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Quality Assurance Report for the National Coastal Condition Assessment 2015 Great Lakes Human Health Fish Fillet Tissue Study

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Chapter 1
Introduction

This report documents the quality of data gathered during the 2015 Great Lakes Human Health Fish Fillet Tissue Study (GLHHFFTS), which was a regional component of EPA's Office of Wetlands, Oceans, and Watersheds (OWOW) National Coastal Condition Assessment (NCCA). The NCCA is a probability-based survey designed to assess the condition of coastal waters of the United States, which includes nearshore waters of the Great Lakes. Multiple EPA offices collaborated to conduct this survey (which is repeated every five years), including the Office of Research and Development (ORD) that developed the survey design and conducted statistical analysis of the fish tissue data, OWOW that provided overall management for implementation of the NCCA, and the Office of Science and Technology (OST) and the Great Lakes National Program Office (GLNPO) that conducted the fish tissue studies under the NCCA.

Section 1.1 Background

Obtaining statistically representative environmental data on mercury, polychlorinated biphenyl (PCB) congeners, and other chemicals of concern is a priority area of interest for EPA. Beginning in 1998, OST partnered with ORD to conduct the first statistically based national-scale assessment of mercury, PCBs, and selected other target chemicals in fish from U.S. lakes and reservoirs. This study was called The National Study of Chemical Residues in Lake Fish Tissue, but it is commonly referred to as the National Lake Fish Tissue Study. The Great Lakes were excluded from the National Lake Fish Tissue Study because assessment of a freshwater system of that magnitude required a separate sampling design. Since 2008, OST has collaborated with OWOW and ORD to conduct a series of probability-based studies of freshwater fish contamination. These include national-scale studies of river fish contamination under the agency's National Rivers and Streams Assessment (NRSA), which are referred to as the 2008-09 NRSA Fish Tissue Study and the 2013-14 NRSA Fish Tissue Study. They also include regional-scale studies of fish contamination in the five Great Lakes, which are referred to as the NCCA 2010 Great Lakes Human Health Fish Tissue Study (2010 GLHHFTS) and the NCCA 2015 Great Lakes Human Health Fish Fillet Tissue Study (2015 GLHHFFTS). OST has been partnering with GLNPO to conduct the Great Lakes fish tissue studies under the NCCA.

The regional Great Lakes fish tissue study component was added to the NCCA sampling design in 2010 and focused on analysis of chemical contaminants in fillet tissue samples (because consumption of fillet tissue is an exposure pathway relevant to human health). As a result, the probability-based Great Lakes sampling design developed for the 2010 NCCA offered the opportunity to conduct the 2010 GLHHFTS as the first statistically representative study of chemical residues in Great Lakes fish relevant to human health. The 2015 GLHHFFTS provided additional Great Lakes basin-wide data on the occurrence and distribution of contaminants in the fillets from Great Lakes fish and, through comparison with the 2010 fillet tissue results, allowed EPA to evaluate temporal changes of these contaminants in Great Lakes fish. Collecting statistically representative data for other contaminants not measured in 2010 (e.g., dioxins and furans) was an additional goal of this 2015 study.

Section 1.2 Study Design

Within OW, OST collaborated with GLNPO and with ORD's Western Ecology Division (now called the Pacific Ecological Systems Division) in Corvallis, Oregon, to conduct the 2015 GLHHFFTS. A total of 152 valid fish samples were collected for the study at a statistical subset of NCCA Great Lakes nearshore sites distributed throughout the five Great Lakes (Figure 1). The majority of fish samples (147) were collected from June through October 2015 and an additional five samples were collected in Lake Michigan during May 2016.

The following were the key design components for the 2015 GLHHFFTS:

- sampling at least 150 randomly selected sites (about 30 sites per lake) in the nearshore regions (depths up to 30 m or distances up to 5 km from shore).
- collecting one fish composite sample for human health applications (i.e., five similarly sized adult fish of the same species that are commonly consumed by humans) from each site.
- shipping whole fish samples to an interim frozen storage facility.
- transferring the whole fish samples to a laboratory for fish sample preparation, which includes filleting the fish, homogenizing the fillet tissue composites, and preparing fillet tissue aliquots for analysis of specific chemicals, along with a series of archive samples that may be used for future analyses of other contaminants.
- analyzing the fillet tissue samples for mercury (total), 209 PCB congeners, 13 perfluorinated compounds that are a subset of the broader group known as per- and polyfluoroalkyl substances (PFAS), 17 2,3,7,8- substituted dioxin and furan congeners (PCDDs/PCDFs), and 38 omega-3 and omega-6 fatty acids.

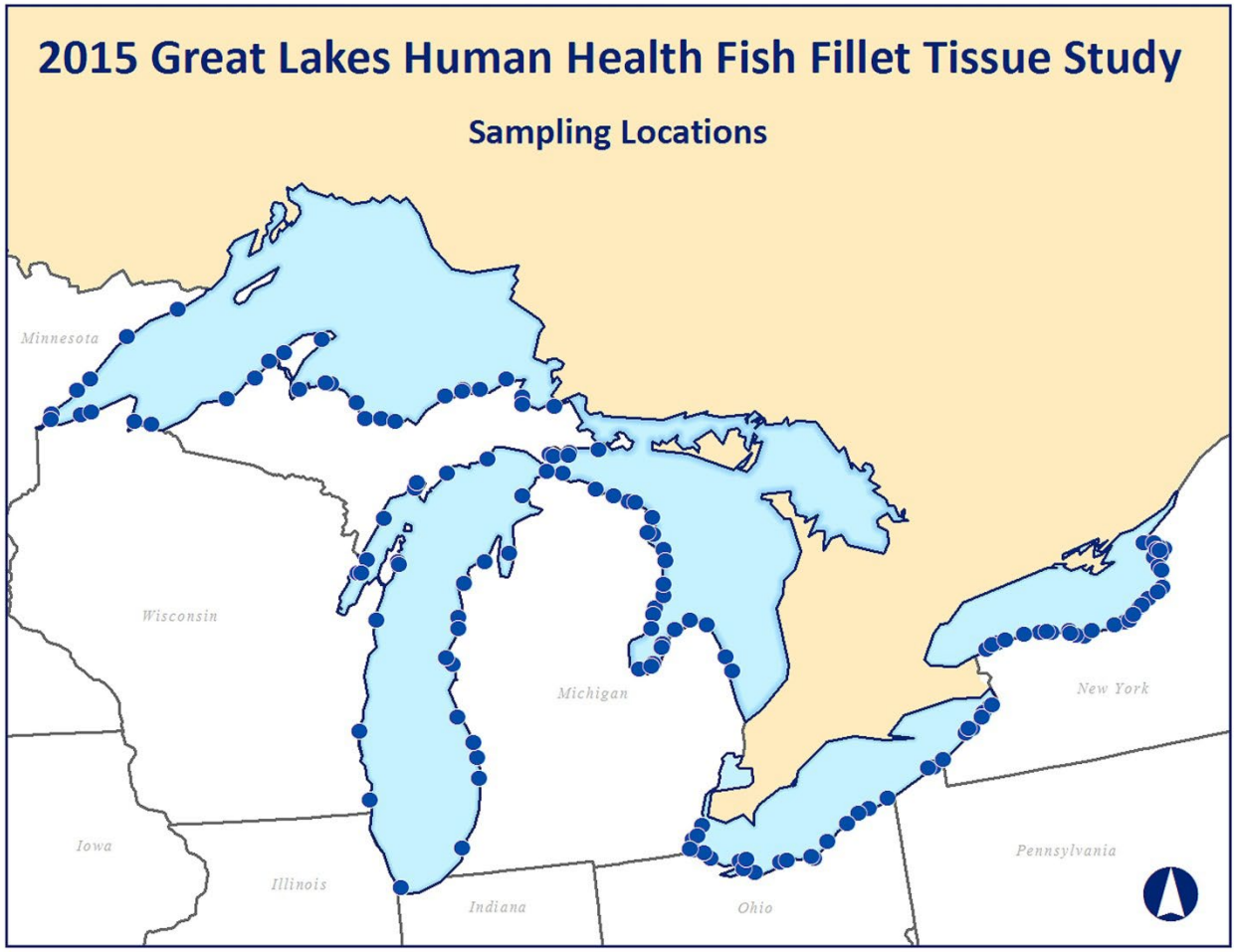

Figure 1. Sampling locations of the 152 valid fish samples collected for the 2015 GLHHFFTS

