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Quality Assurance Report for the National Coastal Condition Assessment 2010 Great Lakes Human Health Fish Tissue Study

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Table of Contents

List of Tables

List of Figures

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Disclaimer

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Chapter 1
Introduction

This report documents the quality of data gathered during the 2010 Great Lakes Human Health Fish Tissue Study (GLHHFTS), which was a regional component of EPA's Office of Wetlands Oceans and Watersheds (OWOW) National Coastal Condition Assessment (NCCA), a probability-based survey designed to assess the condition of coastal waters of the United States. Multiple EPA offices collaborated to conduct this survey, 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 study.

Section 1.1 Background

Obtaining statistically representative environmental data on mercury, polychlorinated biphenyl (PCB) congeners, and other contaminants of concern is a priority area of interest for EPA. Since 1998, EPA's Office of Water (OW) has collaborated with ORD to conduct the first national-scale assessments of mercury in fish tissue through statistically based studies of U.S. lakes and rivers. These studies are referred to as the National Lake Fish Tissue Study and the National Rivers and Streams Assessment, respectively. The Great Lakes, however, were excluded from the National Lake Fish Tissue Study because assessment of such a large freshwater system required a separate sampling design. The probability-based Great Lakes sampling design developed for the National Coastal Condition Assessment offered the opportunity to conduct the GLHHFTS, which is the first representative study of chemical residues in fish relevant to human health for the Great Lakes region. The GLHHFTS also provided the first lake-wide data on the occurrence and distribution of CECs such as per- and polyfluoroalkyl substances (PFAS) in the Great Lakes. In addition, the GLHHFTS was designed to generate species-specific data on omega-3 fatty acids to address an existing data gap and to identify fish with higher omega-3 levels and potentially greater health benefits when used as a human food source.

Section 1.2 Study Design

Within OW, OST collaborated with GLNPO, OWOW, and ORD's Western Ecology Division (now called the Pacific Ecological Systems Division) in Corvallis, Oregon, to conduct the GLHHFTS within the framework of the NCCA. Fish composite samples were collected from June through November 2010 at a statistical subset of NCCA Great Lakes sites, which consisted of over 150 randomly selected nearshore sites distributed throughout the five Great Lakes (see Figure 1, below).

The following were the key design components for the 2010 GLHHFTS:

- sampling at least 150 randomly selected sites (about 30 sites per lake) in the nearshore regions (water depths up to 30 meters or distances of up to 5 kilometers from the 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 sample site.
- shipping whole fish samples to a commercial laboratory for storage and fish sample preparation, which included filleting the fish, homogenizing the fillet tissue composites, and preparing fillet tissue aliquots for analysis of specific contaminants, along with a series of archive samples that could be used for future analyses of other contaminants.
- analyzing the fillet tissue samples for mercury (total), 209 PCB congeners, 52 polybrominated diphenyl ether (PBDE) congeners (and 2 other brominated compounds), 13 perfluorinated compounds that are a subset of the broader group known as per- and polyfluoroalkyl substances (PFAS), and 5 omega-3 fatty acids.

Initially, OWOW designated June through September 2010 as the sampling period for the NCCA, including the Great Lakes region. Field crews in the Great Lakes scheduled fish collection at the majority of the nearshore sites during June and July, which turned out to be a period when many of the target species for the GLHHFTS are difficult to find in shallow waters. Consequently, OST arranged for resampling at over 50 nearshore sites from August through mid-November to complete the goal of collecting fish for the GLHHFTS from at least 150 nearshore sites.

