Quality Assurance Project Plan for

National Coastal Condition Assessment (NCCA)
2020 Great Lakes Human Health Fish Sample Preparation

July 15, 2020

Prepared for:

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

Prepared with support from:

Tetra Tech, Inc.
under
OST Engineering and Analysis Division
Contract No. EP-C-17-024

EPA-822-B-24-003

Quality Assurance Project Plan for National Coastal Condition Assessment (NCCA) 2020 Great Lakes Human Health Fish Sample Preparation

A. PROJECT MANAGEMENT

The U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST) within the Office of Water (OW) prepared this Quality Assurance Project Plan (QAPP) with support from Tetra Tech under EPA Contract No. EP-C-17-024. It presents objectives, procedures, performance requirements, and acceptance criteria for the preparation of fish fillet tissue samples from whole fish composite samples collected by field crews during the 2020 sampling season of the National Coastal Condition Assessment (NCCA). It does not address fish sample collection because that information is included in separate documents (USEPA 2020a and USEPA 2020b) prepared by the Office of Wetlands, Oceans, and Watersheds (OWOW).

This QAPP was prepared in accordance with the most recent version of EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* (USEPA 2001), that was reissued in 2006. It is a dynamic document that is subject to change as project activities progress. Changes to procedures in this QAPP must be reviewed by the OST Project Manager and the OST 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 is 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 information on project background and description is provided in Sections A5 and A6, respectively.

The following information will be added to this QAPP when it becomes available:

- the laboratory designated for mercury analysis of rinsates and blanks (page 27, Section B4.3; page 29, Section B5.2)
- the laboratory designated for PCB analysis of rinsates and blanks (page 27, Section B4.4; page 30, Section B5.3; page B-9, step 27)
- the analytical method used to analyze rinsates and blanks for PCBs (page B-15, Section C)
- the laboratory designated for PFAS analysis of rinsates and blanks (page 33, Section C1.1; page B-9, step 27)
- the analytical method used for PFAS analysis of rinsates and blanks (page 28, Section B4.5)
- the laboratory designated for triplicate and single lipid analysis (page 27, Section B4.2; page 28, Section B5.1; page B-10, step 28)
- the analytical method used for lipid analysis (page 27, Section B4.2; page B-8, step 24)
- the QC acceptance criteria for PCB analysis of aqueous QC samples (page 30, Table 4)
- the QC acceptance criteria for PFAS analysis of aqueous QC samples (page 31, Section B5.4)
- the laboratories designated for analysis of fish tissue aliquots (page B-6, Table 1; page B-7, step 20; page B-8, step 24)

A1. Approvals

Leanne Stahl — 07-15-2020
Leanne Stahl, OST Project Manager, EPA — Date

JOHN HEALEY — Digitally signed by JOHN HEALEY Date: 2020.07.21 13:04:02 -04'00'
John Healey, OST Fish Sample Preparation Technical Leader, EPA — Date

SHARI BARASH — Digitally signed by SHARI BARASH Date: 2020.07.21 12:04:53 -04'00'
Shari Barash, Chief, National Branch, EPA — Date

HARRY KRAMER — Digitally signed by HARRY KRAMER Date: 2020.07.16 14:01:30 -04'00'
Bill Kramer, SHPD QA Coordinator, EPA — Date

MARION KELLY — Digitally signed by MARION KELLY Date: 2020.07.16 16:04:12 -04'00'
Marion Kelly, OST QA Officer, EPA — Date

Brian Lenell — 7/21/2020
Brian Lenell, GLNPO Project Manager, EPA — Date

Louis Blume — July 15, 2020
Louis Blume, GLNPO QA Manager, EPA — Date

Blaine Snyder — 07/17/2020
Blaine Snyder, Tetra Tech Project Leader — Date

Susan Lanberg — 7/17/2020
Susan Lanberg, Tetra Tech QA Officer — Date

A2. Table of Contents

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APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

C	Celsius
DI	Deionized
DQO	Data quality objectives
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
g	Gram
GLHHFFTS	Great Lakes Human Health Fish Fillet Tissue Study
GLNPO	Great Lakes National Program Office
HDPE	High density polyethylene
ID	Identification
IDL	Instrument Detection Limit
IM	Information Management
IR	Infrared
MDL	Method detection limit
mL	Milliliter
NARS	National Aquatic Resource Survey
NCCA	National Coastal Condition Assessment
NRSA	National Rivers and Streams Assessment
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
PDF	Portable Document Format
PFAS	Per- and polyfluoroalkyl substances
PTFE	Polytetrafluoroethylene
QA	Quality assurance
QAPP	Quality Assurance Project Plan
QC	Quality control
RSD	Relative standard deviation
SD	Standard deviation
SHPD	Standards and Health Protection Division
SOP	Standard operating procedure
TBD	To be determined

A3. Distribution List

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A4. Project/Task Organization

This current study of contaminants in Great Lakes fish is referred to as the 2020 Great Lakes Human Health Fish Fillet Tissue Study (GLHHFFTS). The EPA project team for the 2020 GLHHFFTS consists of managers, scientists, and QA personnel in OST and the Great Lakes National Program Office (GLNPO) and statisticians in the Pacific Ecological Systems Division within the Center for Public Health and Environmental Assessment (Corvallis, Oregon) in the Office of Research and Development (ORD). The EPA project team receives scientific, technical, and logistical support from contractors at Tetra Tech and at CSRA General Dynamics Information Technology (GDIT). Tetra Tech provides primarily fisheries support (e.g., fish sampling and fish sample preparation) and CSRA/GDIT provides analytical support for the project team.

Members of the project team responsible for fish fillet sample preparation include the OST Project Manager, the OST Fish Sample Preparation Technical Leader, the GLNPO Project Manager, the OST QA Officer, the SHPD QA Coordinator, the GLNPO QA Manager, the Tetra Tech Project Leader, the Tetra Tech QA Officer, and Tetra Tech staff providing scientific, technical, and logistical support for this activity. The project team organization provides the framework for conducting fish sample preparation to meet study objectives. The organizational structure and function also facilitate project performance and adherence to quality control (QC) procedures and quality assurance (QA) requirements. The project organizational chart is presented in Figure 1. It identifies individuals serving in key roles and the relationships and lines of communication among these project team members. Responsibilities for key members of the project team are described below.

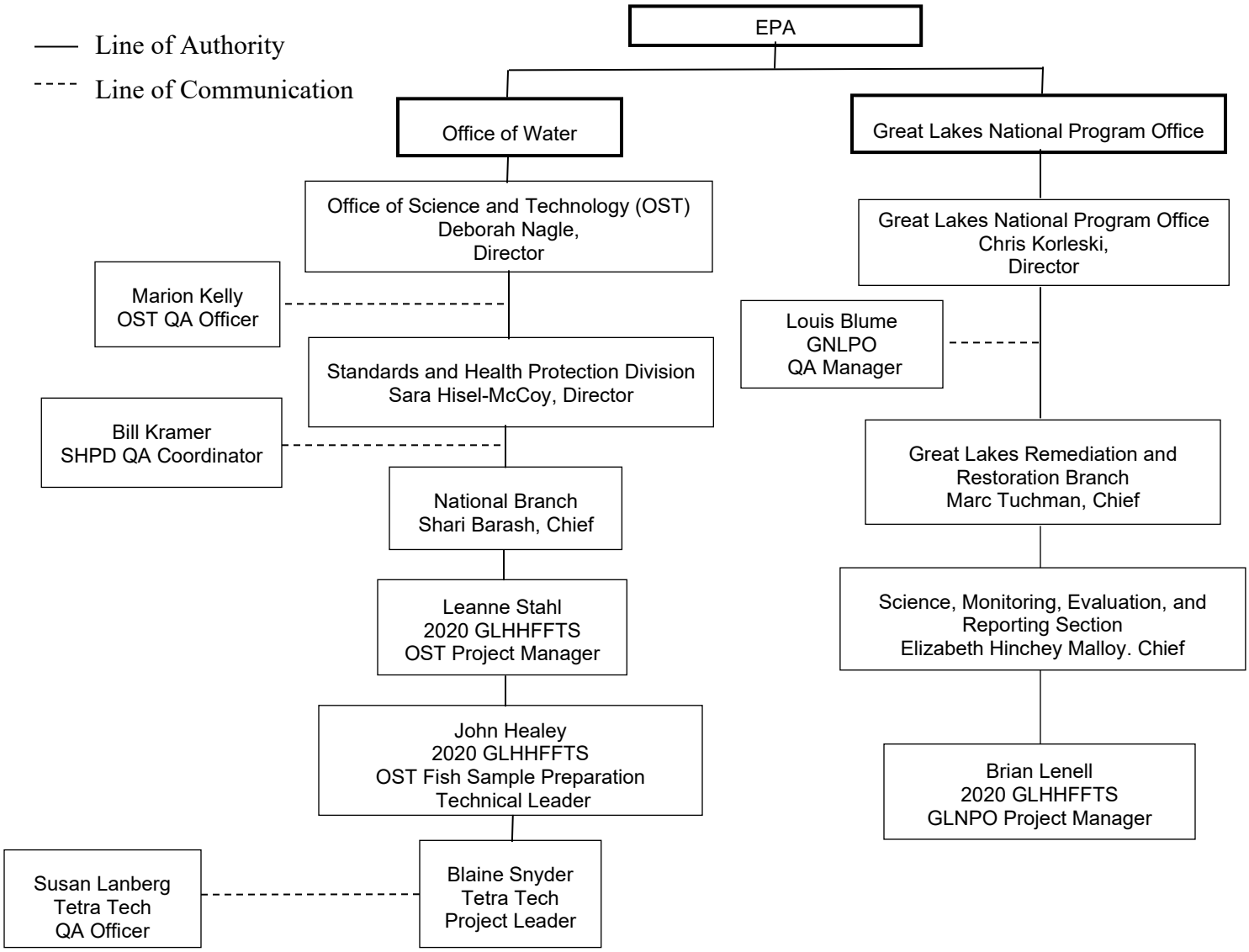

Figure 1. 2020 GLHHFFTS project team organization for fish fillet sample preparation

Leanne Stahl of OST is the **OST Project Manager** who is providing overall direction for planning and implementation of the 2020 GLHHFFTS being conducted under the NCCA. This role involves the following responsibilities related to the 2020 GLHHFFTS:

- developing technical information for whole fish sample collection for fillet analysis that includes preparation of the fish sampling protocols and coordination with the NCCA Project Leader in OWOW to integrate field sampling technical information for the 2020 GLHHFFTS into NCCA documents and training materials (This technical information also applies to the ORD-Duluth 2020 Great Lakes special study.)
- providing technical support to conduct training on the 2020 GLHHFFTS field sampling requirements in coordination with the NCCA Project Leader in OWOW (This training also applies to the ORD-Duluth 2020 Great Lakes special study.)
- developing the fish sample preparation procedures and requirements in coordination with the 2020 GLHHFFTS Fish Sample Preparation Technical Leader (These fish sample preparation procedures and requirements also apply to the ORD-Duluth 2020 Great Lakes special study.)
- managing analysis of fish fillet samples for target chemicals and subsequent activities, including obtaining technical support for chemical analysis of fish fillet tissue samples, directing development of the initial NCCA 2020 Great Lakes human health fish fillet tissue sample analysis QAPP and subsequent QAPP revisions, 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 2020 GLHHFFTS fish tissue results (This series of fillet sample analysis and related activities (including fillet sample analysis QAPP development, fillet sample chemical analysis, analytical data quality review, and data analysis and management activities) also apply to the ORD-Duluth 2020 Great Lakes special study.)
- facilitating communication among 2020 GLHHFFTS project team members and coordinating with all of these individuals to ensure technical quality and adherence to QA/QC requirements (This responsibility for communicating and coordinating with project team members also applies to the ORD-Duluth 2020 Great Lakes special study.)
- developing and managing work assignments and task orders under OST or other EPA contracts to provide technical support for the 2020 GLHHFFTS, providing oversight of contractor activities, and reviewing and approving study deliverables for each work assignment and task order (Contractor support for 2020 Great Lakes human health fish sample collection and analysis activities and associated activities also applies to the ORD-Duluth 2020 Great Lakes special study.)
- scheduling and leading meetings and conference calls with 2020 GLHHFFTS project team members for planning study activities, reporting progress on study tasks, and discussing and resolving technical issues related to the study (This responsibility also applies to the ORD-Duluth 2020 Great Lakes special study.)

- working with QA staff to identify corrective actions necessary to ensure that study quality objectives are met for both Great Lakes studies involving human health fish sample collection and analysis
- managing the development of and/or reviewing and approving all major work products associated with the 2020 GLHHFFTS and various other fish tissue studies, including products prepared by OWOW
- leading the Fish Tissue Study Team for reporting the 2020 GLHHFFTS human health fish fillet indicator results and various other fish tissue study results in technical journal articles and federal technical reports (This responsibility includes collaborating with the ORD-Duluth 2020 Great Lakes special study project team for reporting 2020 Great Lakes human health fish fillet analysis results.)
- coordinating with John Healey (TOCOR) to obtain Tetra Tech support through the task order for preparing fish study briefings and presentations and for providing general technical support; concurring on approval of task order deliverables
- presenting 2020 GLHHFFTS and other fish tissue study briefings for EPA managers and delivering fish tissue study presentations in various forums (e.g., scientific conferences, government meetings, and webinars)

John Healey of OST is the **OST Fish Sample Preparation Technical Leader** who is providing support for planning and implementation of the 2020 GLHHFFTS being conducted under the NCCA. This role involves the following responsibilities related to the 2020 GLHHFFTS:

- developing and managing a task order to provide technical support for preparation of NCCA 2020 Great Lakes human health fish fillet tissue samples for chemical analysis, which includes ensuring training for laboratory processing of NCCA fish samples, providing technical direction for and oversight of fish sample preparation activities (e.g., providing oversight for analysis of fish sample preparation QC samples and single lipid analysis of fillet tissue samples), and reviewing and approving task order deliverables (e.g., fish sample preparation weekly progress reports and results for analysis of QC samples) with OST Project Manager concurrence (This responsibility also applies to the ORD-Duluth 2020 Great Lakes special study.)
- participating in developing, reviewing, and approving the NCCA 2020 Great Lakes Human Health Fish Sample Preparation QAPP (This responsibility also applies to the ORD-Duluth 2020 Great Lakes special study.)
- developing and managing a task order to provide Tetra Tech support for preparing 2020 GLHHFFTS and other fish tissue study presentations and briefings and reviewing and approving task order deliverables with OST Project Manager concurrence and EPA management approval
- coordinating with OST QA staff and 2020 GLHHFFTS project team members to ensure technical quality and adherence to QA/QC requirements for task order deliverables
- obtaining training on the 2020 Great Lakes human health fish sampling requirements in coordination with the OST Project Manager

- participating in meetings and conference calls with Fish Tissue Study Team members for planning 2020 GLHHFFTS and other fish tissue study activities, reporting progress on various fish tissue study tasks, and discussing and resolving technical issues related to the 2020 GLHHFFTS and other fish tissue studies
- attending OWOW weekly NARS meetings and reporting information presented in the meeting (particularly information related to the NCCA and NRSA) to the OST Project Manager and SHPD managers
- managing the development of and/or reviewing and approving all major work products associated with the 2020 GLHHFFTS and various other fish tissue studies, including products prepared by OWOW
- collaborating with Fish Tissue Study Team members for reporting 2020 GLHHFFTS results and results for other fish tissue studies in technical journal articles and federal technical reports
- coordinating with the OST Project Manager to obtain CSRA/GDIT support for preparing materials for fish tissue study briefings and presentations
- participating in presenting 2020 GLHHFFTS and other fish tissue study briefings for EPA managers and delivering fish tissue study presentations in various forums (e.g., scientific conferences, government meetings, and webinars)

Brian Lenell of GLNPO is the **2020 GLHHFFTS GLNPO Project Manager** who is providing support for planning and implementation of this regional Great Lakes study being conducted under the NCCA. This role involves the following responsibilities related to the 2020 GLHHFFTS:

- reviewing and concurring on technical information developed for 2020 GLHHFFTS fish sample collection
- participating in training on the 2020 Great Lakes human health fish sampling requirements in coordination with OST
- arranging additional support for 2020 GLHHFFTS fish sample collection through GLNPO fisheries contacts
- participating in the review of the fish sample preparation and sample analysis QAPPs for the 2020 GLHHFFTS
- managing analysis of fish tissue samples for the fatty acids, including identification of a laboratory for analysis of fillet samples for fatty acids and oversight of fatty acid analysis and reporting results
- coordinating with 2020 GLHHFFTS project team members to ensure technical quality and adherence to QA/QC requirements
- participating in 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

- reviewing and concurring on all major work products associated with the 2020 GLHHFFTS
- collaborating with the 2020 GLHHFFTS project team for reporting the study results in technical journal articles and federal technical reports
- participating in 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 QAPPs that involve scientific work being conducted by OST. Bill Kramer is the **Standards and Health Protection Division (SHPD) QA Coordinator** who is responsible for reviewing and recommending approval of all QAPPs that include scientific work being conducted by SHPD within OST. The OST QA Officer and SHPD QA Coordinator are also responsible for the following QA/QC activities:

- reviewing and approving this QAPP
- reviewing and evaluating the QA/QC requirements and data for all the 2020 GLHHFFTS activities and procedures
- conducting external performance and system audits of the procedures applied for all 2020 GLHHFFTS activities
- participating in Agency QA reviews of the study

Louis Bloom is the **GLNPO Quality Assurance Manager** who is responsible for reviewing and approving all QAPPs that involve scientific work being conducted by GLNPO. The GLNPO QA Manager is 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 2020 GLHHFFTS activities and procedures
- participating in external performance and system audits of the procedures applied for all 2020 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 and logistical support being provided by Tetra Tech staff for the 2020 GLHHFFTS. His specific responsibilities include the following:

- providing direct technical and logistical support for the following 2020 GLHHFFTS activities or providing leadership and oversight for Tetra Tech staff supporting these activities:
    - developing procedures for fish sampling and fish sample preparation
    - preparing 2020 GLHHFFTS training materials and project information to incorporate into NCCA documents developed by OWOW

  - preparing 2020 GLHHFFTS documents specific to the Great Lakes human health fish fillet indicator (including this QAPP)
  - providing fish sampling and fish sample preparation training
  - planning and implementing 2020 GLHHFFTS logistics
  - collecting fish samples at whole fish sampling Great Lakes nearshore sites designated by the OST Project Manager
  - obtaining and performing QC reviews of Great Lakes human health field sampling data related to the human health fish fillet indicator
  - assigning batches for fish sample preparation and preparing fish sample preparation instructions for whole fish samples collected from designated NCCA 2020 GLHHFFTS nearshore sites and ORD-Duluth 2020 Great Lakes special study enhancement sites
  - managing implementation of the fish sample preparation procedures, including obtaining laboratory services for analysis of QC samples generated during fish sample preparation and lipid samples for all Great Lakes human health fillet samples
  - preparing weekly fish sample processing reports and evaluating the reports for adherence to the technical and quality requirements in the fish sample preparation procedures
  - packing and shipping fish fillet tissue and any related samples (e.g., rinsate samples) to analytical laboratories designated for mercury, PCB, PFAS, lipid, and fatty acid analyses
  - preparing project information and graphics for development of project fact sheets, briefings, presentations, and other EPA meeting and outreach materials
  - providing technical support for planning and reviewing statistical analysis of 2020 GLHHFFTS and ORD-Duluth 2020 special study fillet tissue data and reporting the final results
- 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
- ensuring completion of high-quality deliverables within established budgets and time schedules
- participating in meetings and conference calls with project team members for planning study activities, reporting progress on study tasks, and discussing and resolving technical issues related to the study

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

- assisting Tetra Tech's Project Leader with the 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

A5. Problem Definition/Background

Obtaining statistically representative occurrence data on multiple contaminants in fish tissue is a priority area of interest for EPA. Since 2010, OST has collaborated with the Great Lakes National Program Office (GLNPO), Office of Wetlands, Oceans, and Watersheds (OWOW) within the Office of Water (OW), and with the Office of Research and Development (ORD) to conduct a series of regional-scale assessments of chemical contaminants in Great Lakes fish from nearshore areas as part of EPA's National Coastal Condition Assessment (NCCA). This current study of contaminants in Great Lakes fish is referred to as the 2020 Great Lakes Human Health Fish Fillet Tissue Study (GLHHFFTS). It is the third study of Great Lakes fish contamination conducted by OST and GLNPO under the NCCA. The two previous OST/ GLNPO studies of contaminants in Great Lakes fish were the 2010 Great Lakes Human Health Fish Tissue Study and the 2015 Great Lakes Human Health Fish Fillet Tissue Study.

Overall, the 2020 NCCA is a probability-based survey designed to assess the condition of coastal waters of the United States, which includes coastal waters of the Great Lakes. Building on EPA's experience from the 2010 NCCA and the 2015 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 725 estuarine sites along the coasts of the contiguous United States, 226 freshwater sites from nearshore areas throughout the Great Lakes, and 50 enhancement sites in Lake Michigan. OWOW within OW is responsible for managing the planning and implementation of the NCCA.

A6. Project/Task Description

OST and GLNPO began planning and mobilizing for the 2020 GLHHFFTS in 2019. An important new decision during the planning phase for the 2020 GLHHFFTS was to expand human health fish sample collection to the full set of 226 Great Lakes nearshore sites (45 sites per Great Lake except 46 sites in Lake Superior) randomly selected by ORD (Figure 2 and Appendix A). During the previous two Great Lakes human health fish tissue studies in 2010 and 2015, fish sample collection was limited to approximately 150 nearshore sites (about 30 sites per Great Lake). Mobilizing activities for the 2020 GLHHFFTS included updating fish sampling and handling protocols for the NCCA 2020 Field Operation Manual (USEPA 2020b) and Field Sampling QAPP (USEPA 2020a), along with assembling and shipping human health fish sampling kits to the NCCA central supply distribution center in Traverse City, Michigan. During the mobilization phase, OWOW had to develop and implement a significantly different approach for NCCA 2020 field sampling training due to the coronavirus pandemic. Rather than conducting a series of up to 14 onsite training workshops across the U.S., OWOW provided NCCA 2020 field sampling training through a series of virtual training workshops that began in late March and continued until late May 2020.

OST and GLNPO also coordinated with EPA scientists at the ORD facility in Duluth, Minnesota (abbreviated as ORD-Duluth) to add collection and analysis of human health fish samples from 50 enhancement sites in Lake Michigan as part of the ORD-Duluth 2020 Great Lakes special study. These enhancements sites include 38 island nearshore sites in northern Lake Michigan and 12 National Park nearshore sites in southern Lake Michigan (Figure 2 and Appendix A). Collection and preparation of human health whole fish samples from the Lake Michigan enhancement sites will involve procedures that are identical to the fish sample collection and preparation procedures for the 2020 GLHHFFTS.

NCCA 2020 Great Lakes human health whole fish sample collection and preparation for both the 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study involve the following key components:

- Collecting human health whole fish samples at 226 randomly selected Great Lakes nearshore sites and 50 Lake Michigan enhancement sites in the nearshore regions (Appendix A) during 2020 and possibly into 2021 if field crews are subject to travel restrictions in 2020 due to the coronavirus pandemic. Both types of sites have depths up to 30 meters or distances up to 5 kilometers from the shore.

- Obtaining one fish composite sample from each Great Lakes nearshore site and Lake Michigan enhancement site designated for human health fish sampling, which ideally consists of five similarly sized adult fish of the same species that are commonly consumed by humans.

- Shipping Great Lakes human health whole fish samples to freezers at Microbac Laboratories in Baltimore, MD for interim storage.

- Transferring the whole fish samples to the Tetra Tech facility in Owings Mills, MD for fish sample preparation.

- Preparing fillet tissue samples for chemical analysis by scaling and filleting each fish in the composite sample, homogenizing the fillets from all the fish in the sample, and dividing the fillet tissue into aliquots for various chemical analyses and for long-term storage of archived samples in a freezer.

- Shipping fillet tissue samples to laboratories contracted to analyze these samples for mercury, PFAS, PCB congeners, PCBs as Aroclors, lipids, and fatty acids.

This QAPP focuses on fish sample preparation activities for the NCCA 2020 Great Lakes human health whole fish samples, which include the last three study components listed above. Specific fish sample preparation procedures and requirements are described in Appendix B.

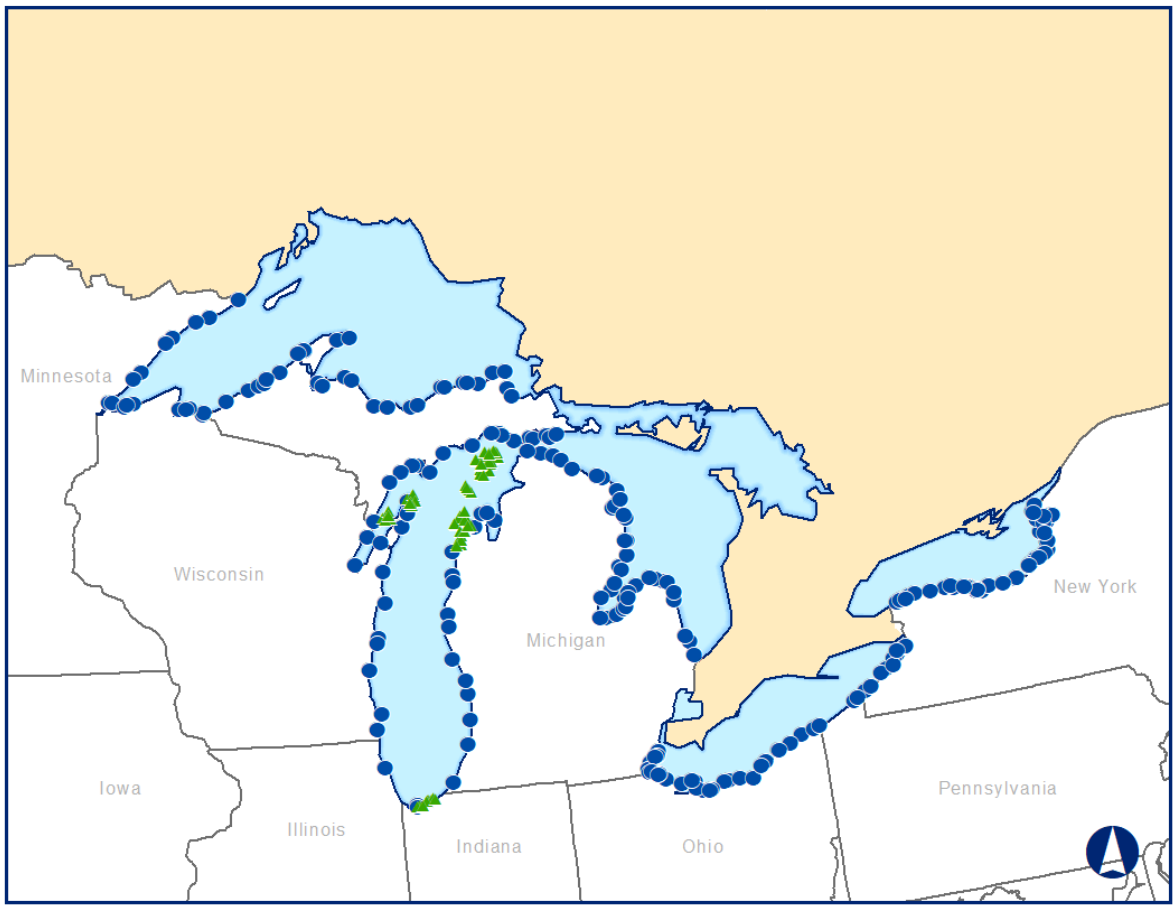

Figure 2. NCCA 2020 Great Lakes human health whole fish sampling locations (226 nearshore sites are blue dots and 50 Lake Michigan enhancement sites are green triangles)

A7. Quality Objectives and Criteria

Data of known and documented quality are essential to the success of any sampling program. Data quality objectives (DQOs) are qualitative and quantitative statements that clarify the intended use of the data, define the type of data needed to support the decision, identify the conditions under which the data should be collected, and specify tolerable limits on the probability of making a decision error due to uncertainty in the data. DQOs are developed by data users to specify the data quality needed to support specific decisions. Sources of error or uncertainty include the following:

- Sampling error: The difference between sample values and *in situ* true values from unknown biases due to collection methods and sampling design.

- Measurement error: The difference between sample values and in situ true values associated with the measurement process.

- Natural variation: Natural spatial heterogeneity and temporal variability in population abundance and distribution.

- Error sources or biases associated with compositing, sample handling, storage, and preservation.

This QAPP addresses activities associated with Great Lakes human health fish sample preparation, so the relevant quality objectives are related to issues involving fillet tissue sample preparation and handling in the laboratory. Table 1 lists the types of fillet tissue sample preparation data needed for the 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study. Methods and procedures described in this document are intended to reduce the magnitude of the sources of uncertainty and their frequency of occurrence by applying the following approaches:

- Use of standardized fish sample preparation procedures (Appendix B)

- Use of trained scientists to perform the fish sample preparation activities

Table 1. Types of Laboratory Data to Be Collected in Association with Great Lakes Human Health Fish Fillet Sample Preparation for the 2020 NCCA

Data Type	Measurement Endpoint(s) or Units
Fish weight	Grams (g)
Unhomogenized fillet weight	Grams (g)
Homogenized fillet weight	Grams (g)
Tissue homogenate recovery	Percent (%)
Tissue aliquot weight	Grams (g)
Tissue archive weight	Grams (g)

Measurement performance criteria are quantitative statistics that are used to interpret the degree of acceptability or utility of the data to the user. These criteria, also known as data quality indicators, include the following:

- Precision
- Accuracy
- Representativeness
- Completeness
- Comparability

Precision

Precision is a measure of internal method consistency. It is demonstrated by the degree of agreement between individual measurements (or values) of the same property of a sample measured under similar conditions. The only analytical testing that is within the scope of this QAPP is the analysis of fish preparation rinsate and solvent blank samples for mercury and PCB

congeners (to ensure that the preparation laboratory environment and equipment are not an extraneous source of mercury or PCBs) and lipid analysis to test the homogeneity of the prepared fish fillet tissue samples and to provide lipid results for the full complement of fish composite samples in each fish sample preparation batch (usually 20 fish samples per batch).

The sample preparation laboratory will prepare two sets of rinsate samples (each consisting of one deionized [DI] water equipment rinsate sample and one DI water blank sample) for mercury and PFAS analysis, one set of rinsate samples (consisting of one hexane equipment rinsate sample and one hexane blank sample) for PCB analysis, and one homogenized fillet sample for triplicate lipid determinations per fish sample preparation batch, as described in Steps 25 through 29 of Appendix B. The batch-specific homogeneity and paired rinsate and solvent blank results are reviewed by EPA against the QC specifications detailed in Section B5.1 (for homogenates), Section B5.2 (for mercury rinsates), and Section B5.3 (for PCB rinsates). The QC requirements for PFAS analysis of rinsate and solvent blank samples will be specified in the NCCA 2020 Great Lakes human health fish fillet sample analysis QAPP, which will be developed at a later date.

Accuracy

Accuracy is defined as the degree of agreement between an observed value and an accepted reference or true value. Accuracy is a combination of random error (precision) and systematic error (bias) introduced during sampling and analytical operations. Bias is the systematic distortion of a measurement process that causes errors in one direction, so that the expected sample measurement is always greater or lesser to the same degree than the sample's true value. Proper sample handling procedures will be followed to minimize sample contamination during fish sample preparation (Section B4.1) and QC analyses (Sections B4.2 through B4.5).

Representativeness

Representativeness expresses the degree to which data represent a characteristic of a population, a parameter, a process condition, an environmental condition, or variations at a sampling point. The representativeness goal for the 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study was addressed during the study design phase and will be satisfied by using experienced field biologists to ensure that the samples collected are actually of the type (species) specified for this study and are from the randomly selected sites specifically identified in the statistical (i.e., probabilistic) study design. Representativeness in the fish sample preparation phase centers on the fish fillet composite samples or fillet tissue aliquots and how accurately they represent the fillet mass for analysis of mercury, PCBs, PFAS, or fatty acids in the fish. Batch-specific rinsate and homogeneity sample analyses (Sections B5.1 through B5.4) are included as QC steps in the fish preparation process to ensure that the fish fillet homogenates are free from any laboratory sources of contamination and are thoroughly mixed, so they are therefore a representative sample of the fillet tissue.

Completeness

Completeness is defined as the percentage of measurements made that are judged to be valid according to specific criteria and entered into the data management system. To optimize

completeness, every effort is made to avoid sample and/or data loss. Accidents during sample transport or lab activities that cause the loss of the original samples will result in irreparable loss of data, which will reduce the ability to perform analyses, integrate results, and prepare reports. Whole fish samples are packed in unbreakable (plastic) shipping containers (i.e., insulated ice chests) to avoid damage during shipment of the samples to the sample repository (i.e. Microbac Laboratories in Baltimore, MD) or during transport of the samples to the fish sample preparation laboratory in Owings Mills, MD. Fillet homogenates are also packed in unbreakable shipping containers for shipment to each analytical laboratory.

Percent completeness (%C) for measurement parameters can be defined as follows:

$$\%C = \frac{v}{T} \times 100$$

Where v = the number of measurements judged valid and
T = the total number of measurements.

Completeness for the NCCA 2020 Great Lakes human health fish fillet indicator sample preparation effort is the number of samples processed relative to the number of samples that are collected and identified as valid samples for analysis. The completeness goal for the sample preparation phase of this study is 100% because processing all valid samples is critical for maintaining the integrity of the statistical design for the study. In some cases, whole fish samples may contain small fish and/or less than five individual specimens, and therefore may not provide sufficient tissue for analysis of the full list of target chemicals. In those cases, the OST Project Manager and the OST Fish Sample Preparation Technical Leader must be notified before sample preparation activities begin, and EPA's priority order for preparing fillet aliquots for analysis must be followed (i.e., highest to lowest priority order is mercury, PFAS, PCB congeners, single lipids, PCBs as Aroclors, and fatty acids). The completeness goals for individual fillet aliquots listed in the bullets below reflect these priorities for analyzing the target chemicals. It should be noted that the total number of sampling locations may change over the course of each Great Lakes study based on location conditions (e.g., accessibility of target locations) and the availability of target species (e.g., natural biological abundance or distribution). Any changes must be approved by the OST Project Manager, and approved changes must be considered when assessing completeness. The completeness goal is achieved when the following requirements are met:

- Fillet samples are collected from each fish identified by the OST Project Manager as a valid specimen for inclusion in the composite sample, and those fillets are homogenized to prepare fish tissue aliquots for mercury, PFAS, PCB congener, PCBs as Aroclors, lipid and fatty acid analyses (completeness goal is 100% for mercury, 100% for PFAS, 90% for PCB congeners, 80% for PCBs as Aroclors and single lipids, and 75% for fatty acids).