EPA stored the 2015 GLHHFFTS whole fish samples in freezers leased by GDIT at Microbac Laboratories in Baltimore, Maryland, prior to transporting them to the sample preparation laboratory. Tetra Tech's Center for Ecological Sciences in Owings Mills, Maryland was the sample preparation laboratory preparing the homogenized fish fillet tissue samples for analysis as outlined in the fourth bullet above, under a purchase order issued from GDIT. The sample preparation laboratory also prepared aliquots of

fillet tissue for mercury, PCBs, PFAS, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs), and omega-3 and omega-6 fatty acids. Commercial environmental laboratories analyzed the 2015 GLHHFFTS fish fillet tissue samples for mercury, PCBs, PFAS, PCDDs/PCDFs, and a university laboratory analyzed the samples for fatty acids, under project-specific purchase orders issued by GDIT. Procedures for handling and shipping homogenized fish tissue samples to the analysis laboratories are described in Appendix B of the *Quality Assurance Project Plan for Fish Sample Preparation and Analysis for the 2015 National Coastal Condition Assessment Great Lakes Human Health Fish Fillet Tissue Study* (USEPA, 2016a).

Section 1.3 Study Participants

The GLHHFFTS project team consisted of managers, scientists, statisticians, and QA personnel in OST, the ORD Western Ecology Division, and GLNPO, along with contractors providing scientific and technical support to OST from GDIT and Tetra Tech, Inc. (Figure 2). Project team members from GLNPO provided support for developing and reviewing technical and program information related to all aspects of the study, including training materials, standard operating procedures, Quality Assurance Project Plans (QAPPs), analytical QA reports, briefings and reports on study results, and outreach materials. Key members of the project team are listed below.

- Leanne Stahl of OST was the GLHHFFTS Project Manager who provided overall direction for planning and implementation of this regional Great Lakes study that was conducted under the NCCA.
- Elizabeth Murphy of GLNPO was a 2015 GLHHFFTS Project Co-Manager who provided overall direction for planning and implementation of this regional Great Lakes study that was conducted under the NCCA.
- Marion Kelly was the OST Quality Assurance Officer who was responsible for reviewing and approving all QAPPs that involve scientific work being conducted by OST with support from Bill Kramer, the SHPD QA Coordinator, and Louis Blume, the GLNPO QA Manager.
- Blaine Snyder was the Tetra Tech Project Leader who was responsible for managing all aspects of the technical support provided by Tetra Tech staff for the GLHHFFTS.
- Susan Lanberg was the Tetra Tech QA Officer.
- Harry McCarty was the GDIT Project Leader who was responsible for managing all aspects of the technical support provided by GDIT staff for the GLHHFFTS.
- Yildiz Chambers-Velarde was the GDIT Project Leader who was responsible for managing all aspects of the administrative support provided by GDIT staff for the GLHHFFTS.
- Marguerite Jones was the GDIT QA Officer.
- Tony Olsen was the Senior Statistician at what was then the ORD Western Ecology Division in Corvallis, Oregon who supported the GLHHFFTS by providing technical expertise for study design planning and statistical analysis of fish tissue data.

The whole fish samples were stored in freezers leased by GDIT at Microbac Laboratories in Baltimore, Maryland. Tetra Tech, in Owings Mills, Maryland, prepared the fish fillet samples and rinsates for analysis. Tetra Tech held multiple aliquots of archived fillet tissue in a freezer at its facility to allow for further analyses of GLHHFFTS samples in the future.

Note: Unless otherwise modified, all references to "fish" and "samples" in this report refer to homogenized fish fillet tissue samples prepared by Tetra Tech.

Four commercial laboratories and one academic laboratory analyzed the GLHHFFTS fish tissue samples for mercury, PCBs, PCDDs/PCDFs, PFAS, and omega-3 and omega-6 fatty acids, under subcontracts to GDIT, as shown below and in Figure 2.

Laboratory	Analysis Type
ALS-Environmental	Mercury
Vista Analytical	PCB congeners
AXYS Analytical	PCDDs/PCDFs
AXYS Analytical	PFAS
Clarkson University	omega-3 and omega-6 Fatty Acids

Section 1.4 Study Results

EPA posted the final analytical results for all of the samples in this study in MS Excel files at:

https://www.epa.gov/fish-tech/2015-great-lakes-human-health-fish-fillet-tissue-study

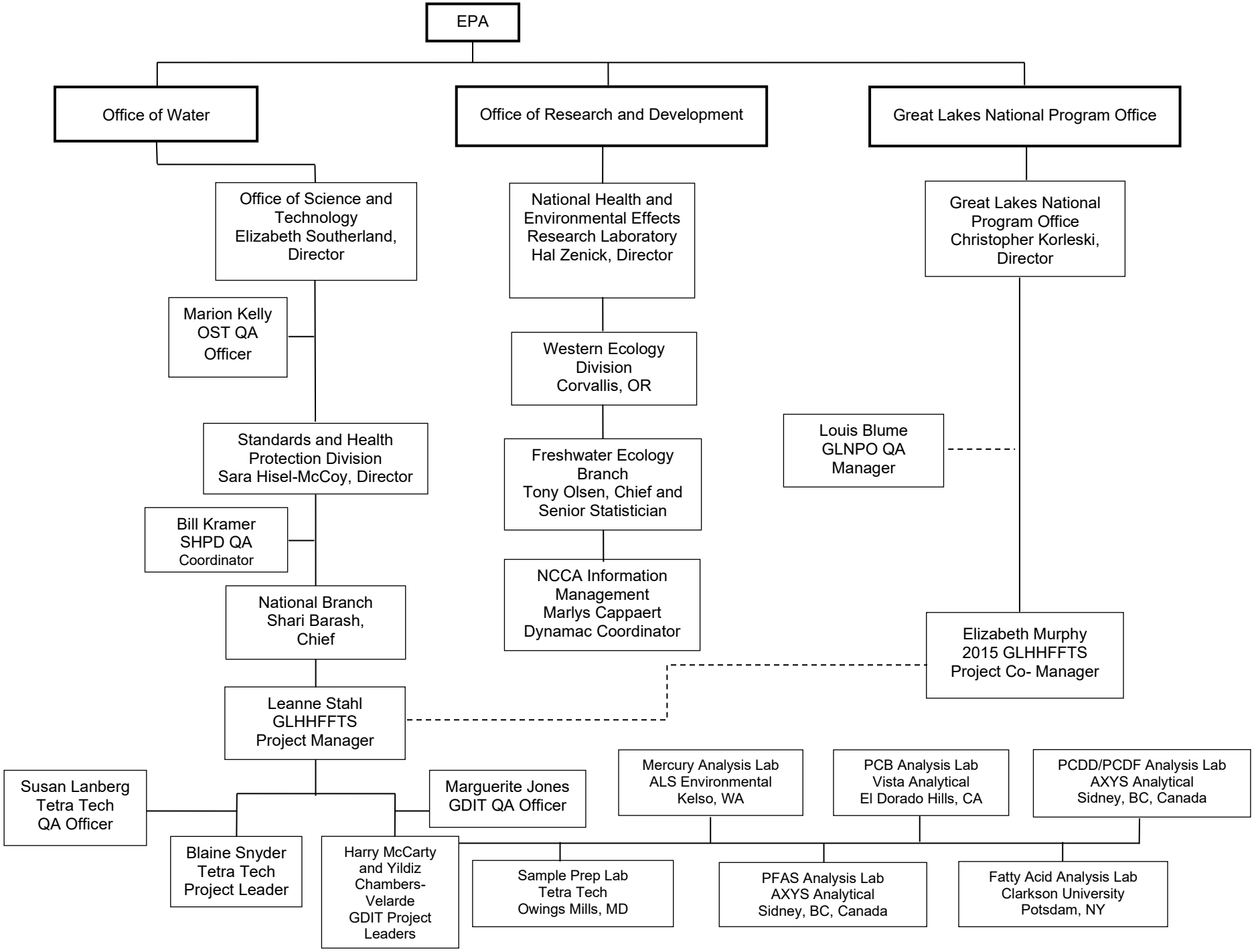

Figure 2. GLHHFFTS project team organization

Chapter 2
Quality Assurance Program

At the beginning of the study, EPA managers recognized that data gathered from the study would be used extensively by individuals responsible for making environmental, economic, and policy decisions. Environmental measurements always contain some level of uncertainty. Decision makers, therefore, must recognize (and have the means to assess) the uncertainty associated with the data on which their decisions are based. In recognition of this, the study managers established a quality assurance (QA) program to ensure that data produced under the study would meet defined standards of quality.