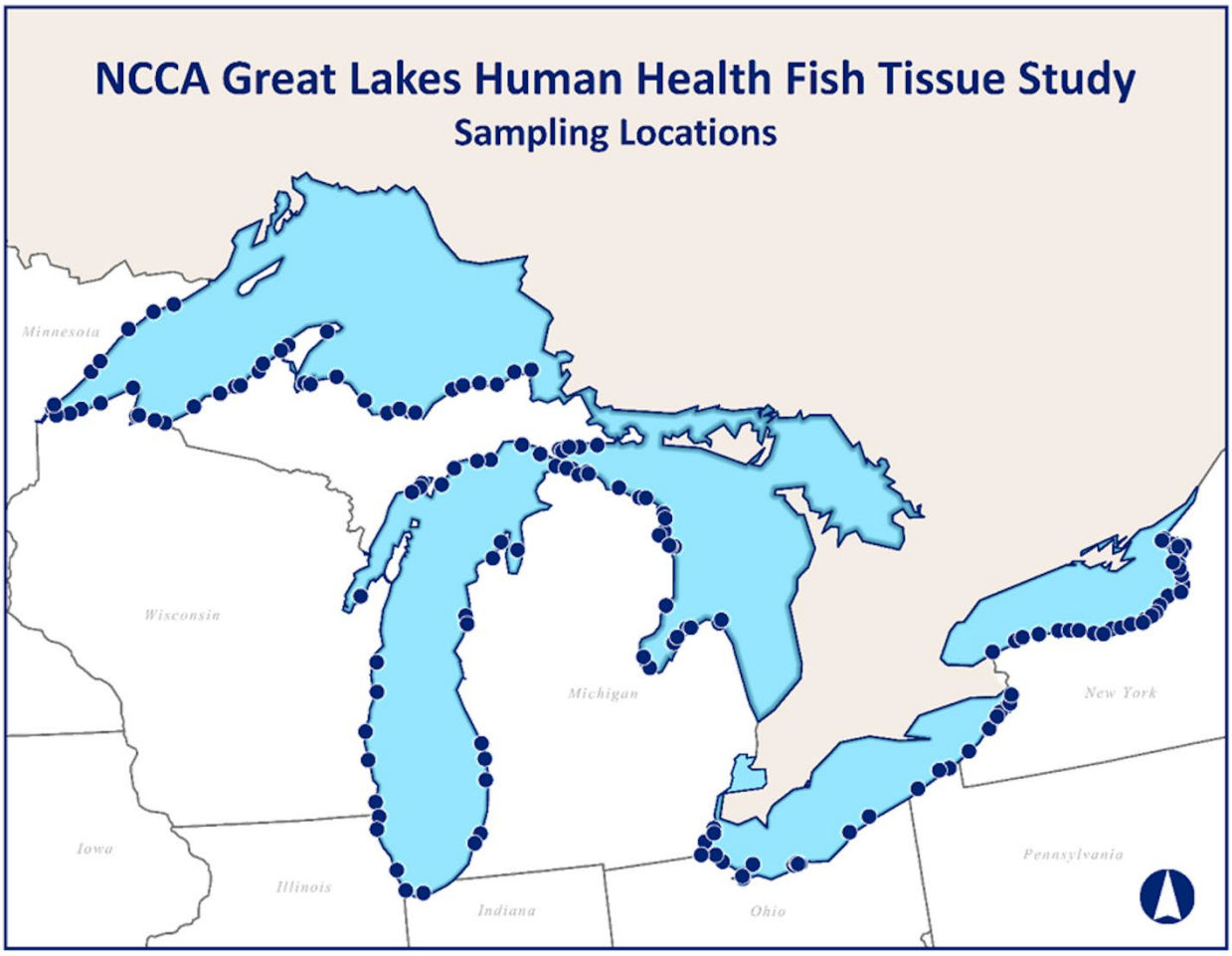


Figure 1. Map of the 157 nearshore Great Lakes sampling locations

Section 1.3 Study Participants

The GLHHFTS 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 GLHHFTS Project Manager who provided overall direction for planning and implementation of this regional Great Lakes study being conducted under the NCCA.
- Marion Kelly was the OST Quality Assurance Officer responsible for reviewing and approving all QAPPs that involve scientific work being conducted by OST.

- Robert Shippen was the Standards and Health Protection Division QA Coordinator responsible for reviewing and recommending approval of all QAPPs that include scientific work being conducted by the Standards and Health Protection Division (SHPD) within OST.
- Blaine Snyder was the Tetra Tech Project Leader responsible for managing all aspects of the technical support being provided by Tetra Tech staff for the GLHHFTS.
- Susan Lanberg was the Tetra Tech QA Officer
- Harry McCarty was the GDIT Project Leader responsible for managing all aspects of the technical support being provided by GDIT staff for the GLHHFTS.
- Marguerite Jones was the GDIT QA Officer.
- Tony Olsen was the Senior Statistician at the ORD Western Ecology Division in Corvallis, Oregon supporting the GLHHFTS by providing technical expertise for study planning and implementation.

Under subcontract to GDIT, Microbac Laboratories in Baltimore, Maryland, stored the GLHHFTS fish samples and prepared the fillet tissue samples for analysis. For each sample, Microbac staff also prepared and held multiple aliquots of archived fillet tissue in a freezer at its facility to allow for further analyses of GLHHFTS samples in the future.

Five other commercial laboratories under subcontract to GDIT analyzed the GLHHFTS fish tissue samples for mercury, PCBs, PBDEs, PFAS, and omega-3 fatty acids, as shown below and in Figure 2.

Laboratory	Analysis Type
Brooks Rand Laboratories	Mercury
AXYS Analytical	PCB congeners
ALS-Canada	Polybrominated Diphenyl Ethers
TestAmerica (Sacramento)	PFAS
Southwest Research Institute	omega-3 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/2010-great-lakes-human-health-fish-tissue-study#results