- All homogenized fillet aliquots are shipped with no errors in documentation or sample handling procedures, which facilitates timely delivery of every shipment of samples and arrival of the samples at the analytical laboratory in good condition.

Comparability

Comparability is an expression of the confidence with which one data set can be compared with another. Comparability is dependent on the proper design of the sampling program and on adherence to accepted sampling techniques, procedures, and quality assurance guidelines. For fish sample preparation, comparability of data is accomplished by standardizing the sample preparation methods and the laboratory training as follows:

- All homogenized fillet samples are prepared by sample preparation laboratory personnel according to the procedures contained in this QAPP (Appendix B).

- All laboratory personnel involved with fish sample preparation will have adequate training and appropriate fillet tissue sample preparation experience (Section A8).

A8. Special Training/Certification

All laboratory staff involved in the preparation of fish fillet tissue samples must be proficient in the associated tasks, as required by the NCCA 2020 Great Lakes Human Health Fish Fillet Sample Preparation, Homogenization, and Distribution Procedures (Appendix B). Specialized training is being provided for laboratory technicians who will be preparing homogenized fillet tissue samples from the whole fish samples collected for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study. This training will be conducted at the Tetra Tech Biological Research Facility in Owings Mills, MD for all laboratory staff involved with fillet tissue sample preparation to accomplish the following objectives:

- Present homogenized fillet tissue sample preparation and distribution procedures as described in Appendix B,

- Demonstrate filleting and homogenizing techniques with practice fish provided by the Tetra Tech laboratory, and

- Provide hands-on opportunities for Tetra Tech laboratory staff to develop proficiency with filleting and homogenizing fish fillet samples, including equipment cleaning procedures and collection of equipment rinsate and solvent blank samples.

A9. Documents and Records

Thorough documentation of all NCCA 2020 Great Lakes human health fish sample preparation activities is necessary for proper sample processing in the laboratory and, ultimately, for the interpretation of study results. The Tetra Tech Biological Research Facility in Owings Mills, MD is serving as the fish sample preparation laboratory, and Tetra Tech is responsible for producing and maintaining the following documents and records:

- The Tetra Tech laboratory must prepare and submit a weekly progress report to the OST Fish Sample Preparation Technical Leader and the OST Project Manager (based on fish processing information recorded on each 2020 GLHHFFTS Fish Sample Preparation

Laboratory Bench Sheet in Appendix C and ORD-Duluth 2020 Great Lakes Special Study Human Health Fish Sample Preparation Laboratory Bench Sheet in Appendix D) to document the status of fish sample preparation activities and provide information specified in the procedures described in Appendix B.

- The Tetra Tech laboratory must report the results for the paired rinsate and solvent blank sample analyses for mercury and PCBs (PFAS rinsates and solvent blanks will be analyzed by the laboratory selected for analysis of NCCA 2020 Great Lake human health fish fillet samples for PFAS) and for the triplicate lipid results associated with each fish sample preparation batch (generally 20 fish samples per batch) to the OST Fish Sample Preparation Technical Leader and the OST Project Manager. The Tetra Tech laboratory is also responsible for reporting the full set of lipid analysis results to the OST Fish Sample Preparation Technical Leader and the OST Project Manager. This includes the single lipid analysis results for 19 of the 20 fish samples in a typical batch and the average triplicate lipid results for one fish sample in the batch.

- The Tetra Tech laboratory must provide shipping information (e.g., tracking number, airbills, and shipping forms) to the OST Fish Sample Preparation Technical Leader and the OST Project Manager for aqueous QC samples and fillet tissue samples sent to designated analytical laboratories.

All documents and records prepared for the NCCA 2020 Great Lakes human health fish fillet sample preparation will be maintained by Tetra Tech for the duration of the study and retained for a period of five years following completion of the study (unless otherwise directed by EPA).

B. DATA GENERATION AND ACQUISITION

B1. Sampling Process Design (Experimental Design)

The target population for the 2020 GLHHFFTS consists of all 226 of the Great Lakes nearshore sites randomly selected for 2020 NCCA sampling. Additionally, there are 50 ORD-Duluth 2020 Great Lakes special study enhancement sites in Lake Michigan identified for human health fish collection. Together these 226 nearshore sites and 50 enhancement sites are designated as the NCCA 2020 Great Lakes human health whole fish sampling sites. The design for selecting the human health whole fish sampling sites incorporated the following objectives:

- Statistically representative data on the concentrations of mercury, PCBs, and PFAS in Great Lakes fish commonly consumed by humans

- Information on the potential for PFAS to bioaccumulate in fish fillet tissue.

- Data to answer questions concerning the occurrence of PFAS in the fillets of fish and the potential for human exposure through fish consumption.

- Species-specific information on fatty acid content of Great Lakes fish that are commonly targeted by fishermen and consumed by humans (analysis of fatty acids will be limited to fillet samples from the 2020 GLHHFFTS).

Fish fillet tissue data from the 2020 GLHHFFTS will also provide EPA with the opportunity to evaluate changes in the levels of Great Lakes fish contamination over time by comparing 2020 GLHHFFTS fillet tissue results to the fillet tissue data generated during the 2015 GLHHFFTS and 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 2020 Field Operations Manual* prepared by OWOW (USEPA 2020b).

Sampling at the 2020 GLHHFFTS nearshore sites and ORD-Duluth 2020 Great Lakes special study enhancement sites in Lake Michigan involves collection of whole fish samples for analysis of fillet tissue samples for mercury, PFAS, PCBs, lipids, and fatty acids (note that analysis of fillet samples from Lake Michigan enhancement sites will not include fatty acids). To meet the study objectives, one fish sample is collected from each nearshore and enhancement site. Ideally, each fish sample is a routine fish composite sample that consists of five fish of adequate size to provide a minimum of 75 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 site, or at least be of consumable size if no legal harvest requirements are in effect.

- All are of similar size, so that the smallest fish specimen in a composite sample is no less than 75% of the total length of the largest specimen.

- 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 sample are available for delivery to the designated laboratory.)

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

The sample collection goal at each NCCA 2020 Great Lakes site designated for whole fish sample collection is to obtain a composite sample of fish that are adequate in size to provide a minimum of 75 grams of fillet tissue for chemical analysis. Field crews are collecting human health fish samples between June and September during the 2020 field season unless additional time is required to complete fish sampling due to constraints imposed on field crews because of the coronavirus pandemic.

B2. Sampling Methods

Sampling method procedures and requirements for collection of human health fish samples are detailed in EPA's *National Coastal Condition Assessment 2020 Quality Assurance Project Plan* (USEPA 2020a) and *National Coastal Condition Assessment 2020 Field Operations Manual* (USEPA 2020b). These sampling procedures and requirements, which apply to human health whole fish sample collection at both the 2020 GLHHFFTS nearshore sites and the ORD-Duluth 2020 Great Lakes special study Lake Michigan enhancement sites, are summarized below.

The sampling objective is for field crews to obtain one representative human health whole fish composite sample from each nearshore and enhancement 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 human health fish composite sample 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. Field teams use this list as the basis for selecting appropriate fish species for the NCCA 2020 Great Lakes human health fish 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.

Table 2. Primary Target Fish Species and Secondary Alternative Fish Species for the 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes Special Study

Primary Fish Species Scientific Name*	Primary Fish Species Common Name
Ambloplites rupestris	Rock bass
Micropterus dolomieu	Smallmouth bass
Micropterus salmoides	Largemouth bass
Pomoxis annularis	White crappie
Pomoxis nigromaculatus	Black crappie
Cyprinus carpio	Common carp
Esox lucius	Northern pike
Esox masquinongy	Muskellunge
Esox niger	Chain pickerel
Ictalurus punctatus	Channel catfish
Lota lota	Burbot
Morone americana	White perch
Morone chrysops	White bass
Perca flavescens	Yellow perch
Sander canadensis	Sauger
Sander vitreus	Walleye
Coregonus clupeaformis	Lake whitefish
Oncorhynchus gorbuscha	Pink salmon

Secondary Fish Species Scientific Name*[a]	Secondary Fish Species Common Name
Carpiodes cyprinus	Quillback
Catostomus catostomus	Longnose sucker
Catostomus commersonii	White sucker
Hypentelium nigracans	Northern hogsucker
Ictiobus cyprinellus	Bigmouth buffalo
Ictiobus niger	Black buffalo
Lepomis cyanellus	Green Sunfish
Lepomis gibbosus	Pumpkinseed
Lepomis gulosus	Warmouth
Lepomis macrochirus	Bluegill
Lepomis megalotis	Longear Sunfish
Ameiurus melas	Black bullhead
Ameiurus natalis	Yellow bullhead
Ameiurus nebulosus	Brown bullhead
Coregonus artedi	Cisco/ lake herring
Coregonus hoyi	Bloater
Prosopium cylindraceum	Round whitefish
Salvelinus fontinalis	Brook trout

Primary Fish Species Scientific Name*	Primary Fish Species Common Name
Oncorhynchus kisutch	Coho salmon
Oncorhynchus tshawytscha	Chinook salmon
Oncorhynchus mykiss	Rainbow trout
Salmo salar	Atlantic salmon
Salmo trutta	Brown trout
Salvelinus namaycush	Lake trout
Aplodinotus grunniens	Freshwater drum

* Minimum acceptable length is 190 mm, TL

Secondary Fish Species Scientific Name*[a]	Secondary Fish Species Common Name

* Minimum acceptable length is 190 mm, TL
[a] Only send if preferred species are not available

In preparing Great Lakes human health fish samples for shipping, field teams record sample number, species name, specimen length, sampling location, and sampling data and time on an electronic Human Health Fish Collection Form in the NCCA 2020 app. 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 Laboratories 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 (i.e., 50 pounds), and to ship them via priority overnight delivery service (i.e., FedEx), so that they arrive at Microbac Laboratories in less than 24 hours from the time of sample collection. Alternatively, field crews may transport Great Lakes human health 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 overnight shipping to Microbac Laboratories on dry ice as described above.

B3. Sample Receipt and Inspection

This section describes the procedures that apply once the Great Lakes human health whole fish (HTIS) samples are shipped from the field to Microbac Laboratories. The Great Lakes human health fish samples are being collected by various organizations participating with EPA in this study, including state and tribal agencies and contractors. Although samples are shipped frozen on dry ice, they must be inspected promptly on receipt. As samples are received, a Microbac Laboratories representative will:

- Check that each cooler 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 degrees Celsius (ºC), or an infra-red (IR) temperature "gun" and report the reading to CSRA/GDIT via email.
- Notify CSRA/GDIT via email about receipt of samples on the day of delivery and report if each sample arrives frozen with dry ice remaining in the cooler.

- Store the coolers in the onsite freezer.

Microbac Laboratories will notify CSRA/GDIT on the day of delivery about any problems encountered upon receipt of samples (e.g., no dry ice left in cooler, fish partly or completely thawed, etc.). CSRA/GDIT subsequently reports sample receipt and inspection issues to the OST Project Manager and OST Fish Sample Preparation Technical Leader, then coordinates with EPA to resolve the issues.

Generally within two weeks of Great Lakes human health whole fish sample deliveries, a CSRA/GDIT staff member acting as a sample custodian will:

- Retrieve coolers containing NCCA 2020 Great Lakes human health whole fish samples from the freezer at Microbac Laboratories.
- Remove whole fish samples from each cooler and record the Site ID and EPA Sample Number.
- Transfer the whole fish samples to trays in the freezer for interim storage and leave empty coolers outside the freezer for Tetra Tech staff to retrieve.
- CSRA/GDIT reports any discrepancies in individual fish label information and receipt of fish samples from sampling sites to the OST Project Manager for resolution.

After completing whole fish sample check-in, CSRA/GDIT stores the whole fish samples in the freezer at Microbac Laboratories where they are kept at temperatures less than or equal to -20°C until they are ready to transfer to the Tetra Tech laboratory in Owings Mills, MD for fish sample preparation. (The freezers are maintained by Microbac Laboratories under a separate agreement with CSRA/GDIT and are continuously monitored by an automated temperature monitoring system.)

B4. Fish Sample Preparation and Analytical Methods

The laboratory at Tetra Tech's Biological Research Facility in Owings Mills, MD is the fish sample preparation laboratory (prep lab) for the NCCA 2020 Great Lakes human health fish samples. In this role, Tetra Tech is responsible for removing scales from each valid fish in the whole fish sample, filleting valid fish in the whole fish samples, homogenizing the fillet tissue, preparing the required number of fish fillet tissue aliquots for analysis and archive, shipping the fillet tissue aliquots for each type of analysis to the designated analytical laboratories, and providing short-term storage for archived fillet tissue samples in a freezer at their facility. The specific procedures for NCCA 2020 Great Lakes human health fish sample preparation activities are described in Appendix B.

This section describes NCCA 2020 Great Lakes human health fish sample preparation methods (i.e., methods for preparing homogenized fillet tissue samples). It also describes methods for analysis of lipids in ground fillet tissue samples for homogeneity testing and lipid content and for analysis of mercury, PCBs, and PFAS in equipment rinsate and solvent blank samples to test the adequacy of equipment cleaning. Lipid analysis of ground fillet tissue samples for homogeneity testing and mercury, PCB, and PFAS analysis of equipment rinsate and solvent blank samples for testing sufficient equipment cleaning are conducted as part of the QC procedures for fish sample preparation. Analytical method requirements for analysis of homogenized fillet samples

for target chemicals (e.g., mercury, PFAS, PCB congeners, PCBs as Aroclors, and fatty acids) are described in the NCCA 2020 Great Lakes human health fish fillet tissue sample analysis QAPP, which will be developed at a later date.

B4.1 Fish Sample Preparation

Trained laboratory staff at Tetra Tech's Biological Research Facility in Owings Mills, MD are responsible for preparation of homogenized fillet tissue samples from NCCA 2020 Great Lakes human health whole fish samples. Preparing the homogenized fillet tissue samples involves removing scales from each valid fish specimen in a sample, filleting the individual fish to be included in the tissue composite sample, homogenizing the fillet tissue from each whole fish sample, preparing the required number of fish fillet tissue aliquots for analysis and archive, shipping the fillet tissue aliquots for each type of analysis to the designated analytical laboratories, and storing archived fillet tissue samples on an interim basis in a freezer at their facility. The specific procedures for fillet tissue sample preparation activities are summarized below and fully described in Appendix B.

Homogenized Fillet Sample Preparation

The filleting process involves removing the fillet (with skin on and "belly flap" or ventral muscle attached) from both sides of each valid fish in the whole fish sample. The combined fillets from all valid fish in the whole fish sample are weighed to the nearest gram (wet weight) before they are homogenized together. An electric meat grinder is used to prepare each homogenized fillet sample. The entire set of fillets (with skin and belly flap) from both sides of every valid fish in the whole fish sample (i.e., ideally 5 fish per sample) are homogenized, and the entire homogenized volume is used to prepare the fillet tissue sample aliquots. Grinding of the fillet tissue is repeated until the tissue consists of a uniform color and finely ground texture. Homogeneity is confirmed by conducting triplicate analyses of the lipid content in one fish sample from each fish sample preparation batch (generally one in 20 fish samples). The collective weight of the homogenized fillet tissue from the whole fish sample is recorded to the nearest gram (wet weight) after processing. Tetra Tech lab technicians prepare fillet tissue sample aliquots for chemical analysis and archive according to specifications in Table 1 of Appendix B.

Fish Sample Preparation Batches

Each NCCA 2020 Great Lakes human health fish sample preparation batch generally consists of 20 whole fish samples. The number of whole fish samples in the final fish sample preparation batch (or two) may be adjusted to include a few more than 20 or fewer than 20, depending on what fraction of 20 whole fish samples are left near the end of fish processing for assignment to a batch. Tetra Tech staff will develop fish sample preparation instructions that include all valid fish samples available for processing. Processing may not begin until the OST Fish Sample Preparation Technical Leader and the OST Project Manager review the draft instructions and the OST Fish Sample Preparation Technical Leader approves the final instructions and batch assignments with OST Project Manager concurrence.

B4.2 Lipid Analysis

Tetra Tech will procure the services of an analytical laboratory (to be determined or TBD) to conduct one set of triplicate lipid analyses per fish sample preparation batch (see definition in Section B4.1) as described in Steps 28 and 29 of Appendix B. A single homogenized fillet tissue aliquot is analyzed for lipid content for each of the remaining samples in the batch (usually 19).

Lipids are extracted from each fillet tissue sample using a method proposed by the TBD analytical laboratory. (This QAPP will be amended to include a description of this method after the analytical laboratory is selected and their proposed method for lipid analysis is approved.)

