without corrective actions. The result must be flagged with a "B" flag that indicates the presence of the analyte in the associated blank and the data package narrative must discuss the comparison of the blank and sample results for that sample. 3. If the result for the analyte in the field sample is less than or equal to 10 times the level found in the method blank, then re-extraction and reanalysis of the affected sample is **required** (but not samples that meet the conditions in #1 and #2 above) in conjunction with a new method blank and all other method-specified QC samples. CSRA will work with the laboratory to schedule any required reanalyses in a manner that does not delay analyses of subsequent batches of field samples. 4. If the results of the re-extraction and reanalysis of the field sample do not resolve the problem, i.e., the background levels in the method blank are still a concern, CSRA will require that the laboratory provide information on historical levels of blank contaminants for similar matrices. CSRA and EPA will evaluate those historical results and the reanalysis results on a case-by-case basis to determine if there is a pattern of blank contamination that is indicative of a broader problem and if any further corrective actions are required by the laboratory.

B6. Instrument/Equipment Testing, Inspection, and Maintenance

There are no analytical instruments used in the preparation of the fillet tissue samples. However, the balances used to weigh the whole fish, the fillets, the homogenized fillet tissue, and the fillet tissue aliquots are inspected and serviced on a regular schedule (Tetra Tech, 2013) and the homogenization equipment (i.e., meat grinder) will be inspected when it is reassembled after cleaning between samples.

All analytical instrumentation associated with the rinsate analyses and fillet tissue sample analyses will be inspected and maintained as described in the respective analysis methods and laboratory SOPs.

B7. Instrument/Equipment Calibration and Frequency

The balances used to weigh the whole fish and the fillet tissue during the various stages of homogenization and aliquot preparation will be calibrated on a regular schedule and calibrations are verified at the beginning of each day on which the balances are used.

All analytical instrumentation associated with the rinsate analyses and fillet tissue sample analyses will be calibrated as described in the respective analysis methods. The methods in Table 3 for the rinsate analyses require multi-point initial calibrations and periodic calibration verifications, and all the methods contain QC acceptance criteria for calibration.

The mercury analysis method for tissue samples, Method 1631E, specifies calibration with at least five calibration standards and multiple blanks, as described in Section B5.2 above. The mercury analysis method for the rinsate samples, Method 245.1, also specifies calibration with five calibration standards.

The PCB analysis method for tissue samples, Method 1668C, specifies calibration with at least five calibration standards, as described in Section B5.3 above. The PCB analysis method for the rinsate samples, SW-846 Method 8082A, specifies calibration with at least five calibration standards.

The PFC analysis procedure from AXYS Analytical for tissue and rinsate analyses specifies calibration with at least six calibration standards, as described in Section B5.4 above.

The fatty acid analysis procedure from Clarkson University for tissue analyses specifies calibration with five calibration standards, as described in Section B.5.5 above.

The dioxin and furan analysis method for tissue samples, Method 1613B, specifies calibration with at least five calibration standards, as described in Section B5.6 above. However, AXYS Analytical will add a sixth standard at one-fifth of the concentration of the lowest standard specified in the original method, in order to meet the project's requirements for sensitivity.

B8. Inspection/Acceptance of Supplies and Consumables

The inspection and acceptance of any laboratory supplies and consumables associated with the rinsate analyses and fillet tissue sample analyses are addressed in the individual laboratory operating procedures to be used, and/or in the laboratory's existing overall quality system documentation. 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 data management practices used for EPA's National Lake Fish Tissue Study and other OST fish contamination studies (e.g., the 2010 Great Lakes Human Health 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 by CSRA (CSRA 2015). 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 2015 GLHHFFTS, are summarized below.

Laboratory Data Management

Laboratory data management procedures include the following:

- The fish sample preparation laboratory is required to maintain all records and documentation associated with the preparation of fillet tissue samples and analysis of rinsate QC samples for a minimum period of five years after completion of the study. Similarly, each analytical laboratory is required to maintain all records and documentation associated with the analysis fillet tissue samples and rinsate samples (if applicable) 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 in electronic format, as specified in the analytical laboratory statements of work from CSRA.
- 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 will be incorporated in their overall quality system documentation (e.g., their quality management plan).

CSRA Data Management

Data management procedures employed by CSRA 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 subsequent OST fish contamination studies) 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, CSRA will

modify existing data review guidelines as necessary to reflect the methods, method modifications, and data quality objectives for the 2015 GLHHFFTS and provide those modifications to all CSRA data review staff for implementation. Descriptions of any modifications will be retained in CSRA'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. CSRA data review chemists use this multi-stage process to verify the quality of each laboratory submission under the 2015 GLHHFFTS. If an error is detected in any stage of the review, the CSRA data review chemist and the CSRA 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 CSRA's project records and reported to the OST Project Manager.

Concurrent with the performance of data quality audits, CSRA 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, matrix spike, etc.)
- fish species (scientific and common names)
- fish specimen number
- length of fish specimen
- weight of fish specimen
- inclusion of the fish specimen in the homogenized sample
- year samples were collected
- sample collection date
- Great Lake from which samples were collected
- state where site is located
- latitude/longitude where site is located
- ecological group for fish samples (predator or bottom dweller)
- sample analysis date
- measured value for each target analyte
- fish tissue lipid content measurements

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

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 CSRA 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 CSRA. Corrective actions will be taken as needed to resolve deficiencies. Following completion of the data quality review, the CSRA data review chemist and the CSRA 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 CSRA data review chemist to verify database accuracy before submission to EPA. These reports are maintained in CSRA'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 (Tetra Tech 2013, ALS 2014, Vista 2014, AXYS 2015, and Clarkson 2016). These materials have been be reviewed by CSRA during the solicitations for each of the analyses, as part of an assessment of laboratory capabilities. Copies of these QA plans are being maintained on file at CSRA and will be made available to EPA for review on request.

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 2015 GLHHFFTS are conducted as prescribed and that the performance criteria defined for the study are met.

C1.1 Surveillance

The CSRA Project Leader will schedule and track all analytical work performed by laboratories for mercury, PCB, PFC, dioxin and furan, and fatty acid analyses. The Project Leader will coordinate with staff at the sample preparation laboratory 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 CSRA if the shipments do not arrive intact as scheduled. Within 24 hours of scheduled sample receipt, CSRA 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.

CSRA's project leader will obtain fish sample processing instructions for each batch of 20 samples from the OST Project Manager and transmit those instructions to the sample preparation

laboratory by email (the number of samples per analysis batch may vary slightly). The sample preparation laboratory may not begin processing any samples until this QAPP is approved and CSRA provides the sample processing instructions.

CSRA also will communicate periodically with laboratory staff by telephone or email to monitor the progress of tissue sample preparation, lipid and rinsate analyses, analytical sample preparation, tissue sample analyses, and data reporting. If technical problems are encountered during sample preparation and analysis, CSRA will identify a technical expert within CSRA to assist in resolving the problem, and work with EPA to identify and implement a solution to the problem. The sample preparation laboratory will be permitted to work two batches ahead of the CSRA/EPA review of the lipid and rinsate analyses to ensure that the homogenization and equipment cleaning procedures are adequate. If the sample preparation laboratory fails to deliver data on time, or if the laboratory notifies CSRA of anticipated reporting delays, CSRA will notify the OST Project Manager of the situation. To the extent possible, CSRA will adjust schedules and shift resources within CSRA as necessary to minimize the impact of laboratory delays on EPA schedules. CSRA also will immediately notify the OST Project Manager of any laboratory delays that are anticipated to impact EPA schedules.
The mercury tissue analysis laboratory will be permitted to work one batch ahead of the CSRA/EPA review of the QC results associated with the tissue analyses. CSRA also will immediately notify the OST Project Manager of any mercury laboratory delays that are anticipated to impact EPA schedules.

The PCB tissue analysis laboratory will be permitted to work one batch ahead of the CSRA/EPA review of the QC results associated with the tissue analyses. CSRA also will immediately notify the OST Project Manager of any PCB laboratory delays that are anticipated to impact EPA schedules.

The PFC tissue analysis laboratory will be permitted to work one batch ahead of the CSRA/EPA review of the QC results associated with the tissue analyses. CSRA also will immediately notify the OST Project Manager of any PFC laboratory delays that are anticipated to impact EPA schedules. The PFC rinsate samples will be submitted to the analysis laboratory as one batch at the end of the tissue analysis effort.

The fatty acid analysis laboratory will be permitted to complete two batches of 10 samples prior to CSRA/EPA review of the QC results associated with the tissue analyses. CSRA also will immediately notify the OST Project Manager of any fatty acid laboratory delays that are anticipated to impact EPA schedules.

The dioxin and furan tissue analysis laboratory will be permitted to work one batch ahead of the CSRA/EPA review of the QC results associated with the tissue analyses. CSRA also will immediately notify the OST Project Manager of any dioxin and furan laboratory delays that are anticipated to impact EPA schedules. The dioxin and furan rinsate samples will be submitted to the analysis laboratory as one batch at the end of the tissue analysis effort.

Finally, the CSRA 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, CSRA 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, CSRA will meet with the EPA OST Project Manager to discuss an appropriate solution.

C1.2 Product Review

Product reviews for validated analytical data packages will be performed within CSRA to verify that the CSRA 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 CSRA 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. Product reviews will be conducted on at least 10% of the data packages for a given analysis. Qualified product reviewers will include any staff members that have been trained in CSRA 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, CSRA staff will review 100% of the data qualifier flags entered into the project database.

The EPA Project Co-Managers, SHPD QA Coordinator, and GLNPO QA Manager will review the analytical QA report developed by CSRA, and the OST Project Manager will approve the final analytical QA report. The 2015 GLHHFFTS data files prepared by CSRA for statistical analysis of the data will be reviewed internally by CSRA staff and independently by the OST Project Manager with support from Tetra Tech before being forwarded to ORD statisticians at NHEERL-Corvallis, who will complete statistical analysis of the 2015 GLHHFFTS data and deliver the results to the OST 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 2015 GLHHFFTS mercury, PCB, PFC, dioxin and furan, and fatty acid analyses portion of the NCCA 2015.

C1.4 Readiness Review

A readiness review of the sample preparation laboratory's capability to produce homogeneous tissue sample aliquots will begin with the kick-off meeting with the laboratory. This effort will include the initial demonstration of capabilities described in Appendix B. Routine processing of fish tissue samples will not begin until the laboratory demonstrates its competency through acceptable performance in the initial demonstration of capabilities. Records of these reviews and any corrective actions will be maintained by CSRA. CSRA staff will document their findings and recommendations concerning the readiness review as part of a written analytical QA report to EPA.

A readiness review of the mercury tissue analysis laboratory's capability to produce acceptable sample results began with a review of materials submitted by the laboratory during the solicitation process and continued during a kick-off conference call with the laboratory (ALS-Environmental). The requested materials included information about the laboratory's capacity, past experience with tissue analyses, and accreditations or certifications for mercury analyses in tissue and other matrices. The laboratory is accredited for mercury analyses in tissue by at least six states, including the Oregon Environmental Laboratory Accreditation Program, and by the Department of Defense Environmental Laboratory Accreditation Program. The laboratory also is certified by the International Standards Organization (ISO 17205). These materials were reviewed during the solicitation process to assess the laboratory's competency and will be kept on file by CSRA.

A similar readiness review of the PCB tissue analysis laboratory's capability to produce acceptable sample results began with a CSRA review of materials submitted by the laboratory during the solicitation process and continued during a kick-off conference call with the laboratory (Vista Analytical). The requested materials included information about the laboratory's capacity, past experience with tissue analyses, and accreditations or certifications for PCB analyses in tissue and other matrices. The laboratory is accredited for PCB analyses in tissue by the Oregon Environmental Laboratory Accreditation Program and by the Department of Defense Environmental Laboratory Accreditation Program. These materials were reviewed during the solicitation process to assess the laboratory's competency and will be kept on file by CSRA.

A readiness review of the PFC tissue and rinsate analysis laboratory's capability to produce acceptable sample results began with a CSRA review of materials submitted by the laboratory during the solicitation process and continued during a kick-off conference call with the laboratory (AXYS Analytical). The requested materials included information about the laboratory's capacity, past experience with tissue analyses, and accreditations or certifications for PFC analyses in tissue and other matrices. The laboratory is accredited for PFC analyses in tissue by the Canadian Association for Laboratory Accreditation, the Department of Defense Environmental Laboratory Accreditation Program, the New Jersey Department of Environmental Services, and the Minnesota Department of Health. These materials were reviewed during the solicitation process to assess the laboratory's competency and will be kept on file by CSRA.

A readiness review of the fatty acid analysis laboratory's capability to produce acceptable sample results began with a CSRA review of materials submitted by the laboratory during the solicitation process and will continue during a kick-off conference call with the laboratory (Clarkson University). Unlike the procurements for the other types of analyses in this project, SHPD and GLNPO requested that CSRA utilize a sole-source procurement for the fatty acid analyses. EPA made this request because Clarkson University has developed and tested sample preparation and analysis procedures for fatty acids in fish tissues, and has conducted research and generated a substantial body of data for GLNPO under Grant No. GL 00E01505. The data from this study will be used by EPA to evaluate the fatty acid content of fish tissues from the Great Lakes and GLNPO's efforts will be greatly enhanced by ensuring comparability with the existing data generated by Clarkson.