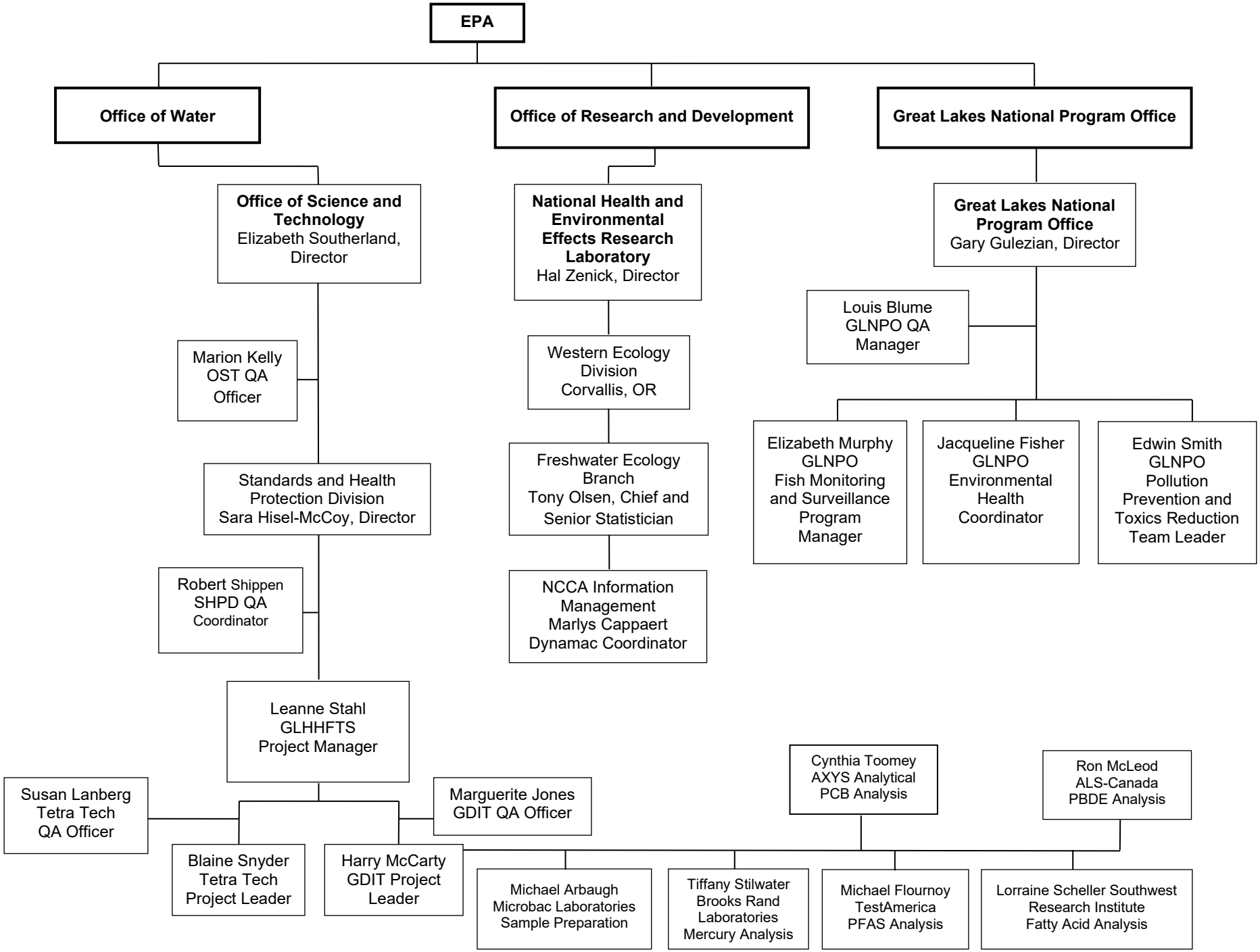


Figure 2. GLHHFTS project team organization

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 2010, OWOW developed the NCCA Quality Assurance Project Plan (USEPA 2010a) 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 GLHHFTS 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 June 2011, OST developed the QAPP that covers laboratory activities associated with GLHHFTS fish sample preparation and analysis of fillet tissue for mercury, PFAS, and fatty acids (USEPA 2011a). That QAPP was revised twice as funding became available to carry out additional types of analyses of the filet tissue samples. The first revision of the OST QAPP added PBDE analyses and was approved in October 2011 (USEPA 2011b). The second revision added PCB analyses and was approved in April 2012 (USEPA 2012).

The OST QAPP for the study presented performance criteria, acceptance criteria, and objectives for the analysis of mercury, PCBs, PBDEs, PFAS, and fatty acids in fish composites collected for the GLHHFTS. The QAPP also described the methods and procedures to be followed during the GLHHFTS to ensure that the criteria and objectives are met. The QAPP addressed mercury, PCBs, PBDEs, PFAS, and fatty acid analytical activities only. The QAPP was prepared in accordance with the most recent version of EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* (USEPA 2001), 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 Microbac in Baltimore, Maryland, on February 23, 2011, for all laboratory staff involved with GLHHFTS fish tissue sample preparation, to accomplish the following objectives:

- present GLHHFTS fish tissue preparation, homogenization and distribution procedures described in Appendix B of *NCCA GLHHFTS Tissue Preparation, Homogenization, and Distribution Procedures*,
- demonstrate filleting and homogenizing techniques with fish from invalid GLHHFTS 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 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 analytical activities QAPP (USEPA 2011a, 2011b, and 2012)
- 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 PBDE 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 QAPP (USEPA 2011a, 2011b, and 2012)
- 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 analytical activities 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, 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 Leader scheduled and tracked all analytical work performed by laboratories for mercury, PCB, PBDE, PFAS, and fatty acids analyses. The GDIT Project Leader also coordinated with staff at Microbac 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 samples collected during 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, composite, homogenize, and aliquot samples in a strictly controlled, contaminant-free environment. The methods employed by the sample preparation laboratory and by the five analysis laboratories are described below.

Section 3.1 Preparation of Fish Tissue Samples

Microbac served as the fish sample preparation laboratory for the study. In this role, Microbac 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 analysis to the designated analytical laboratory, and storing archived fish tissue samples in a freezer at its facility. The specific procedures for all GLHHFTS fish sample preparation activities are described in Appendix B of the QAPP for the study.

Fish were filleted by qualified technicians using thoroughly clean utensils and cutting boards (cleaning procedures are detailed in Appendix B of the QAPP for the study). Each fish was weighed to the nearest gram wet weight, rinsed with deionized water, and filleted on a glass cutting board. For the GLHHFTS, fillets from both sides of each fish 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).

An electric meat grinder was used to prepare homogenate 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. Microbac prepared fillet tissue aliquots according to the specifications listed in Step 15 of the fish sample preparation procedures in Appendix B of the QAPP for the study.

Section 3.2 Analysis of Fish Tissue Samples for Mercury

The fish tissue samples were initially analyzed using a combination of SW-846 Method 3051, a strong acid digestion procedure for solid matrices, and SW-846 Method 7470A, a cold-vapor atomic absorption procedure. However, the detection limit achieved by the laboratory for these samples resulted in a small number of fish tissue samples being reported as "not detected."

In response, EPA had all 157 samples reanalyzed by Brooks Rand Laboratories, using EPA Method 1631E (USEPA 2002), a more sensitive atomic fluorescence procedure that was utilized in the National Lake Fish Tissue Study, and the appendix to EPA Method 1631B (USEPA 2001) that describes the procedures for acid digestion and bromine monochloride (BrCl) oxidation of tissue samples prior to analysis. Mercury was detected in all 157 of the fish tissue samples during these later analyses. 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 AXYS Analytical Services, in general accordance with EPA Method 1668C (USEPA 2010c) 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 AXYS SOP deviates from the published EPA method in several aspects, including:

- Use of more ^{13}C-labeled extraction standards than called for in the method
- Modifications to the glassware cleaning procedures
- Modifications to the preparation procedures for some reagents
- Minor changes to the concentrations and volumes used for spiking solutions
- Extracting tissue samples with dichloromethane rather than toluene, to minimize the loss of some of the mono- through trichlorinated analytes
- Multi-point calibration of the gas chromatography/mass spectrometry system with all 209 PCB congeners, not just the 27 congeners specified in the method

The entire list of modifications is presented in detail in the study 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 PBDEs

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

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

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

The target analytes included 52 PBDE congeners and two other brominated analytes. Of the 47 PBDE congeners, 41 were determined as individual congeners and 6 were determined as coeluting pairs that could not be separated chromatographically. MDLs and MLs for the target PBDEs are listed in Appendix C of the study QAPP.

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, fish tissue samples were analyzed by the TestAmerica - Sacramento laboratory using procedures developed, tested, and documented in that

laboratory. The SOPs for those procedures are considered proprietary by the laboratory. However, the SOPs have been reviewed by GDIT and the analytical procedures are briefly described below.

Approximately 1 to 5 g of fish tissue were required for analysis. Samples were spiked with 13 isotopically labeled standards and extracted by shaking the tissue in a caustic solution of methanol, water, and sodium hydroxide. The hydroxide solution broke down the tissue and allowed the PFAS to be extracted into the methanol/water solution.