Percent lipid content is calculated by dividing the lipid weight by the initial fillet tissue aliquot weight and multiplying that result by 100. The batch-specific lipid results for homogeneity evaluations are reviewed initially by Tetra Tech and independently by CSRA/GDIT against the QC specifications detailed in Section B5.1.

B4.3 Mercury Rinsate Analysis

Tetra Tech laboratory technicians prepare one set of paired rinsate and solvent blank samples per fish sample preparation batch (see definition in Section B4.1) for mercury analysis as described in Steps 25 through 27 of Appendix B. This set of paired QC samples consists of one deionized (DI) water equipment rinsate sample and one DI water blank sample. Tetra Tech will procure the services of an analytical laboratory (TBD) to conduct mercury analysis of these QC samples using EPA Method 245.1 (USEPA 1994). This method was developed to measure total mercury (organic and inorganic) in aqueous samples. The flameless atomic absorption procedure is a physical method based on the absorption of radiation at 253.7 nanometers by mercury vapor. Mercury is first reduced to its elemental state using a potassium permanganate digestion procedure. The samples/standards and a stannous chloride reagent are then pumped into the mercury analyzer and mixed. Argon gas is introduced into the solution stream. Absorbance (peak height) is measured as a function of mercury concentration and recorded as ppb of mercury. Results of the batch-specific mercury rinsate and solvent blank analyses are reviewed initially by Tetra Tech and independently by CSRA/GDIT against the QC specifications detailed in Section B5.2.

B4.4 PCB Rinsate Analysis

Tetra Tech will procure the services of an analytical laboratory (TBD) to conduct analyses of paired rinsate and solvent blank samples for a subset of PCB congeners that contain at least PCB congeners 52, 66, 105, 118, 141, 146, 170, 174, 177, and 187. These congeners represent those that EPA has found frequently and at relatively high concentrations in other fish tissue studies. Laboratories responding to Tetra Tech's solicitation will propose a PCB method that can meet the analytical specifications in the solicitation. This QAPP will be amended to include a description of the method after the analytical laboratory is selected and their proposed method for PCB analysis is approved.

B4.5 PFAS Rinsate Analysis

The PFAS paired rinsate and solvent blank samples will not be analyzed during the fish sample preparation process. Instead, they will be analyzed by the laboratory selected for PFAS analysis of the 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study fish fillet tissue samples. A description of the method used for analysis of the aqueous PFAS rinsate and solvent blank samples will be provided in the NCCA 2020 Great Lakes human health fish fillet tissue sample analysis QAPP, which will be developed at a later date.

B5. Fish Sample Preparation Quality Control Requirements

The procedures associated with the NCCA 2020 Great Lakes human health fish sample preparation process include the following: preparation of homogenized fillet tissue samples, which includes triplicate lipid analyses of homogenized fillet tissue samples to test for homogeneity (Section B5.1) and analytical testing for mercury, PCBs, and PFAS in equipment rinsate and solvent blank samples (Sections B5.2, B5.3, and B5.4, respectively) for QC purposes. The QC procedures are performed for one whole fish sample in each fish sample preparation batch (usually one in a batch of 20 fish samples).

B5.1 Homogenized Fillet Samples

Lipid content analysis is used as a surrogate to confirm homogeneity of the homogenized fish fillet samples that are prepared in the Tetra Tech Biological Research Facility laboratory. A laboratory (TBD) under contract to Tetra Tech will conduct triplicate lipid analyses of ground fillet tissue aliquots from one whole fish sample in each fish sample preparation batch and use the lipid content of those 3 fillet tissue aliquots to confirm that the ground fillet tissue is homogeneous. Laboratory technicians prepare this triplicate lipid aliquot (30 to 35 g) of homogenized fillet tissue mass for lipid analysis following the specific procedures described in Appendix B for placing the aliquot in a container, labeling it, and storing it in a freezer (refer to Step 19 in Appendix B). All homogenized fillet tissue aliquots for lipid analysis are shipped on dry ice and under chain of custody to the designated analytical laboratory. The results of the homogeneity testing are delivered to Tetra Tech for their initial review and forwarded to the OST Fish Sample Preparation Technical Leader and OST Project Manager for independent review by CSRA/GDIT and EPA.

From the triplicate lipid results, CSRA/GDIT calculates 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, and reports the results to the OST Fish Sample Preparation Technical Leader and the OST Project Manager.

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

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

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

If the RSD of the triplicate lipid results is less than or equal to 15% for mean % lipid measurements that are greater than or equal to 2.5% or if the RSD of the triplicate lipid results is less than or equal to 20% for mean % lipid measurements less than 2.5%, then EPA will notify Tetra Tech that the homogenization effort is sufficient for all of the homogenized fillet tissue samples (usually 20) in each analysis batch (refer to Step 29 in Appendix B).

Tetra Tech laboratory staff may continue to process up to two additional fish sample preparation batches. However, the laboratory may not continue to process batches beyond that third fish sample preparation batch until receiving notification from the OST Fish Sample Preparation Technical Leader (after OST Project Manager concurrence) that review of homogeneity test results from the initial batch is complete and the results are deemed satisfactory.

B5.2 Mercury Analysis of Rinsate Samples

The Tetra Tech laboratory prepares one set of DI water equipment rinsate and DI water blank samples during processing of each fish sample preparation batch and a subcontracted laboratory (TBD) analyzes each set of rinsate and blank samples for total mercury using EPA Method 245.1, which is a cold-vapor atomic absorption procedure applicable to water samples (Section B4.3). The pair of blank and rinsate samples are analyzed individually, not in batches of up to 20, in order to provide timely feedback of the cleanliness of the homogenization equipment.

EPA Method 245.1 requires daily instrument calibration and analysis of two quality control samples, an instrument blank and a laboratory control sample. The rinsates are prepared in reagent water, so there is little chance of a "matrix effect." Each laboratory control sample, which is also prepared in reagent water, provides sufficient information on the performance of the method and the laboratory. The QC sample requirements, including the acceptance criteria and corrective actions, are summarized in Table 3 below.

Table 3. QC Samples and Acceptance Criteria for Mercury Analysis of Rinsates

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

The batch-specific rinsate results are reviewed initially by Tetra Tech and forwarded to the OST Fish Sample Preparation Technical Leader and the OST Project Manager for independent review by CSRA/GDIT. The rinsate results are evaluated based on the mass of mercury detected and the assumption that all of the apparent contamination could be transferred to a nominal 465-g

mass of homogenized tissue. If review of the results shows that the rinsate samples are below the acceptance limit for mercury, i.e., 0.2 µg/L for total mercury based on the method detection limit for an aqueous sample, then the equipment cleaning effort is sufficient for all samples in that fish sample preparation batch.

Rinsate results for mercury above the reporting limit mentioned above may cause a need for corrective actions by the Tetra Tech laboratory. These corrective actions may include revisions to the laboratory's equipment cleaning procedures, followed by a successful demonstration of the revised cleaning procedures through preparation and analysis of additional rinsate samples.

Tetra Tech laboratory staff may continue to process up to two additional fish sample preparation batches. However, laboratory staff may not continue to process batches beyond that third fish sample preparation batch until receiving notification from the OST Fish Sample Preparation Technical Leader (with OST Project Manager concurrence) that review of rinsate test results from the initial fish sample preparation batch is complete and the results are deemed satisfactory.

B5.3 PCB Analysis of Rinsate Samples

The Tetra Tech laboratory prepares one set of hexane equipment rinsate and solvent blank samples during processing of each fish sample preparation batch. A subcontracted laboratory (TBD) analyzes each set of rinsate and blank samples for a selected subset of PCB congeners using a PCB method proposed and approved during the laboratory solicitation process.

For the NCCA 2020 Great Lakes human health fish preparation process, PCB rinsate analyses will focus on at least PCB congeners 52, 66, 105, 118, 141, 146, 170, 174, 177, and 187. These congeners represent those that EPA has found frequently and at relatively high concentrations in other fish tissue studies. As with the mercury rinsate analysis, the PCB 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. When PCB method information becomes available, this QAPP will be amended to identify QC samples and acceptance criteria in Table 4 below.

Table 4. QC Samples and Acceptance Criteria for PCB Analysis of Rinsates

QC Sample	Frequency	Acceptance Criteria

The batch-specific rinsate results are reviewed initially by Tetra Tech and forwarded to the OST Fish Sample Preparation Technical Leader and the OST Project Manager for independent review by CSRA/GDIT. If review of the results shows that rinsate samples are below the acceptance limit for PCBs, i.e., 0.5 ng/mL per congener (based on the instrument detection limit for a 1.0-mL final volume of solvent concentrated from the original 100-mL rinsate sample), then the equipment cleaning effort is sufficient for all samples in that fish sample preparation batch. Rinsate results for PCBs above the reporting limit mentioned above may cause a need for corrective actions by the Tetra Tech laboratory. These corrective actions may include revisions

to the laboratory's equipment cleaning procedures, followed by a successful demonstration of the revised cleaning procedures through preparation and analysis of additional rinsate samples.

The Tetra Tech laboratory may continue to process up to two additional fish sample preparation batches. However, the laboratory may not continue to process batches beyond that third fish sample preparation batch until receiving notification from the OST Fish Sample Preparation Technical Leader that review of PCB rinsate results from the initial fish sample preparation batch is complete and the results are deemed satisfactory.

B5.4 PFAS Analysis of Rinsate Samples

The QC requirements for PFAS analysis of rinsate samples will be specified in the NCCA 2020 Great Lakes human health fish fillet tissue sample analysis QAPP, which will be developed at a later date.

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 and the fillet tissue sample aliquots are inspected daily and the homogenization equipment (meat grinder) is inspected when it is reassembled after cleaning between samples.

All analytical instruments associated with fish sample preparation operations are inspected and maintained as described in the respective analytical methods and laboratory Standard Operating Procedures (SOPs). This includes the instruments involved with the fish fillet homogeneity (lipid) testing and with analyses of aqueous rinsate and solvent blank samples for mercury, PCBs, and PFAS and fillet tissue samples for percent lipids.

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 are 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 with fillet homogeneity (lipid) testing and percent lipid analyses will be calibrated as described in the respective analytical methods. The methods cited in Sections B4 and B5 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 the rinsate samples, Method 245.1, specifies calibration with five calibration standards. The PCB analysis method for the rinsate samples specifies calibration with three to five calibration standards depending on the proposed method.

B8. Inspection/Acceptance of Supplies and Consumables

Careful and thorough planning is necessary to ensure the efficient and effective completion of the fillet tissue sample preparation tasks. Fish preparation laboratory equipment and supplies are described in Appendix B. All fish sample packaging and shipping supplies are provided by Tetra Tech. It is the responsibility of the Tetra Tech laboratory technicians to procure, compile, and inspect the necessary fillet sample preparation equipment and supplies prior to commencement of fillet tissue sample preparation activities, and to inspect packaging and shipping supplies before fillet tissue samples are shipped to the respective analytical laboratories for analysis.

B9. Non-direct Measurements

Non-direct measurements are not required for this project. The analytical results from the 2010 GLHHFTS or the 2015 GLHHFFTS to which any new data are to be compared are primary data that EPA generated under an approved QAPP for each study.

B10. Data Management

Data management practices employed in this study are based on standard data management practices used for EPA's National Lake Fish Tissue Study and other OST fish contamination studies (e.g., the 2008-09 NRSA, 2010 Great Lakes Human Health Fish Tissue Study, 2013-14 NRSA, 2015 Great Lakes Human Health Fish Fillet Tissue Study, and 2018-19 NRSA). The data management (i.e., sample tracking, data tracking, data inspection, data quality assessment, and database development) procedures have been regularly applied to other technical studies by Tetra Tech and CSRA/GDIT.

Fish Sample Collection Data

Samples are documented and tracked through the use of standardized human health fish tissue (HTIS) fields in the 2020 NCCA app, Whole Fish Sample Identification Labels, and 2020 NCCA HTIS tracking spreadsheets. Specific fish sample collection data requirements are detailed in the Field Operations Manual (USEPA 2020b) prepared by OWOW. Whole fish samples are shipped to Microbac Laboratories (Baltimore, MD) by an overnight air delivery service that provides constant tracking of shipments (i.e., FedEx).

The Tetra Tech laboratory retains a copy of the 2020 NCCA HTIS tracking spreadsheet sample information that is received electronically from the NARS IM group upon shipment of each 2020 NCCA whole fish sample. Tetra Tech staff perform a data QC check on each sample tracking spreadsheet, compile each individual sample tracking spreadsheet into a combined current master sample tracking spreadsheet, and forward it to the OST Project Manager and the OST Fish Sample Preparation Technical Leader, along with documentation reporting the field data QC review (consistent with field data QC documentation provided for previous EPA fish tissue studies). All electronic files related to fish sample collection that are produced and retained by Tetra Tech are maintained in a project file during the active phase of this project, and for a period of 5 years following completion of the project (unless otherwise directed by EPA).

Upon completion of sampling activities, Tetra Tech develops a Fish Sample Master Spreadsheet based on information recorded by all field sampling teams in the 2020 NCCA app and provided by the NARS IM group in sample tracking spreadsheets. This field data is entered into Excel spreadsheets to create separate master spreadsheets for the NCCA 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study. All data entries are checked for errors in transcription and computer input by qualified persons (minimum of two) who did not originally enter the data. If there is any indication that requirements for sample integrity or data quality have not been met, the Tetra Tech QA Officer is notified immediately (with an accompanying explanation of the problems encountered) for discussion and resolution of quality issues before delivery of the Fish Sample Master Spreadsheet to the OST Project Manager and the OST Fish Sample Preparation Technical Leader.

Fish Sample Preparation Data

The Tetra Tech laboratory is required to maintain all records and documentation associated with the preparation of 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study human health fish samples (e.g., weekly reports containing fillet tissue sample preparation data and fillet tissue aliquot documentation), the analyses of fillet tissue samples for lipids for homogeneity testing and lipid content, and the analyses of rinsate samples for mercury and PCBs. All required analytical laboratory reports and documentation, including raw data, must be sequentially paginated and clearly labeled with the laboratory name, and associated sample numbers. Any electronic media submitted must be similarly labeled. The sample preparation laboratory and analytical laboratories contracted for homogeneity testing and rinsate analyses 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 (USEPA 1995) or with commonly employed data management procedures approved by the National Environmental Laboratory Accreditation Conference (NELAC).

Data Retention

All computer files associated with the NCCA 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study are stored in a project subdirectory by Tetra Tech and are copied to network storage for archive for the 5 years subsequent to project completion (unless otherwise directed by the OST Project Manager).

C. ASSESSMENT AND OVERSIGHT

C1. Assessments and Response Actions

C1.1 Fish Sample Preparation

The Tetra Tech laboratory supporting fish sample preparation for this study and the analytical laboratories responsible for lipid testing and rinsate analyses each have a comprehensive QA program in place and operating at all times. In performing fish sample preparation and QC and lipid sample analyses for this study, each laboratory will adhere to the requirements of those respective QA programs. Copies of those plans are maintained on file at Tetra Tech.

If any technical problems are encountered during operations at the Tetra Tech laboratory, the Tetra Tech Project Leader will consult with the Tetra Tech Laboratory Manager, the OST Fish Sample Preparation Technical Leader, and the OST Project Manager to identify corrective actions. The Tetra Tech Project Leader is responsible for ensuring that the corrective actions are successfully implemented. Section B5 of this QAPP identifies corrective actions for any lipid, mercury, or PCB analysis results generated by the analytical laboratory (or laboratories) that do not meet the QC acceptance criteria. The Tetra Tech Project Leader is responsible for ensuring that each analytical laboratory implements the required corrective actions.

Analysis of the QC rinsate samples for PFAS will be conducted by a TBD laboratory selected for PFAS analysis of the 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study homogenized fillet tissue samples. PFAS analyses of both the QC rinsate samples (from the fish sample preparation process) and the fillet tissue samples are included under a separate 2020 Great Lakes human health fish fillet tissue sample analysis QAPP that will be developed at a later date.

C1.2 Performance Audits

Performance audits are qualitative checks on different segments of project activities. For the 2020 GLHHFFTS, performance audit techniques include checks on post-collection review of field measurements and the use of triplicate lipid analyses of one homogenized sample in every fish sample preparation batch as a check on homogeneity. The Tetra Tech Project Leader is responsible for overseeing work as it is performed and for periodically conducting QC checks during fillet sample preparation for this project. Results of these checks are reported to the Tetra Tech Quality Assurance Officer, the OST Fish Sample Preparation Technical Leader, and the OST Project Manager.

C1.3 System Audits

System audits are qualitative reviews of project activities to check that the overall quality program is functioning and that the appropriate QC measures identified in the QAPP are being implemented. If the results of the performance audits described in Section C1.2 indicate problems, the Tetra Tech QA Officer will conduct an internal system audit during the project and report the results to the OST Fish Sample Preparation Technical Leader and the OST Project Manager. If QA/QC deficiencies are discovered, additional internal system audits are conducted until the Tetra Tech QA Officer, the OST Fish Sample Preparation Technical Leader, and the OST Project Manager conclude that overall project quality requirements are being met.