Section 2.1 Quality Assurance Project Plans

Two separate Quality Assurance Project Plans (QAPPs) are associated with this study. In 2014, OWOW and OST coordinated to develop the NCCA Quality Assurance Project Plan (USEPA 2014a) that describes the procedures and associated quality assurance/quality control (QA/QC) activities for collecting and shipping NCCA samples of all types. It includes the human health fish collection and shipping procedures that OST developed for the GLHHFFTS based on the protocols used for the National Lake Fish Tissue Study, as well as procedures for the collection of other types of samples.

In February 2016, OST developed the QAPP that covered the activities associated with GLHHFFTS fish sample preparation and analysis of samples (USEPA 2016a). That QAPP was revised four times as funding became available to carry out additional types of analyses of the fillet tissue samples. The first revision of the OST QAPP added mercury analyses, and it was approved in April 2016 (USEPA 2016b). The second revision added PCB and PFAS analyses, and it was approved in June 2016 (USEPA 2016c). The third revision added fatty acid analysis, and it was approved in February 2017 (USEPA 2017a). The final revision added PCDD/PCDF analysis, and it was approved in April 2017 (USEPA 2017b).

The OST QAPP for the study presented performance criteria, acceptance criteria, and objectives for the analysis of mercury, PCBs, PCDDs/PCDFs, PFAS, and fatty acids in fish collected for the GLHHFFTS. The QAPP also described the methods and procedures to be followed during the study to ensure that the criteria and objectives are met. The QAPP addressed mercury, PCBs, PCDDs/PCDFs, PFAS, and fatty acid analytical activities. The QAPP was prepared in accordance with the most recent version of EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* (USEPA 2001a), which was reissued in 2006.

Section 2.2 Training

Fish Tissue Sample Preparation

Specialized training was provided for laboratory technicians who prepared fish tissue fillets and homogenates for the study. This training was conducted at Tetra Tech in Owings Mills, Maryland, on March 2, 2016 for all laboratory staff involved with GLHHFFTS fish tissue sample preparation, to accomplish the following objectives:

- present the GLHHFFTS fish tissue preparation, homogenization and distribution procedures that are described in the standardized operating procedure (SOP) found in Appendix B of the sample preparation and analysis QAPP (USEPA 2016a)
- demonstrate filleting and homogenizing techniques with fish from invalid GLHHFFTS samples, and
- provide hands-on opportunities for fish preparation laboratory staff to become proficient at filleting and homogenizing fish samples.

Analysis of Fish Tissue Samples

All laboratory staff involved in the analysis of fish tissue samples were required to be proficient in the associated tasks, as required by each analytical laboratory's existing quality system. All GDIT staff involved in analytical data review and assessment were already proficient in data review, so no specialized training was required for data reviewers for this project.

Section 2.3 Sample Preparation and Analysis QA/QC

EPA integrated various QA/QC activities into the study to ensure data comparability and generate analytical data of known quality during preparation and analysis of the fish fillet tissue samples and evaluation of analytical data quality. There were separate QA/QC activities associated with the preparation of the fish fillet samples and the analyses of those samples.

Following is a summary of the critical QA/QC components associated with the sample preparation process:

- Development and implementation of the sample preparation activities in the QAPP (USEPA 2016a, 2016b, 2016c, 2017a, and 2017b)
- Use of one laboratory for sample preparation (filleting, tissue homogenization, and preparation of tissue aliquots)
- Requirement for triplicate lipid analyses to test for tissue homogeneity during sample preparation
- Requirement for preparation equipment rinsate samples with each batch of fish fillet tissue samples prepared
- Requirement for analyses of the rinsate samples for mercury and selected PCDD/PCDF and PCB congeners
- Review and acceptance of rinsate results by EPA before proceeding with preparation of additional samples

Following is a summary of the critical QA/QC components associated with the sample analysis process:

- Development and implementation of the analytical activities in the QAPP (USEPA 2016a, 2016b, 2016c, 2017a, and 2017b)
- Use of one laboratory for the analyses of a given class of analytes
- Identification of quantifiable measurement quality objectives
- Use of pure and traceable reference standards
- Demonstration of instrument calibration and system performance
- Periodic calibration verification
- Analysis of QC samples to assess performance of analytical methods
- Specification of method detection limits (MDLs) and method/chemical QC acceptance criteria that applied throughout the study
- Use of a standardized data quality assessment process

The general measurement quality objective (MQO) for the study was to satisfy method-specific performance criteria. The sample preparation and analysis QAPP provides a summary of the method performance criteria and specifies MQOs and QC acceptance criteria to assess the bias and precision associated with the analytical methods used for this study. Chapter 4 of this report describes the process for data quality assessment and presents the results of these assessments, which includes data from the following laboratory QC samples or measures: blanks, recoveries for spiking surrogate chemicals into field-based tissue samples, matrix spiking (matrix spike/matrix spike duplicate [MS/MSD]), laboratory

control samples (LCS), and calibration verifications. Chapter 4 also includes a discussion of data completeness for the study.

Section 2.4 QA Oversight of Laboratory Operations

The GDIT Project Technical Leader scheduled and tracked all analytical work performed by laboratories for mercury, PCB, PCDD/PCDF, PFAS, and fatty acids analyses. The GDIT Project Leader also coordinated with staff at the Tetra Tech fish sample preparation laboratory regarding fish tissue sample shipments.

When samples were shipped to an analytical laboratory, the GDIT Project Leader contacted designated laboratory staff by email to notify them of the forthcoming shipment(s) and request that they contact GDIT if the shipments did not arrive intact, as scheduled. Within 24 hours of scheduled sample receipt, GDIT contacted the laboratory to verify that the samples arrived in good condition, and if problems were noted, it worked with the laboratory and EPA to resolve any problems as quickly as possible to minimize data integrity problems.

GDIT communicated periodically with laboratory staff by telephone or email to monitor the progress of analytical sample preparation, sample analysis, and data reporting. If any technical problems were encountered during sample preparation and analysis, GDIT identified a technical expert within GDIT to assist in resolving the problem, and work with EPA to identify and implement a solution to the problem. In cases in which the laboratory failed to deliver data on time, or if the laboratory notified GDIT of anticipated reporting delays, GDIT notified the EPA Project Manager. To the extent possible, GDIT adjusted schedules and shifted resources within GDIT as necessary to minimize the impact of any laboratory delays on EPA schedules. GDIT also immediately notified the Project Manager of any laboratory delays that were anticipated to affect EPA schedules.

Finally, the GDIT Project Leader monitored the progress of the data quality audits (data reviews) and database development to ensure that each laboratory data submission was reviewed in a timely manner. In the event that dedicated staff were not able to meet EPA schedules, GDIT identified additional staff who were qualified and capable of reviewing the data so that EPA schedules could be met. In cases when such resources could not be identified, and if training new employees was not feasible, GDIT met with the EPA Project Manager to discuss an appropriate solution.

Chapter 3
Preparation and Analysis Methods

To control variability among tissue sample results, all fillet samples prepared for the study were analyzed by a single set of methods, and all analyses performed with a given method were performed by only one laboratory. Further control of variability was ensured by utilizing a single laboratory to prepare (i.e., fillet, composite, homogenize, and aliquot) samples in a strictly controlled, contaminant-free environment. The methods employed by the sample preparation laboratory and by the five analytical laboratories are described below.