After extraction, the solution was centrifuged to remove the solids and the supernatant liquid was diluted with dilute hydrochloric acid (HCl) to a pH < 2. That diluted extract was processed by solid-phase extraction (SPE). The PFAS were eluted from the SPE cartridge and the eluant was spiked with additional labeled recovery standards and analyzed by high performance liquid chromatography-tandem mass spectrometry.

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

Tissue sample results were reported based on the wet weight of the tissue sample, in micrograms per kilogram (µg/kg), which are equivalent to the units of 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 products and not environmental contaminants. However, there are procedures for analysis of fats and oils available from some VCSBs, including the Association of Official Analytical Chemists (AOAC). The fatty acid samples for the study were analyzed by Southwest Research Institute (SwRI) using a combination of an extraction procedure from the literature and an AOAC analytical method, as described below.

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

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

Tissue results initially were reported as the fractional percentage of each fatty acid methyl ester, based on the wet weight of the sample. After consultation with EPA and representatives from the Great Lakes States, GDIT converted the fatty acid results to units of milligram per gram (mg/g). MDLs and MLs for the fatty acids are listed in Appendix C of the study QAPP.

Section 3.7 Analysis of Rinsates and Solvent Blanks

As noted in Section 2.3, Microbac prepared equipment rinsate samples and solvent blanks with each batch of fish fillet tissue samples. Rinsates and solvent blanks were prepared for mercury, PCBs and PBDEs (as one rinsate), PFAS, and fatty acids. Microbac analyzed the aqueous rinsate samples and solvent blanks for mercury using EPA Method 245.1, a cold-vapor atomic absorption procedure applicable to water samples. Rinsate results and solvent blanks for mercury were reported based on the volume of the sample, in micrograms (µg). Microbac also analyzed the hexane rinsates and solvent blanks for selected PCB and PBDE congeners using EPA Method 608, a gas chromatography procedure with an electronic capture detector, by calibrating the procedure for selected PCB and PBDE congeners specified by EPA. Rinsate results for PCBs and PBDEs were reported in micrograms (µg).

Microbac prepared the aqueous rinsate samples and solvent blanks for PFAS and held them until EPA and GDIT had obtained the services of the PFAS analysis laboratory, TestAmerica - Sacramento. TestAmerica extracted the aqueous samples using a solid-phase extraction (SPE) procedure and analyzed the extracts with a high performance liquid chromatography (HPLC) procedure that employs a tandem mass spectrometer detection system (MS-MS) and isotope dilution quantitation. Rinsate and solvent blank results for PFAS were reported in units of nanograms per liter (ng/L). GDIT converted those results to nanograms, based on the volume of the sample.

Microbac prepared the hexane rinsate samples and solvent blanks for fatty acids and held them until EPA and GDIT had obtained the services of the fatty acid analysis laboratory, Southwest Research Institute (SWRI). SWRI evaporated the fatty acid rinsates and solvent blanks to dryness with nitrogen and the fatty acids were derivatized following AOAC Method 991.39 (AOAC 1995). That method used boron trifluoride (BF_3) to derivatize the fatty acids to their methyl esters. The methyl esters were extracted with isooctane, concentrated, and analyzed by gas chromatography with a flame ionization detection system (GC/FID). Rinsate and solvent blank results for the omega-3 fatty acids were reported in units of micrograms per liter (µg/L).

Section 3.8 Quality Control Procedures

Fish Tissue Analyses

The analytical procedures applied by the laboratories designated for analysis of GLHHFTS 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
	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
	Laboratory control sample	One per sample batch
	Duplicate sample	One per sample batch
	Labeled compounds	Spiked into every field sample
PBDEs	Method blank	One per sample batch
	Laboratory control sample	One per sample batch
	Duplicate sample	One per sample batch
	Labeled compounds	Spiked into every field sample
PFAS	Method blank	One per sample batch
	Laboratory control sample	One per sample batch
	Matrix spike and matrix spike duplicate samples	One pair per sample batch
	Labeled compound recovery	Every field and QC sample
Fatty Acids	Method blank	One per sample batch
	Surrogate	Every field and QC sample
	Laboratory control sample	One per sample batch
	Reference material (NIST SRM 1946 Lake Superior fish tissue)	One per sample batch
	Duplicate sample	One per sample batch

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 several exceptions. First, the rinsate and solvent blank analyses for mercury, PCBs and PBDEs were prepared and analyzed individually, not in batches of up to 20, and analyzed at 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 fatty acids were held for later analyses and therefore were grouped together in batches, each with its own associated QC activities.) Secondly, because the rinsates for PCBs, PBDEs, and fatty acids were prepared in an organic solvent, 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 and solvent blank samples are described in Table 2.

Table 2. Quality Control Activities for Analysis of Rinsates and Solvent Blanks		
Analyte Type	**Quality Control Sample**	**Frequency**
Mercury	Instrument blank	With each rinsate sample
	Laboratory control sample	With each rinsate sample
PCBs and PBDEs	Instrument blank	With each rinsate sample
	Surrogate	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
Fatty Acids	Method blank	With each batch of rinsate samples
	Surrogate	Every field and QC sample
	Laboratory control sample	With each batch of rinsate samples

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 PCBs and PBDEs 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 is used for the fatty acids to assess the performance of the derivatization process applied to the analytes.

GDIT reviewed the results for the mercury, PCB, and PBDE rinsates and solvent blanks as soon as they were available from Microbac 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 Microbac to continue processing fish tissue samples.

A similar review approach was utilized for the PCB and PBDE rinsates and solvent blanks. None of the PCB congeners or PBDE congeners were detected in the rinsates samples, and "worst case" estimates similar to those described above for the mercury rinsate results were sufficient to authorize Microbac to continue processing fish tissue samples.