C2. Surveillance

C2.1 Whole Fish Sample Shipment

When NCCA 2020 Great Lakes human health whole fish samples are shipped to Microbac Laboratories (Baltimore, MD), the NARS Information Management (IM) Group contacts the OST Project Manager, OST Fish Sample Preparation Technical Leader, Tetra Tech Project Leader, and CSRA/GDIT via email distribution of the sample tracking spreadsheet to notify them of the upcoming fish sample deliveries, and CSRA/GDIT contacts the OST Project

Manager and the OST Fish Sample Preparation Technical Leader when the samples arrive. Within 24 hours of sample receipt, CSRA/GDIT notifies the OST Project Manager and the OST Fish Sample Preparation Technical Leader of sample condition. If problems with the shipment are noted, Tetra Tech and CSRA/GDIT will work with the OST Project Manager to resolve any problems as quickly as possible to minimize data integrity problems.

C2.2 Fish Sample Preparation

The content of fish sample preparation batches and the process for forming the batches are described in Section B4.1. The Tetra Tech laboratory may not begin processing any 2020 GLHHFFTS and ORD-Duluth 2020 Great Lakes special study whole fish samples until this QAPP is approved and the laboratory personnel responsible for fish sample preparation have been trained on the fish sample preparation procedures and requirements described in this QAPP.

The Tetra Tech Project Leader coordinates with the OST Fish Sample Preparation Technical Leader and the OST Project Manager regarding fish tissue sample shipments to other laboratories (i.e., the analytical laboratories responsible for mercury, PCB, and PFAS analysis of fish sample preparation equipment rinsate and solvent blank QC samples and for lipid analysis of homogenized fillet tissue samples, including triplicate lipid analysis of one fish sample per fish sample preparation batch for homogeneity testing) once analysis contracts are in place. Tetra Tech communicates periodically with laboratory staff by telephone or email to monitor the progress of lipid, mercury, and PCB rinsate analyses. If technical problems are encountered during fish sample preparation or during lipid, mercury, or PCB analyses, the Tetra Tech Project Leader will identify a technical expert within Tetra Tech to assist in resolving the problem, and work with the OST Fish Sample Preparation Technical Leader and the OST Project Manager to identify and implement a solution to the problem. The Tetra Tech laboratory is permitted to work two batches ahead of the delivery and review of batch-specific QC results that indicate if the homogenization and equipment cleaning procedures for each fish sample preparation batch are adequate.

If the fish sample preparation (Tetra Tech) or analytical (TBD) laboratories fail to deliver QC data on time, or if an analytical laboratory notifies Tetra Tech of anticipated reporting or sample processing delays, Tetra Tech notifies the OST Fish Sample Preparation Technical Leader and the OST Project Manager of the situation. To the extent possible, Tetra Tech will adjust schedules and shift resources as necessary to minimize the impact of laboratory delays on EPA schedules. Tetra Tech will immediately notify the OST Fish Sample Preparation Technical Leader and the OST Project Manager of any laboratory delays that are anticipated to impact EPA schedules.

C3. Reports to Management

Upon completion of weekly fish sampling and sample preparation activities, the Tetra Tech Project Leader provides the OST Project Manager and OST Fish Sample Preparation Technical Leader with reports of fish sampling team and Tetra Tech laboratory progress for the preceding week when these activities are occurring. These weekly progress reports include specific details about the fish sample collection and fillet sample preparation activities and note any concerns about sample quality and resolution of those concerns. Following completion of fish sampling

and fillet sample preparation activities, Tetra Tech prepares a fish collection effort summary (which details all sampling participants, sampling locations, and specimens collected) and a sample preparation summary (which lists all samples processed and identifies all fillet tissue aliquots prepared) for review by the OST Project Manager and the OST Fish Sample Preparation Technical Leader.

D. DATA VALIDATION AND USABILITY

D1. Data Review, Verification, and Validation

The processes for data review, verification, and validation provide an approach for standardized data quality assessment. These processes are also important for determining the usability and limitations of the whole fish sample collection and fillet tissue sample preparation data generated for the NCCA 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study. Processes for each step in the data quality assessment are described below.

D1.1 Data Review

Fish Sample Collection

Tetra Tech reviews data entries in the 2020 GLHHFFTS whole fish and ORD-Duluth 2020 Great Lakes special study whole fish sample tracking spreadsheets and individual fish sample labels for completeness, correctness, and consistency among the fish sampling records. Any errors or omissions identified during this review are reported to the OST Project Manager and corrected by contacting the 2020 NCCA Field Logistics Coordinator (Chris Turner of GLEC) or the field crew leader who initially made the data entries, if necessary. The Tetra Tech Project Leader is responsible for ensuring that all errors or omissions are addressed in the fish sampling records before the fish samples are transferred from Microbac Laboratories to the Tetra Tech Laboratory for fish sample preparation.

Analysis of Lipid and Fish Sample Preparation QC Samples

The Laboratory Managers at each analytical laboratory designated for analysis of lipid and fish sample preparation QC samples review all laboratory results and calculations prior to submission of a data package. Any errors identified during this peer review are returned to the lab analyst for correction. Following correction of the errors, each Laboratory Manager verifies that the final data package is complete and compliant with the contract, signs each data submission to certify that the package was reviewed and determined to be in compliance with the terms and conditions of the contract, and submits the data package to the Tetra Tech Project Leader.

D1.2 Data Verification

The basic goal of data verification is to ensure that project participants know what data were produced, if these data are complete, if the data are contractually compliant, and if the data meet the objectives of the study and the QA requirements described in this QAPP.

Fish Sample Collection

Tetra Tech staff independent of fish sampling teams verify fish sample collection data reviewed and submitted by each field team leader. This involves verifying that all data entries in the sample tracking spreadsheets for NCCA 2020 Great Lakes human health whole fish samples and whole fish sample labels are complete, correct, and consistent among the fish sampling records. The data verifier reports any discrepancies identified during this process to the Tetra Tech Project Leader. The Tetra Tech Project Leader is responsible for reconciling any discrepancies reported during data verification with the appropriate associated field personnel and for notifying the data verifier about the resolution of these discrepancies. The data verifier is responsible for documenting resolution of these data entry discrepancies.

Analysis of Lipid and Fish Sample Preparation QC Samples

The Tetra Tech Laboratory Manager conducts initial reviews of the fish sample preparation QC sample analysis results for each Great Lakes human health fish sample preparation batch and for the single lipid analysis results associated with each fish sample to verify the completeness and accuracy of these data and their compliance with QC acceptance criteria in Section B5 of this QAPP. Verification of these analytical results involves review of data for percent lipid measurements (including the triplicate lipid analysis results for the homogeneity testing of one fish sample per fish sample preparation batch, along with lipid analysis results for the remaining fish samples) and review of sample processing equipment rinsate and corresponding solvent blank QC sample data. The Tetra Tech Project Leader verifies the summary level results for these QC samples and remaining lipid samples, determines if they meet the project objectives in this QAPP, and reports the verification findings to the OST Fish Sample Preparation Technical Leader and the OST Project Manager. The CSRA/GDIT analytical chemist supporting the 2020 GLHHFFTS conducts an independent review of analytical results for lipids and for fish sample preparation QC samples and reports the verification findings to the OST Fish Sample Preparation Technical Leader and the OST Project Manager. The OST Project Manager and OST Fish Sample Preparation Technical Leader work with the Tetra Tech Project Leader and the CSRA/GDIT analytical chemist to resolve any differences in their respective verification findings.

D1.3 Data Validation

Data validation is the process of evaluating the quality of the results relative to their intended use. This process is applied to fish sample collection and fish sample preparation as described below.

Fish Sample Collection

Evaluating the quality of fish sample collection results involves comparing these results to the fish sampling requirements described in the NCCA 2020 Field Operations Manual (USEPA 2020b) prepared by OWOW. These requirements include collecting fish samples from specific waterbodies and obtaining particular fish species and numbers of fish to meet study objectives.

Fish Sample Preparation

The data validation process for fillet tissue sample preparation is more limited than the process applied during validation of analytical data from analysis of fillet tissue samples for the study-specific target chemicals. It focuses on evaluating the clarity and accuracy of required information in the fish sample preparation weekly progress reports (e.g., percentage of total fillet mass compared to the total body mass, tissue mass requirements for specified tissue aliquots, etc.).

D2. Verification and Validation Methods

Fish Sample Collection Data

The initial step in the process for data verification involves Tetra Tech staff independent of fish sampling operations conducting reviews of all data related to fish sample collection as a means of identifying any discrepancies among sampling data and related information entered in the sample tracking spreadsheets for 2020 Great Lakes human health whole fish samples and whole fish sample labels. Results from each review are documented in a series of data verification forms and compiled in a data review file that is submitted to the OST Project Manager after the end of the final field sampling season. There will be separate fish sample collection data QC files for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study. Each fish sample collection data QC file includes the study-specific collective Great Lakes human health sample tracking spreadsheet for whole fish samples, as well as detailed results of the QC review of the study-specific fish sampling data and sample description information that are recorded on standard forms (e.g., the Data Review Form and the Sample Description Review Form). Any discrepancies among the fish sampling records for each of the 2020 Great Lakes studies and resolution of these discrepancies are reported to the OST Project Manager.

Lipid Data and Fish Sample Preparation QC Sample Data

The first stage in the data verification process involves the Tetra Tech Laboratory Manager performing a completeness check in which all elements in each analytical laboratory submission are 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 Tetra Tech Lab Manager will transmit the analytical laboratory submission to the OST Fish Sample Preparation Technical Leader, the OST Project Manager, and the CSRA/GDIT data review chemist for independent review.

The second stage of the data verification process focuses on an instrument performance check in which the CSRA/GDIT data review chemist verifies that calibrations, calibration verifications, standards, and calibration blanks, as they apply to either lipid analysis or analysis of fish sample preparation QC samples, were analyzed at the appropriate frequency and met method or study performance specifications. If errors are noted at this stage, CSRA/GDIT will identify corrective action procedures for Tetra Tech to initiate with the analytical laboratory immediately.

Stage three of the data verification process focuses on a laboratory performance check in which the CSRA/GDIT data review chemist verifies 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 laboratory control samples and blanks, as they apply to either analysis of lipid samples or fish sample preparation QC samples. CSRA/GDIT will provide corrective action procedures for Tetra Tech to initiate with the analytical laboratory to resolve any deficiencies identified.

D3. Reconciliation with User Requirements

Fish Sample Collection Data

As soon as possible following completion of fish sampling operations during the 2020 NCCA field season, the Tetra Tech Project Leader assesses fish sample collection data for completeness, precision, and representativeness by comparing these data with the criteria discussed for each of these measures in Section A7 of this QAPP. This represents the final determination of whether the fish samples collected for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study are of the correct type, quantity, and quality to support their intended use for this study. The Tetra Tech Project Leader will report any problems encountered in meeting the performance criteria (or uncertainties and limitations in the use of the data) to the OST Project Manager, and work with the OST Project Manager to reconcile the problems, if possible.

Lipid Data and Fish Sample Preparation QC Sample Data

The QC results for lipids from the homogeneity testing and for the mercury and PCB rinsate analyses from homogenization of fillet tissue samples for each fish sample preparation batch are assessed by the CSRA/GDIT data review chemist against the QC acceptance criteria in Section B5 of this QAPP. Although the Tetra Tech laboratory will be permitted to work two fish sample preparation batches ahead of the delivery of the batch-specific QC results, the Tetra Tech Project Leader will track laboratory performance, notify the OST Fish Sample Preparation Technical Leader and the OST Project Manager of any issues, initiate corrective actions, and track progress by the fish sample preparation laboratory.

References

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Appendix A

Target Lists of NCCA 2020 Great Lakes Human Health Whole Fish Sampling Locations

2020 Great Lakes Human Health Fish Fillet Tissue Study
Nearshore Target Sampling Locations (226)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Erie	MI	NGL20_MI-10001	41.855488	-83.371811
Lake Erie	MI	NGL20_MI-10002	41.978389	-83.226068
Lake Erie	MI	NGL20_MI-10003	41.775408	-83.424598
Lake Erie	MI	NGL20_MI-10004	41.928531	-83.250994
Lake Erie	MI	NGL20_MI-10005	41.920233	-83.297047
Lake Erie	MI	NGL20_MI-10006	41.739698	-83.421714
Lake Erie	NY	NGL20_NY-10001	42.732124	-78.970972
Lake Erie	NY	NGL20_NY-10002	42.538288	-79.275335
Lake Erie	NY	NGL20_NY-10003	42.681456	-79.086131
Lake Erie	NY	NGL20_NY-10004	42.504435	-79.383478
Lake Erie	NY	NGL20_NY-10005	42.753035	-78.929682
Lake Erie	NY	NGL20_NY-10006	42.645265	-79.138888
Lake Erie	NY	NGL20_NY-10007	42.771058	-78.910310
Lake Erie	NY	NGL20_NY-10008	42.318041	-79.692515
Lake Erie	NY	NGL20_NY-10009	42.358800	-79.581425
Lake Erie	NY	NGL20_NY-10010	42.566027	-79.158291
Lake Erie	NY	NGL20_NY-10011	42.730634	-79.073758
Lake Erie	OH	NGL20_OH-10001	41.746250	-83.379173
Lake Erie	OH	NGL20_OH-10002	41.510475	-82.139115
Lake Erie	OH	NGL20_OH-10003	41.500632	-82.214543
Lake Erie	OH	NGL20_OH-10004	41.975683	-80.615216
Lake Erie	OH	NGL20_OH-10005	41.633941	-83.168247
Lake Erie	OH	NGL20_OH-10006	41.428897	-82.581778
Lake Erie	OH	NGL20_OH-10007	41.488651	-81.741749
Lake Erie	OH	NGL20_OH-10008	41.566691	-82.765201
Lake Erie	OH	NGL20_OH-10009	41.779146	-81.271315
Lake Erie	OH	NGL20_OH-10010	41.919821	-80.859975
Lake Erie	OH	NGL20_OH-10011	41.712038	-83.249070
Lake Erie	OH	NGL20_OH-10012	41.552818	-82.700842
Lake Erie	OH	NGL20_OH-10013	41.472286	-82.692721
Lake Erie	OH	NGL20_OH-10014	41.837711	-81.051557
Lake Erie	OH	NGL20_OH-10015	41.426038	-82.438730
Lake Erie	OH	NGL20_OH-10016	41.549394	-82.924082
Lake Erie	OH	NGL20_OH-10017	41.961265	-80.629612
Lake Erie	OH	NGL20_OH-10018	41.516712	-81.951277
Lake Erie	OH	NGL20_OH-10019	41.569624	-82.744472
Lake Erie	OH	NGL20_OH-10020	41.668449	-81.492888
Lake Erie	OH	NGL20_OH-10021	41.495360	-81.733241
Lake Erie	OH	NGL20_OH-10022	41.422845	-82.470374
Lake Erie	OH	NGL20_OH-10023	41.994433	-80.541792

2020 Great Lakes Human Health Fish Fillet Tissue Study
Nearshore Target Sampling Locations (226)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Erie	OH	NGL20_OH-10024	41.693706	-83.281403
Lake Erie	OH	NGL20_OH-10025	41.773549	-81.262087
Lake Erie	OH	NGL20_OH-10026	41.629780	-81.589718
Lake Erie	PA	NGL20_PA-10001	42.216060	-79.908285
Lake Erie	PA	NGL20_PA-10002	42.248563	-79.833229
Lake Huron	MI	NGL20_MI-10020	43.661172	-83.813745
Lake Huron	MI	NGL20_MI-10021	43.246154	-82.464205
Lake Huron	MI	NGL20_MI-10022	45.750362	-84.563951
Lake Huron	MI	NGL20_MI-10023	44.839345	-83.242500
Lake Huron	MI	NGL20_MI-10024	43.691878	-83.607161
Lake Huron	MI	NGL20_MI-10025	45.963069	-84.714304
Lake Huron	MI	NGL20_MI-10026	45.378104	-83.647971
Lake Huron	MI	NGL20_MI-10027	44.005051	-83.227579
Lake Huron	MI	NGL20_MI-10028	45.939652	-84.669392
Lake Huron	MI	NGL20_MI-10029	45.006597	-83.359057
Lake Huron	MI	NGL20_MI-10030	43.879908	-83.436643
Lake Huron	MI	NGL20_MI-10031	44.013500	-82.767393
Lake Huron	MI	NGL20_MI-10032	45.960709	-84.419150
Lake Huron	MI	NGL20_MI-10033	45.186507	-83.333887
Lake Huron	MI	NGL20_MI-10034	44.262727	-83.475720
Lake Huron	MI	NGL20_MI-10035	45.365339	-83.558984
Lake Huron	MI	NGL20_MI-10036	43.933393	-83.375104
Lake Huron	MI	NGL20_MI-10037	44.058141	-82.890460
Lake Huron	MI	NGL20_MI-10038	44.975283	-83.442236
Lake Huron	MI	NGL20_MI-10039	43.987738	-83.643622
Lake Huron	MI	NGL20_MI-10040	43.762320	-82.615251
Lake Huron	MI	NGL20_MI-10041	45.700899	-84.357468
Lake Huron	MI	NGL20_MI-10042	44.380129	-83.305156
Lake Huron	MI	NGL20_MI-10043	43.998848	-82.682268
Lake Huron	MI	NGL20_MI-10044	45.637261	-84.224843
Lake Huron	MI	NGL20_MI-10045	44.220559	-83.436314
Lake Huron	MI	NGL20_MI-10046	44.560749	-83.269783
Lake Huron	MI	NGL20_MI-10047	43.325180	-82.522957
Lake Huron	MI	NGL20_MI-10048	45.931044	-84.370602
Lake Huron	MI	NGL20_MI-10049	43.747200	-83.482180
Lake Huron	MI	NGL20_MI-10050	43.069218	-82.418883
Lake Huron	MI	NGL20_MI-10051	45.510818	-84.053375
Lake Huron	MI	NGL20_MI-10052	44.995087	-83.402484
Lake Huron	MI	NGL20_MI-10053	43.763117	-83.450645
Lake Huron	MI	NGL20_MI-10054	43.866042	-83.384851