A readiness review of the dioxin and furan tissue and rinsate analysis laboratory's capability to produce acceptable sample results began with a CSRA review of materials submitted by the laboratory during the solicitation process and will continue during a kick-off conference call with

the laboratory (AXYS Analytical). The requested materials included information about the laboratory's capacity, past experience with tissue analyses, and accreditations or certifications for dioxin and furan analyses in tissue and other matrices. The laboratory is accredited for dioxin and furan analyses in tissue by the Canadian Association for Laboratory Accreditation, the Department of Defense Environmental Laboratory Accreditation Program, the Florida Department of Health, the New Jersey Department of Environmental Services, and the Virginia Department of General Services. These materials were reviewed during the solicitation process to assess the laboratory's competency and will be kept on file by CSRA.

Readiness reviews will be performed by CSRA data reviewers. If problems are identified during these reviews, CSRA will work with the laboratory, to the extent possible, to resolve the problem prior to awarding an analysis contract. If the problem cannot be resolved within the time frame required by EPA, CSRA will notify the OST Project Manager immediately. Records of these reviews and any corrective actions are maintained by CSRA separate from the analytical results for the field samples. CSRA 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, sample processing, rinsate and tissue 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 OST Project Manager and/or the SHPD QA Coordinator (or a qualified EPA staff member designated by the OST QA Officer) and a CSRA 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), CSRA will create an analytical database that contains all field sample results from the 2015 GLHHFFTS (see Section B10). At selected intervals and upon completion of the study, CSRA'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. CSRA 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

The sample preparation laboratory will provide CSRA with a weekly status report that describes all of the fish samples processed during the previous week. CSRA will review those reports for completeness and then forward them to the OST Project Manager. CSRA will track the receipt of data submissions for the mercury, PCB, PFC, dioxin and furan, and fatty acid fillet tissue analyses and advise the OST Project Manager of progress on a monthly basis.

Following data verification and validation of all project analytical data, CSRA will apply standardized data qualifier flags to the fish tissue 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 Study 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.

CSRA will provide a monthly report to the OST Project Manager that describes the status of all current analysis and data review activities, during each month in which analyses and data review are conducted. CSRA will provide periodic status reports on database activities and revisions, as needed.

D. DATA VALIDATION AND USABILITY

This QAPP addresses the preparation of fish tissue samples and the generation of data for mercury, PCBs, PFCs, dioxins and furans, and fatty acids in fish tissue samples. Sections D1, D2, and D3 of this QAPP apply to all of the analytical data generation for the 2015 GLHHFFTS.

D1. Data Review, Verification, and Validation

The data review, verification, and validation aspects of the fillet tissue sample preparation effort are more limited than those that will be applied to the mercury, PCB, PFC, dioxin and furan, and fatty acid analysis efforts. However, the procedures described below apply to both types of data.

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 CSRA 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, PCB, PFC, dioxin and furan, and fatty acid 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.

CSRA staff will also conduct reviews of the QC sample results for homogenized fish tissue samples prepared by the fish sample preparation laboratory. This will involve review of data for percent lipid measurements that serve as a surrogate for homogeneity testing and review of the results from any rinsates of the sample processing equipment and the paired solvent blanks. The CSRA 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. CSRA data review chemists will validate all of the mercury, PCB, PFC, dioxin and furan, and fatty acid 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, CSRA 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 CSRA 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 CSRA 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 CSRA 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 CSRA 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 CSRA data review chemist will immediately implement corrective actions.

D2.2 Validation Methods

CSRA 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.

During this assessment, data qualifier flags are applied to the project results to identify any results that did not meet the method- or project-specific requirements, CSRA 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, blank contamination, 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.

D3. Reconciliation with User Requirements

The QC results for lipids from the homogeneity testing and the rinsate analysis for each batch of fish tissue samples prepared will be assessed against the QC acceptance criteria. Although the sample preparation laboratory will be permitted to work "two batches ahead" of the delivery of the batch-specific QC results, CSRA will track laboratory performance, notify the OST Project Manager of any issues, initiate corrective actions, and track progress by the sample preparation laboratory.

The QC results for the analyses of the fish tissue samples for mercury, PCB congeners, and PFCs will be assessed against the QC acceptance criteria for those analyses. Although the analytical laboratories will be permitted to work "one batch ahead" of the delivery of the batch-specific QC

results (with the exception of the fatty acid analysis laboratory, as noted in C1.1), CSRA will track laboratory performance, notify the OST Project Manager of any issues, initiate corrective actions, and track progress by the sample analysis laboratories.

The QC results for the analyses of the fish tissue samples for fatty acids will be assessed against the QC acceptance criteria for those analyses. CSRA will track laboratory performance, notify the OST Project Manager of any issues, initiate corrective actions, and track progress by the fatty acid analysis laboratory.

The QC results for the analyses of the fish tissue samples for dioxins and furans will be assessed against the QC acceptance criteria for those analyses. CSRA will track laboratory performance, notify the OST Project Manager of any issues, initiate corrective actions, and track progress by the dioxin and furan analysis laboratory.

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Appendix A
List of 2015 GLHHFFTS Sampling Locations

List of 2015 GLHHFFTS Sampling Locations				
Lake	**State**	**Site ID**	**Latitude**	**Longitude**
Lake Erie	MI	GLNS15-1156	41.8554879	-83.37181083
Lake Erie	MI	GLNS15-1164	41.9783891	-83.22606826
Lake Erie	MI	GLNS15-2145	41.8847289	-83.30824563
Lake Erie	MI	GLNS15-2153	41.7555907	-83.43777643
Lake Erie	NY	GLNS15-1154	42.7321235	-78.97097200
Lake Erie	NY	GLNS15-1155	42.5382884	-79.27533466
Lake Erie	NY	GLNS15-1158	42.6814562	-79.08613143
Lake Erie	NY	GLNS15-1166	42.5044351	-79.38347809
Lake Erie	NY	GLNS15-2139	42.6345223	-79.11702333
Lake Erie	NY	GLNS15-2141	42.5446796	-79.32787183
Lake Erie	NY	GLNS15-2143	42.7375442	-78.94866342
Lake Erie	NY	GLNS15-2152	42.2749308	-79.75374642
Lake Erie	OH	GLNS15-1152	41.7462498	-83.37917303
Lake Erie	OH	GLNS15-1153	41.5104748	-82.13911480
Lake Erie	OH	GLNS15-1157	41.5006316	-82.21454330
Lake Erie	OH	GLNS15-1159	41.9756827	-80.61521617
Lake Erie	OH	GLNS15-1160	41.6339413	-83.16824722
Lake Erie	OH	GLNS15-1161	41.4288967	-82.58177787
Lake Erie	OH	GLNS15-1163	41.4886506	-81.74174857
Lake Erie	OH	GLNS15-1165	41.5666907	-82.76520113
Lake Erie	OH	GLNS15-2140	41.9074408	-80.88457322
Lake Erie	OH	GLNS15-2142	41.4826889	-82.73135356
Lake Erie	OH	GLNS15-2144	41.8708241	-81.03970010
Lake Erie	OH	GLNS15-2147	41.5105015	-81.78188419
Lake Erie	OH	GLNS15-2149	41.7009558	-83.25070278
Lake Erie	OH	GLNS15-2150	41.5723313	-82.68277916
Lake Erie	OH	GLNS15-2151	41.7873146	-81.21470025
Lake Erie	OH	GLNS15-2154	41.5036096	-82.12039313
Lake Erie	OH	GLNS15-2163	41.6403488	-81.52632327
Lake Erie	PA	GLNS15-1162	42.2160603	-79.90828532
Lake Erie	PA	GLNS15-2148	42.2147739	-79.98687777
Lake Huron	MI	GLNS15-1197	43.6611723	-83.81374505
Lake Huron	MI	GLNS15-1199	45.7503624	-84.56395123
Lake Huron	MI	GLNS15-1200	44.8393453	-83.24250009
Lake Huron	MI	GLNS15-1201	43.6918781	-83.60716071
Lake Huron	MI	GLNS15-1202	45.9630690	-84.71430350
Lake Huron	MI	GLNS15-1203	45.3781040	-83.64797083
Lake Huron	MI	GLNS15-1205	45.9396516	-84.66939243
Lake Huron	MI	GLNS15-1206	45.0065969	-83.35905655
Lake Huron	MI	GLNS15-1207	43.8799081	-83.43664297
Lake Huron	MI	GLNS15-1208	44.0134998	-82.76739263
Lake Huron	MI	GLNS15-1209	45.9607088	-84.41915005
Lake Huron	MI	GLNS15-1210	45.1865067	-83.33388705
Lake Huron	MI	GLNS15-1211	44.2627273	-83.47572041
Lake Huron	MI	GLNS15-1212	45.3653385	-83.55898448
Lake Huron	MI	GLNS15-2093	44.7284588	-83.23523123
Lake Huron	MI	GLNS15-2094	45.9464816	-83.99380482
Lake Huron	MI	GLNS15-2095	44.3757456	-83.32763202
Lake Huron	MI	GLNS15-2096	44.0904944	-82.99978198
Lake Huron	MI	GLNS15-2097	44.4839004	-83.31300610
Lake Huron	MI	GLNS15-2098	45.9335052	-84.44576418
Lake Huron	MI	GLNS15-2099	44.1898411	-83.50796398
Lake Huron	MI	GLNS15-2100	44.0153668	-83.22928536

List of 2015 GLHHFFTS Sampling Locations				
Lake	**State**	**Site ID**	**Latitude**	**Longitude**
Lake Huron	MI	GLNS15-2101	45.0339592	-83.43878281
Lake Huron	MI	GLNS15-2102	45.5450881	-84.11359735
Lake Huron	MI	GLNS15-2103	43.6939822	-83.59829374
Lake Huron	MI	GLNS15-2104	43.6615767	-82.56605811
Lake Huron	MI	GLNS15-2106	43.6695815	-83.64148419
Lake Huron	MI	GLNS15-2107	44.0505958	-83.56432289
Lake Huron	MI	GLNS15-2108	43.5076996	-82.50602794
Lake Huron	MI	GLNS15-2116	43.8440331	-83.44960901
Lake Huron	MI	GLNS15-2117	45.4602306	-83.85656002
Lake Michigan	IN	GLNS15-2051	41.6912384	-87.47797869
Lake Michigan	MI	GLNS15-1245	45.7690891	-86.74217938
Lake Michigan	MI	GLNS15-1246	43.3753939	-86.46277176
Lake Michigan	MI	GLNS15-1247	45.0007100	-85.47704522
Lake Michigan	MI	GLNS15-1248	44.9440326	-85.84071470
Lake Michigan	MI	GLNS15-1249	44.3960289	-86.30882045
Lake Michigan	MI	GLNS15-1250	45.8880900	-86.25743836
Lake Michigan	MI	GLNS15-1252	43.9188985	-86.45743667
Lake Michigan	MI	GLNS15-1253	42.9441948	-86.24677400
Lake Michigan	MI	GLNS15-1254	45.7975030	-84.79227265
Lake Michigan	MI	GLNS15-1255	43.1023971	-86.27176684
Lake Michigan	MI	GLNS15-2047	42.0341486	-86.57656027
Lake Michigan	MI	GLNS15-2050	43.9931668	-86.54477996
Lake Michigan	MI	GLNS15-2052	45.4863928	-87.23726690
Lake Michigan	MI	GLNS15-2053	45.8198774	-86.71584769
Lake Michigan	MI	GLNS15-2054	44.2800180	-86.32644731
Lake Michigan	MI	GLNS15-2055	45.9858327	-85.64287553
Lake Michigan	MI	GLNS15-2056	44.7392551	-86.17727931
Lake Michigan	MI	GLNS15-2057	45.5693026	-85.18340886
Lake Michigan	MI	GLNS15-2061	42.7323492	-86.24231456
Lake Michigan	WI	GLNS15-1243	45.0291466	-87.09338281
Lake Michigan	WI	GLNS15-1244	42.6147009	-87.81057710
Lake Michigan	WI	GLNS15-1251	43.3289178	-87.86407149
Lake Michigan	WI	GLNS15-1256	44.9480270	-87.69860745
Lake Michigan	WI	GLNS15-2048	45.0815094	-87.54124320
Lake Michigan	WI	GLNS15-2058	44.4525419	-87.49285025
Lake Michigan	WI	GLNS15-2059	44.9447586	-87.64326803
Lake Michigan	WI	GLNS15-2060	44.9992570	-87.09029184
Lake Ontario	NY	GLNS15-1288	43.9682677	-76.11540359
Lake Ontario	NY	GLNS15-1289	43.9135954	-76.18341189
Lake Ontario	NY	GLNS15-1290	43.3581994	-78.70273346
Lake Ontario	NY	GLNS15-1291	43.3379735	-77.67321462
Lake Ontario	NY	GLNS15-1292	43.5062224	-76.48771714
Lake Ontario	NY	GLNS15-1293	43.9121875	-76.28412354
Lake Ontario	NY	GLNS15-1294	43.3119737	-78.88899885
Lake Ontario	NY	GLNS15-1295	43.2547999	-77.48872710
Lake Ontario	NY	GLNS15-1296	43.5875910	-76.25064947
Lake Ontario	NY	GLNS15-1297	44.0758817	-76.37699745
Lake Ontario	NY	GLNS15-1298	43.3812749	-78.08532368
Lake Ontario	NY	GLNS15-1299	43.4314528	-76.62717846
Lake Ontario	NY	GLNS15-1300	43.8033817	-76.25181977
Lake Ontario	NY	GLNS15-1301	44.0053209	-76.18596095
Lake Ontario	NY	GLNS15-1302	43.3613768	-77.93097380
Lake Ontario	NY	GLNS15-1303	43.3191314	-76.87900567