As noted earlier, the PFAS rinsate and solvent blank samples were analyzed after the end of the preparation of all of the fish samples and thus were not used to determine if Microbac could proceed with preparing additional batches of fish. Of the 13 PFAS constituents targeted in the study, 10 of them were detected in at least one of the eight rinsate samples, as presented in Table 3, but always at very low levels. The detection frequencies are show below:

Table 3. PFAS Detections in Rinsates			
PFAS	**# Rinsate Detections**	**PFAS**	**# Rinsate Detections**
PFBA	4	PFOS	2
PFPeA	2	PFNA	2
PFHxA	2	PFDA	1
PFHxS	1	PFUnA	1
PFOA	2	PFDoA	1

None of the PFAS were detected in two of the eight rinsates. Four of the other rinsates had two PFAS detected and one rinsate had two PFAS detected. One rinsate had detections for seven of the ten PFAS listed above. However, for the 10 PFAS that were reported, the "worst case" estimates of the potential contamination were 34 to 112 times below that corresponding tissue sample MDLs for those analytes. Therefore, in no instance was there any risk that the PFAS results reported in the fish tissue samples were the result of inadequate equipment cleaning.

As with PFAS, the fatty acid rinsate and solvent blank samples were analyzed after the end of the preparation of all of the fish samples and thus were not used to determine if Microbac could proceed with preparing additional batches of fish. None of the five fatty acids were ever detected in any of the rinsate or solvent blank samples.

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.

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 reanalyses, 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 developed a database to capture results for each sample and entered results of the data reviews directly in the database through the application of standardized data qualifier flags and descriptive comments concerning the reliability of the flagged results. Table 4 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 4. Individual SCC Codes Applied to the GLHHFTS 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.
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, 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.
HMSR	High Matrix Spike Recovery	High matrix spike (MS) recovery indicated a positive interference or a high bias. Isolated instances of high recovery are not uncommon, and patterns across multiple MS samples are more of a concern. When high matrix spike recovery was observed for an analyte, the results for that analyte in all of the samples in the batch with the matrix spike sample were qualified.
HMSR, RNAF	High Matrix Spike Recovery, Result Not Affected	High matrix spike (MS) recovery indicated a positive interference or a high bias, but the analyte was not detected in the sample, so there was no high bias concern for the specific sample and the RNAF flag was applied.
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, J	High CALVER, Estimated	The results for the calibration verification associated with the analyte were above the acceptance limit, suggesting a possible high bias. Detected analytes also are considered estimated values.
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.
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.

Table 4. Individual SCC Codes Applied to the GLHHFTS Results		
SCC Code	**Comments**	**Implication**
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.
LLCS	Low LCS result	The lab control sample (LCS) was a clean reference matrix. If recovery in the LCS was low, there may be a low bias for that analyte.
LMSR	Low Matrix Spike Recovery	Low recovery in the matrix spike indicated a potential low bias for the analyte, possibly due to poor extraction efficiency in the sample matrix. Isolated instances of low recovery are not uncommon, and patterns across multiple MS samples are more of a concern. When low matrix spike recovery was observed for an analyte, the results for that analyte in all of the samples in the batch with the matrix spike sample were qualified.
LSRM	Low Standard Reference Material Result	Applied only to the omega-3 fatty acid results, where NIST SRM1946, a Lake Superior fish tissue, was analyzed as the Standard Reference Material with each batch of field samples. Results for the SRM were compared to the "certified" values from NIST. A low result for the SRM indicated a potential low bias for the analyte, possibly due to poor extraction efficiency or loss during the derivatization process. Note that NIST does not provide a certified value for eicosatrienoic acid (ETE), only "reference" 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 rule described in Table 4 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

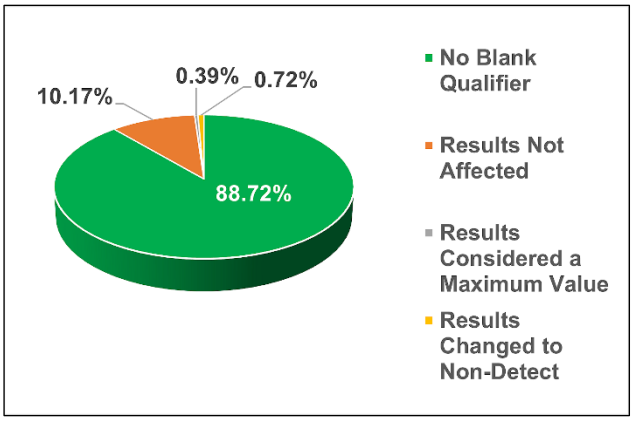


Figure 3. Impacts of Blank Contamination on the PCB Results

As shown in Figure 3, more than 98% of the PCB results were not affected by blank contamination, either because the analyte was not detected in the blank (88.72%) or because the concentration in the sample was more than 5 times the level observed in the blank (10.17%). For 0.39% 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. Only 0.72% of the PCB results were changed to nondetects (RNON) because of blank contamination.

4.2.3 Blanks for PBDE Analysis

As with the PFAS, there were very few data quality issues with PBDEs in the blanks, as illustrated in the figure to the right. Figure 4 shows that more than 99% of the PBDE results were not affected by blank contamination, either because the analyte was not detected in the blank (96.84%) or because the concentration in the sample was more than 5 times the level observed in the blank (2.93%). For 0.01% 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. Only 0.22% of the PBDE results were changed to nondetects (RNON) because of blank contamination.