2020 Great Lakes Human Health Fish Fillet Tissue Study
Nearshore Target Sampling Locations (226)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Huron	MI	NGL20_MI-10055	45.399225	-83.640746
Lake Huron	MI	NGL20_MI-10056	44.881911	-83.263937
Lake Huron	MI	NGL20_MI-10057	43.672811	-83.905286
Lake Huron	MI	NGL20_MI-10058	44.082561	-82.990724
Lake Huron	MI	NGL20_MI-10059	44.563966	-83.305080
Lake Huron	MI	NGL20_MI-10060	43.915173	-83.840608
Lake Huron	MI	NGL20_MI-10061	43.857134	-82.597289
Lake Huron	MI	NGL20_MI-10062	44.062607	-83.575569
Lake Huron	MI	NGL20_MI-10063	45.079141	-83.300411
Lake Huron	MI	NGL20_MI-10064	45.961854	-84.254088
Lake Michigan	IL	NGL20_IL-10001	42.141568	-87.745414
Lake Michigan	IN	NGL20_IN-10001	41.663612	-87.266719
Lake Michigan	IN	NGL20_IN-10002	41.626684	-87.268386
Lake Michigan	MI	NGL20_MI-10088	45.937953	-84.994048
Lake Michigan	MI	NGL20_MI-10089	45.769089	-86.742179
Lake Michigan	MI	NGL20_MI-10090	43.375394	-86.462772
Lake Michigan	MI	NGL20_MI-10091	45.000710	-85.477045
Lake Michigan	MI	NGL20_MI-10092	44.944033	-85.840715
Lake Michigan	MI	NGL20_MI-10093	44.396029	-86.308820
Lake Michigan	MI	NGL20_MI-10094	45.888090	-86.257438
Lake Michigan	MI	NGL20_MI-10095	43.918899	-86.457437
Lake Michigan	MI	NGL20_MI-10096	42.944195	-86.246774
Lake Michigan	MI	NGL20_MI-10097	45.797503	-84.792273
Lake Michigan	MI	NGL20_MI-10098	43.102397	-86.271767
Lake Michigan	MI	NGL20_MI-10099	45.934508	-85.719973
Lake Michigan	MI	NGL20_MI-10100	45.097762	-85.699194
Lake Michigan	MI	NGL20_MI-10101	44.312549	-86.299545
Lake Michigan	MI	NGL20_MI-10102	46.051344	-85.241341
Lake Michigan	MI	NGL20_MI-10103	44.678209	-86.261122
Lake Michigan	MI	NGL20_MI-10104	45.128687	-87.553117
Lake Michigan	MI	NGL20_MI-10105	45.772779	-86.809326
Lake Michigan	MI	NGL20_MI-10106	42.327680	-86.334775
Lake Michigan	MI	NGL20_MI-10107	46.023363	-85.195092
Lake Michigan	MI	NGL20_MI-10108	45.708931	-87.015603
Lake Michigan	MI	NGL20_MI-10109	45.107563	-85.596550
Lake Michigan	MI	NGL20_MI-10110	42.629003	-86.255722
Lake Michigan	MI	NGL20_MI-10111	45.667295	-86.518665
Lake Michigan	MI	NGL20_MI-10112	46.068962	-85.381571
Lake Michigan	MI	NGL20_MI-10113	45.590173	-87.221718
Lake Michigan	MI	NGL20_MI-10114	43.778261	-86.456345

2020 Great Lakes Human Health Fish Fillet Tissue Study
Nearshore Target Sampling Locations (226)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Michigan	MI	NGL20_MI-10115	41.884623	-86.630807
Lake Michigan	WI	NGL20_WI-10001	45.029147	-87.093383
Lake Michigan	WI	NGL20_WI-10002	42.614701	-87.810577
Lake Michigan	WI	NGL20_WI-10003	43.328918	-87.864071
Lake Michigan	WI	NGL20_WI-10004	44.948027	-87.698607
Lake Michigan	WI	NGL20_WI-10005	45.336088	-86.956190
Lake Michigan	WI	NGL20_WI-10006	43.719069	-87.657049
Lake Michigan	WI	NGL20_WI-10007	43.331739	-87.865814
Lake Michigan	WI	NGL20_WI-10008	44.513063	-87.479199
Lake Michigan	WI	NGL20_WI-10009	44.863781	-87.481783
Lake Michigan	WI	NGL20_WI-10010	44.132949	-87.500974
Lake Michigan	WI	NGL20_WI-10011	45.188723	-86.979138
Lake Michigan	WI	NGL20_WI-10012	43.653379	-87.685584
Lake Michigan	WI	NGL20_WI-10013	42.798074	-87.717127
Lake Michigan	WI	NGL20_WI-10014	44.621561	-87.961059
Lake Ontario	NY	NGL20_NY-10032	43.968268	-76.115404
Lake Ontario	NY	NGL20_NY-10033	43.913595	-76.183412
Lake Ontario	NY	NGL20_NY-10034	43.358199	-78.702733
Lake Ontario	NY	NGL20_NY-10035	43.337974	-77.673215
Lake Ontario	NY	NGL20_NY-10036	43.506222	-76.487717
Lake Ontario	NY	NGL20_NY-10037	43.912188	-76.284124
Lake Ontario	NY	NGL20_NY-10038	43.311974	-78.888999
Lake Ontario	NY	NGL20_NY-10039	43.254800	-77.488727
Lake Ontario	NY	NGL20_NY-10040	43.587591	-76.250649
Lake Ontario	NY	NGL20_NY-10041	44.075882	-76.376997
Lake Ontario	NY	NGL20_NY-10042	43.381275	-78.085324
Lake Ontario	NY	NGL20_NY-10043	43.431453	-76.627178
Lake Ontario	NY	NGL20_NY-10044	43.803382	-76.251820
Lake Ontario	NY	NGL20_NY-10045	44.005321	-76.185961
Lake Ontario	NY	NGL20_NY-10046	43.361377	-77.930974
Lake Ontario	NY	NGL20_NY-10047	43.319131	-76.879006
Lake Ontario	NY	NGL20_NY-10048	43.334502	-78.808970
Lake Ontario	NY	NGL20_NY-10049	43.256623	-77.569462
Lake Ontario	NY	NGL20_NY-10050	43.470100	-76.579153
Lake Ontario	NY	NGL20_NY-10051	43.380115	-78.595466
Lake Ontario	NY	NGL20_NY-10052	43.294845	-77.351292
Lake Ontario	NY	NGL20_NY-10053	43.684236	-76.239633
Lake Ontario	NY	NGL20_NY-10054	43.313321	-78.918995
Lake Ontario	NY	NGL20_NY-10055	43.985167	-76.060517
Lake Ontario	NY	NGL20_NY-10056	43.822188	-76.317569

2020 Great Lakes Human Health Fish Fillet Tissue Study
Nearshore Target Sampling Locations (226)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Ontario	NY	NGL20_NY-10057	43.340606	-77.703826
Lake Ontario	NY	NGL20_NY-10058	43.331946	-78.852610
Lake Ontario	NY	NGL20_NY-10059	43.684749	-76.220903
Lake Ontario	NY	NGL20_NY-10060	43.972557	-76.335932
Lake Ontario	NY	NGL20_NY-10061	43.288035	-77.553622
Lake Ontario	NY	NGL20_NY-10062	43.373254	-78.324593
Lake Ontario	NY	NGL20_NY-10063	43.546523	-76.314592
Lake Ontario	NY	NGL20_NY-10064	44.144023	-76.327305
Lake Ontario	NY	NGL20_NY-10065	43.516782	-76.433882
Lake Ontario	NY	NGL20_NY-10066	43.330161	-78.766097
Lake Ontario	NY	NGL20_NY-10067	43.913117	-76.242845
Lake Ontario	NY	NGL20_NY-10068	43.278913	-77.545576
Lake Ontario	NY	NGL20_NY-10069	43.332665	-76.863334
Lake Ontario	NY	NGL20_NY-10070	43.794633	-76.246004
Lake Ontario	NY	NGL20_NY-10071	43.986909	-76.206235
Lake Ontario	NY	NGL20_NY-10072	43.436884	-76.619956
Lake Ontario	NY	NGL20_NY-10073	43.387118	-77.986445
Lake Ontario	NY	NGL20_NY-10074	43.347117	-77.755232
Lake Ontario	NY	NGL20_NY-10075	44.059480	-76.369895
Lake Ontario	NY	NGL20_NY-10076	43.298707	-77.100180
Lake Superior	MI	NGL20_MI-10130	47.388639	-87.924763
Lake Superior	MI	NGL20_MI-10131	46.532907	-87.389569
Lake Superior	MI	NGL20_MI-10132	46.887188	-88.324722
Lake Superior	MI	NGL20_MI-10133	47.283798	-88.517410
Lake Superior	MI	NGL20_MI-10134	46.685295	-86.169696
Lake Superior	MI	NGL20_MI-10135	46.924509	-87.843784
Lake Superior	MI	NGL20_MI-10136	46.793415	-85.233590
Lake Superior	MI	NGL20_MI-10137	47.042887	-88.981274
Lake Superior	MI	NGL20_MI-10138	46.512006	-87.148603
Lake Superior	MI	NGL20_MI-10139	46.589141	-85.020580
Lake Superior	MI	NGL20_MI-10140	46.487506	-86.740911
Lake Superior	MI	NGL20_MI-10141	46.720289	-85.762836
Lake Superior	MI	NGL20_MI-10142	46.730769	-89.968199
Lake Superior	MI	NGL20_MI-10143	46.846226	-89.573089
Lake Superior	MI	NGL20_MI-10144	46.686942	-85.506656
Lake Superior	MI	NGL20_MI-10145	46.582070	-90.406321
Lake Superior	MI	NGL20_MI-10146	46.898957	-89.388481
Lake Superior	MI	NGL20_MI-10147	46.708512	-85.708076
Lake Superior	MI	NGL20_MI-10148	46.918146	-89.272481
Lake Superior	MI	NGL20_MI-10149	47.393787	-87.701592

2020 Great Lakes Human Health Fish Fillet Tissue Study
Nearshore Target Sampling Locations (226)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Superior	MI	NGL20_MI-10150	47.296755	-88.548888
Lake Superior	MI	NGL20_MI-10151	46.508743	-87.146502
Lake Superior	MI	NGL20_MI-10152	46.604595	-90.378582
Lake Superior	MI	NGL20_MI-10153	46.839705	-88.262902
Lake Superior	MI	NGL20_MI-10154	46.485322	-84.958745
Lake Superior	MI	NGL20_MI-10155	46.689560	-86.114568
Lake Superior	MI	NGL20_MI-10156	46.967084	-89.234385
Lake Superior	MI	NGL20_MI-10157	46.876538	-87.730033
Lake Superior	MI	NGL20_MI-10158	46.790886	-85.026494
Lake Superior	MI	NGL20_MI-10159	47.259431	-88.643379
Lake Superior	MI	NGL20_MI-10160	46.494254	-86.608618
Lake Superior	MN	NGL20_MN-10001	47.141140	-91.450357
Lake Superior	MN	NGL20_MN-10002	47.556276	-90.867735
Lake Superior	MN	NGL20_MN-10003	46.790489	-92.044779
Lake Superior	MN	NGL20_MN-10004	47.771646	-90.180874
Lake Superior	MN	NGL20_MN-10005	47.480891	-90.983105
Lake Superior	MN	NGL20_MN-10006	47.973099	-89.635062
Lake Superior	MN	NGL20_MN-10007	46.793050	-91.995742
Lake Superior	MN	NGL20_MN-10008	47.062637	-91.592607
Lake Superior	MN	NGL20_MN-10009	47.727800	-90.426800
Lake Superior	WI	NGL20_WI-10022	46.770510	-91.622243
Lake Superior	WI	NGL20_WI-10023	46.672803	-90.816963
Lake Superior	WI	NGL20_WI-10024	46.729253	-91.787980
Lake Superior	WI	NGL20_WI-10025	46.680720	-90.632506
Lake Superior	WI	NGL20_WI-10026	46.754410	-91.731229
Lake Superior	WI	NGL20_WI-10027	46.666176	-90.692698

[1] This list of sites is subject to change as the project proceeds. For example, access to some sites may not be granted by property owners. Other sites may not yield fish of suitable size or species. OST maintains the list of valid sites, and this QAPP will **not** be revised just to address changes in the list of sites.

ORD-Duluth 2020 Great Lakes Special Study
Lake Michigan Enhancement Target Sampling Locations (50)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Michigan	MI	ISA20-01	45.022576	-85.955361
Lake Michigan	MI	ISA20-02	44.992944	-86.143602
Lake Michigan	MI	ISA20-03	45.810411	-85.536973
Lake Michigan	MI	ISA20-04	45.732114	-85.586710
Lake Michigan	MI	ISA20-05	45.100510	-86.075701
Lake Michigan	MI	ISA20-06	45.754047	-85.396307
Lake Michigan	MI	ISA20-07	45.744411	-85.518414
Lake Michigan	MI	ISA20-08	45.406036	-85.865609
Lake Michigan	MI	ISA20-09	45.373191	-86.950673
Lake Michigan	MI	ISA20-10	45.806034	-85.336755
Lake Michigan	MI	ISA20-11	45.690690	-85.460965
Lake Michigan	MI	ISA20-12	45.457843	-85.912753
Lake Michigan	MI	ISA20-13	45.386882	-86.829426
Lake Michigan	MI	ISA20-14	45.821972	-85.334852
Lake Michigan	MI	ISA20-15	45.627184	-85.633541
Lake Michigan	MI	ISA20-16	45.161108	-87.409663
Lake Michigan	MI	ISA20-17	44.994629	-86.163159
Lake Michigan	MI	ISA20-18	45.740626	-85.583030
Lake Michigan	MI	ISA20-19	45.071318	-86.101963
Lake Michigan	MI	ISA20-20	45.733368	-85.438622
Lake Michigan	MI	ISA20-21	45.780002	-85.549848
Lake Michigan	MI	ISA20-22	45.367833	-85.835910
Lake Michigan	MI	ISA20-23	45.418739	-86.835769
Lake Michigan	MI	ISA20-24	45.803657	-85.395611
Lake Michigan	MI	ISA20-25	45.600707	-85.478461
Lake Michigan	MI	ISA20-26	45.243421	-87.297889
Lake Michigan	MI	ISA20-27	45.333378	-86.809511
Lake Michigan	MI	ISA20-28	45.798403	-85.577589
Lake Michigan	MI	ISA20-29	45.748655	-85.684087
Lake Michigan	MI	ISA20-30	45.164952	-87.285285
Lake Michigan	MI	ISA20-31	45.032575	-86.005719
Lake Michigan	MI	ISA20-32	45.758626	-85.313298
Lake Michigan	MI	ISA20-33	45.791583	-85.527481
Lake Michigan	MI	ISA20-34	45.577667	-85.641267
Lake Michigan	MI	ISA20-35	45.037425	-85.997924
Lake Michigan	MI	ISA20-36	45.742621	-85.459929
Lake Michigan	MI	ISA20-37	45.321337	-86.892611
Lake Michigan	MI	ISA20-38	45.829346	-85.393017

ORD-Duluth 2020 Great Lakes Special Study
Lake Michigan Enhancement Target Sampling Locations (50)[1]

LAKE	STATE	SITE ID	LATITUDE	LONGITUDE
Lake Michigan	MI	NPA20-01	44.914807	-86.099659
Lake Michigan	MI	NPA20-02	44.799368	-86.090591
Lake Michigan	IN	NPA20-03	41.646178	-87.217218
Lake Michigan	IN	NPA20-04	41.699221	-87.039672
Lake Michigan	MI	NPA20-05	44.859089	-86.088796
Lake Michigan	MI	NPA20-06	44.749306	-86.096894
Lake Michigan	IN	NPA20-07	41.632908	-87.216226
Lake Michigan	IN	NPA20-08	41.699639	-87.078886
Lake Michigan	MI	NPA20-09	44.772349	-86.171933
Lake Michigan	MI	NPA20-10	44.971318	-85.896002
Lake Michigan	IN	NPA20-11	41.635451	-87.266269
Lake Michigan	IN	NPA20-12	41.699258	-87.087526

[1] This list of sites is subject to change as the project proceeds. For example, access to some sites may not be granted by property owners. Other sites may not yield fish of suitable size or species. OST maintains the list of valid sites, and this QAPP will **not** be revised just to address changes in the list of sites.