Section 3.1 Preparation of Fish Fillet Tissue Samples

Tetra Tech served as the fish sample preparation laboratory for the study. In this role, Tetra Tech was responsible for filleting each valid fish sample, homogenizing the fillet tissue, preparing the required number of fish tissue aliquots for analysis and archive, shipping the fish tissue aliquots for each type of analysis to the designated analytical laboratory, storing archive fish tissue samples temporarily in a freezer at its facility, and transferring archive fish tissue samples to Microbac Labs for long-term storage. The specific procedures for all GLHHFFTS fish sample preparation activities are described in Appendix B of the sample preparation and analysis QAPP for the study (USEPA 2016a).

Fish were filleted by qualified technicians using thoroughly clean utensils and cutting boards (cleaning procedures are detailed in Appendix B of that QAPP). Each fish was weighed to the nearest gram wet weight, rinsed with deionized water, and filleted on a glass cutting board. For the GLHHFFTS, fillets from both sides of each fish were prepared with scales removed, skin on, and belly flap (ventral muscle and skin) attached. Fillets were composited using the "batch" method, in which all of the individual specimens that comprise the sample were homogenized together, regardless of each individual specimen's proportion to one another (as opposed to the "individual" method, in which equal weights of each specimen are added together), as described in USEPA 2000.

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

Section 3.2 Analysis of Fish Tissue Samples for Mercury

The mercury samples were prepared and analyzed by ALS-Environmental (Kelso, WA), using EPA Procedure I from "Appendix to Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation" from Revision B of Method 1631 (1631B) for sample preparation (USEPA 2001b), and Revision E of Method 1631 (1631E) for the analysis of mercury in fish tissue samples (USEPA 2002). Mercury was detected in all 152 of the fish tissue samples. Fillet tissue sample results were reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g).

Section 3.3 Analysis of Fish Tissue Samples for PCBs

The PCB samples were prepared and analyzed by Vista Analytical Laboratory, in general accordance with EPA Method 1668C (USEPA 2010a) and as detailed in the laboratory's SOP. The samples were

analyzed for all 209 PCB congeners, and reported as either individual congeners or coeluting groups of congeners. The Vista SOP deviates from the published EPA method in several aspects, including:

- Use of sodium sulfate as the reference matrix for QC samples instead of vegetable oil due to traces of PCBs found in the vegetable oil
- Use of sodium hydroxide to adjust the pH of the solution in the back extraction procedure rather than potassium hydroxide
- Use of mid-level calibration standard (CS-3) that contains all 209 congeners instead of the subset of congeners listed in the method
- Use of 44 ^{13}C-labeled compounds in each sample which is five more than the 39 specified in the method

The entire list of modifications is presented in detail in the QAPP. These changes fall within the method's established allowance for flexibility, and EPA accepted these deviations from Method 1668C for the purposes of the study. Tissue sample results were reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g).

Section 3.4 Analysis of Fish Tissue Samples for PCDD/PCDF

The PCDD/PCDF samples were prepared and analyzed by AXYS Analytical Services Ltd. (Sidney, BC, Canada) using Revision B of EPA Method 1613 (1613B), Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS (USEPA 1994) and as detailed in the laboratory's SOP. The samples were analyzed for the 17 2,3,7,8-substituted PCDD/PCDF congeners listed in Appendix C of the QAPP. The AXYS SOP deviates from the published EPA method in several aspects, including:

- Approximately 25 g of fish tissue was used for the analysis
- A 6-point instrument calibration was performed using an additional low-level standard, (CS0.2) at 0.1-0.5 ng/mL, which lowers the method-specified initial calibration range by a factor of 5
- The cleanup standard ($^{37}Cl_4$-2,3,7,8-TCDD) was not used due to the low detection limits required

These changes fall within the method's established allowance for flexibility, and EPA accepted these deviations from Method 1613B for the purposes of the study. Tissue sample results were reported based on the wet weight of the tissue sample, in picograms per gram (pg/g).

Section 3.5 Analysis of Fish Tissue Samples for PFAS

At the time of this study, there were no formal analytical methods from EPA or any voluntary consensus standard bodies (VCSBs) for PFAS analyses of tissues. Therefore, PFAS samples were analyzed by AXYS Analytical Services, Ltd. (Sidney, BC, Canada) using procedures developed, tested, and documented in that laboratory. The SOP for the procedure is considered proprietary by the laboratory, but was reviewed by GDIT prior to the study and the analytical procedure is briefly described below.

Approximately 2 g of fish tissue are required for analysis. (If matrix-related analytical problems are identified during the analysis of a given fish tissue sample, a sample aliquot of 1 g may be used to minimize those problems.) The sample is spiked with 10 isotopically labeled standards and extracted by shaking the tissue in a caustic solution of methanol, water, and potassium hydroxide. The hydroxide solution breaks down the tissue and allows the PFAS to be extracted into the methanol/water.

After extraction, the solution is centrifuged to remove the solids and the supernatant liquid is diluted with reagent water and processed by solid-phase extraction (SPE). The PFAS are eluted from the SPE

cartridge and the eluant is spiked with additional labeled recovery standards and analyzed by high performance liquid chromatography with tandem mass spectrometry.

The concentration of each PFAS was determined using the responses from one of the ^{13}C- or ^{18}O-labeled standards added prior to sample extraction, applying the technique known as isotope dilution. As a result, all of the target analyte concentrations were corrected for the recovery of the labeled standards, thus accounting for extraction efficiencies and losses during cleanup. Because a labeled standard for perfluorobutanesulfonic acid was not commercially available at the time of the study, this target analyte was quantified using the response for ^{18}O-labeled perfluorohexanesulfonic acid, a closely related compound. Tissue sample results were reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g).

Section 3.6 Analysis of Fish Tissue Samples for Fatty Acids

There are no formal analytical methods from EPA for the fatty acids, largely because they are natural substances and not environmental contaminants. The samples were analyzed for fatty acids by Clarkson University (Potsdam, NY), using procedures developed, tested, and documented in that laboratory and employed under GLNPO Grant No. GL 00E01505. The SOP for the procedure is considered proprietary by the laboratory but was reviewed by GDIT prior to the study and the analytical procedure is briefly described below.

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

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

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

Section 3.7 Analysis of Rinsates and Solvent Blanks

As noted in Section 2.3, Tetra Tech prepared equipment rinsate samples with each batch of fish fillet tissue samples. Aqueous rinsates were prepared for mercury and PFAS, and hexane rinsates were prepared for PCBs and PCDDs/PCDFs. Paired rinsate and solvent blank samples were analyzed for mercury and PCBs by subcontract laboratories under the control of Tetra Tech. ALS (Kelso, WA) analyzed the rinsate and solvent blank samples for mercury using EPA Method 245.1 (USEPA 1983), while Pace Analytical Services analyzed the rinsate and solvent blank samples for PCBs using SW-846 Method 8082A (USEPA 2007). Results for mercury were reported in micrograms per liter (µg/L), and PCBs were reported in nanograms per liter (ng/L).

Tetra Tech stored the aqueous rinsate and solvent blank samples for PFAS analyses and the hexane rinsate and solvent blank samples for PCDD/PCDF analyses until EPA obtained the funding for the tissue analysis laboratories. PFAS and PCDD/PCDF rinsate and solvent blank samples were analyzed by

AXYS Analytical Services (Sydney, BC, Canada) at the same time as the analyses of the fish fillet tissue samples. Rinsate and solvent blank sample results were reported in ng/L for PFAS and PCDDs/PCDFs.

Rinsates and solvent blanks were not prepared for the fatty acids because they are naturally occurring components of fish tissue that are present at much higher concentrations than the contaminants of interest in this study. Based on experience with prior studies, EPA concluded that the rinsate results for contaminants such as mercury and PCBs would be sufficient to demonstrate that the equipment cleaning procedures employed for the study were effective for fatty acids as well.

Section 3.8 Quality Control Procedures

Fish Tissue Analyses

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

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

Other quality control activities for fish tissue samples varied by the analysis type, as described in Table 1.