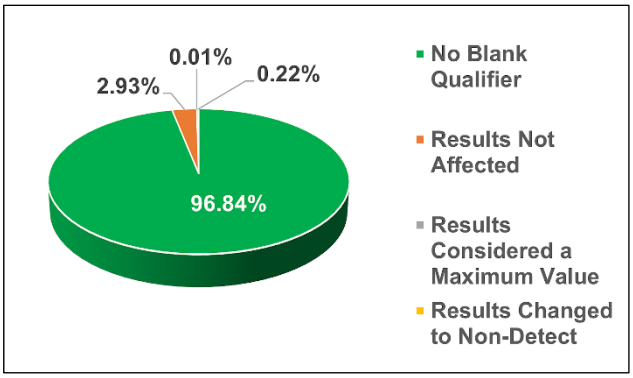


Figure 4. Impacts of Blank Contamination on the PBDE Results

4.2.4 Blanks for PFAS Analysis

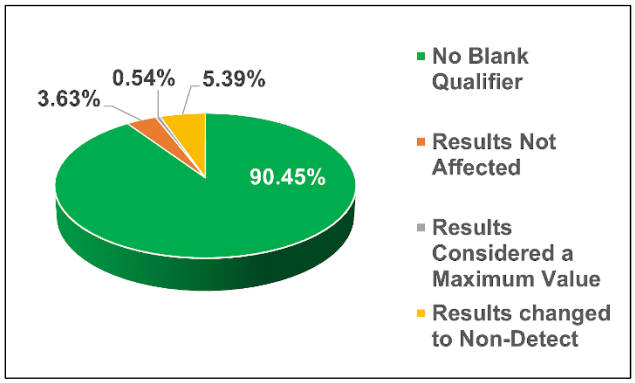


Figure 5. Impacts of Blank Contamination on the PFAS Results

Overall, there were few data quality issues with the blanks from the PFAS analyses, as illustrated in the figure to the right. Figure 5 shows that more than 94% of the PFAS results were not affected by blank contamination, either because the analyte was not detected in the blank (90.45%) or because the concentration in the sample was more than 5 times the level observed in the blank (3.63%). For 0.54% 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. The remaining 5.39% of the PFAS results were changed to nondetects (RNON) because of blank contamination, affecting only three analytes, PFBA, PFOA, and PFUnA, with all of those affected results initially reported by the laboratory between the MDL and the ML for the analyte.

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 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 limit. Therefore, no LCS qualifiers were applied to the PCB results for the study.

4.3.3 PBDE LCS Results

There were few data quality issues with the LCS results for the PBDE analyses. Figure 6 shows that 98.95% of the PBDE results were not affected by LCS issues. Only 1.05% of the PBDE results were qualified because of a low LCS result that might reflect a low bias in the results. All of the LCS qualifiers were applied to results for BDE-7, BDE-10, or BDE-116, and the vast majority of those results were reported as nondetects. Of the six detected results that were affected, five were results for BDE-7 that were already considered estimates because they were between the MDL and the ML for the sample. The one remaining result was for BDE-116, and that result may exhibit a low bias, based on the LCS results.

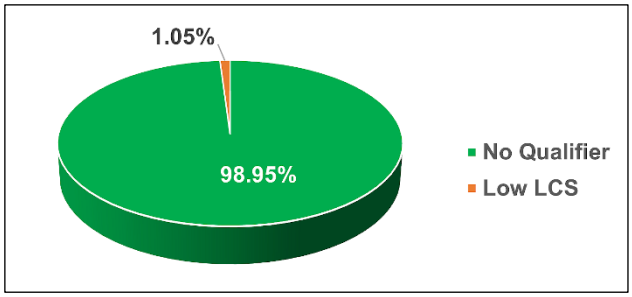


Figure 6. Impacts of LCS on the PBDE Results

4.3.4 PFAS LCS Results

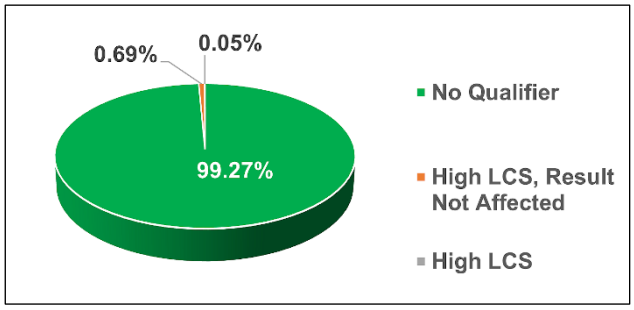


Figure 7. Impacts of LCS on the PFAS Results

There were very few data quality issues with the LCS results for the PFAS analyses, as illustrated in the figure to the right. Figure 7 shows that 99.95% of the PFAS results were not affected by LCS issues, either because the LCS results met the acceptance criteria (99.27%) or because the LCS results were above the acceptance limit, but the analyte was not detected in the sample (0.69%). Only 0.045% (0.05% after rounding) of the PFAS results were qualified because of a high LCS result that might reflect a high bias in the results. This represented one result for one PFAS analyte in one sample.

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, and compares the results to method- or project-specific acceptance criteria to provide information on the effects of the sample matrix on method performance.

A 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. 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 much greater chance of generating useful data on method and laboratory precision.

Alternatively, EPA methods such as those used for the PCBs and PBDEs 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 required that the laboratories performing analyses of fish tissue samples prepare and analyze MS/MS, and or duplicate samples with each batch of field samples as presented in Table 5.

Table 5. Matrix Spike, Matrix Spike Duplicate, and Duplicate Sample Requirements by Analysis Type

Analysis Type	Matrix Spike	Matrix Spike Duplicate	Duplicate
Mercury	X		X
PCBs			X
PBDEs			X
PFAS*	X	X	
Fatty acids			X

* The PFAS method developed by laboratory included the use of both labeled compounds and MS/MSD analysis at no additional cost. Therefore, EPA opted to use the laboratory's default QC approach of the MS/MSD instead of just a laboratory duplicate sample.

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 99.92% of the PCB results not affected by duplicate issues. Given that only 0.08% of results were affected by high RPD values, a pie chart has not been included in this section because the tiny sliver of affected results would not be visible.

4.4.3 PBDE Duplicate Sample Results

The PBDE laboratory duplicate analyses exhibited very good precision. Figure 8 shows that approximately 97.82% of the PBDE results were not affected by duplicate issues, either because the duplicate results met the acceptance criteria (97.43%) or because the duplicate results were above the acceptance limit, but the analyte was not detected in the sample (0.39%). The remaining 2.19% (after rounding) of the results may have a slightly greater uncertainty because of the observed duplicate precision.