Appendix B

NCCA 2020 Great Lakes Human Health Fish Fillet Sample Preparation, Homogenization, and Distribution Procedures

Appendix B

NCCA 2020 Great Lakes Human Health Fish Fillet Sample Preparation, Homogenization, and Distribution Procedures

I. PURPOSE

This document describes the procedures that the fish sample preparation laboratory (Tetra Tech laboratory at Owings Mills, MD) follows when preparing fish fillet tissue samples for EPA's National Coastal Condition Assessment (NCCA) 2020 Great Lakes Human Health Fish Fillet Tissue Study (GLHHFFTS) and the ORD-Duluth 2020 Great Lakes special study. Adherence to these procedures ensures that fillet tissue sample preparation activities at the Tetra Tech laboratory are performed consistently across all study samples and in a manner consistent with previous EPA fish tissue studies. The effort is divided into two primary components:

- Fish fillet tissue sample preparation and distribution procedures, including quality control steps (e.g., triplicate lipid analysis of homogenized fillet tissue aliquots), for all fish sample preparation batches.
- Preparation and analyses of rinsate and solvent blank samples for mercury, PCBs, and PFAS for each fish sample preparation batch.

Each of these components is described in detail below.

II. FISH FILLET TISSUE PROCESSING AND DISTRIBUTION PROCEDURES

The procedures for processing 2020 GLHHFFTS whole fish samples and distributing fillet tissue samples for mercury, PFAS, PCB, lipid, and fatty acid analyses are described below. This process description is organized into the following components, including the quality control (QC) procedures:

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

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

Fillet Tissue Processing Definitions

- **Whole Fish Composite Sample:** A whole fish composite sample for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study consists of 5 fish (ideally) of the same species that are similar in size and typically consumed by humans (See Section B1). One human health whole fish composite sample is collected from each viable Great Lakes nearshore site and Lake Michigan enhancement site whole fish sampling location. There are 226 Great Lakes nearshore sites that are designated as whole fish sampling locations for the 2020 GLHHFFTS, as well as 50 enhancement sites in Lake Michigan that are designated as whole fish sampling locations for the ORD-Duluth 2020 Great Lakes special study.

- **Fish sample preparation batch:** Each fish sample preparation batch consists of 20 whole fish composite samples. The number of whole fish composite samples in the final fish sample preparation batch (or two) may be adjusted to include a few more than 20 or fewer than 20, depending on what fraction of 20 whole fish composite samples remain for assignment to a batch. Note that Tetra Tech will assign separate fish sample preparation batches for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study.

- **Analytical batch:** An analytical batch consists of the 20 fillet tissue aliquots generated for each target chemical (i.e., mercury, PFAS, PCB congeners, PCBs as Aroclors, lipids and fatty acids) during processing of a fish sample preparation batch. The number of fillet tissue samples in the final analytical batch (or two) may be adjusted to include a few more than 20 or fewer than 20, depending on what fraction of 20 fillet sample aliquots remain for assignment to a batch. Note that analytical batches correspond to fish sample preparation batches, so there will be separate analytical batches for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study.

II.A. Sample Receipt and Storage

Field crews are collecting NCCA 2020 Great Lakes human health fish samples from June through September (or possibly through October or early November) during 2020. A total of 226 Great Lakes nearshore sites and 50 enhancement sites in Lake Michigan are designated as human health whole fish sampling locations (Appendix A).

Whole fish samples are shipped by priority overnight delivery service from field locations in the Great Lakes to the sample repository at Microbac Laboratories in Baltimore, MD, where they are held in freezers for interim storage at a temperature of less than or equal to -20 °C. All samples are subsequently hand-delivered to the Tetra Tech laboratory in Owings Mills, MD for preparation of fillet tissue samples and interim storage of fillet samples until sample shipment to designated analytical laboratories. The Tetra Tech laboratory must have sufficient freezer space to store **at least 3 batches of unprocessed fish samples** at a temperature of less than or equal to -20 °C from the time of receipt until completion of sample processing and sufficient freezer space to store **homogenized fillet tissue aliquots from up to 60 processed samples** (e.g., up to 600 homogenized tissue jars) prior to distribution.

1. Although whole fish samples are delivered frozen, on dry ice, they must be inspected promptly on receipt. As samples are received at Microbac Laboratories, a laboratory representative 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.
   - Transfer the samples to the freezer for long-term storage.

2. Notify CSRA/GDIT immediately about any problems encountered upon receipt of samples. CSRA/GDIT will communicate these problems to the OST Project Manager for resolution.

   Section B3 of the QAPP contains details about sample inspection by the CSRA/GDIT sample custodian before whole fish samples are transferred to the Tetra Tech laboratory for fillet tissue sample preparation. Following fillet sample processing, the Tetra Tech laboratory must store homogenized fillet tissue sample aliquots frozen to less than or equal to -20 °C until they are distributed to the laboratories designated for fillet tissue analysis.

II.B. Sample Handling

The whole fish samples collected for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study must remain frozen at less than or equal to -20 ºC until the Tetra Tech laboratory receives direction from the OST Fish Sample Preparation Technical Leader to begin fish sample preparation. Fish samples must be retrieved from the freezer, with their associated paperwork, and allowed to partially thaw before they can be processed to prepare fillet tissue samples.

3. Prior to beginning fish sample processing, Tetra Tech prepares an Excel spreadsheet with draft fish sample processing instructions and preparation batch assignments for each of the 2020 Great Lakes studies and submits these spreadsheets to the OST Fish Sample Preparation Technical Leader and OST Project Manager for review. The OST Fish Sample Preparation Technical Leader will approve the fish sample preparation batch assignments and fish sample processing instructions with OST Project Manager concurrence.

Processing for a fish sample preparation batch involves the following for each fish sample in the batch:

- Preparation of one homogenized fillet tissue sample (consisting of fillets from both sides of each fish in the sample) according to the sample processing instructions approved by the OST Fish Sample Preparation Technical Leader with OST Project Manager concurrence.

Note: Processing a fish sample preparation batch produces a total of 20 homogenized fillet tissue samples that are subdivided into aliquots for target chemical analyses and for long-term storage of archived tissue (Section II.D). Each set of 20 target chemical aliquots (mercury, PFAS, PCB congeners, PCBs as Aroclors, lipids, and fatty acids) constitutes an analysis batch for the fish fillet indicator.

4. When retrieving samples from the freezer, the Tetra Tech 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 fish specimen to the fish sample preparation batch spreadsheet and notify the OST Fish Sample Preparation Technical Leader and the OST Project Manager of any discrepancies between the sample labels and the Excel file of sample processing instructions. Problems involving sample paperwork, sample integrity, or custody information inconsistencies for all fish samples should be reported to the OST Fish Sample Preparation Technical Leader and the OST Project Manager in writing (e.g., by email) within one business day following sample retrieval and inspection. **Do not proceed with sample processing until discrepancies are resolved.**

II.C. Filleting and Homogenization Procedures

The target chemical analyses for mercury, PFAS, PCB congeners, PCBs as Aroclors, lipids and fatty acids are performed on aliquots of homogenized fillet tissue samples prepared from the NCCA 2020 human health whole fish samples. Steps 5 - 9 below must be completed before beginning processing and preparing any fillet samples in the laboratory.

5. Prior to preparing any fillet 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 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

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 a whole fish sample for fillet removal and tissue homogenization. After unwrapping, inspect each fish specimen in the sample 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 applicable Fish Sample Preparation Laboratory Bench Sheet (Appendix C for 2020 GLHHFFTS nearshore sites and Appendix D for ORD-Duluth 2020 Great Lakes special study Lake Michigan enhancement sites) and notify the OST Fish Sample Preparation Technical Leader and the OST Project Manager before proceeding further.

7. Weigh each fish to the nearest gram (wet weight) prior to any sample processing. Enter weight information for each specimen into the applicable Fish Sample Preparation Laboratory Bench Sheet (Appendix C for nearshore sites and Appendix D for enhancement sites). Individual specimen weights will be transferred to spreadsheets for submission to the OST Fish Sample Preparation Technical Leader and the OST Project Manager.

8. Rinse each fish in the sample with DI water as a precautionary measure to treat for possible contamination from sample handling in the field. Use HDPE wash bottles (not PTFE) for rinsing fish and for cleaning homogenization equipment and utensils.

9. Before beginning the scaling process for each fish in the composite sample, put on new powder-free nitrile gloves. (Gloves must be changed *between* whole fish composite samples.) 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.

10. Continue scaling all of the other fish in the whole fish sample as described in Step 9 above. Filleting of the fish in the sample can proceed after all scales have been removed from the skin and a separate clean cutting board and fillet knife are prepared or available.

11. Put on new powder-free nitrile gloves. Place each fish on a clean glass cutting board in preparation for the filleting process. Note that filleting should be conducted under the supervision of an experienced fisheries biologist. 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 the 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 bench sheet 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.

12. Whole fillet samples (consisting of the entire right and left fillets) are weighed to the nearest gram (wet weight) and the weight is recorded on the bench sheet prior to homogenization. These samples should be homogenized partially frozen for ease of grinding.

13. Process each whole fillet 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 the fish must be homogenized. Mix the tissues thoroughly until they are completely homogenized as

evidenced by fillet tissue that consists of a uniform color and finely ground 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.

Note: The dry ice pellets used for homogenizing the fillet tissue are classified as food grade and meet the specifications for substances Generally Regarded As Safe as a direct food ingredient in the Food and Drug Administration regulation FDA 184.1240 (21 CFR 184.1240).

14. Grind the entire fillet 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 homogenized fillet sample must consist of finely ground tissue of uniform color and texture. If there are obvious differences in color or texture, grind the entire sample a third time or more to ensure uniform homogenization.

15. Measure the collective weight of the homogenized fillet tissue from each fish sample to the nearest gram (wet weight) after processing and record the total homogenate weight on the laboratory bench sheet. The total weights of the fillets and weights of the homogenized fillet tissue from each fish sample are transferred to spreadsheets for submission to the OST Fish Sample Preparation Technical Leader and the OST Project Manager. At least 465 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 465 g of homogenized tissue, contact the OST Project Manager via email immediately and await instructions.** As appropriate, place any remaining homogenized fillet tissue in the freezer while waiting for instructions, which are likely to involve preparing fewer archive aliquots.

16. After the final (second, third, or higher number) grinding, clean the **grinding equipment and all other sample preparation equipment** using the procedures described in Step 22.

17. Once in every fish sample preparation batch (generally containing 20 whole fish samples), verify the continued absence of equipment contamination and uniformity of homogenization using the procedures described in Steps 25 to 29.

II.D. Aliquoting and Distribution Procedures

18. The sample preparation laboratory prepares the bulk homogenate tissue from one whole fish sample and uses it to fill the pre-cleaned sample containers specified for each type of aliquot listed in Table 1, following the procedures described in Step 19. **Except as noted in Table 1, all containers are provided by the fish sample preparation laboratory.** Documentation of their cleanliness provided by the vendor (i.e., certificates of analysis) must be retained by the fish sample preparation laboratory and provided to EPA on request. The target masses listed in Table 1 are designed to provide enough tissue for multiple analyses of each sample, including tissue for QC purposes, as needed. The fish sample preparation laboratory should not exceed those aliquot target masses when filling the containers. The order of the containers and target masses in Table 1 are important (i.e., they indicate priority order for aliquots) and are designed to ensure that adequate tissue is available for all analyses, as well as for archiving.

Table 1. NCCA 2020 Great Lakes Human Health Fish Fillet Tissue Sample Aliquot Requirements

Analysis	Target Mass	Container Type	Destination
Mercury	5 - 10 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	TBD
PFAS	10 - 15 g	100-mL HDPE straight-sided jar with **foil-lined lid**, or conical HDPE tube with snap top. ***PTFE lid liners not allowed.***	TBD
PCB congeners	20 - 25 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid	TBD
Lipids, Fish 1	30-35 g (prepare 3 aliquots containing at least 10 g each)	TBD laboratory's choice	TBD
Lipids, Fish 2 - 20	10 – 15 g	TBD laboratory's choice	TBD
PCBs as Aroclors	20 – 25 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid	TBD
Fatty acids	10 - 15 g	125-mL straight-sided amber or clear glass jar with PTFE-lined lid	TBD
Small Archive 1	50 - 60 g	125-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSRA/GDIT Sample Repository
Small Archive 2	50 - 60 g	125-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSRA/GDIT Sample Repository
Bulk Archive 1	240 - 250 g	500-mL straight-sided amber or clear glass jar with **foil-lined lid**	CSRA/GDIT 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**	CSRA/GDIT Sample Repository
Total (to the nearest gram)[a]	465 – 740 g	*Assumes at least 50 g of tissue is available for Bulk Archive 2*	

[a] In the event that insufficient fish tissue mass exists to prepare the required number of aliquots, contact the OST Project Manager for instructions as per Step 15.

19. Prepare the homogenized sample aliquots for **mercury, PFAS, PCB congeners, lipids, PCBs as Aroclors, and fatty acids** (see Step 21 for lipid aliquot preparation). Weigh an appropriate clean sample container (Table 1) to the nearest 0.5 g and record the weight. Transfer sufficient homogenized fillet tissue 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 fish sample preparation laboratory must use foil-lined lids for jars containing the fillet tissue aliquots for PFAS analysis and the archived fillet tissue samples, as specified in Table 1.**

    Note: The archive sample jars are not filled until after sufficient volume for lipids determination, PCBs as Aroclors, and fatty acids have been collected, as described in Step 21. The archive jars are not filled until the triplicate lipid aliquot (30-35 g), the PCBs as Aroclors aliquot (20-25 g), and the fatty acids aliquots (10-15 g) are collected (see Step 28 for triplicate lipid aliquot preparation, which is used for homogeneity testing).

    When filling jars, leave sufficient space at the top of each jar before sealing with the designated lid to allow for expansion of the tissue as it freezes. *In no case should jars be filled beyond 80% capacity,*

as this may result in breakage on freezing. Wipe off the outside of the jars to remove any tissue residue or moisture. Fill out a label for each container using a waterproof marker. Include the following information (at a minimum) on each label:

- sample identification number,
- tissue sample type (i.e., homogenized fillet),
- analysis type (e.g., mercury, PFAS, PCB congeners, lipids, PCBs as Aroclors, or fatty acids),
- aliquot weight (to the nearest 0.5 gram),
- preparation batch ID, and
- preparation date (e.g., mm/dd/yyyy).

Affix the label to the container with clear wide tape. Place each container inside one heavy-weight food-grade self-sealing plastic freezer bag to avoid sample loss due to breakage. Freeze the tissue samples at -20 ºC and maintain samples in the freezer until directed by the OST Fish Sample Preparation Technical Leader with OST Project Manager concurrence to ship them to the analytical laboratories. (The OST Fish Sample Preparation Technical Leader will not issue these instructions until equipment rinsate and homogeneity tests described in Steps 24 to 29 have been completed, reported, evaluated, and determined to be acceptable.)

20. After filling the containers with the tissue aliquots for mercury, PFAS, and PCB congeners, remove 30 to 35 g of homogenized fillet tissue from one sample in the batch (for triplicate lipid analysis) and 10 to 15 g of homogenized fillet tissue from all other samples in the batch (for single lipid analysis) to be used to determine the lipid content of each fillet composite sample. Place these aliquots in clean glass or plastic containers of suitable size (provided by the TBD analytical laboratory) and label each of them with the sample ID number. Store the lipid aliquots in the freezer at -20 °C until they are ready to be shipped to the designated analytical laboratory to perform the lipid determinations in Steps 24, 28, and 29. After preparing the lipid aliquots, follow the procedures in Step 19 to prepare the PCBs as Aroclors aliquot and the fatty acids aliquot.

21. The archive sample jars are not filled until after sufficient volume for determining lipids, PCBs as Aroclors, and fatty acids have been collected. Once the aliquots for mercury, PFAS, PCB congeners, lipids, PCBs as Aroclors, and fatty acids have been collected, the remaining tissue mass is used to create the four archive samples. Begin by transferring 50 - 60 g of tissue to the first small archive sample container. Continue by transferring a 50 - 60 g aliquot to the remaining small archive container. Ideally, sufficient homogenized fillet tissue mass will remain to produce two bulk archive containers. Therefore, transfer 240 - 250 g of tissue to the first bulk archive sample container. Continue by transferring up to 250 g of tissue to the second bulk archive container. However, if less than 250 g of tissue is available, transfer all of the remaining homogenized tissue to the bulk archive container and weigh it to determine the tissue mass in the last archive container. Seal and label the containers as described in Step 19 for the other aliquots.

    Note: Step 15 requires that the laboratory contact the OST Project Manager whenever a homogenized fillet sample does not yield at least 465 g of tissue. The OST Fish Sample Preparation Technical Leader will provide direction to the laboratory with OST Project Manager concurrence regarding samples yielding less than 465 g of tissue that must be followed at this point in the procedure.

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

II.E. Equipment Cleaning between Composite Samples

22. All of the homogenization equipment must be thoroughly cleaned between each individual fish sample. Once both of the fillets from the individual 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

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

II.F. Lipid Determination for Every Homogenized Fillet Sample

The first fish sample in each preparation batch is designated for triplicate lipid analysis for the homogeneity testing process described in Steps 28 and 29. The procedures for determining the lipid content of homogenized fillet tissue from all other fish samples in a fish sample preparation batch are described in Step 24 below.