Table 1. Quality Control Activities for Analysis of Fish Tissue Samples

Analyte Type	Quality Control Sample	Frequency
Mercury	Bubbler blank	3 blanks run during calibration and with each analytical batch of up to 20 field samples
	Method blank	3 method blanks per batch of up to 20 field samples, with analyses interspersed among the samples in the analysis batch
	Laboratory control sample	Once per batch of up to 20 field samples, prior to the analysis of any field samples, and again at the end of each analytical batch, spiked at 4.0 ng
	QC Sample	Once per batch of up to 20 field samples
	Matrix spike and matrix spike duplicate samples	Once per every 10 field samples (e.g., twice per 20 samples in a preparation batch)
PCBs	Method blank	One per sample batch of up to 20 field samples
	Laboratory control sample	One per sample batch of up to 20 field samples
	Laboratory duplicate sample	One per sample batch of up to 20 field samples
	Labeled compounds	Spiked into every field sample
PCDDs/PCDFs	Method blank	One per sample batch of up to 20 field samples
	Laboratory control sample	One per sample batch of up to 20 field samples
	Laboratory duplicate sample	One per sample batch of up to 20 field samples
	Labeled compounds	Spiked into every field sample
PFAS	Method blank	One per sample batch of up to 20 field samples
	Laboratory control sample	One per sample batch of up to 20 field samples
	Laboratory duplicate sample	One per sample batch of up to 20 field samples
	Labeled compounds	Every field and QC sample before extraction
Fatty Acids	Method blank	One per sample batch of up to 10 field samples
	Surrogate	Every field and QC sample
	Reference material	One per sample batch
	Laboratory duplicate sample	One per sample batch of up to 10 field samples

Rinsate and Solvent Blank Analyses

The quality control activities associated with the rinsate and solvent blank analyses were generally similar to those for the tissue analyses, with the following exceptions. First, the rinsate and solvent blank samples for mercury and PCBs were prepared and analyzed as individual pairs, not in batches of up to 20 samples, and analyzed by laboratories under subcontract to the sample preparation laboratory, in order to provide timely feedback of the cleanliness of the homogenization equipment. The rinsates and solvent blanks for PFAS and PCDDs/PCDFs were held for later analyses, so they were grouped together in batches, each with its own associated QC activities. Secondly, because the rinsates for PCBs and PCDDs/PCDFs were prepared in an organic solvent (hexane), there were no sample extraction procedures required, so the typical QC procedures relevant to the sample extraction procedure were modified. The common quality control activities for rinsate samples are described in Table 2.

Table 2. Quality Control Activities for Analysis of Rinsates

Analyte Type	Quality Control Sample	Frequency
Mercury	Instrument blank	With each rinsate sample
	Laboratory control sample	With each rinsate sample
PCBs and PCDDs/PCDFs	Instrument blank	With each rinsate sample
	Surrogates or labeled compounds	Added to every rinsate sample
PFAS	Method blank	With each batch of rinsate samples
	Laboratory control sample	With each batch of rinsate samples
	Labeled compound recovery	Every rinsate sample

Because the mercury rinsates and the PFAS rinsates were prepared in reagent water, there was little chance of a "matrix effect" and the laboratory control sample, which was also prepared in reagent water, provided sufficient information on the performance of the method and the laboratory in reagent water, so a separate matrix spike sample was not required.

Because the rinsates for PCB, PCDD/PCDF and fatty acids were prepared from hexane and no sample extraction was required, "matrix effects" were not possible. Therefore, matrix spike and duplicate samples were not required for these rinsate samples. A laboratory control sample was used for the fatty acids to assess the performance of the derivatization process applied to the analytes.

GDIT reviewed the results for the mercury and PCB rinsates as soon as they were available from Tetra Tech's subcontracted laboratories and relayed the review findings to EPA and Tetra Tech within hours of receipt of the results. Mercury was never detected above the subcontracted laboratory's MDL in any of the rinsate or aqueous (solvent) blank samples from the study. However, in making its assessments of the rinsate results, GDIT took a conservative approach and assumed that mercury could be present in the rinsate sample at exactly the MDL. Based on this assumption, GDIT calculated the total mass of mercury that theoretically might be transferred to the smallest bulk homogenized tissue sample in the sample batch (due to inadequate cleaning of the homogenization equipment). That "worst case" estimate was then compared to the MDL for mercury in tissues and was always at least 6 times lower than the tissue sample MDL. Therefore, in no instance was there any risk that the mercury reported in the fish tissue samples was the result of inadequate equipment cleaning, and EPA authorized Tetra Tech to continue processing fish tissue samples.

A similar review approach was utilized for the PCB rinsates and solvent blanks. Overall, only one of the ten PCB congeners that were monitored was ever detected in the eight pairs of rinsates and solvent blanks. PCB-118 was detected in one rinsate sample, at a concentration that was over 9,000 times lower than the tissue sample MDL, based on the smallest bulk homogenized tissue sample in that sample batch.

The rinsate results for the PCDDs/PCDFs were reviewed after all of the fish tissue samples had been analyzed and were assessed using a similar approach as described for mercury and the PCBs. There were only three instances of a PCDD or PCDF congener being reported in a rinsate sample. The rinsates for Batches 1 and 2 contained 2,3,7,8-TCDF at sub-picogram levels and 1,2,3,4,6,7,8-HpCDD was reported in the rinsate for Batch 4. However, in all three instances, the levels in the rinsates were more than 200 times lower than the tissue sample MDL, based on the smallest bulk homogenized tissue sample in each sample batch.

The PFAS rinsates were reviewed in the same manner. PFOA was reported in two of the rinsates and one solvent blank not paired with either of those rinsates and PFOSA was reported in one rinsate. When assessed against their tissue MDLs in the smallest tissue sample in each of those batches, the levels were 300 to 900 times lower than could have been detected in a tissue sample.

Overall, the rinsate results demonstrate that the equipment cleaning procedures employed for the study were more than adequate to ensure that cross contamination between tissue samples was not occurring during processing.

Chapter 4
Data Quality Assessment

Section 4.1 Data Review

All of the data from the study were subjected to two levels of review. First, all laboratory results and calculations were reviewed by the respective laboratory manager for that analysis prior to submission. Any errors identified during this peer review were returned to the analyst for correction prior to submission of the data package. Following correction of the errors, the laboratory manager verified that the final package was complete and compliant with the contract, and signed each data submission to certify that the package was reviewed and determined to be in compliance with the terms and conditions of the GDIT subcontract.

For the second level of review, GDIT data reviewers examined the results for each field-based tissue sample and the available quality control data to assess and document the quality of the data relative to the objectives of the study. Each data package was thoroughly reviewed by GDIT to ensure the following:

- All samples were analyzed, and results were provided for each sample analyzed, including results for any dilutions and re-analyses, and for all associated QC samples.
- All required QC samples were analyzed, and these QC samples met specified acceptance criteria.
- Data reporting forms and/or electronically formatted data were provided for each of the field-based tissue samples and/or associated QC analyses.
- Raw data associated with each field-based tissue sample and QC sample were provided with each data package, and the instrument output (peak height, area, or other signal intensity) was traceable from the raw data to the final result reported.
- Any problems encountered and corrective actions taken were clearly documented.

When anomalies were identified, GDIT contacted the laboratory and asked them to provide the missing data, clarifications, and/or explanations so that a comprehensive data review could be performed to verify the quality of their results.

GDIT data reviewers documented their findings by adding standardized data qualifier flags and descriptive comments concerning the reliability of the flagged results to the electronic data deliverables (EDDs) submitted by each laboratory. Following an internal review of the flagged EDD, GDIT imported the results into project-specific database. Table 3 contains the individual data qualifiers that were applied to results from the study and provides an explanation of the implications of each qualifier for the use of the data.