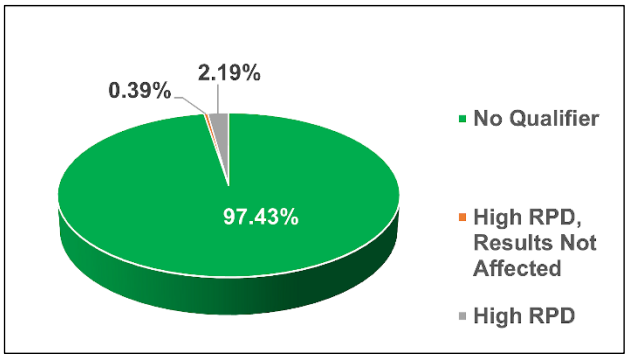
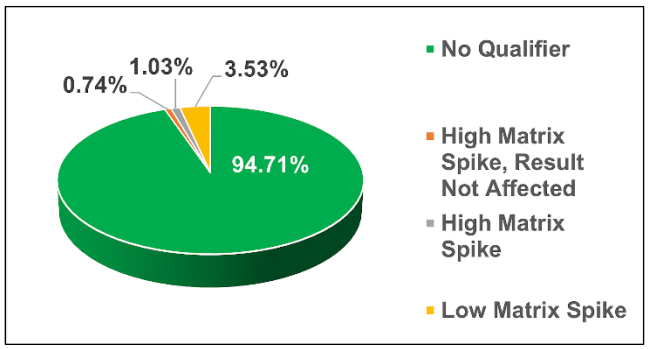


Figure 8. Impacts of Duplicates on the PBDE

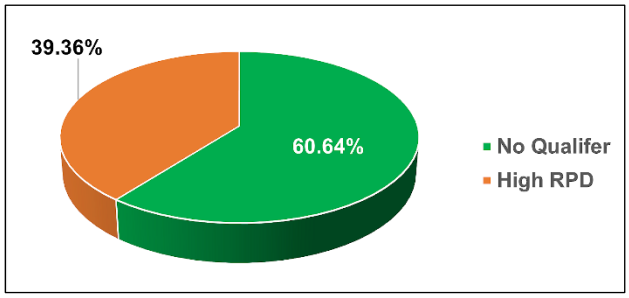
4.4.4 PFAS Matrix Spike and Matrix Spike Duplicate Sample Results



There were very few data quality issues with the MS/MSD results for the PFAS analyses, as illustrated in the figure to the right. Figure 9 shows that 95.45% of the PFAS results were not affected by MS/MSD issues, either because the MS/MSD results met the acceptance criteria for spike recovery (94.71%) or because the MS/MSD results were above the acceptance limit, but the analyte was not detected in the sample (0.74%). Only 1.03% of the detected PFAS results were associated with high matrix spike recoveries that suggest a high bias. The remaining 3.53% of the PFAS results were associated with low matrix spike recoveries that suggest a low bias. Of the results associated with low matrix spike recoveries, 20 of the 21 results were for one analyte, PFBS. All of the MS/MSD analyses for PFAS met the acceptance criterion for precision.

4.4.5 Fatty Acid Duplicate Sample Results

The duplicate sample results for the fatty acids presented the greatest data quality concerns of this study. Overall, as shown in Figure 10, only 60.64% of the results were associated with a laboratory duplicate sample that meet the QC acceptance criteria. The remaining 39.36% of the results were associated with duplicate analyses that did not meet the criterion. GDIT and the laboratory examined the fatty acid results in detail, searching for a potential cause for the high relative percent differences (RPDs) observed in many of the laboratory duplicates. No obvious cause was apparent, but several factor may have contributed to the issues.



First, because there were no formal methods for the fatty acids readily available, it was difficult to establish the QC acceptance criterion for duplicate precision. The laboratory estimated that the

instrumental analysis itself could likely achieve an RPD of less than or equal to 20%. GDIT and EPA utilized that estimate in preparing the project QAPP and reviewing the fatty acid results, but in practice, it may have been too optimistic for a naturally occurring component of these fish tissues. A total of 309 individual fatty acid results were qualified due to a high RPD in one of the eight laboratory duplicate samples analyzed during the course of the study (e.g., one duplicate sample per batch of 20 field samples). Of those 309 qualified results, 100 were instances where the RPD between the original sample and its duplicate was between 20 and 24%. Had the acceptance limit been set at 25% instead of 20%, these 100 results would not have been qualified.

Another 56 instances involved the situation where one result for either the original or duplicate sample was reported as a nondetect and the other result was detected at a low level. In such instances, the RPD cannot be calculated, and the associated field sample results are qualified as failing to meet the acceptance criterion. This situation might have been overcome through the use of paired MS/MSD analyses in which the spiked analytes would eliminate the chance of a nondetect. However, in developing the QAPP, GDIT did not believe that the fatty acids could be spiked into the ground tissue samples in a manner that would reflect their natural occurrence within the tissue itself.

Ultimately, because the fatty acids are not environmental contaminants, GDIT and EPA agreed that the results could be presented as generated, and that the data users would be provided with the data necessary to understand the potential implications of the observed duplicate precision.

Section 4.5 Surrogates and Labeled Compounds

A surrogate is a compound that is chemically similar to the analytes of interest but 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 3.4, the fatty acid laboratory added a known amount of triheneicosanoin, a C_{21}-triglyceride, 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 compound 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 provide 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 29 ^{13}C-labeled PCBs to each sample before extraction. The PBDE laboratory added known amounts of 14 ^{13}C-labeled PBDEs and a ^{13}C-labeled compound for one additional analyte (HBB) to each sample before extraction. The PFAS laboratory added known amounts of 12 ^{13}C-labeled PFAS and one ^{18}O-labeled PFAS to each sample before extraction.

The QAPP for the study 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.

No surrogates or labeled compounds were employed for the mercury analyses.

4.5.1 PCB Labeled Compound Recoveries

Virtually all of the labeled compound recoveries for the PCB samples met the QC acceptance limits. Given that only 0.004% 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.

4.5.2 PBDE Labeled Compound Recoveries

Likewise, the vast majority (99.04%) of the labeled compound recoveries for the PBDE samples met the QC acceptance limits. Given that only 0.96% 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

Recovery of the labeled compounds during the PFAS analyses presented a greater challenge than for any other of the analysis types. As can be seen in Figure 11, only 82.22% (after rounding) of the PFAS results are associated with labeled compound recoveries that met the acceptance criteria. The remaining 17.79% of the PFAS results had recoveries of one or more labeled compounds that fell below the acceptance criteria. Approximately one third of those low labeled compound recoveries were for PFOSA. Early in the course of the study, the PFAS laboratory noted a relationship between low recoveries and the appearance of the sample extracts. Samples that exhibited low recoveries of labeled compounds were ones where the solvent extract from the sample had a milky layer floating on top of the solvent. The effect appeared to be related to the fish species, but not the lipid content of the samples. Many of the lowest recoveries occurred in non-salmonid species such as yellow perch, walleye, and freshwater drum.