24. For samples 2 through 20 in each fish sample preparation batch, use the 10 to 15 g aliquot of homogenized tissue collected in Step 20 to determine the lipid content of the sample. The analytical laboratory (TBD) will extract the aliquot using an appropriate method (TBD) approved by EPA to determine the lipid content of that aliquot, which is recorded in units of percent (i.e., grams of lipid per gram of tissue x 100). This QAPP will be amended to include a description of this method after the analytical laboratory is selected and their proposed method for lipid analysis is approved.

II.G. Quality Control (QC) Procedures

The QC procedures for fish sample preparation include preparation and testing of equipment rinsate samples and homogeneity testing, using lipids as a surrogate.

During the fish sample preparation process, the Tetra Tech laboratory prepares three sets of aqueous rinsate and solvent blank samples for mercury, PCB congener, and PFAS analyses, respectively, (Attachment 1) and one homogenized fillet tissue aliquot for triplicate lipid determinations from each fish sample preparation batch, as described in Steps 25 to 28 below. The batch-specific rinsate and homogeneity results are reviewed by Tetra Tech and CSRA/GDIT. The Tetra Tech laboratory doing fish sample preparation may continue to process up to 2 additional batches during the QC sample analysis and review process. However, the Tetra Tech laboratory may **not** continue beyond the third batch of fish samples until receiving notification from the OST Fish Sample Preparation Leader (with OST Project Manager concurrence) that the review of initial batch rinsate and homogeneity test results is complete, and the results were deemed satisfactory.

Continued sample processing is dependent on both the quality of the Tetra Tech laboratory's efforts and on the timeliness of their delivery of QC results.

Rinsate and Blank Sample Production

25. Once per batch (of usually 20 fish samples) during the fish sample preparation operations, prepare three sets of rinsate and blank solvent samples (see Attachment 1) prior to reassembling the homogenization equipment (Step 23), as follows:

PCB rinsate and blank samples:

- Prepare a **hexane rinsate sample** by pouring a 100-mL portion of pesticide-grade hexane over all parts of homogenization equipment, including the cutting boards and knives, and collect it in a clean glass container. Place an additional 100-mL aliquot of clean hexane in a similar glass container for use as a solvent blank. Allow the solvent to evaporate from the equipment. This set of rinsate and solvent blank samples will be analyzed for selected PCB congeners (see Attachment 1). Label and store the PCB rinsate and blank samples as described Step 26.

Mercury rinsate and blank samples:

- Once the hexane has evaporated from the equipment, prepare the **first DI water rinsate** using 250 mL of DI water. Collect the DI water rinsate in a clean glass or HDPE container. Place a second aliquot of DI water in a separate similar clean container for use as a blank. Acidify these two samples to pH < 2 with nitric acid. Label and store each sample as described in Step 26. These rinsate and blank samples will be analyzed for mercury (see Attachment 1).

PFAS rinsate and blank samples:

- Prepare the **second DI water rinsate** using an additional 250 mL of DI water. Collect this rinsate in a clean glass container **with a non-PTFE lid liner**. Place a second aliquot of DI water in a separate similar clean glass container for use as a blank. This set of rinsate and blank samples will be analyzed by the laboratory selected at a later date to analyze fillet samples for PFAS, thus the non-PTFE lid liners are essential. The OST Project Manager will provide the Tetra Tech laboratory with the PFAS laboratory name and shipping information as soon as it is available. Label and store these PFAS rinsate and blank samples as described in Step 26.

Note: In order to minimize the number of project samples that might be affected by cross contamination, collect the rinsate and blank samples on the first day that fish samples in a sample preparation 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.).

26. Label each container as either "rinsate -[insert the name of the solvent, either hexane or DI water]" or "blank –[insert the name of the solvent, either hexane or DI water]," and include the date it was prepared (mm/dd/yyyy), the analysis type (Hg, PCBs, or PFAS), and the preparation batch identifier. Store the rinsate and blank samples in a refrigerator at a temperature of <6 °C.

Rinsate and Blank Sample Analyses

27. During the fish sample preparation operations, laboratories under contract to Tetra Tech (to be determined for mercury and PCBs) and CSRA/GDIT (PFAS) will analyze one set of rinsate and blank samples per batch for:
    - mercury using EPA Method 245.1, a cold-vapor atomic absorption procedure (Details for this method are described in Attachment 1),
    - PCBs using an appropriate method proposed by the TBD laboratory and approved by EPA (Note that this QAPP will be amended to include a description of this method after the analytical laboratory is selected and their proposed method is approved), and

- PFAS using an appropriate method proposed by the TBD laboratory and approved by EPA (Note that the PFAS rinsate samples will be analyzed by the laboratory selected for PFAS analysis of the 2020 GLHHFFTS fish fillet tissue samples).

Corrective Actions for Rinsates

The rinsate results will be evaluated based on the mass of each analyte detected, and assuming that all of the apparent contamination could be transferred to a nominal 465-g mass of homogenized tissue. Results for mercury and PCBs above the anticipated reporting limits for these analytes in homogenized fillet tissue samples may be cause for corrective actions by the fish sample preparation (Tetra Tech) laboratory. These corrective actions may include revisions to the 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

28. For one sample in each fish sample preparation batch of generally 20 samples, a laboratory under contract to Tetra Tech will use the 30 - 35 g aliquot of homogenized fillet tissue to conduct triplicate analyses of the lipid content of homogenized fillet tissue samples to confirm that they are homogeneous. As with the collection of rinsate samples, the Tetra Tech laboratory should identify and process the fish sample for homogeneity testing during the first day of fish sample preparation operations for each batch.

    Remove 30 to 35 g of fillet homogenate from the fish sample designated for homogeneity testing before filling the archive sample containers. Place this aliquot in a glass or plastic container of suitable size and label it with the sample ID number. Transfer the lipid aliquot to the TBD analytical laboratory for triplicate lipid determination. This laboratory will use 5 to 10 g aliquots of fillet tissue for each of the 3 lipid analyses.

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

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

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

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

If the RSD of the triplicate results is less than or equal to 15% for triplicate lipid samples with mean lipid values at or above 2.5%, or if the RSD of the triplicate results is less than or equal to 20% for triplicate lipid samples with mean lipid values below 2.5%, 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 lipid value reported for that sample, following the requirements described in Step 24.

Corrective Actions for Homogeneity

If the RSD is greater than 15% for triplicate lipid samples with mean lipid values at or above 2.5%, or if the RSD is greater than 20% for triplicate lipid samples with mean lipid values below 2.5%, then corrective action is required for all samples in that preparation batch. Corrective actions will be determined by EPA in direct consultation with the laboratory and Tetra Tech, but the default corrective action consists of regrinding all of the aliquots from each whole fish 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 13. In these instances, all of the equipment cleaning procedures will be repeated between each whole fish sample, new lipid results will be determined for each fish sample, and a new homogenization QC determination (triplicate lipids for one fish sample per batch) will be performed. New sample containers are required for any rehomogenized samples.

II.H. Reporting Requirements

30. The fish sample preparation laboratory prepares a separate weekly progress report to document the status of fish preparation activities for the 2020 GLHHFFTS and the ORD-Duluth 2020 Great Lakes special study and forwards each report electronically to the OST Fish Sample Preparation Technical Leader and the OST Project Manager. The format of each weekly progress report will be an Excel spreadsheet using the 2015 GLHHFFTS fish sample preparation reports as a guide for organization of each spreadsheet. For each homogenized 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 sample,
    - common name for the fish species (provided to the laboratory in the processing instructions from EPA),
    - field-determined length and lab-determined weight of each specimen in a whole fish sample,
    - total whole fillet (unhomogenized) weight (to the nearest gram),
    - total homogenized fillet composite sample (i.e., homogenate) weight (to the nearest gram),
    - analysis type (e.g., mercury, PFAS, PCB congeners, lipids, PCBs as Aroclors, fatty acids, and archive samples),
    - fillet tissue aliquot weight (to the nearest 0.5 gram),
    - fish sample preparation batch ID,
    - preparation date (e.g., mm/dd/yyyy),
    - QC sample identifiers associated with the batch of homogenized fillet samples, and
    - lipid results for each fish sample.

    Weekly progress reports will be due by COB Monday (or one day later in the case of holidays), and each report will document fish sample preparation progress for the previous week.

    In addition, the laboratory must report the results of the rinsate analyses for mercury, PCB congeners, and the triplicate lipid results associated with the sample batch. Those results **must** be reported to the OST Fish Sample Preparation Technical Leader and the OST Project Manager as soon after the analyses as practical to facilitate timely review of the data from the QC samples and to minimize delays in receiving approval from the OST Fish Sample Preparation Technical Leader (with OST Project Manager concurrence) to process future batches.

Note: As specified in the QC section of this QAPP (Section B5.1), the fish sample preparation laboratory may **not** continue beyond the series of 3 fish sample preparation batches until receiving notification from the OST Fish Sample Preparation Technical Leader (with OST Project Manager concurrence) that the review of initial batch (in the series of 3 batches) rinsate and homogeneity test results is complete, and the results were deemed satisfactory.

II.I. Shipping Samples

31. **No samples (except fish sample preparation mercury, PCB, and triplicate lipid QC samples) may be shipped until the OST Fish Sample Preparation Technical Leader and the OST Project Manager have reviewed the fish sample preparation batch homogeneity testing and rinsate results and authorized shipment of samples to designated analytical laboratories in writing.** The OST Project Manager will notify the Tetra Tech laboratory by email when specific batches of samples may be shipped, and to whom.

    Samples are shipped in batches (one batch per cooler) to each designated analytical laboratory. When shipping batches of pre-frozen fillet tissue aliquots, keep the individual containers bagged in the food-grade plastic freezer bags. Place these bags in a cooler with adequate space for the tissue containers, packing materials, and dry ice blocks.

    Secure each of the tissue containers with packing materials (e.g., bubble wrap or foam) before adding the block dry ice. Place a layer of bubble wrap and a plastic cooler liner on top of the containers before adding the dry ice, as this can prevent cracking the lids.

    The amount of dry ice required for shipping will depend on the number of homogenized 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 30 pounds of dry ice per cooler for up to 10 pounds of fillet tissue samples). Only blocks of dry ice are allowed for shipping fish tissue samples. **Do not use dry ice pellets for shipping fillet samples.**

    Record the samples contained in the cooler on a shipping form provided by CSRA/GDIT 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 the OST Project Manager, and attach a dry ice (dangerous goods) label. Ship the cooler via an overnight express carrier on a date that will allow delivery of the cooler to the analytical laboratory 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 CSRA/GDIT, the OST Project Manager, and the OST Fish Sample Preparation Leader via email on the day that the shipment occurs. **CSRA/GDIT will provide the Tetra Tech laboratory with a third-party FedEx account to which each shipment will be billed.**

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ATTACHMENT 1
ANALYSES OF RINSATES AND BLANKS FOR MERCURY AND PCBs

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

A. EQUIPMENT AND MATERIALS:

- Mercury analyzer suitable for aqueous samples using cold-vapor atomic absorption (CVAA) instruments compatible with EPA Method 245.1 (or other suitable analytical procedure and detector system capable of achieving a method detection limit (MDL) of approximately 1 µg/L).
- 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 1.0 to 10 mL.
- 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 can 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

The three sets of rinsate and blank samples include:

- One deionized (DI) water rinsate sample and one DI water blank sample for mercury analysis.
- One hexane rinsate sample and one hexane blank sample for analysis of PCB congeners.
- One DI water rinsate sample and one DI water blank sample for PFAS analysis.

During fish sample preparation efforts, the Tetra Tech laboratory will prepare each set of rinsate and blank samples at a frequency of one set for each batch of generally 20 fish samples prepared.

The analytical laboratory (to be determined) will digest and analyze the mercury rinsate and blank samples by CVAA. For each analysis, the laboratory will determine the mass of mercury in the total volume of each rinsate or blank sample, rather than the concentration of mercury. The analytical laboratory will either perform a method detection limit (MDL) study for mercury in aqueous samples or use existing aqueous MDL data for the CVAA instrument employed. The laboratory must be able to achieve a MDL of approximately 1 µg/L. Mercury results will be reported down to the mass equivalent to the mass at the MDL for aqueous samples.

For PCB rinsates and blanks, the analytical laboratory will concentrate the 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.

The Tetra Tech laboratory will not be responsible for analysis of rinsate and blank samples for PFAS. Tetra Tech will hold these samples in temporary storage until the OST Project Manager identifies the laboratory selected to analyze 2020 GLHHFFTS fillet samples for PFAS, provides shipping information for this laboratory, and notifies Tetra Tech when they can ship these samples.

C. QUALITY CONTROL

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

- MDL and IDL studies, as described above
- Instrument calibration (see Method 245.1 and TBD PCB method for procedures and acceptance criteria, or consult the specifications in any other procedures proposed)
- Instrument blanks for mercury and PCB analyses
- Calibration verification (once per analysis batch) for mercury and PCB analyses
- Laboratory control sample (LCS) once per analysis batch (for mercury analysis only)

The MDL and IDL results are reviewed by CSRA/GDIT and EPA as soon as they become available for each fish sample preparation batch, and the Tetra Tech laboratory will not be authorized to prepare fish tissue samples beyond a series of 3 fish sample preparation batches until that review is complete and the results are acceptable for the initial batch in each series of 3 fish sample preparation batches.

The matrix for the mercury rinsates is reagent (deionized) 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 EPA in an Excel file. That file must contain the following information, at a minimum:

- Batch ID - assigned by EPA (numerical sequence beginning at 1)
- Sample ID - as described in the instructions for preparing the rinsates (Step 26 in Appendix B)
- Lab sample ID - unique internal identifier used by the laboratory, if any

- Prep date - Date (MM/DD/YYYY) on which the rinsate and solvent blank samples were prepared
- Analysis type - “Mercury” or “PCBs”
- Analysis date - Date (MM/DD/YYYY) on which the rinsate and solvent blank samples were analyzed
- Analyte name – Mercury (total) or PCBs
- Mass of analyte found - in micrograms for mercury and 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 mercury (i.e., the MDL for this study) and PCBs (i.e., the IDL for this study) - in the same mass units used for the mercury and PCB 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).

Provide Excel files for the mercury and PCB analysis results to the Tetra Tech Project Leader. The laboratory may submit a separate Excel file for each type of analysis. Raw data supporting mercury and PCB analyses (e.g., instrument printouts) must be retained by the laboratory and made available to EPA when requested, at no additional cost. If requested, raw data may be submitted in hard copy, or as a PDF file.

Appendix C

2020 Great Lakes Human Health
Fish Fillet Tissue Study
Fish Sample Preparation Laboratory Bench Sheet

2020 GLHHFFTS Fish Sample Preparation Laboratory Bench Sheet

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Site ID:		Prep Date (MMDDYYY):		Sum of Fillet Mass (g):	Fish _____
Sample ID:		Filleter:		Homogenate Tissue Mass (g):	
EPA Batch ID:		Fish Processer:		Fillet & Homogenate Mass Difference (g):	

Specimen ID	Species	Fish Length (mm)	Fish Mass (g)	Fillet Mass (g)	Fillet Tissue Recovery (%)	Notes
.01						
.02						
.03						
.04						
.05						
.06						
.07						
.08						
.09						
.10						

Sample Jar	Hg Mass	PFAS	PCB Congeners	Lipids, Fish 1	Lipids, Fish 2-20	PCBs as Aroclors	Fatty Acids	Small Archive 1	Small Archive 2	Bulk Archive 1	Bulk Archive 2
Target Sample Mass (g)	5 - 10 g	10 - 15 g	20 - 25 g	30 - 35 g (prepare 3 aliquots)	10 - 15 g	20 - 25 g	10 - 15 g	50 - 60 g	50 - 60 g	240 - 250 g	All remaining mass up to 250 g
Sample Mass (g)				1. 2. 3.							

Tetra Tech, Inc.
Ecological Testing Facility

Data Checked and Approved _____

2020

Appendix D

ORD-Duluth 2020 Great Lakes Special Study
Human Health
Fish Sample Preparation Laboratory Bench Sheet

2020 Great Lakes Special Study Human Health Fish Sample Preparation Laboratory Bench Sheet

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Site ID:		Prep Date (MMDDYYY):		Sum of Fillet Mass (g):	Fish _____
Sample ID:		Filleter:		Homogenate Tissue Mass (g):	
EPA Batch ID:		Fish Processer:		Fillet & Homogenate Mass Difference (g):	

Specimen ID	Species	Fish Length (mm)	Fish Mass (g)	Fillet Mass (g)	Fillet Tissue Recovery (%)	Notes
.01						
.02						
.03						
.04						
.05						
.06						
.07						
.08						
.09						
.10						

Sample Jar	Hg Mass	PFAS	PCB Congeners	Lipids, Fish 1	Lipids, Fish 2-20	PCBs as Aroclors	Fatty Acids	Small Archive 1	Small Archive 2	Bulk Archive 1	Bulk Archive 2
Target Sample Mass (g)	5 - 10 g	10 - 15 g	20 - 25 g	30 - 35 g (prepare 3 aliquots)	10 - 15 g	20 - 25 g	10 - 15 g	50 - 60 g	50 - 60 g	240 - 250 g	All remaining mass up to 250 g
Sample Mass (g)				1. 2. 3.							

Tetra Tech, Inc.
Ecological Testing Facility

Data Checked and Approved _____

2020