List of 2015 GLHHFFTS Sampling Locations				
Lake	**State**	**Site ID**	**Latitude**	**Longitude**
Lake Ontario	NY	GLNS15-2185	43.3485453	-78.79364249
Lake Ontario	NY	GLNS15-2186	43.3037081	-77.03285603
Lake Ontario	NY	GLNS15-2187	43.2845819	-77.59522436
Lake Ontario	NY	GLNS15-2188	44.0700546	-76.39230424
Lake Ontario	NY	GLNS15-2189	43.3668013	-78.59714382
Lake Ontario	NY	GLNS15-2190	43.3240576	-76.80657331
Lake Ontario	NY	GLNS15-2191	43.3126415	-77.66985422
Lake Ontario	NY	GLNS15-2192	44.0444897	-76.23753409
Lake Ontario	NY	GLNS15-2193	43.3734281	-78.00235682
Lake Ontario	NY	GLNS15-2194	43.5497595	-76.33100239
Lake Ontario	NY	GLNS15-2195	43.3014963	-77.36346305
Lake Ontario	NY	GLNS15-2196	43.9806235	-76.22676044
Lake Ontario	NY	GLNS15-2197	43.3904285	-78.31651411
Lake Ontario	NY	GLNS15-2198	43.4551540	-76.59470128
Lake Ontario	NY	GLNS15-2199	43.7622461	-76.21926519
Lake Ontario	NY	GLNS15-2200	43.9594273	-76.18538067
Lake Ontario	NY	GLNS15-2201	43.3758047	-76.73319466
Lake Superior	MI	GLNS15-1335	47.3886385	-87.92476313
Lake Superior	MI	GLNS15-1336	46.5329073	-87.38956934
Lake Superior	MI	GLNS15-1338	46.8871877	-88.32472239
Lake Superior	MI	GLNS15-1339	47.2837977	-88.51741039
Lake Superior	MI	GLNS15-1340	46.6852954	-86.16970000
Lake Superior	MI	GLNS15-1341	46.9245090	-87.84378000
Lake Superior	MI	GLNS15-1343	46.7934146	-85.23358964
Lake Superior	MI	GLNS15-1345	47.0428873	-88.98127413
Lake Superior	MI	GLNS15-1346	46.5120060	-87.14860340
Lake Superior	MI	GLNS15-1347	46.5891412	-85.02057986
Lake Superior	MI	GLNS15-2001	46.7273946	-85.88538969
Lake Superior	MI	GLNS15-2002	46.7132846	-85.63315410
Lake Superior	MI	GLNS15-2003	46.9369677	-87.92977336
Lake Superior	MI	GLNS15-2005	46.7110774	-85.90873956
Lake Superior	MI	GLNS15-2006	46.5078013	-85.03641076
Lake Superior	MI	GLNS15-2007	47.2055949	-88.74795916
Lake Superior	MI	GLNS15-2009	46.7039225	-87.48890625
Lake Superior	MI	GLNS15-2011	46.8475917	-89.42212616
Lake Superior	MI	GLNS15-2013	46.4695412	-86.93968035
Lake Superior	MI	GLNS15-2025	46.4462755	-84.56226687
Lake Superior	MN	GLNS15-1333	47.1411404	-91.45035656
Lake Superior	MN	GLNS15-1334	47.5562763	-90.86773508
Lake Superior	MN	GLNS15-1342	46.7904886	-92.04477864
Lake Superior	MN	GLNS15-2004	47.0224337	-91.64884580
Lake Superior	MN	GLNS15-2010	46.7370448	-92.07052213
Lake Superior	MN	GLNS15-2012	47.8107006	-90.06487150
Lake Superior	WI	GLNS15-1337	46.7705100	-91.62224256
Lake Superior	WI	GLNS15-1344	46.6728033	-90.81696326
Lake Superior	WI	GLNS15-2008	46.7992707	-91.46272308
Lake Superior	WI	GLNS15-2015	46.6342693	-90.57572954

Appendix B
Revised 2015 GLHHFFTS Fish Tissue Preparation, Homogenization, and Distribution Procedures

The contents of this appendix have *not* been revised to reflect the name change of CSGov to CSRA because the sample preparation work described herein was completed prior to that change taking place and the version of the procedure presented here accurately reflects the work performed at the time.

Revised 2015 National Coastal Condition Assessment
Great Lakes Human Health Fish Fillet Tissue Study
Tissue Preparation, Homogenization, and Distribution Procedures

I. PURPOSE

This document describes the procedures that the sample preparation laboratory will follow when preparing fish tissue samples for EPA's 2015 National Coastal Condition Assessment Great Lakes Human Health Fish Fillet Tissue Study (2015 GLHHFFTS) under contract to CSGov. 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 Office of Science and Technology (OST) fish tissue studies. The effort is divided into four components:

- A kickoff meeting and workshop involving all study participants, including the sample preparation laboratory staff, EPA/OST, CSGov, and Tetra Tech (OST's fish sampling support contractor)
- An initial demonstration of capabilities, also referred to as the QA study
- Normal fish tissue processing and distribution procedures, including quality control steps
- Special handling requirements for up to 100 single-specimen fish samples and selected individual specimens from other composite fish samples
- Preparation and analyses of rinsate samples and blanks for mercury and selected polychlorinated biphenyls (PCBs), and preparation of rinsate samples and blanks for perfluorinated compounds (PFCs) to be analyzed by a laboratory under a separate CSGov purchase order.

Each of these components is described in detail below.

EPA will prepare a quality assurance project plan (QAPP) for this project which will include the details of fish tissue sample preparation processes described in this SOW, including the description of the analytical procedures and the QC acceptance criteria. After award, CSGov will provide the laboratory with a copy of EPA's QAPP for the project.

II. KICKOFF MEETING AND WORKSHOP

Following award of a purchase order, CSGov will schedule a kickoff meeting and workshop to be held at the sample preparation laboratory at a mutually agreed upon date and time. Staff from all study participants, including the sample preparation laboratory, EPA/OST, CSGov, and Tetra Tech, will meet at the sample preparation laboratory to review the overall 2015 GLHHFFTS 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, CSGov will provide whole fish samples that will be used during a hands-on workshop on the specific procedures for fish sample preparation. All the sample preparation laboratory staff involved in the preparation of fish samples must attend the kickoff meeting and workshop.

The kickoff meeting and workshop will be billable to the CSGov subcontract as a fixed price line item.

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 2015 GLHHFFTS project, the sample preparation laboratory 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 23). In between each fish, the sample preparation laboratory will prepare the equipment rinsate samples and blanks described in Section IV, Steps 27 and 28, and analyze the rinsates and blanks for mercury and

PCBs (Steps 29 and 30, and Attachment 1). The sample preparation laboratory will perform triplicate determinations of lipids on each test sample, as described in Steps 31 and 32. The results of the QA study will be reported to CSGov.

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

The sample aliquots prepared from these QA study samples will be stored frozen at the sample preparation laboratory for possible future use by EPA, or until CSGov authorizes their disposal. Each of the samples prepared for the QA study will be billable under the CSGov subcontract at the cost for a normal project sample.

IV. FISH TISSUE PROCESSING AND DISTRIBUTION PROCEDURES

The procedures for processing and distributing 2015 GLHHFFTS fish tissue samples are described below. The process description is organized into the following ten components, including the quality control (QC) procedures:

A. Sample Receipt and Storage
B. Sample Handling
C. Filleting and Homogenization Procedures
D. Special Handling Requirements for up to 100 Single-specimen Samples and Other Selected Fish Specimens
E. Aliquoting and Distribution Procedures
F. Equipment Cleaning between Fish Samples
G. Lipid Determination on Every Homogenized Fillet Composite Sample
H. Quality Control (QC) Procedures
I. Reporting Requirements
J. Shipping Samples

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

Note: The sample preparation laboratory may **not** process any fish tissue samples until directed by CSGov to proceed. No samples collected from 2015 GLHHFFTS sampling sites may be processed until after the kickoff meeting and workshop and until CSGov reviews the results of the initial demonstration of capabilities (QA study) described in Section III above.

Fish Sample Classifications

For the purposes of the 2015 GLHHFFTS, EPA has classified each valid sample as a "routine" fish sample, or a "non-routine" fish sample, based on the following definitions:

- **Routine sample** – A routine fish 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 sample 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 fish sample is any sample that does not meet the definition of a routine sample, including those that do not meet the 75% length rule and those with fewer or greater than five fish. When non-routine samples are sent to the sample preparation laboratory, EPA and CSGov will provide instructions for processing the non-routine samples. For non-routine samples

containing two or more fish, 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 sample.

Each of the five fish in the routine samples must be filleted before homogenization. **For non-routine samples, 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 fish tissue sample 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 tissue samples.

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 2015 GLHHFFTS, and CSGov will not ship those samples to the laboratory. However, it remains imperative that the sample preparation laboratory not process any sample without specific instructions from CSGov. Therefore, samples will be retained in frozen storage and processed only upon receipt of CSGov-issued instructions. If the status of any fish sample in the instructions is not clear, contact CSGov and wait for clarification.

IV.A Sample Receipt and Storage

Fish samples for the 2015 GLHHFFTS are being collected by various organizations cooperating with EPA in this study, including state agencies, other federal agencies, and contractors. Sample collection began in June 2015 and continued through November 2015. Ultimately, EPA anticipates the collection of composite samples from up to 160 sites by the end of the collection effort in late 2015. CSGov is storing samples at our existing sample repository in Baltimore, Maryland, and will subsequently ship the valid whole fish samples to the sample processing laboratory in batches of 20 samples.

The sample preparation laboratory must have sufficient freezer space to store:

- **up to 60 unprocessed fish composite samples** (e.g., 60 5-fish composites) at a temperature of less than or equal to -20 °C from the time of receipt until completion of sample processing,
- **homogenized tissue aliquots from up to 100 processed samples** (e.g., up to 900 jars) prior to distribution, and
- The filleted carcasses of up to 100 single fish specified by CSGov (see Step 19)

CSGov will provide advance notice of shipments of whole fish samples from our repository, along with sample shipping paperwork relevant to each specific shipment.

1. Although samples will be shipped frozen, on dry ice, they must be inspected promptly on receipt. As samples are received, the sample custodian must:

    - Check that each shipping container has arrived undamaged and verify that samples are still frozen and in good condition.
    - Check the temperature of one of the samples in the cooler using a thermometer that reads to at least -20 ºC, or an infra-red (IR) temperature "gun" and record the reading.
    - Verify that all associated paperwork is complete, legible, and accurate.
    - Compare the information on the label on each individual fish specimen to the sample tracking form for each fish sample and verify that each specimen was included in the shipment and is properly wrapped and labeled.
    - Notify CSGov of the fact that samples were received and report any discrepancies in the paperwork identified above.
    - Transfer the samples to the freezer for long-term storage.

2. Notify CSGov immediately about any problems encountered upon receipt of samples. Problems involving sample integrity, conformity, or inconsistencies for fish samples should be reported to CSGov in writing (e.g., by email) as soon as possible following sample receipt and inspection.

Following sample processing, the sample preparation laboratory must store the sample aliquots and the carcasses of the fish specimens that CSGov has designated to be retained frozen to less than or equal -20 °C until they are distributed to the laboratories performing analyses under separate CSGov purchase orders (see Sec IV.I).

IV.B Sample Handling

The whole fish collected for the 2015 GLHHFFTS must remain frozen at less than or equal to -20 ºC until the sample processing laboratory receives sample-specific processing instructions from CSGov. Samples to be processed must be retrieved from the freezer, with their associated paperwork, and allowed to partially thaw before they can be processed.

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

    - Site ID
    - Date of collection
    - Sample ID (XXXXXX.YY, where YY usually ranges from 1 to 5 specimens in the composite, but can range up to 10)
    - Scientific name for the fish species
    - Common name for the fish species
    - Measured length of each specimen in mm
    - Sample type (predator or bottom dweller)
    - Sample classification (Routine or Non-Routine)
    - Deviation (e.g., why it is not routine)
    - Instructions (sample-specific details about which fish to process)

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

    - Verify that all associated 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 CSGov 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 samples should be reported to CSGov 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 sent with the fish samples does *not* contain all of the information in the Excel instruction files. Therefore, lack of information on hardcopy field paperwork regarding the sample type, sample classification, or deviation is *not* a discrepancy that must be reported.

IV.C. Filleting and Homogenization Procedures

5. Prior to preparing any samples, 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.

6. 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 CSGov before proceeding further.

7. The sample collection personnel measured the total length of each fish specimen in the field and recorded those lengths on the sample tracking form. The label applied to each wrapped specimen also includes the fish length.

   Begin processing the specimens by laying them out in order by specimen number (the portion of the sample ID after the decimal point) and allowing them to partially thaw to the point that each specimen can be laid relatively flat. Using the length data on the sample tracking form, confirm that the specimen ID and length for each specimen recorded on the tracking form is the same as the specimen ID and length recorded on the label. This check is important for confirming that the field crews attached the correct label to each fish in the sample. If discrepancies are observed, document them in the laboratory project logsheet and notify CSGov before proceeding further.

8. 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 (either paper or electronic). Individual specimen weights eventually will be transferred to spreadsheets for submission to CSGov.

9. 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.

10. Before beginning the scaling process for the first fish in the sample, put on new powder-free nitrile gloves. (Gloves must be changed *between* samples, but the same gloves may be used for all fish *within* a given sample.) Fish with scales must be scaled (and any adhering slime should be removed) prior to filleting. Scale the first designated 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.