Note: *The presence of data qualifiers is not intended to suggest that data are not useable; rather, the qualifiers are intended to caution the user about an aspect of the data that does not meet the acceptance criteria established in the project QAPP.*

Table 3. Individual SCC Codes Applied to the GLHHFFTS Results		
SCC Code	**Comments**	**Implication**
B, RMAX	Blank Contamination, Result is a Maximum Value	Blank contamination was observed and the target analyte was reported in the sample at a concentration between 5 and 10 times higher than the blank value. The result was considered to be of acceptable quality, but data users are cautioned that it may be a maximum value due to possible influence of contamination.
B, RNAF	Blank Contamination, Result Not Affected	Blank contamination was present but was not considered to adversely impact the sample result. The presence of the analyte in the blank is not considered to adversely affect the data in cases where the sample results are more than 10 times the associated blank results or where the analyte is not detected in associated samples.
B, RNON	Blank Contamination, Result Reported as a Non-detect	When the sample result is less than five times the blank result, there are no means by which to ascertain whether or not the presence of the analyte may be attributed to contamination. Therefore, the result is reported in the database as a non-detect at the MDL, adjusted for sample size and dilution.
CONF	Confirmed Result	The result was confirmed on the method-specific second GC column. This only applies to the analysis of 2,3,7,8-TCDD, one of the PCDD/PCDF analytes, and does not imply a data quality issue, but identifies that the presence of the analyte was confirmed as described in the method.
HIAR, J	High Ion Abundance Ratio, Estimated	Each analyte is identified and quantified based on the instrumental response for two specific ions and the ratio of those two ions was above the upper acceptance limit, suggesting a potential interference that may affect the sample result. Therefore, the result also is flagged as an estimated value.
HLBL, J	High Labeled Compound Recovery, Estimated	The labeled analog of the target analyte was recovered above acceptance criteria, suggesting the possible presence of matrix interferences. Isolated instances of high recovery are not uncommon, and patterns across multiple samples are more of a concern. If the analyte was detected in a field sample, the result is considered an estimate and the J is added to the HLBL flag.
HLBL, RNAF	High Labeled Compound Recovery, Result Not Affected	The labeled analog of the target analyte was recovered above acceptance criteria, suggesting the possible presence of matrix interferences. Isolated instances of high recovery are not uncommon, and patterns across multiple samples are more of a concern. If the analyte was not detected in a field sample, there is no concern and the RNAF is added to the HLBL flag.
HLCS	High Lab Control Sample Recovery	The lab control sample (LCS) was a clean reference matrix. If recovery in the LCS was high, there may be a high bias for that analyte.
HLCS, RNAF	High Lab Control Sample Recovery, Result Not Affected	The recovery in the LCS was high, but the analyte was not detected in the associated tissue sample, so there was no high bias concern and the RNAF flag was applied.
HSRM	High Standard Reference Material Recovery	Reference standard had high recovery, results for that analyte in any of the associated samples were qualified as estimated values.
HRPD, J	High RPD, Estimated	The relative percent difference (RPD) between the results in the parent sample and the laboratory duplicate is above the acceptance limit. This may be due to inhomogeneity in the bulk sample or analytical variability. When high RPD was observed for an analyte, all the detected results for that analyte in any of the samples in the batch with the duplicate sample were qualified as estimated values.
HRPD, RNAF	High RPD, Result Not Affected	The relative percent difference (RPD) between the results in the parent sample and the laboratory duplicate is above the acceptance limit. This may be due to inhomogeneity in the bulk sample or analytical variability. However, when high RPD was observed for an analyte, the non-detected results for that analyte were not affected, and the RNAF flag was applied.
HVER, RNAF	High CALVER, Result Not Affected	The results for the calibration verification associated with the analyte were above the acceptance limit, suggesting a possible high bias. The non-detected results for that analyte were not affected, and the RNAF flag was applied.
J	Estimated	When applied alone, this code indicates that the result is at or above the MDL, but below the QL. This flag also may be applied in conjunction with other flags to indicate the potential for greater uncertainty.

Table 3. Individual SCC Codes Applied to the GLHHFFTS Results		
SCC Code	**Comments**	**Implication**
LIAR, J	Low Ion Abundance Ratio, Estimated	Each analyte is identified and quantified based on the instrumental response for two specific ions and the ratio of those two ions was below the lower acceptance limit, suggesting a potential interference that may lower the sample result. Therefore, the result also is flagged as an estimated value.
LLBL	Low Labeled Compound Recovery	The labeled analog of the target analyte was recovered below acceptance criteria, suggesting the possible presence of matrix interferences or incomplete recovery of both the labeled compound and target analyte during the extract cleanup processes used in the analytical procedure. The use of isotope dilution quantitation automatically corrects the results for the target analyte, even when the labeled compound recovery is below expectations.
LLBL, J	Low Labeled Compound Recovery, Result is an Estimate	The labeled analog of the target analyte was recovered below acceptance criteria, suggesting the possible presence of matrix interferences or incomplete recovery of both the labeled compound and target analyte during the extract cleanup processes used in the analytical procedure. The use of isotope dilution quantitation automatically corrects the results for the target analytes. For detects, results considered an estimate.
NASA, J	No Authentic Standard Available	There is no authentic standard available for calibration. The result is considered an estimated value. This flag only applies to some of the fatty acid analytes and does not imply a data quality issue
REXC, J	Result Exceeds Calibration	The result exceeded the calibration range; however, sample dilution was not practical. The result is considered an estimated value.

Section 4.2 Analysis of Blanks

Blanks are used to verify the absence of contamination that may occur at any point in the measurement process. The data reviewers evaluated each sample result in comparison to the result for that analyte in the method blank prepared in the same extraction batch. For those analytes reported as present in the method blank, the data reviewers applied the 5x and 10x rules (described in the first three SCC codes of Table 3) to determine the potential impact of the blank contamination on the study results. The impacts of blank contamination are discussed separately for each analyte class in Sections 4.2.1 to 4.2.5.

4.2.1 Blanks for Mercury Analysis

Mercury was never detected above the QC acceptance limit of 0.4 nanograms (ng) in any of the three method blanks associated with each batch of samples. Therefore, no method blank qualifiers were applied to the mercury results for the study.

4.2.2 Blanks for PCB Analysis

The method blanks associated with the analytical batches showed occasional minor PCB contamination. More than 99.97% of the PCB results were not affected by the blank contamination, either because the analytes were not detected in the sample (99.42%) or because the concentration was more than 5 times the level observed in the blank (0.564%). For 0.0162% of these results, the data reviewers judged that the sample result is likely a maximum value (RMAX) because there is some chance that the sample result was inflated by the background contamination from the laboratory that is evident in the blank. This flag was applied to the PCB-11 results for four samples. Only 0.0203% of the results for those congeners were changed to non-detects (RNON) due of blank contamination. This flag was applied to the PCB-11 results for five samples. Because the percent of results affected was below 1%, a pie chart has not been added to the report as the slivers will be barely visible.

4.2.3 Blanks for PCDD/PCDF Analysis

There were a few data quality issues with PCDDs/PCDFs in the blanks, as illustrated in the figure to the right. Figure 3 shows that 96.33% of the results were not affected by blank contamination, either because the analyte was not detected in the blank (95.09%) or because the concentration in the sample was more than 10 times the level observed in the blank (1.24%). For 0.77% of the results, the data reviewers judged that the sample result is likely a maximum value (RMAX) because there is some chance that the sample result was inflated by the background contamination from the laboratory that is evident in the blank. A total of 2.86% of the PCDD/PCDF results were changed to non-detects (RNON) because the sample results were less than 5 times the concentration in the method blank. Only seven of those results were for 2,3,7,8-TCDD and the others were largely hexa-, hepta, and octa-chlorinated congeners.

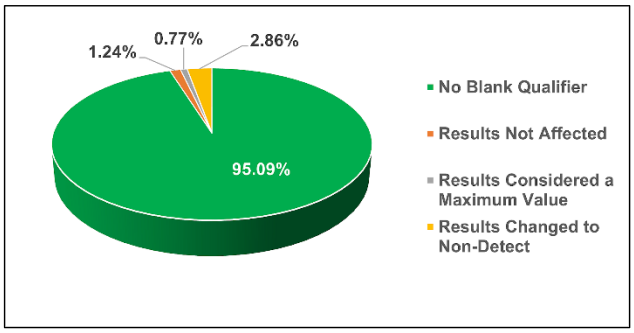

Figure 3. Impacts of Blank Contamination on the PCDD/PCDF Results

4.2.4 Blanks for PFAS Analysis

No PFAS analytes were detected above the MDL in any of the method blanks associated with each batch of samples. Therefore, no method blank qualifiers were applied to the results for the study.