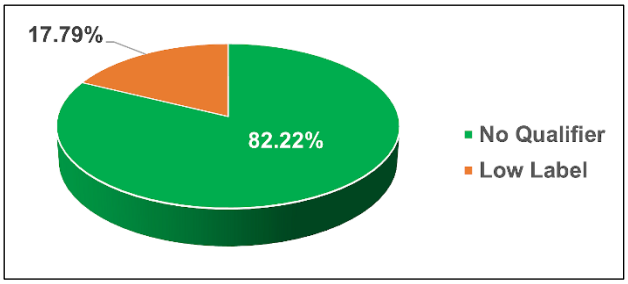


Figure 11. Impacts of Labeled Compound Recovery on the PFAS Results

The laboratory consulted GDIT and EPA and agreed on a plan to re-extract and reanalyze a modest number of fish tissue samples using different initial masses of tissue and different final extract volumes. After review of those results, the PFAS procedure was modified to reduce the tissue mass that is extracted from 5 g to 1 g, yet concentrate the extract to a smaller final volume to maintain the overall sensitivity of the method. Samples analyzed during the early part of the study that low labeled compound recoveries were re-extracted using the smaller tissue mass and generally exhibited improved labeled compound recoveries.

Given the constraints on time and funding, it was not possible to more fully investigate the potential effect of fish species on labeled compound recovery. Because the labeled compounds behave similarly to their unlabeled ("native") counterparts, they are generally extracted with the same efficiency as the target analytes and any loss of the target analytes during subsequent cleanup procedures will be mirrored by similar loss of the labeled compounds. Therefore, using isotope dilution quantitation procedures, the final

results for each target analyte includes a correction for the recovery of the corresponding labeled compound that compensates for such losses during processing. Isotope dilution quantitation functions well even when the recovery of the labeled compound is below a consensus-type acceptance criterion. The results from this study suggest that if EPA pursues development of a formal EPA method for PFAS in fish tissue, that effort should look at performance in many different species of fish and that QC acceptance criteria for labeled compound recovery be based on studies of typical fish tissue samples, not dried reference materials or simulated tissue matrices.

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 PBDEs 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 PBDEs and PCBs 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 PBDEs and PCBs in Sections 4.6.1 and 4.5.2.

4.6.1 PCB Ion Abundance Ratios

The PCB results exhibited fewer ion abundance ratio concerns, with 98.56% of the results meeting the acceptance criteria. The remaining 1.46% (after rounding) of the PCB results were divided among those with higher than expected ion abundance ratios (0.94%) and those with lower than expected ion abundance ratios (0.51%). Given that only 1.46% of results were affected by ion abundance ratio concerns, a pie chart has not been included in this section.

4.6.2 PBDE Ion Abundance Ratios

Overall, 90.42% of the PBDE results were not qualified because of ion abundance ratio concerns. As shown in Figure 12, the remaining 9.58% (after rounding) of the PBDE results were approximately equaling divided among those with higher-than-expected ion abundance ratios (4.60%) and those with lower-than-expected ion abundance ratios (4.97%). The roughly equal distribution of high and low ratios suggests that any interferences being extracted from the fish tissue samples are not systematically affecting the numerical results for the PBDEs in one direction or the other.

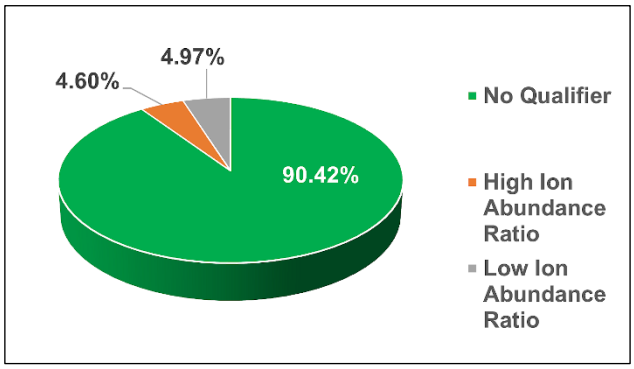


Figure 12. Impacts of Ion Abundance Ratio on the PBDE Results

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 1946, which is a frozen fish tissue homogenate which was prepared from lake trout (*Salvelinus namaycush namaycush*) collected from Lake Superior. The NIST certificate of analysis provides "certified concentration values" for PCB congeners, chlorinated pesticides, and fatty acids. Those fatty acids include four of the five target analytes in this study. NIST does not provide a certified value for eicosatrienoic acid (ETE), only a "reference" value that may not reflect the same degree of numerical certainty as the "certified values" for the other four fatty acids.

During data review, the results from the analysis of NIST SRM 1946 associated with each batch of field samples in this study were compared to the four certified values and the one reference value for the fatty acids. The implications of the SRM results on data quality for the fatty acids are illustrated in Figure 13. Overall, 87.64% of the fatty acid results are associated with SRM results that agreed with the certified values for the analyte. The remaining 12.36% of the results were associated with lower SRM results. All of those lower values were for ETE, the analyte for which NIST only provides a "reference value" rather than a "certified value." Many of those ETE results are only a small percentage outside of that reference value. Moreover, the ETE results for 60 of the 157 samples in the study are associated with SRM results for ETE that meet the acceptance criteria.

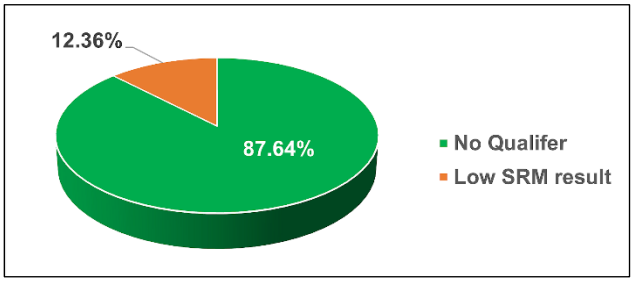


Figure 13. Impact of SRMs on the Fatty Acid Results

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 in this study is 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, PCBs, PBDEs, PFAS, and fatty acids) yields a total of 230 measured results for each sample (based on 159 results that cover all 209 PCB congeners). For the 157 samples collected for the GLHHFTS, the total number of sample/analyte combinations is 36,110.

Despite the data quality concerns outlined in this report, all 157 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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