11. Continue scaling all the other fish in the sample composite as described in Step 10 above. Filleting can proceed after all scales have been removed from the skin and a separate clean cutting board and fillet knife are prepared or available.

12. 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 individual experienced with filleting fish, 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 using the tip of the fillet knife or a clean pair of forceps. Prior to homogenizing the fillets, place all of the fillets from the sample in a suitable clean container, weigh the total fillet mass on a top-loading balance to the nearest gram (wet weight), and record the total mass of the fillets on the laboratory project logsheet. The collective weight of the fillets will be transferred to spreadsheets for submission to CSGov.

13. Samples should be homogenized partially frozen for ease of grinding. Composite the 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).

14. 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 sample will be used to prepare the fillet tissue 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 small pieces or pellets of dry ice may also keep the tissue from sticking to the equipment. Pellets of dry ice also may be added to the tissue as it enters the grinder.

15. 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.

16. Measure the collective weight of the homogenized fillet 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 (either paper or electronic). The collective weight of the homogenized tissue from each sample will be transferred to spreadsheets for submission to CSGov. At least 485 g of homogenized tissue will be needed to fill all of the containers in Table 1 below with their minimum acceptable masses. **If a sample does not yield at least 485 g of homogenized tissue, contact CSGov via email immediately and await instructions.** CSGov will consult with EPA before communicating the final decisions. As appropriate, place any homogenized samples with less than 485 g in the freezer while waiting for instructions, which are likely to involve preparing fewer archive aliquots.

17. After the final (second or third) grinding, carefully remove the recoverable tissue and clean the **grinding equipment and all other sample preparation equipment** using the procedures described in Step 24.

18. Once in every batch of 20 samples, verify the continued absence of equipment contamination using the procedures described in Steps 27 through 30, and verify the uniformity of homogenization using the procedures described in Steps 31 and 32.

IV.D. Special Handling of Single-Specimen Samples and Other Selected Fish Specimens

EPA/OST is cooperating with other researchers in a study of microplastic particles in the digestive tracts of fish from the Great Lakes by providing the carcasses of up to 100 individual fish specimens collected during the 2015 GLHHFFTS. These specimens will include both fish from single-specimen samples and individual specimens from selected multi-specimen composite samples. The sample preparation instructions sent to the laboratory will clearly identify all the individual fish specimens that require special handling of the carcasses. Where practical, CSGov and EPA may group the samples requiring special handling together into 20-sample preparation batches.

19. For each single-specimen sample or selected other fish specimen identified by CSGov/EPA in the batch instructions, the sample preparation laboratory must use extra caution to ensure that the filleting process does not damage the internal organs of the specimen. Once the fillets from both sides are removed in Step 12 above, carefully set the carcass aside while the homogenization process is undertaken (e.g., Steps 13 to 17). After completing the grinding process and before aliquoting the ground tissue into containers in Step 20, use the supplies provided by CSGov/EPA to wrap, label, and store fish carcasses as follows:

    - Wrap the carcass of each specimen in two layers of **heavy duty** aluminum foil (provided by CSGov/EPA). Please do not use solvent-rinsed foil since the solvent could contact the open body cavity. Take care to avoid spines and bones piercing the foil.
    - Cut a sufficient length of food-grade polyethylene tubing (provided by CSGov/EPA) to allow the carcass to lay flat, and allow adequate space to close each end of the tubing with a cable tie.
    - Place the foil-wrapped carcass in the tubing and seal one end of the tubing with a cable tie (provided by CSGov/EPA).
    - Tape the preprinted label for the specimen to a Tyvek® tag (provided by CSGov/EPA), thread a second cable tie through the tag, and seal the other end of the tubing.
    - Transfer the carcass to a freezer maintained at -20 °C until CSGov requests that the carcass be shipped to the laboratory performing the microplastics analysis.

IV.E. Aliquoting and Distribution Procedures

20. The sample preparation laboratory will prepare one bulk homogenate tissue aliquot per fish 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 21. A**ll containers will be provided by the sample preparation laboratory.** Documentation of their cleanliness provided by the vendor (i.e., certificates of analysis) must be retained by the sample preparation laboratory and provided to CSGov on request. The target masses listed in Table 1 are designed to provide enough tissue for multiple analyses of each sample and analyte type, including tissue for QC purposes, as needed. The sample preparation laboratory should not exceed those target masses when filling the containers. The order of the containers and target masses in Table 1 are important and are designed to ensure that adequate tissue is available for all analyses, as well as archiving. The order that the tissue aliquots are listed in Table 1 also indicates the priority order for preparing the aliquots for chemical analyses in cases where samples do not provide sufficient tissue to prepare the full complement of ten tissue aliquots.

Table 1. 2015 GLHHFFTS Initial Tissue Sample Aliquot Requirements

Analysis	Target Mass	Container Type	Destination
Mercury	5 - 10 g	50-mL HDPE straight-sided jar with PTFE- or foil-lined lid, or conical HDPE tube with snap top	TBD
PCBs	30 - 35 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid	TBD

Analysis	Target Mass	Container Type	Destination
PFCs	10 - 15 g	50-mL HDPE straight-sided jar with **foil-lined lid**, or conical HDPE tube with snap top. ***PTFE lid liners not allowed.***	TBD
Dioxins/furans	25 - 30 g	125-mL straight-sided amber or clear glass jar with **foil-lined lid**. *Note: This aliquot does not require a foil-lined lid, but this type of lid may be used if it has already been purchased.*	TBD
Fatty acids	10 - 15 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid	TBD
Lipids	10 - 15 g	Laboratory's choice, as this aliquot will be used in-house to determine the lipid content of the sample	In-house
Other CECs	50 - 75 g	125-mL straight-sided amber or clear glass jar with **foil-lined lid**	TBD
Small Archive	40 - 50 g	125-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSGov Sample Repository
Bulk Archive 1	240 - 250 g	500-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSGov Sample Repository
Bulk Archive 2	All remaining mass up to 250 g	500-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSGov Sample Repository
Total (to the nearest gram)*	485 - 765 g	*The 485-g minimum assumes at least 50 g of tissue is available for Bulk Archive 2*	

* In the event that insufficient fish tissue mass exists to prepare the required number of aliquots, contact CSGov for instructions, per Step 16.

21. Prepare the sample aliquots for mercury, PFCs, PCBs, dioxins/furans, fatty acids, lipids (see Step 22 for lipid aliquot preparation) and other CECs in the order indicated in Table 1. 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 target 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 sample preparation laboratory must use foil-lined lids for jars containing the tissue aliquots for PFC and CEC analyses and for 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 22) and the aliquot for other CECs has been prepared. For the sample used for homogeneity testing, the other CECs jar and the archive jars are not filled until triple the lipid mass is collected (see Step 22).

    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. Print a label for each container or complete a label using a waterproof marker. Include the following information (at a minimum) on each label:

    - site identification number,
    - sample identification number,
    - analysis type (e.g., mercury, PFCs, dioxins/furans, fatty acids, etc.),
    - aliquot weight (to the nearest 0.5 gram),
    - preparation batch ID, and

- preparation date (e.g., mm/dd/yyyy)

(Other information may be included on the label at the laboratory's discretion, provided that CSGov is given an explanation of each additional field.)

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 CSGov to ship them to the analytical laboratories. (CSGov will not issue such instructions until equipment rinsate and homogeneity tests described in Steps 26 to 32 have been completed, reported, evaluated, and determined to be acceptable.)

22. After filling all of the containers for the aliquots for mercury, PFCs, PCBs, dioxins/furans, and fatty acids, and **before** filling the container for other CECs, remove 10 to 15 g of homogenized tissue to be used by the sample preparation laboratory 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. Homogeneity testing is required for one sample in each preparation batch, using lipids as a surrogate as described in Step 31. Therefore, collect two additional 10- to 15-g aliquots from one sample selected by the laboratory for homogeneity testing. Transfer the lipid aliquot(s) to the appropriate staff performing the lipid determinations described in Steps 26, 31, and 32.

23. The archive sample jars are not filled until after sufficient volume for lipids determination has been collected and the aliquot for other CECs has been prepared. Once the aliquots for mercury, PFCs, PCBs, dioxins/furans, fatty acids, lipids, and other CECs have been collected, the remaining tissue mass is used to create up to three archive samples. Begin by transferring 40 - 50 g aliquot to the small archive container. Continue by transferring 240 - 250 g of tissue to the first bulk archive sample container, thus ensuring that at least one large volume (bulk) aliquot is archived. Ideally, sufficient homogenized fillet tissue mass will remain to produce a second bulk archive container. Therefore, transfer 240 - 250 g of tissue to the second bulk archive sample container. However, if less than 240 g of tissue is available, transfer all of the remaining homogenized tissue to the second bulk archive container. Seal and label the containers as described in Step 21 for the other aliquots.

    Note: Step 16 requires that the laboratory contact CSGov whenever a sample does not yield at least 485 g of tissue, which will provide enough mass for all the samples and the first two archive containers. After consultation with EPA, CSGov will provide direction to the laboratory regarding samples yielding less than 485 g of tissue that must be followed at this point in the procedure.

Any tissue that remains after filling the second bulk archive jar may be discarded.

IV.F. Equipment Cleaning between Fish Samples

24. All of the homogenization equipment must be thoroughly cleaned between each fish sample (which may contain one to ten specimens). Once all of the fillets from the individual specimens in a given fish 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

25. Reassemble the homogenization equipment and proceed with homogenization of the next fish sample in the batch (e.g., begin with Step 6 above).

IV.G. Lipid Determination on Every Homogenized Fillet Composite Sample

The procedures for determining the lipid content of every fillet composite sample are described in Step 26 below. (Additional lipid determinations are required for one sample in every preparation batch, as described in Steps 31 and 32.)

26. Use the 5 to 10 g of homogenized tissue collected in Step 22 to determine the lipid content of the sample. Extract the aliquot using the method of the laboratory's choice. (This method must be pre-approved by CSGov 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) to two decimal places, and provide the results to CSGov by email, as described in Section IV.I.

IV.H. 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, the sample preparation laboratory 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, the sample preparation laboratory staff will clean all of the sample preparation equipment as described in Step 24 above. After each cleaning, the sample preparation laboratory staff will prepare the entire series of equipment rinsates and solvent blanks described in Step 27 below.

The sample preparation laboratory 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 31 below.

The results of the analyses of the rinsates and the homogeneity testing (three sets each) will be submitted to CSGov for review. The sample preparation laboratory may **not** begin 2015 GLHHFFTS sample preparation until CSGov and EPA determine that the sample preparation laboratory has successfully demonstrated proficiency in meeting QC requirements for equipment cleaning and tissue homogenization.

Normal Operations: During normal sample preparation efforts, the sample preparation laboratory will prepare one set of rinsate samples and will conduct one set of triplicate lipid determinations per batch of 20 fish samples, as described in Steps 27 to 32, below. The batch-specific rinsate and homogeneity results will be reviewed by CSGov and EPA. The sample preparation laboratory may continue to process up to two additional batches of 20 samples (based on sample preparation instructions provided by CSGov) during that review process. However, the sample preparation laboratory may **not** continue beyond that second additional batch of samples until receiving notification from CSGov that review of the batch rinsate and homogeneity test results for the batch before those two additional batches is complete

and the results were deemed satisfactory. Thus, continued sample processing is dependent on both the quality of the sample preparation laboratory's efforts and on the timeliness of their delivery of QC results.

Rinsate and Blank Sample Production

27. Prior to reassembling the homogenization equipment (Step 25) between each of the samples processed during the initial demonstration of capabilities, and once per batch during normal operations, prepare four rinsate samples, as follows:

    - Prepare **two hexane rinsate samples** by pouring 200-mL portion of pesticide-grade hexane over all parts of homogenization equipment, including the cutting boards and knives, and dividing it equally between two clean glass containers. Place a 100-mL aliquot of clean hexane in each of two similar glass containers for use as solvent blanks. Allow the solvent to evaporate from the equipment. One of these rinsates and one of these solvent blanks will be analyzed for selected PCBs and the other pair will be analyzed for dioxins/furans by another laboratory under contract to CSGov at a later date. Label, store, and analyze the PCB and dioxin/furan rinsates and blanks as described in Steps 28 and 29.

    - Once the hexane has evaporated, prepare the **first DI water rinsate** (for mercury analysis) 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. Label, store, and analyze the mercury rinsate and blank as described in Steps 28 and 30.

    - Prepare the **second DI water rinsate** (for PFC analysis) using an additional 250 mL of DI water. Collect this rinsate in a clean glass or HDPE container **with a non-PTFE lid liner**. Place a second aliquot of DI water in a separate similar clean glass or HDPE container **with a non-PTFE lid liner** 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. CSGov will provide the sample preparation laboratory 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 28.

    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.).

28. 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, PFCs, PCBs, dioxins/furans), and the preparation batch identifier. Store the rinsates and blanks cold (<6 ºC).

Rinsate Analyses

29. As part of the initial demonstration of capabilities, the sample preparation laboratory will analyze three sets of hexane rinsate and blank samples for PCBs using a GC/ECD procedure or other proposed and approved procedure (e.g., one set prepared after each tissue sample prepared during the initial demonstration process) as described in Attachment 1. During normal operations, the sample preparation laboratory will analyze one set of the hexane rinsate and blank samples per batch for PCBs. Requirements for the PCB analyses are provided in Attachment 1 of this document.