4.2.5 Blanks for Fatty Acid Analysis

None of the fatty acids were detected above the MDL in any of the method blanks associated with the samples. Therefore, no method blank qualifiers were applied to the fatty acid results for the study.

Section 4.3 Analysis of Laboratory Control Samples

A laboratory control sample (LCS) is a mass or volume of a clean reference matrix into which the laboratory spikes the analytes of interest. In some EPA methods, it is also known as the ongoing precision and recovery (OPR) sample. The laboratory analyzes the LCS or OPR using the same sample preparation and analysis techniques that are applied to the field samples, and compares the results to method- or project-specific acceptance criteria to demonstrate that the laboratory can perform the analysis acceptably in the absence of matrix-specific interferences.

The QAPP for the study (USEPA 2017b) required that each laboratory performing analyses of fish tissue samples prepare and analyze one LCS for each batch of 20 or less field samples. The impacts of LCS results are discussed separately for each analyte class in Sections 4.3.1 to 4.3.5.

4.3.1 Mercury LCS Results

The LCS results associated with each batch of samples analyzed for mercury met the QC acceptance limit. Therefore, no LCS qualifiers were applied to the mercury results for the study.

4.3.2 PCB LCS Results

The LCS results associated with each batch of samples analyzed for PCBs met the QC acceptance limits. Therefore, no LCS qualifiers were applied to the PCB results for the study.

4.3.3 PCDD/PCDF LCS Results

There were few data quality issues with the LCS results for the PCDD/PCDF analyses. Almost 99.2% of the results were not affected by LCS issues. Only 0.81% of the results were qualified because of a high LCS result that might reflect a high bias in the results. Given that such small percentage of results were affected by high LCS values, a pie chart has not been included in this section because the tiny sliver of affected results would not be visible. All of the LCS qualifiers were applied to 21 results for 1,2,3,4,6,7,8-HpCDF. A total of 17 results were reported as estimated. Of those, fifteen were already considered estimates because they were between the MDL and the ML for the sample. The four remaining results were not detected and therefore are not affected.

4.3.4 PFAS LCS Results

The LCS results associated with each batch of samples analyzed for PFAS met the QC acceptance limit. Therefore, no LCS qualifiers were applied to the PFAS results for the study.

4.3.5 Fatty Acid LCS Results

The LCS results associated with each batch of samples analyzed for fatty acids met the QC acceptance limit. Therefore, no LCS qualifiers were applied to the fatty acid results for the study.

Section 4.4 Analysis of Matrix Spike, Matrix Spike Duplicate, and Laboratory Duplicate Samples

A matrix spike sample (MS) is a mass or volume of a field sample into which the laboratory spikes the analytes of interest. The laboratory analyzes the MS using the same sample preparation and analysis techniques that are applied to the field samples, then compares the results to method- or project-specific acceptance criteria to provide information on the effects of the sample matrix on method performance.

A laboratory duplicate sample is a second aliquot of one field sample that is prepared and analyzed to provide information on the precision of the analytical method. Laboratory duplicate samples are routinely used for analytes such as metals that are expected to be found in most or all samples. However, other types of analytes, particularly organic contaminants, are not detected as frequently in field samples, and the analysis of an unspiked duplicate sample often will not yield useful data on analytical precision when both the original sample and the duplicate are reported as “not detected.” Therefore, EPA methods for organic contaminants often require that a second spiked aliquot of the sample matrix be prepared as a matrix spike duplicate (MSD). By spiking the analytes into both, the MS and MSD aliquots, there is a greater chance of generating useful data on method and laboratory precision.

Alternatively, EPA methods such as those used for the PCBs and PCDDs/PCDFs, spike labeled compounds into every sample and the results for those labeled compounds provide sample-specific data on method performance, as opposed to the batch-specific data generated from one MS/MSD pair per batch.

The QAPP for the study (USEPA 2017b) required that the laboratories performing analyses of fish tissue samples prepare and analyze MS/MSD and/or duplicate samples with each batch of field samples as follows:

Table 4. Matrix Spike, Matrix Spike Duplicate, and Laboratory Duplicate Sample Requirements by Analysis Type			
Analysis Type	**Matrix Spike**	**Matrix Spike Duplicate**	**Laboratory Duplicate**
Mercury	X	X	
PCBs			X
PCDDs/PCDFs			X
PFAS*			X
Fatty acids			X

* The method developed by the PFAS laboratory includes the use of both labeled compounds and duplicate analysis.

The data reviewers evaluated the results for each MS, MSD, and/or laboratory duplicate sample. The impacts are discussed separately for each analyte class in Sections 4.4.1 to 4.4.5.

4.4.1 Mercury Matrix Spike and Duplicate Sample Results

The matrix spike and duplicate sample results associated with each batch of samples analyzed for mercury met the QC acceptance limit. Therefore, no matrix spike or duplicate sample qualifiers were applied to the mercury results for the study.

4.4.2 PCB Duplicate Sample Results

The PCB laboratory duplicate analyses exhibited excellent precision, with approximately 99.51% of the PCB results not affected by duplicate issues. Given that only 0.49% of results were qualified due to high RPD values, a pie chart has not been included in this section because the tiny sliver of affected results would barely be visible. Of the 120 results with high RPD values, 16 were non-detect results, and therefore not affected. An additional 11 results were already classified as estimated values because they were between the MDL and the ML.

4.4.3 PCDD/PCDF Duplicate Sample Results

The PCDD/PCDF laboratory duplicate analyses exhibited excellent precision with each batch of samples analyzed. Therefore, no duplicate sample qualifiers were applied to the results for the study.

4.4.4 PFAS Duplicate Sample Results

The PFAS laboratory duplicate analysis exhibited excellent precision with each batch of samples analyzed. Therefore, no duplicate sample qualifiers were applied to the results for the study.

4.4.5 Fatty Acid Duplicate Sample Results

The fatty acids laboratory duplicate analysis exhibited excellent precision with each batch of samples analyzed. Therefore, no duplicate sample qualifiers were applied to the results for the study.

Section 4.5 Surrogates and Labeled Compounds

A surrogate is a compound that is chemically similar to the analytes of interest, but one that is not expected to occur in an environmental sample. A known amount of a surrogate is added to each sample before any sample processing steps and the amount of the surrogate recovered during the analysis provides information about the overall extraction and analysis process applied to each sample. As noted

in Section 3.6, the fatty acid laboratory added a known amount of nonadecanoic acid, C19:0, to each sample before extraction, as a surrogate for the target analytes.

Some methods for organic contaminants use analogs of the target analytes that contain a stable (nonradioactive) isotope of one or more of the atoms that make up the contaminant. These compounds are referred to as "labeled compounds" and often incorporate multiple atoms of naturally occurring, but less common isotopes such as ^{13}C, ^{18}O, or ^{37}Cl. For example, because ^{13}C makes up 1.1% of the carbon in nature, some PCBs in the environment may contain a single occurrence of ^{13}C among the 12 carbon atoms that make up the basic PCB structure. However, if the labeled compound is synthesized with all 12 atoms of the more common isotope ^{12}C replaced by ^{13}C, there is virtually no chance that the $^{13}C_{12}$ labeled compound will be present in an environmental sample. Therefore, the labeled compound is ideally suited for use as a quantitation reference standard during the analysis of PCBs.

The labeled compounds in such methods serve two functions. First, their responses can be used to quantify the responses for the unlabeled target analytes in each sample through the technique known as isotope dilution. Secondly, the measured recovery of each labeled compound provides information about the overall extraction and analysis process applied to each sample in a similar fashion as the surrogate used for the fatty acids. Other labeled compounds are often added to each sample extract before any cleanup steps to provide information on the performance of those cleanups as well.

The PCB laboratory added known amounts of 44 ^{13}C-labeled PCBs to each sample before extraction. The PCDD/PCDF laboratory added known amounts of 6 ^{13}C-labeled dioxin and 9 ^{13}C-labeled compounds for furans to each sample before extraction. The PFAS laboratory added known amounts of 8 ^{13}C-labeled PFAS and one ^{18}O-labeled PFAS to each sample before extraction.

No surrogates or labeled compounds are required for the mercury analyses.