30. As part of the initial demonstration of capabilities, the sample preparation laboratory will analyze three sets of DI water rinsate and blank samples for mercury using a cold-vapor atomic absorption

procedure or other proposed and approved procedure (e.g., one set prepared after each tissue sample prepared during the initial demonstration process). During normal operations, the sample preparation laboratory will analyze one set of the DI water rinsate and blank samples for mercury. Requirements for the mercury analyses are provided in Attachment 1 of this document.

Corrective Actions for Rinsates

CSGov 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 485-g mass of homogenized tissue. Results for mercury or any PCBs above the anticipated reporting limits for these analytes in tissue samples may be cause for corrective actions by the sample preparation laboratory. Such corrective actions may include revisions to the sample preparation laboratory'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

31. 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, the sample preparation laboratory 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 (30 to 45 g). Therefore, unless otherwise directed by CSGov for a particular batch of samples, the sample preparation laboratory will select one sample processed on the first day of every batch that will provide well over 485 g of total tissue mass.

    As noted in Step 22, from that sample, remove three 5- to 10-g aliquots of tissue before filling the other CEC container and 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 laboratory's choice. Transfer the lipid aliquot to the appropriate staff performing the lipid determination.

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

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

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

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

    If the RSD of the triplicate results is less than or equal to 15.0%, 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 26.

Corrective Actions for Homogeneity

If the RSD is greater than 15.0%, then corrective action is required for all samples in that preparation batch. Corrective actions will be determined by CSGov 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 14. 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.I. Reporting Requirements

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

- site identification number,
- sample identification number,
- specimen numbers of the fish homogenized for the fillet composite,
- common name for the fish species (provided to the laboratory in the instructions from EPA),
- field-determined lengths and lab-determined weights of individual specimens that were filleted and homogenized,
- total weight of the fillet tissue before homogenization (to the nearest gram)
- total composite sample (i.e., homogenate) weight (to the nearest gram),
- analysis type (e.g., mercury, PFCs, PCBs, dioxins/furans, fatty acids, other CECs, 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 fillet composite samples, and
- Name of the analyst who prepared each sample.

(Much of the sample-specific information above will be provided to the sample preparation laboratory electronically in the fish sample processing instructions from CSGov.)

The weekly report will be due by COB Monday, or as agreed to in writing by CSGov after consultation with the laboratory in the cases of holidays, 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 PCBs, and the individual lipid results for each sample, as well as the triplicate lipid results associated with the sample batch. Those results **must** be reported to CSGov within 2 business days of their generation to facilitate CSGov's timely review and to minimize delays in receiving instructions to process future batches.

Note: As specified in the QC section of this document, the sample preparation laboratory may **not** continue beyond the next two batches of samples until receiving notification from CSGov that review of the prior batch (the batch before the two additional batches) rinsate and homogeneity test results is complete and the results were deemed satisfactory.

IV.J. Shipping Samples

34. **No samples may be shipped until CSGov and EPA have reviewed the sample homogeneity testing and rinsate results.** CSGov will notify the sample preparation laboratory by email when specific samples may be shipped, and to whom.

    When shipping batches of pre-frozen fillet tissue aliquots, keep the individual containers bagged in bubblewrap bags. Place these bags in a sturdy clean plastic or metal cooler *supplied by the laboratory* with adequate space for the tissue containers, packing materials, and dry ice. Multiple coolers may be used for each shipment. 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.

    Use blocks of dry ice for shipping, NOT pellets, because the pellets sublimate too quickly. The amount of dry ice required for shipping will depend on the number of fillet 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 fillet tissue samples).

    Record the samples contained in the cooler on a shipping form provided by CSGov 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 CSGov, and attach a dry ice (dangerous goods) label. Ship the cooler via FedEx on a date that will allow priority delivery of the cooler to the analytical laboratory in the morning on a normal business day (e.g., **no Saturday deliveries and no deliveries on U.S. Federal holidays**). Provide the air bill number for each shipment to CSGov via email on the day that the shipment occurs. **CSGov will provide the sample preparation laboratory with a third-party FedEx account to which each shipment will be billed.**

35. The sample preparation laboratory will be responsible for shipping the carcasses from the single-specimen samples and selected other fish specimens designated by EPA that were retained in Step 19. As with the jars of ground tissue, the carcasses must be shipped frozen, on dry ice, using the following procedures:

    - Open a large plastic trash bag and place it in a 70-quart cooler (supplied by CSGov/EPA from among those used to ship the whole fish to the sample preparation laboratory) as a liner.
    - Place each wrapped frozen carcass flat in the bag in the cooler, filling the cooler no more than one half full with carcasses. (Use multiple coolers, as needed, for each shipment requested by CSGov.)
    - Gather the plastic bag liner together, twist the top to minimize the air trapped inside, and knot the top of the bag, or seal it with packing tape.
    - Add 50 pounds of block dry ice to the cooler.
    - Pack crumbled newspaper or other packaging materials around and on top of the dry ice to insulate the contents and protect the inside lid of the cooler.
    - Insert the CSGov sample shipping form provided by CSGov with the shipping instructions into a plastic bag, seal the bag, and place bag in the top of the cooler.
    - Close the cooler lid and secure the cooler with a band of packing tape at each end. Form the band at each end of the cooler by wrapping the tape up one side, across the lid, down the other side,

under the cooler, up the first side again, across the lid again, and stop part way down the other side (i.e., wrap one and a half times around the cooler).

- Attach the FedEx airbill to a plastic hang tag and attach the hang tag to the handle at one end of the cooler.
- Complete a UN Dry Ice label, noting the weight of the dry ice used, attach it to another hang tag, and attach that to the handle at the other end of the cooler.
- Arrange for FedEx pickup or deliver to a FedEx drop off location.

As with the aliquoted tissue samples, CSGov will provide shipping forms and a third-party FedEx billing account for these shipments. Carcasses must be stored frozen at less than or equal to -20 °C until CSGov directs the laboratory to ship them.

CSGov Contact Information

Primary CSGov Contact
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V. DELIVERABLES

Item	Deliverable Description	Mechanism	Schedule
1	Kickoff meeting and workshop	--	TBD
2	Initial demonstration of capabilities	--	Begin within **3 business days** of receipt of test samples from Tetra Tech
3	Results of the initial demonstration, to include three sets of rinsate and solvent blank results for mercury and PCBs, plus three sets of triplicate lipid determinations	Email	Close of Business (COB) **2 business days** after results are generated
4	Confirmation of receipt of sample processing instructions, identifying any specific sample discrepancies	Email	COB on the day of receipt
5	Notification of samples that do not yield at least 485 g of homogenized fillet tissue	Email/phone	Immediately upon discovery during sample preparation
6	First completed batch of 20 homogenized fillet samples, ready for shipment at CSGov's direction (i.e., sample turnaround time)	--	**21 calendar days** from receipt of sample processing instructions from CSGov
7	Each subsequent batch of 20 homogenized fillet samples	--	**14 calendar days** from completion of the previous batch, or **14 calendar days** from receipt of sample processing instructions from CSGov, whichever is longer.
8	Mercury and PCB results for rinsates and solvent blanks and lipid RSD results, and individual sample lipid results	Email	**2 business days** after the results are generated
9	Weekly status report	Email	COB Monday of each week
10	Homogenized sample shipments	FedEx overnight	Within **3 business days** of receipt of shipping information from CSGov.
11	Retained carcasses for microplastics analysis	FedEx overnight	Within **3 business days** of receipt of shipping information from CSGov.

Item	Deliverable Description	Mechanism	Schedule
12	Shipping information (airbills, shipping forms, etc.) for tissue or rinsate samples	Email	COB on day samples ship to other labs
13	Copies of all bench sheets, sample preparation records, and other project records	Hard copy or PDF	As directed by CSGov after the completion of the project

VI. STAFFING

The laboratory must have personnel that meet the qualifications specified below:

- Project manager - Minimum of three years of experience in management of laboratory projects and communicating with clients,
- Sample custodian - Demonstrated experience in receiving laboratory samples from outside sources, completing and returning required paperwork, appropriately storing samples, and shipping outgoing samples,
- Sample preparation specialist - Either (1) demonstrated professional experience in the preparation of fish tissue samples from whole fish, including filleting and grinding of tissues, or (2) experience in filleting fish as a recreational fisher and a demonstrated aptitude for similar laboratory work,
- Equipment cleaning specialist - Demonstrated experience in cleaning and decontaminating sample preparation equipment, particularly that used for tissue samples.

EPA, CSGov, and Tetra Tech will provide project-specific training to the sample preparation specialist and equipment cleaning specialist during the kick-off meeting and workshop described earlier in this SOW. Staff filling these two positions will be cross-trained in both sets of project-specific duties. Therefore, the laboratory may not substitute other staff in these two critical positions without advance notice to CSGov. In such cases, CSGov reserves the right to halt further sample preparation until additional training options are evaluated in consultation with EPA and implemented.

As noted above, EPA/OST, CSGov, and Tetra Tech will provide project-specific training during the kick-off meeting and workshop. However, that training cannot encompass all of the fish species of interest in this study. Therefore, CSGov and EPA/OST reserve the right to provide additional oversight and training during the course of this project, particularly with regard to "sensitive species" of fish that may require special handling, as well as the handling of the single-specimen samples and other selected fish specimens for which the carcasses are to be retained. To that end, the laboratory must be willing to allow staff from CSGov and Tetra Tech to be present in the laboratory on a mutually agreeable schedule, and to allow them to provide laboratory staff with additional direction and training at no cost to CSGov. As also noted earlier, where practical, CSGov and EPA/OST will group such samples requiring additional attention into batches to minimize the disruption of routine laboratory operations. CSGov will work with the laboratory to schedule such on-site efforts sufficiently far in advance to minimize travel costs.

ATTACHMENT 1
ANALYSES OF RINSATES AND BLANKS FOR MERCURY AND PCBs

This attachment describes the analyses of rinsate samples and blanks generated during the 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.

Note: Depending on the laboratory's expertise and analytical capabilities, the sample preparation laboratory may subcontract the mercury and/or PCB analyses to another laboratory. The use of a subcontractor must be disclosed to CSGov/EPA during the bid process, and CSGov must receive details on the subcontractor laboratory's qualifications with the bid package. The sample preparation laboratory is responsible for the timeliness and quality of the subcontractor's work and the subcontractor may not be changed without prior approval from CSGov. The costs of any subcontracted analyses are the responsibility of the sample preparation laboratory.

A. EQUIPMENT AND MATERIALS:

- Mercury analyzer suitable for aqueous samples. Cold-vapor atomic absorption (CVAA) instruments compatible with EPA Method 245 are acceptable. The analyzer must be capable of achieving an MDL of approximately 1 µg/L. Cold-vapor atomic fluorescence spectroscopy (CVAFS) instrumentation (e.g., EPA Method 1631E) is not required, but may be employed.
- Gas chromatograph with an electron-capture detector (GC/ECD) and two dissimilar GC columns suitable for analysis of PCB congeners, or other suitable analytical procedures and detector systems (e.g., low resolution or high resolution GC/MS). The laboratory must be able to achieve an IDL for each congener on the order of 0.5 ng/mL, for a 1-mL final volume.
- Solvent concentration equipment suitable for reducing hexane rinsates to final volumes of 1 to 10 mL for analysis (other volumes may be proposed for other detector systems).
- A PCB standard solution containing at least the following PCB congeners: **52, 66, 105, 118, 141, 146, 170, 174, 177, and 187**, to be used to establish retention times and perform at least a 3-point calibration of the GC/ECD, or to calibrate other detector systems that do not rely solely on retention times for identification. (Additional congeners may be included by the laboratory. These congeners represent those that EPA has found frequently, at relatively high concentrations, in other fish tissue studies.)
- Assorted glassware, syringes, etc.

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 sample for analysis of mercury and for analysis of PFCs.
- One hexane rinsate sample and one hexane blank sample for analysis of PCBs.

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 11 sets of rinsates are anticipated (including 3 for the IDC).

The laboratory will digest and analyze the mercury rinsates and blanks by CVAA or another proposed and approved procedure. The laboratory will either perform a method detection limit (MDL) study for mercury in aqueous samples, or use existing aqueous MDL data for the instrument employed. The

laboratory must be able to achieve an MDL of approximately 1 µg/L. Mercury results will be reported down to the mass equivalent of the mass at the method detection limit (MDL) for aqueous samples. The laboratory will concentrate the PCB rinsates and blanks to a suitable final volume for the analytical procedure and analyze the concentrated samples. Because the PCB 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 PCBs listed above. The laboratory will determine the standard deviation of results for each PCB across all 7 analyses, and multiply the standard deviation times 3.143, which is 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, or a total mass of 0.5 ng.

If using a GC/ECD procedure, PCB 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). If using another proposed and approved procedure such as GC/MS, the congeners will be identified based on the requirements in that procedure.

PCB results in the rinsates and blanks will be reported down to the mass equivalent of the IDL. If using a GC/ECD procedure, any PCBs 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. For each analysis, the laboratory will determine the mass of each PCB congener in the total volume of each rinsate or blank sample, rather than the concentration of each analyte.

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, or consult the specifications in any other procedures that are proposed)
- Instrument blanks for mercury and PCB analyses
- Calibration verification (once per analysis batch) for mercury and PCB analyses
- Laboratory control sample (LCS) for mercury once per analysis batch

The MDL and IDL results will be reviewed by CSGov 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 PCB rinsates do not involve extraction of an environmental matrix, matrix spike samples are not applicable. Likewise, laboratory control samples are not applicable to PCBs.

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

D. DELIVERABLES

Summary data from the rinsate analyses are to be delivered to CSGov 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, if any
- Prep date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was prepared
- Analysis type - "Mercury" or "PCBs"
- Analysis date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was analyzed
- Mass of analyte found - in micrograms for mercury and in nanograms for PCBs
- 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 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 PCBs, 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).

Raw data supporting each analysis (e.g., instrument printouts) must be retained by the laboratory and made available to CSGov when requested, at no additional cost. If requested, raw data may be submitted in hard copy or as a PDF file.

In addition to the data in Excel files, the laboratory must provide copies of the analytical methods used for mercury and for PCB congeners. These may be copies of published methods, or SOPs developed by the laboratory. These may be delivered as hard copy documents or as PDF files.

Appendix C
2015 GLHHFFTS Detection and Quantitation Limits for Tissue Analysis

Method Detection Limits (MDLs) and Minimum Levels (MLs) for 2015 GLHHFFTS Target Analytes

Mercury *(based on a 0.5-g sample)*	
MDL[a] (ng/g)	**ML** (ng/g)
0.06	0.20

[a] The MDL is based on the EPA procedure described at 40 CFR 136, Appendix B.

In order to ensure comparability of the data from the 2015 GLHHFFTS PCB analyses with those from earlier EPA studies, EPA used the method detection limits (MDLs) and minimum levels (MLs) from the National Lake Fish Tissue Study (NLFTS) as metrics for assessing the capabilities of prospective laboratories to support this study. The table below presents the MDLs and MLs from Vista Analytical Laboratory and those from the NLFTS.

PCB MDLs and MLs *(in congener order, based on a 10-g sample)*				
Congener	**Vista MDL (ng/g)[1]**	**Vista ML (ng/g)[2]**	**NLFTS MDL (ng/g)[3]**	**NLFTS ML (n/ng)[3]**
1	0.06	0.2	0.8	2
2	0.09	0.5	0.8	2
3	0.10	0.5	0.6	2
4	0.10	0.5	0.5	2
5	0.07	0.5	0.7	2
6	0.11	0.5	0.3	1
7	0.09	0.5	0.4	1
8	0.10	0.5	1.3	5
9	0.10	0.5	0.5	2
10	0.08	0.5	0.7	2
11	0.33	1.0	1	2
12/13	0.20	1.0	1	2
14	0.09	0.5	0.5	2
15	0.13	0.5	0.4	1
16	0.17	0.5	1	2
17	0.07	0.5	1	2
18/30	0.17	0.5	1.8	5
19	0.15	0.5	0.1	0.5
20/28	0.23	1.0	4.2	10
21/33	0.20	1.0	1	2
22	0.11	0.5	0.2	0.5
23	0.10	0.5	0.6	2
24	0.13	0.5	0.8	2
25	0.09	0.5	0.6	2
26/29	0.20	1.0	3.5	10
27	0.09	0.5	0.7	2
31	0.14	0.5	0.5	2
32	0.08	0.5	0.6	2
34	0.12	0.5	0.5	2
35	0.16	0.5	0.4	1
36	0.11	0.5	0.5	2
37	0.17	0.5	0.2	0.5
38	0.13	0.5	0.4	1
39	0.17	0.5	0.5	2

PCB MDLs and MLs *(in congener order, based on a 10-g sample)*				
Congener	**Vista MDL (ng/g)[1]**	**Vista ML (ng/g)[2]**	**NLFTS MDL (ng/g)[3]**	**NLFTS ML (n/ng)[3]**
40/41/71	0.44	2.0	1.6	5
42	0.16	0.5	0.7	2
43	0.15	0.5	1.4	5
44/47/65	0.34	1.0	4.3	10
45/51	0.26	1.0	2.2	5
46	0.15	0.5	1.2	5
48	0.12	0.5	0.8	2
49/69	0.29	1.0	1.4	5
50/53	0.18	1.0	3.2	10
52	0.11	0.5	4.3	10
54	0.10	0.5	0.3	1
55	0.12	0.5	0.5	2
56	0.13	0.5	0.5	2
57	0.14	0.5	0.6	2
58	0.11	0.5	0.5	2
59/62/75	0.34	1.0	2	5
60	0.10	0.5	0.4	1
61/70/74/76	0.43	2.0	2.3	10
63	0.10	0.5	0.7	2
64	0.16	0.5	0.6	2
66	0.17	1.0	5.2	20
67	0.11	0.5	0.6	2
68	0.18	1.0	0.4	1
72	0.10	0.5	0.5	2
73	0.11	0.5	0.7	2
77	0.26	1.0	4.9	20
78	0.19	1.0	0.7	2
79	0.15	0.5	0.8	2
80	0.12	0.5	0.8	2
81	0.20	1.0	0.5	2
82	0.16	0.5	0.6	2
83/99	0.21	1.0	1	2
84	0.16	0.5	0.9	2
85/116/117	0.35	2.0	1	2
86/87/97/108/119/125	0.59	2.0	4.5	20
88/91	0.24	1.0	0.8	2
89	0.10	0.5	0.6	2
90/101/113	0.30	1.0	3.7	10
92	0.11	0.5	0.1	0.5
93/98/100/102	0.44	2.0	2.1	5
94	0.14	0.5	0.6	2
95	0.12	0.5	--	--
96	0.12	0.5	1	2
103	0.11	0.5	0.4	1
104	0.10	0.5	3.6	10
105	0.19	1.0	5.3	20
106	0.32	1.0	0.4	1
107/124	0.23	1.0	1	2
109	0.15	0.5	--	--
110/115	0.26	1.0	0.5	2
111	0.16	0.5	0.4	1
112	0.09	0.5	0.6	2
114	0.16	0.5	0.4	1

PCB MDLs and MLs *(in congener order, based on a 10-g sample)*				
Congener	**Vista MDL (ng/g)[1]**	**Vista ML (ng/g)[2]**	**NLFTS MDL (ng/g)[3]**	**NLFTS ML (n/ng)[3]**
118	0.19	1.0	4.9	20
120	0.12	0.5	0.5	2
121	0.13	0.5	0.6	2
122	0.12	0.5	0.3	1
123	0.13	0.5	1.1	2
126	0.30	1.0	5.1	20
127	0.22	1.0	0.5	2
128/166	0.13	0.5	3.7	10
129/138/160/163	0.98	5.0	5.4	20
130	0.12	0.5	0.4	1
131	0.10	0.5	0.6	2
132	0.08	0.5	0.8	2
133	0.07	0.5	0.5	2
134/143	0.19	1.0	0.8	2
135/151	0.22	1.0	4	10
136	0.14	0.5	0.6	2
137	0.23	1.0	0.4	1
139/140	0.15	0.5	0.8	2
141	0.13	0.5	0.7	2
142	0.20	1.0	0.5	2
144	0.10	0.5	0.7	2
145	0.12	0.5	0.5	2
146	0.06	0.2	0.4	1
147/149	0.21	1.0	0.6	2
148	0.10	0.5	0.6	2
150	0.12	0.5	0.4	1
152	0.10	0.5	0.4	1
153/168	0.19	1.0	4.1	10
154	0.12	0.5	--	--
155	0.13	0.5	0.3	1
156/157	0.41	2.0	0.5	2
158	0.09	0.5	0.4	1
159	0.13	0.5	0.4	1
161	0.11	0.5	0.5	2
162	0.16	0.5	0.4	1
164	0.13	0.5	0.3	1
165	0.09	0.5	0.4	1
167	0.16	0.5	0.2	1
169	0.25	1.0	0.3	1
170	0.17	1.0	5	20
171/173	0.23	1.0	0.8	2
172	0.10	0.5	0.5	2
174	0.17	1.0	0.6	2
175	0.16	0.5	0.6	2
176	0.12	0.5	0.5	2
177	0.10	0.5	0.3	1
178	0.14	0.5	0.8	2
179	0.13	0.5	0.4	1
180/193	0.21	1.0	4.5	10
181	0.10	0.5	0.5	2
182	0.13	0.5	0.8	2
183/185	0.19	1.0	1.1	5
184	0.15	0.5	0.6	2

PCB MDLs and MLs *(in congener order, based on a 10-g sample)*				
Congener	**Vista MDL (ng/g)[1]**	**Vista ML (ng/g)[2]**	**NLFTS MDL (ng/g)[3]**	**NLFTS ML (n/ng)[3]**
186	0.12	0.5	0.7	2
187	0.17	1.0	4.3	10
188	0.08	0.5	4.6	10
189	0.19	1.0	0.4	1
190	0.11	0.5	0.3	1
191	0.12	0.5	0.5	2
192	0.16	0.5	0.3	1
194	0.12	0.5	1.1	5
195	0.15	0.5	4.9	20
196	0.14	0.5	0.8	2
197	0.19	1.0	0.8	2
198/199	0.24	1.0	0.8	2
200	0.29	1.0	--	--
201	0.10	0.5	4.9	20
202	0.14	0.5	0.5	2
203	0.12	0.5	0.8	2
204	0.10	0.5	0.9	2
205	0.14	0.5	0.5	2
206	0.13	0.5	4.5	10
207	0.08	0.5	0.5	2
208	0.09	0.5	0.5	2
209	0.13	0.5	5	20

[1] The Vista MDL values were provided as part of the bid response from the laboratory and will be used for the purposes of this study. The MDLs in the table above are rounded to two decimal places.

[2] The Vista ML values were derived by CSRA and EPA from the laboratory's MDL values. The ML values shown above are equivalent to 3.18 times the MDL (as reported by the laboratory to four decimal places), rounded up to the nearest multiple of 1, 2, or 5. The ML values are displayed to one decimal place.

[3] The NLFTS MDL and ML values are shown for reference purposes only and were taken from Method 1668A. Entries of " – " indicate a congener that can be separated at Vista but that was not separated in the original method, hence the NLFTS did not list an MDL or ML value.

The PFCs to be determined in this project are listed in the table below, along with their common abbreviations and the method detection and quantitation limits are provided by the laboratory as part of its bid submission. The quantitation limits in the table below are "minimum levels" (MLs), based on the lowest calibration standard analyzed by the laboratory.

Name	Abbreviation	Tissue Samples (ng/g)[1]		Rinsate Samples (ng/L)[2]	
		MDL	ML	MDL	ML
Perfluorobutyric acid	PFBA	0.21	0.25	0.70	2
Perfluoropentanoic acid	PFPeA	0.24	0.25	0.31	2
Perfluorohexanoic acid	PFHxA	0.13	0.25	0.18	2
Perfluoroheptanoic acid	PFHpA	0.13	0.25	0.18	2
Perfluorooctanoic acid	PFOA	0.23	0.25	0.51	2
Perfluorononanoic acid	PFNA	0.12	0.25	0.81	2
Perfluorodecanoic acid	PFDA	0.16	0.25	0.31	2
Perfluoroundecanoic acid	PFUnA	0.17	0.25	0.64	2
Perfluorododecanoic acid	PFDoA	0.091	0.25	0.36	2
Perfluorobutanesulfonic acid	PFBS	0.36	0.50	0.88	2
Perfluorohexanesulfonic acid	PFHxS	0.63	1.25	1.49	2
Perfluorooctanesulfonic acid	PFOS	0.52	1.25	1.32	2
Perfluorooctanesulfonamide	PFOSA	0.13	0.25	0.79	2

[1] Based on a tissue sample size of 2 g, with the ML based on the lowest calibration standard analyzed.
[2] Based on a rinsate sample size of 250 mL, with the ML based on the lowest calibration standard analyzed.

The fatty acids to be determined in this project are listed in the table below, along with the method detection and quantitation limits that were provided by the laboratory as part of its bid submission. The quantitation limits in the table below are three times the MDL and rounded to two decimal places.

Fatty Acid	Lipid Number Abbreviation*	MDL (µg/g)	QL (µg/g)
Methyl decanoate	C10:0	0.24	0.71
Methyl undecanoate	C11:0	0.24	0.71
Methyl dodecanoate	C12:0	0.44	1.32
Methyl tridecanoate	C13:0	0.09	0.27
Methyl myristate	C14:0	1.89	5.68
Methyl myristoleate	C14:1	0.18	0.54
Methyl pentadecanoate	C15:0	0.36	1.09
Methyl *cis*-10-pentadecenoate	C15:1	0.09	0.27
Methyl palmitate	C16:0	6.72	20.2
Methyl palmitoleate	C16:1	7.14	21.4
Methyl heptadecanoate	C17:0	0.33	0.99
Methyl *cis*-10-heptadecenoate	C17:1	0.09	0.27
Methyl stearate	C18:0	0.69	2.08
Methyl oleate	C18:1n9c	10.6	31.8
Methyl elaidate	C18:1n9t	0.30	0.89
Methyl vaccenoate	C18:1w7	2.26	6.79
Methyl linoleate	C18:2n6c	1.60	4.81
Methyl linolelaidate	C18:2n6t	0.93	2.80
Methyl linolenate	C18:3n3	3.50	10.5
Methyl *gamma*-linolenate	C18:3n6	0.34	1.01
Methyl octadecatetraenoate	C18:4n3	2.49	7.47
Methyl arachidate	C20:0	0.09	0.27
cis-11-Eiconsenoic acid methyl ester	C20:1n9	3.50	10.5
cis-11,14-Eicosadienoic acid methyl ester	C20:2	0.16	0.48
cis-11,14,17-Eicosatrienoic acid methyl ester	C20:3n3	2.04	6.13
cis-8,11,14-Eicosatrienoic acid methyl ester	C20:3n6	0.32	0.96
Methyl arachidonate	C20:4n6	0.14	0.41
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	C20:5n3	0.20	0.59
Methyl heneicosanoate	C21:0	0.12	0.36
Methyl behenate	C22:0	0.09	0.27
Methyl erucate	C22:1n9	1.67	5.00
Methyl cetoleoate	C22:1n11	2.49	7.47
cis-13,16-Docosadienoic acid methyl ester	C22:2	1.84	5.51
Methyl docosapentaenotate	C22:5n3	1.29	3.88
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	C22:6n3	2.49	7.47
Methyl tricosanoate	C23:0	0.15	0.44
Methyl tetracosanoate	C24:0	0.09	0.27
Methyl *cis*-15-tetracosanoate	C24:1n9	0.63	1.89

* Lipid numbers take the form C:DnX, where C is the number of carbon atoms in the fatty acid and D is the number of double bonds. Where applicable, the fatty acid double bond location is identified by nX, where X is the carbon number of the first double bond relative to the terminal alkyl end of the fatty acid.