The QAPP for the study (USEPA 2017b) includes acceptance criteria for the recoveries of the various surrogates and labeled compounds. The impacts of surrogate or labeled compound results are discussed separately for each analyte class in Sections 4.5.1 to 4.5.4.

4.5.1 PCB Labeled Compound Recoveries

Virtually all (over 99.8%) of the labeled compound recoveries for the PCB samples met the QC acceptance limits. Given that only 0.106% of results were affected by high labeled compound recoveries, a pie chart has not been included in this section because the tiny sliver of affected results would not be visible.

4.5.2 PCDD/PCDF Labeled Compound Recoveries

Likewise, virtually all (99.96%) of the labeled compound recoveries for the PCDD/PCDF samples met the QC acceptance limits. Given that only 0.04% of results were affected by low labeled compound recoveries, a pie chart has not been included in this section because the tiny sliver of affected results would not be visible.

4.5.3 PFAS Labeled Compound Recoveries

Some labeled compounds for the PFAS analyses had high recoveries and some had low recoveries. Over 99.75% of sample results were not affected by the recoveries of the labeled compounds due to either the compounds not being outside limits (99.6%) or because the associated native compounds were not detected and therefore the results were not affected (0.15%). Only 0.10% of samples with high labeled compound recoveries were qualified as estimated values, while 0.15% of samples with low labeled

compound recoveries were affected and qualified as estimated values. Because the percent of results affected by labeled compound recoveries was small, a pie chart was not added to this report.

4.5.4 Fatty Acid Surrogate Recoveries

The surrogate results associated with each batch of samples analyzed for fatty acids met the QC acceptance limit. Therefore, no surrogate qualifiers were applied to the fatty acid results for the study.

Section 4.6 Ion Abundance Ratio

The methods for PCBs and PCDDs/PCDFs utilize a high-resolution mass spectrometer to detect the target analytes and differentiate them from potential interferences. As part of those methods, the instrument monitors the signals from two ions produced for each analyte. The resolution of the mass spectrometer is sufficient to distinguish ions that differ in mass by a few ten-thousandths of an atomic mass unit. The ratio of the abundances of these two ions is used as one of four criteria to identify the analyte. The methods include QC acceptance criteria for the ion abundance ratios for each target analyte that are based on the theoretical occurrence of each of the component atoms in nature, plus and minus some percentage (e.g., ± 15%).

In some cases, the observed ion abundance ratio may fall outside of the consensus-based acceptance limit. That does not mean that the analyte is not present, but it suggests that there may be some contribution to the response from an ion with a very similar mass produced by an interference. A higher-than-expected ion abundance ratio suggests an interference with the ion in the pair for the target analyte with the smaller mass, while a lower-than-expected ion abundance ratio suggests an interference with the ion in the pair for the target analyte with the larger mass. When the exceedance from the acceptance limit is small (e.g., a few percent), the methods for PCBs and PCDDs/PCDFs allow the analyst to report the results in such instances with a qualifier flag that alerts the data user to the situation. During the data review process, any results reported with an ion abundance ratio issue are reviewed in more depth. If all of the other identification criteria in the method are met, the results are reported for the analyte with the appropriate qualifier flag. The impacts of ion abundance ratio concerns are discussed separately for the PCBs and PCDDs/PCDFs in Sections 4.6.1 and 4.6.2.

4.6.1 PCB Ion Abundance Ratios

The PCB results did not exhibit ion abundance ratio concerns and therefore, no ion abundance ratio qualifiers were applied to the results for the study.

4.6.2 PCDD/PCDF Ion Abundance Ratios

Overall, 76.78% of the PCDD/PCDF results were not qualified because of ion abundance ratio concerns. As shown in Figure 4, the remaining 23.22% of the results were almost equally divided among those with higher-than-expected ion abundance ratios (10.29%) and those with lower-than-expected ion abundance ratios (12.93%). Because the areas of both monitored ions are used to calculate the concentration of the analyte, the direction of the ion abundance ratio failure does not reflect a similar bias in the reported sample result, but each such value is considered an estimated value.

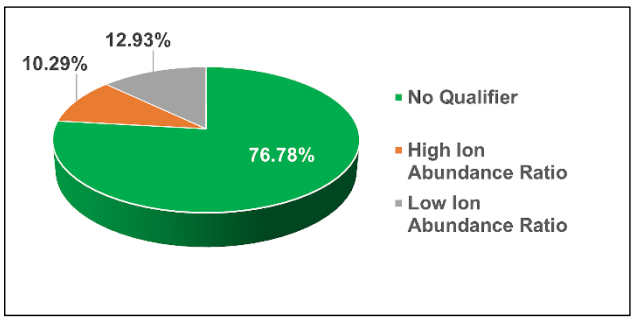

Figure 4. Impacts of Ion Abundance Ratios on the PCDD/PCDF Results

Section 4.7 Standard Reference Material for Fatty Acids

A reference material is a special type of sample that has been well characterized in terms of its physical and chemical makeup. Unlike a laboratory control sample that is spiked with the analytes of interest, a reference material is generally prepared by an outside organization and characterized by analyses from a number of independent laboratories. Reference materials can be obtained from various sources, some of them governmental bodies. In the U.S., the National Institute of Standards and Technology (NIST) has trademarked the name "Standard Reference Material," or "SRM," and sells reference material for a wide variety of matrices, including fish tissues. Other organizations provide what are referred to a "Certified Reference Materials," or "CRMs," to differentiate them from the NIST products.

As part of the fatty acid analyses, the laboratory analyzed an aliquot of NIST SRM 1947, which is a frozen fish tissue homogenate which was prepared from lake trout (*Salvelinus namaycush*) collected from Lake Michigan. The NIST certificate of analysis provides "certified concentration values" for PCB congeners, chlorinated pesticides, and fatty acids. Those fatty acids include only four of the 38 target analytes in this study.

During data review, the results from the analysis of NIST SRM 1947 associated with each batch of field samples in this study were compared to the reference values for the fatty acids. For the purposes of this assessment, the SRM results were viewed in context of all 38 of the fatty acids analyzed in this study. The implications of the SRM results on data quality for the fatty acids are illustrated in Figure 5.

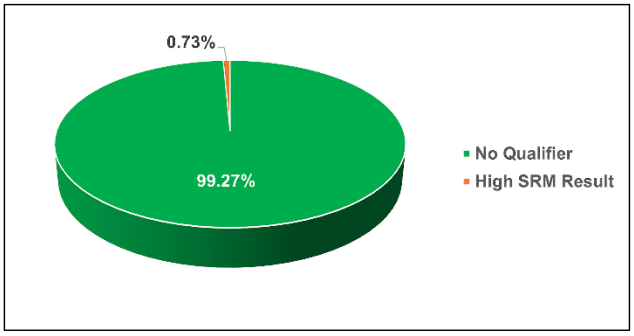

Figure 5. Impacts of SRMs on the Fatty Acid Results

Overall, 99.27% of the fatty acid results are associated with SRM results that agreed with the certified values for the four analytes. The remaining 0.73% of the results were associated with higher SRM results and therefore are considered estimated values. All high SRM recoveries were for heptadecanoic acid and docosahexaenoic acid.

Section 4.8 Completeness

Completeness is a measure of the amount of data that are collected and deemed to be acceptable for use the intended purpose. The completeness goal established in the QAPP for this study (USEPA 2016a) was to obtain valid measurements from 95% of the samples analyzed.

For multi-analyte methodologies, analytical completeness is best calculated on the basis of the number of possible sample/analyte combinations. Otherwise, a problem with a single analyte could be seen as invalidating an entire field sample.

Combining the number of target analytes for the five types of analyses (mercury, PCB, PCDD/PCDF, PFAS, and fatty acids) yields a total of 231 measured results for each sample (based on 162 results that cover all 209 PCB congeners). For the 152 samples collected for the GLHHFFTS, the total number of sample/analyte combinations is 35,112.

Despite the data quality concerns outlined in this report, all 152 samples were successfully analyzed for all of the target analytes. Following an intensive review of the project data, none of the results were excluded from consideration based on data quality concerns. Therefore, analytical completeness is 100%, and OST met its completeness goal.

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