The dioxins and furans to be determined in this project are listed in the table below, along with the method detection and quantitation limits that were provided by the laboratory as part of its bid submission. The quantitation limits in the table below are "minimum levels" (MLs), based on the lowest calibration standard analyzed by the laboratory.

Analyte	CAS Number	Tissue Samples (pg/g)[1]		Rinsate Samples (ng/L)[2]	
		MDL	ML	MDL	ML
Dioxins					
2,3,7,8-TCDD	1746-01-6	0.01	0.08	0.004	0.02
1,2,3,7,8-PeCDD	40321-76-4	0.02	0.40	0.005	0.10
1,2,3,4,7,8-HxCDD	39227-28-6	0.02	0.40	0.005	0.10
1,2,3,6,7,8-HxCDD	57653-85-7	0.03	0.40	0.005	0.10
1,2,3,7,8,9-HxCDD	19408-74-3	0.02	0.40	0.005	0.10
1,2,3,4,6,7,8-HpCDD	35822-46-9	0.02	0.40	0.005	0.10
OCDD	3268-87-9	0.05	0.80	0.005	0.20
Furans					
2,3,7,8-TCDF	51207-31-9	0.01	0.08	0.003	0.02
1,2,3,7,8-PeCDF	57117-41-6	0.02	0.40	0.005	0.10
2,3,4,7,8-PeCDF	57117-31-4	0.02	0.40	0.005	0.10
1,2,3,4,7,8-HxCDF	70648-26-9	0.03	0.40	0.005	0.10
1,2,3,6,7,8-HxCDF	57117-44-9	0.02	0.40	0.005	0.10
1,2,3,7,8,9-HxCDF	72918-21-9	0.02	0.40	0.005	0.10
2,3,4,6,7,8-HxCDF	60851-34-5	0.03	0.40	0.005	0.10
1,2,3,4,6,7,8-HpCDF	67562-39-4	0.02	0.40	0.005	0.10
1,2,3,4,7,8,9-HpCDF	55673-89-7	0.02	0.40	0.005	0.10
OCDF	39001-02-0	0.06	0.80	0.005	0.20

[1] Based on a tissue sample size of 25 g, with the ML based on the lowest calibration standard analyzed.

[2] Based on a rinsate sample size of 250 mL, with the ML based on the lowest calibration standard analyzed.

Appendix D

2015 GLHHFFTS Quality Control Acceptance Criteria for PCB Analysis of Tissue Samples

Quality Control Acceptance Criteria for PCB Analysis of Tissue Samples

QC Acceptance Criteria for VER[1], OPR[2], and Labeled Compounds[3] in Samples

Congener Name	Congener Number	VER (%)	OPR Recovery (%)	Labeled Compound Recovery in Samples (%)
2-MonoCB	1	75-125	60-135	
3-MonoCB	2	75-125	60-135	
4-MonoCB	3	75-125	60-135	
2,2'-DiCB/2,6-DiCB	4/10	75-125	60-135	
2,3-DiCB/2,4'-DiCB	5/8	75-125	60-135	
2,3'-DiCB	6	75-125	60-135	
2,4-DiCB/2,5-DiCB	7/9	75-125	60-135	
3,3'-DiCB	11	75-125	60-135	
3,4-DiCB/3,4'-DiCB	12/13	75-125	60-135	
3,5-DiCB	14	75-125	60-135	
4,4'-DiCB	15	75-125	60-135	
2,2',3-TrCB/2,4',6-TrCB	16/32	75-125	60-135	
2,2',4-TrCB	17	75-125	60-135	
2,2',5-TrCB	18	75-125	60-135	
2,2',6-TrCB	19	75-125	60-135	
2,3,3'-TrCB/2,3,4-TrCB/2',3,4-TrCB	20/21/33	75-125	60-135	
2,3,4'-TrCB	22	75-125	60-135	
2,3,5-TrCB	23	75-125	60-135	
2,3,6-TrCB/2,3',6-TrCB	24/27	75-125	60-135	
2,3',4-TrCB	25	75-125	60-135	
2,3',5-TrCB	26	75-125	60-135	
2,4,4'-TrCB	28	75-125	60-135	
2,4,5-TrCB	29	75-125	60-135	
2,4,6-TrCB	30	75-125	60-135	
2,4',5-TrCB	31	75-125	60-135	
2,3',5'-TrCB	34	75-125	60-135	
3,3',4-TrCB	35	75-125	60-135	
3,3',5-TrCB	36	75-125	60-135	
3,4,4'-TrCB	37	75-125	60-135	NA
3,4,5-TrCB	38	75-125	60-135	
3,4',5-TrCB	39	75-125	60-135	
2,2',3,3'-TeCB	40	75-125	60-135	
2,2',3,4-TeCB/2,3,4',6-TeCB/2,3',4',6-TeCB/ 2,3',5,5'-TeCB	41/64/71/72	75-125	60-135	
2,2',3,4'-TeCB/2,3,3',6-TeCB	42/59	75-125	60-135	
2,2',3,5-TeCB/2,2',4,5'-TeCB	43/49	75-125	60-135	
2,2',3,5'-TeCB	44	75-125	60-135	
2,2',3,6-TeCB	45	75-125	60-135	
2,2',3,6'-TeCB	46	75-125	60-135	
2,2',4,4'-TeCB	47	75-125	60-135	
2,2',4,5-TeCB/2,4,4',6-TeCB	48/75	75-125	60-135	
2,2',4,6-TeCB	50	75-125	60-135	
2,2',4,6'-TeCB	51	75-125	60-135	
2,2',5,5'-TeCB/2,3',4,6-TeCB	52/69	75-125	60-135	
2,2',5,6'-TeCB	53	75-125	60-135	
2,2',6,6'-TeCB	54	75-125	60-135	
2,3,3',4-TeCB	55	75-125	60-135	
2,3,3',4'-TeCB/2,3,4,4'-TeCB	56/60	75-125	60-135	
2,3,3',5-TeCB	57	75-125	60-135	
2,3,3',5'-TeCB	58	75-125	60-135	
2,3,4,5-TeCB/2,3',4',5-TeCB	61/70	75-125	60-135	
2,3,4,6-TeCB	62	75-125	60-135	
2,3,4',5-TeCB	63	75-125	60-135	
2,3,5,6-TeCB	65	75-125	60-135	
2,3',4,5-TeCB	67	75-125	60-135	
2,3',4,5'-TeCB	68	75-125	60-135	

QC Acceptance Criteria for VER[1], OPR[2], and Labeled Compounds[3] in Samples

Congener Name	Congener Number	VER (%)	OPR Recovery (%)	Labeled Compound Recovery in Samples (%)
2,3',4',5-TeCB	70	75-125	60-135	NA
2,3',5',6-TeCB	73	75-125	60-135	
2,4,4',5-TeCB	74	75-125	60-135	
2',3,4,5-TeCB/2,3',4,4'-TeCB	76/66	75-125	60-135	
3,3',4,5-TeCB	77	75-125	60-135	
3,3',4,5'-TeCB	78	75-125	60-135	
3,3',5,5'-TeCB	79	75-125	60-135	
3,4,4',5-TeCB	80	75-125	60-135	
2,2',3,3',4-PeCB	81	75-125	60-135	
2,2',3,3',5-PeCB	82	75-125	60-135	
2,2',3,3',5-PeCB	83	75-125	60-135	
2,2',3,3',6-PeCB/2,2',3,5,5'-PeCB	84/92	75-125	60-135	
2,2',3,4,4'-PeCB/2,3,4,5,6-PeCB	85/116	75-125	60-135	
2,2',3,4,5-PeCB	86	75-125	60-135	
2,2',3,4,5'-PeCB/2,3,4',5,6-PeCB/ 2',3,4,5,6'-PeCB	87/117/125	75-125	60-135	
2,2',3,4,6-PeCB/2,2',3,4',6-PeCB	88/91	75-125	60-135	
2,2',3,4,6'-PeCB	89	75-125	60-135	
2,2',3,4',5-PeCB/2,2',4,5,5'-PeCB	90/101	75-125	60-135	
2,2',3,5,6-PeCB	93	75-125	60-135	
2,2',3,5,6'-PeCB	94	75-125	60-135	
2,2',3,5',6-PeCB/2,2',3',4,6-PeCB/ 2,2',4,5,6'-PeCB	95/98/102	75-125	60-135	
2,2',3,6,6'-PeCB	96	75-125	60-135	
2,2',3,4',5-PeCB	97	75-125	60-135	
2,2',4,4',5-PeCB	99	75-125	60-135	
2,2',4,4',6-PeCB	100	75-125	60-135	
2,2',4,5',6-PeCB	103	75-125	60-135	
2,2',4,4,6'-PeCB	104	75-125	60-135	
2,3,3',4,4'-PeCB	105	75-125	60-135	
2,3',4,4',5-PeCB/2,3,3',4,5-PeCB	118/106	75-125	60-135	
2,3,3',4',5-PeCB/2,3,3',4,6-PeCB	107/109	75-125	60-135	
2,3,3',4,5'-PeCB/2,3,3',5,6-PeCB	108/112	75-125	60-135	
2,3,3',4',6-PeCB	110	75-125	60-135	
2,3,3',5,5'-PeCB/2,3,4,4',6-PeCB	111/115	75-125	60-135	
2,3,3',5',6-PeCB	113	75-125	60-135	
2,3,4,4',5-PeCB	114	75-125	60-135	
2,3',4,4',6-PeCB	119	75-125	60-135	
2,3',4,5,5'-PeCB	120	75-125	60-135	
2,3',4,5',6-PeCB	121	75-125	60-135	
2,3,3',4',5'-PeCB	122	75-125	60-135	
2,3',4,4',5'-PeCB	123	75-125	60-135	
2,3',4',5,5'-PeCB	124	75-125	60-135	
3,3'4,4',5-PeCB	126	75-125	60-135	
3,3',4,5,5'-PeCB	127	75-125	60-135	
2,2',3,3',4,4'-HxCB/2,3,3',4',5,5'-HxCB	128/162	75-125	60-135	
2,2',3,3',4,5-HxCB	129	75-125	60-135	
2,2',3,3',4,5'-HxCB	130	75-125	60-135	
2,2',3,3',4,6-HxCB	131	75-125	60-135	
2,2',3,3',4,6'-HxCB/2,3,3',4,5',6-HxCB	132/161	75-125	60-135	
2,2',3,3',5,5'-HxCB/2,2',3,4,5,6-HxCB	133/142	75-125	60-135	
2,2',3,3',5,6-HxCB/2,2',3,4,5,6'-HxCB	134/143	75-125	60-135	
2,2',3,3',5,6'-HxCB	135	75-125	60-135	
2,2',3,3',6,6'-HxCB	136	75-125	60-135	
2,2',3,4,4',5-HxCB	137	75-125	60-135	
2,2',3,4,4',5'-HxCB/2,3,3',4',5,6-HxCB/ 2,3,3',4',5',6-HxCB	138/163/164	75-125	60-135	
2,2',3,4,4',6-HxCB/2,2',3,4',5',6-HxCB	139/149	75-125	60-135	
2,2',3,4,4',6'-HxCB	140	75-125	60-135	

QC Acceptance Criteria for VER[1], OPR[2], and Labeled Compounds[3] in Samples

Congener Name	Congener Number	VER (%)	OPR Recovery (%)	Labeled Compound Recovery in Samples (%)
2,2',3,4,5,5'-HxCB	141	75-125	60-135	NA
2,2',3,4,5',6-HxCB	144	75-125	60-135	
2,2',3,4,6,6'-HxCB	145	75-125	60-135	
2,2',3,4',5,5'-HxCB/2,3,3',5,5',6-HxCB	146/165	75-125	60-135	
2,2',3,4',5,6-HxCB	147	75-125	60-135	
2,2',3,4',5,6'-HxCB	148	75-125	60-135	
2,2',3,4',6,6'-HxCB	150	75-125	60-135	
2,2',3,5,5',6-HxCB	151	75-125	60-135	
2,2',3,5,6,6'-HxCB	152	75-125	60-135	
2,2',4,4',5,5'-HxCB	153	75-125	60-135	
2,2',4,4',5,6'-HxCB	154	75-125	60-135	
2,2',4,4',6,6'-HxCB	155	75-125	60-135	
2,3,3',4,4',5-HxCB	156	75-125	60-135	
2,3,3',4,4',5'-HxCB	157	75-125	60-135	
2,3,3',4,4',6-HxCB/2,3,3',4,5,6-HxCB	158/160	75-125	60-135	
2,3,3',4,5,5'-HxCB	159	75-125	60-135	
2,3,4,4',5,6-HxCB	166	75-125	60-135	
2,3',4,4',5,5'-HxCB	167	75-125	60-135	
2,3',4,4',5',6-HxCB	168	75-125	60-135	
3,3',4,4',5,5'-HxCB	169	75-125	60-135	
2,2',3,3',4,4',5-HpCB	170	75-125	60-135	
2,2',3,3',4,4',6-HpCB	171	75-125	60-135	
2,2',3,3',4,5,5'-HpCB	172	75-125	60-135	
2,2',3,3',4,5,6-HpCB	173	75-125	60-135	
2,2',3,3',4,5,6'-HpCB	174	75-125	60-135	
2,2',3,3',4,5',6-HpCB	175	75-125	60-135	
2,2',3,3'4,6,6'-HpCB	176	75-125	60-135	
2,2',3,3',4',5,6-HpCB	177	75-125	60-135	
2,2',3,3',5,5',6-HpCB	178	75-125	60-135	
2,2',3,3',5,6,6'-HpCB	179	75-125	60-135	
2,2',3,4,4',5,5'-HpCB	180	75-125	60-135	
2,2',3,4,4',5,6-HpCB	181	75-125	60-135	
2,2',3,4,4',5,6'-HpCB/2,2',3,4',5,5',6-HpCB	182/187	75-125	60-135	
2,2',3,4,4',5',6-HpCB	183	75-125	60-135	
2,2',3,4,4',6,6'-HpCB	184	75-125	60-135	
2,2',3,4,5,5',6-HpCB	185	75-125	60-135	
2,2',3,4,5,6,6'-HpCB	186	75-125	60-135	
2,2',3,4',5,6,6'-HpCB	188	75-125	60-135	
2,3,3',4,4',5,5'-HpCB	189	75-125	60-135	
2,3,3',4,4',5,6-HpCB	190	75-125	60-135	
2,3,3',4,4',5',6-HpCB	191	75-125	60-135	
2,3,3',4,5,5',6-HpCB	192	75-125	60-135	
2,3,3',4',5,5',6-HpCB	193	75-125	60-135	
2,2',3,3',4,4',5,5'-OcCB	194	75-125	60-135	
2,2',3,3',4,4',5,6-OcCB	195	75-125	60-135	
2,2',3,3',4,4',5,6'-OcCB/2,2',3,4,4',5,5',6-OcCB	196/203	75-125	60-135	
2,2',3,3',4,4',6,6'-OcCB	197	75-125	60-135	
2,2',3,3',4,5,5',6-OcCB	198	75-125	60-135	
2,2',3,3',4,5,5',6'-OcCB	199	75-125	60-135	
2,2',3,3',4,5,6,6'-OcCB	200	75-125	60-135	
2,2',3,3',4,5',6,6'-OcCB	201	75-125	60-135	
2,2',3,3',5,5',6,6'-OcCB	202	75-125	60-135	
2,2',3,4,4',5,6,6'-OcCB	204	75-125	60-135	
2,3,3',4,4',5,5',6-OcCB	205	75-125	60-135	
2,2',3,3',4,4',5,5',6-NoCB	206	75-125	60-135	
2,2',3,3',4,4',5,6,6'-NoCB	207	75-125	60-135	
2,2',3,3',4,5,5',6,6'-NoCB	208	75-125	60-135	
DeCB	209	75-125	60-135	

QC Acceptance Criteria for VER[1], OPR[2], and Labeled Compounds[3] in Samples

Congener Name	Congener Number	VER (%)	OPR Recovery (%)	Labeled Compound Recovery in Samples (%)
Labeled Compounds				
^{13}C-2-MonoCB	1L	50-145	15-145	5-145
^{13}C-4-MonoCB	3L	50-145	15-145	5-145
^{13}C-2,2'-DiCB	4L	50-145	15-145	5-145
^{13}C-2,5-DiCB	9L	50-145	15-145	5-145
^{13}C-3,3'-DiCB	11L	50-145	15-145	5-145
^{13}C- 2,2',6-TrCB	19L	50-145	15-145	5-145
^{13}C-2,4,4'-TrCB	28L	50-145	15-145	5-145
^{13}C-2,4',6-TrCB	32L	50-145	15-145	5-145
^{13}C-3,4,4'-TrCB	37L	50-145	15-145	10-145
^{13}C-2,2',4,4'-TeCB	47L	50-145	40-145	10-145
^{13}C-2,2',5,5'-TeCB	52L	50-145	40-145	10-145
^{13}C-2,2',6,6'-TeCB	54L	50-145	40-145	5-145
^{13}C-2,3',4',5-TeCB	70L	30-135	15-145	10-145
^{13}C-3,3',4,4'-TeCB	77L	50-145	40-145	10-145
^{13}C-3,4,4',5-TeCB	80L	50-145	40-145	10-145
^{13}C-3,3',4,4'-TeCB	81L	50-145	40-145	10-145
^{13}C-2,2',3,5',6-PeCB	95L	50-145	40-145	10-145
^{13}C-2,2',3,4',5-PeCB	97L	50-145	40-145	10-145
^{13}C-2,2',4,5,5'-PeCB	101L	50-145	40-145	10-145
^{13}C-2,2',4,6,6'-PeCB	104L	50-145	40-145	10-145
^{13}C-2,3,3',4,4'-PeCB	105L	50-145	40-145	10-145
^{13}C-2,3,4,4',5-PeCB	114L	50-145	40-145	10-145
^{13}C-2,3',4,4',5-PeCB	118L	50-145	40-145	10-145
^{13}C-2',3,4,4',5-PeCB	123L	50-145	40-145	10-145
^{13}C-3,3',4,4',5-PeCB	126L	50-145	40-145	10-145
^{13}C-3,3',4,5,5'-PeCB	127L	50-145	40-145	10-145
^{13}C-2,2',3,4,4',5'-HxCB	138L	50-145	40-145	10-145
^{13}C-2,2',3,4,5,5'-HxCB	141L	50-145	40-145	10-145
^{13}C-2,2',4,4',5,5'-HxCB	153L	50-145	40-145	10-145
^{13}C- 2,2',4,4',6,6'-HxCB	155L	50-145	40-145	10-145
^{13}C-2,3,3',4,4',5-HxCB	156L	50-145	40-145	10-145
^{13}C-2,3,3',4,4',5'-HxCB	157L	50-145	40-145	10-145
^{13}C-2,3,3',4,5,5'-HxCB	159L	50-145	40-145	10-145
^{13}C-2,3',4,4',5,5'-HxCB	167L	50-145	40-145	10-145
^{13}C-3,3',4,4',5,5'-HxCB	169L	50-145	40-145	10-145
^{13}C-2,2',3,3',4,4',5-HpCB	170L	50-145	40-145	10-145
^{13}C-2,2',3,4,4',5,5'-HpCB	180L	50-145	40-145	10-145
^{13}C- 2,2',3,4',5,6,6'-HpCB	188L	50-145	40-145	10-145
^{13}C- 2,3,3',4,4',5,5'-HpCB	189L	50-145	40-145	10-145
^{13}C-2,2',3,3',4,4',5,5'-OcCB	194L	50-145	40-145	10-145
^{13}C-2,2',3,3',5,5',6,6'-OcCB	202L	50-145	40-145	10-145
^{13}C-2,2',3,3',4,4',5,5',6-NoCB	206L	50-145	40-145	10-145
^{13}C-2,2',3,3',4,5,5',6,6'-NoCB	208L	50-145	40-145	10-145
^{13}C-DeCB	209L	50-145	40-145	10-145
Cleanup Standards				
^{13}C-3,3',4,5'-TeCB	79L	50-145	40-145	10-145
^{13}C-2,2'3,3'5,5'6-HpCB	178L	50-145	40-145	10-145

[1]VER = Calibration verification
[2]OPR = Ongoing precision and recovery
[3]The suffix "L" in a congener number indicates an isotopically labeled compound.

Appendix E

2015 GLHHFFTS Quality Control Acceptance Criteria for PFC Analysis of Tissue and Rinsate Samples

Quality Control Acceptance Criteria for PFC Analysis of Tissue and Rinsate Samples

Calibration Verification (VER), Laboratory Control Sample (LCS), and Labeled Compound Recovery QC Acceptance Criteria for PFCs in Tissue and Rinsate Samples					
		LCS Recovery (%)		Labeled Compound Recovery in Samples (%)	
Analyte	**VER (%)**	**Tissues**	**Rinsates**	**Tissues**	**Rinsates**
PFBA	70-130	70-130	80-120	NA	NA
PFPeA	70-130	70-130	80-120		
PFHxA	70-130	70-130	80-120		
PFHpA	70-130	70-130	80-120		
PFOA	70-130	70-130	80-120		
PFNA	70-130	70-130	80-120		
PFDA	70-130	70-130	80-120		
PFUnA	70-130	70-130	80-120		
PFDoA	70-130	70-130	80-120		
PFBS	70-130	70-130	70-130		
PFHxS	70-130	70-130	70-130		
PFOS	70-130	70-130	70-130		
PFOSA	70-130	70-130	70-130		
Quantitation Standards					
$^{13}C_4$-PFBA	20-150	20-150	20-150	20-150	20-150
$^{13}C_2$-PFHxA	40-150	40-150	40-150	40-150	40-150
$^{13}C_2$-PFOA	40-150	40-150	40-150	40-150	40-150
$^{13}C_5$-PFNA	40-150	40-150	40-150	40-150	40-150
$^{13}C_2$-PFDA	40-150	40-150	40-150	40-150	40-150
$^{13}C_2$-PFDoA	40-150	40-150	40-150	40-150	40-150
$^{18}O_2$-PFHxS	40-150	40-150	40-150	40-150	40-150
$^{13}C_4$-PFOS	20-130	20-130	40-150	20-130	40-150
Cleanup Standard					
$^{13}C_8$-PFOA	40-150	40-150	40-150	40-150	NA

Appendix F

2015 GLHHFFTS Quality Control Acceptance Criteria for Dioxin and Furan Analysis of Tissue and Rinsate Samples

Quality Control Acceptance Criteria for Dioxin and Furan Analysis of Tissue and Rinsate Samples

The QC acceptance criteria in this appendix are taken from Tables 6 and 7 of Method 1613B and apply to all fish tissue sample analyses. The criteria for IPR, OPR, and calibration verification (VER) are shown in the table below. Note that only the VER criteria from Table 6 and the labeled compound recovery criteria from Table 7 apply to the rinsate sample analyses.

Analyte	Test Conc. (ng/mL)	IPR		OPR (ng/mL)	VER (ng/mL)
		S (ng/mL)	X (ng/mL)		
2,3,7,8-TCDD	10	2.8	8.3–12.9	6.7–15.8	7.8–12.9
2,3,7,8-TCDF	10	2.0	8.7–13.7	7.5–15.8	8.4–12.0
1,2,3,7,8-PeCDD	50	7.5	38–66	35–71	39–65
1,2,3,7,8-PeCDF	50	7.5	43–62	40–67	41–60
2,3,4,7,8-PeCDF	50	8.6	36–75	34–80	41–61
1,2,3,4,7,8-HxCDD	50	9.4	39–76	35–82	39–64
1,2,3,6,7,8-HxCDD	50	7.7	42–62	38–67	39–64
1,2,3,7,8,9-HxCDD	50	11.1	37–71	32–81	41–61
1,2,3,4,7,8-HxCDF	50	8.7	41–59	36–67	45–56
1,2,3,6,7,8-HxCDF	50	6.7	46–60	42–65	44–57
1,2,3,7,8,9-HxCDF	50	6.4	42–61	39–65	45–56
2,3,4,6,7,8-HxCDF	50	7.4	37–74	35–78	44–57
1,2,3,4,6,7,8-HpCDD	50	7.7	38–65	35–70	43–58
1,2,3,4,6,7,8-HpCDF	50	6.3	45–56	41–61	45–55
1,2,3,4,7,8,9-HpCDF	50	8.1	43–63	39–69	43–58
OCDD	100	19	89–127	78–144	79–126
OCDF	100	27	74–146	63–170	63–159
$^{13}C_{12}$-2,3,7,8-TCDD	100	37	28–134	20–175	82–121
$^{13}C_{12}$-2,3,7,8-TCDF	100	35	31–113	22–152	71–140
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	39	27–184	21–227	62–160
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	34	27–156	21–192	76–130
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	38	16–279	13–328	77–130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	41	29–147	21–193	85–117
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	38	34–122	25–163	85–118
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	43	27–152	19–202	76–131
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	35	30–122	21–159	70–143
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	40	24–157	17–205	74–135
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	100	37	29–136	22–176	73–137
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	35	34–129	26–166	72–138
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	41	32–110	21–158	78–129
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	40	28–141	20–186	77–129
$^{13}C_{12}$-OCDD	200	95	41–276	26–397	96–415

[1] All specifications are given as concentration in the final extract, assuming a 20-µL volume.
[2] s = standard deviation of the concentration
[3] X = average concentration

The criteria for labeled compound recovery in fish tissue and rinsate samples are shown in the table below.

Labeled Compound	Recovery Range (%)
$^{13}C_{12}$-2,3,7,8-TCDD	25–164
$^{13}C_{12}$-2,3,7,8-TCDF	24–169
$^{13}C_{12}$-1,2,3,7,8-PeCDD	25–181
$^{13}C_{12}$-1,2,3,7,8-PeCDF	24–185
$^{13}C_{12}$-2,3,4,7,8-PeCDF	21–178
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	32–141
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	28–130
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	26–152
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	26–123
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	29–147
$^{13}C_{12}$-2,3,4,6,7,8,-HxCDF	28–136
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	23–140
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	28–143
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	26–138
$^{13}C_{12}$-OCDD	17-157