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Quality Assurance Report for the National Lakes Assessment 2022 Fish Tissue Study

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Acknowledgements

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Chapter 1
Introduction

This report documents the quality of data gathered during the 2022 National Lakes Assessment Fish Tissue Study, which was a component of EPA's Office of Wetlands Oceans and Watersheds (OWOW) 2022 National Lakes Assessment (NLA), a probability-based survey designed to assess the condition of lakes in the United States, excluding the Great Lakes. 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 NLA, and within the Office of Water (OW), the Office of Science and Technology (OST) that conducted the fish tissue study under the NLA.

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. That 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. Since 2008, OST has collaborated with OWOW and ORD to conduct a series of probability-based studies of freshwater fish contamination. These include three national-scale studies of river fish contamination, three regional-scale studies of fish contamination in the five Great Lakes, and this study, the 2022 National Lakes Assessment (NLA). The 2022 NLA is the first national study to analyze fish fillet tissue from inland lakes for PFAS.

Section 1.2 Study Design

OST collaborated with ORD's Pacific Ecological Systems Division (ORD-PESD) in Corvallis, Oregon, to conduct the 2022 NLA Fish Tissue Study within the broader framework of the 2022 NLA. The following were the key design components for the 2022 NLA Fish Tissue Study:

- Sampling up to 636 lakes designated as fish fillet tissue contaminants indicator (FTIS) sites (which are equivalent to NLA 2022 Fish Tissue Study sampling sites)
- 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, and 40 per- and polyfluoroalkyl substances (PFAS)

A total of 413 valid fish samples were collected for the study at a statistical subset of the 636 NLA sites (Figure 1). All of fish samples were collected between May and September 2022.

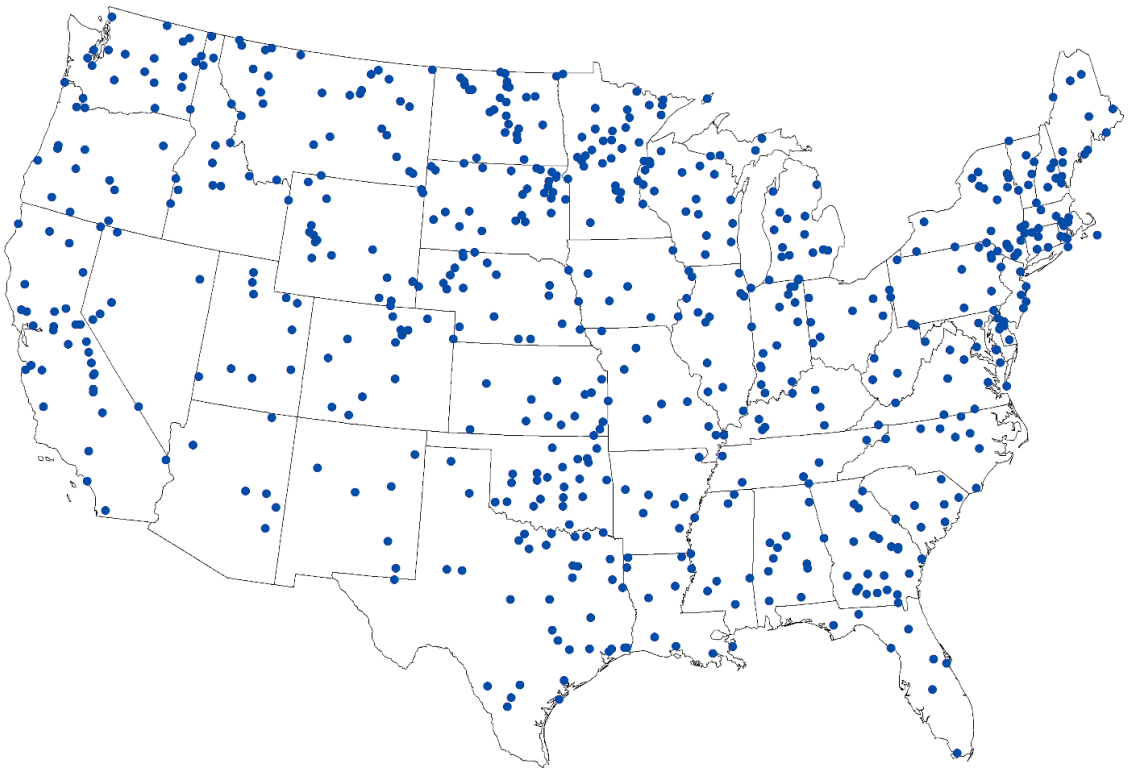

Figure 1. 2022 NLA Sampling Locations

EPA stored the 2022 NLA 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. Tetra Tech prepared the homogenized fish fillet tissue samples for analysis as outlined in the fourth bullet above. The sample preparation laboratory prepared aliquots of fillet tissue for mercury, PCBs, and PFAS analyses, and as archive tissue samples. Commercial environmental laboratories analyzed the 2022 NLA fish fillet tissue samples for mercury, PCB congeners, and PFAS 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 Analysis of the 2022 National Lakes Assessment Fish Fillet Samples for Mercury, Per- and Polyfluoroalkyl Substances, and Polychlorinated Biphenyls* (USEPA 2022b).

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

Section 1.3 Study Participants

The 2022 NLA Fish Study project team consisted of managers, scientists, statisticians, and QA personnel from OST and the ORD Pacific Ecological Systems Division, along with contractors providing scientific and technical support to OST from Tetra Tech, Inc. and GDIT (Figure 2). Key members of the project team are listed below.

- John Healey (OST) was the 2022 NLA Fish Study Project Manager who provided overall direction for planning and implementation of this study.

- Joe Beaman 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.
- 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 2022 NLA.
- Tara Cohen was the Tetra Tech staff member responsible for preparing and distributing sampling supplies, preparing sample shipments to the analytical laboratories, and assisting with the sample preparation.
- Susan Lanberg was the Tetra Tech QA Officer.
- Yildiz Chambers-Velarde was the GDIT Task Order Manager who was responsible for managing all aspects of the administrative support provided by GDIT staff for the 2022 NLA.
- Harry McCarty was the GDIT Project Leader who was responsible for managing all aspects of the technical support provided by GDIT staff for the 2022 NLA.
- Emily Surpin was the GDIT QA Coordinator.
- Tony Olsen was the Senior Statistician at the ORD Pacific Ecological Systems Division in Corvallis, Oregon who supported the 2022 NLA Fish Study by providing technical expertise for study design planning and statistical analysis of fish tissue data.

Three commercial laboratories analyzed the 2022 NLA Fish Study fish tissue samples for mercury, PCBs, and PFAS, under purchase orders from GDIT, as shown below and in Figure 2, on the next page.

Laboratory	Analysis Type
ALS-Environmental	Mercury
SGS AXYS Analytical	PFAS
Enthalpy Analytical	PCB congeners

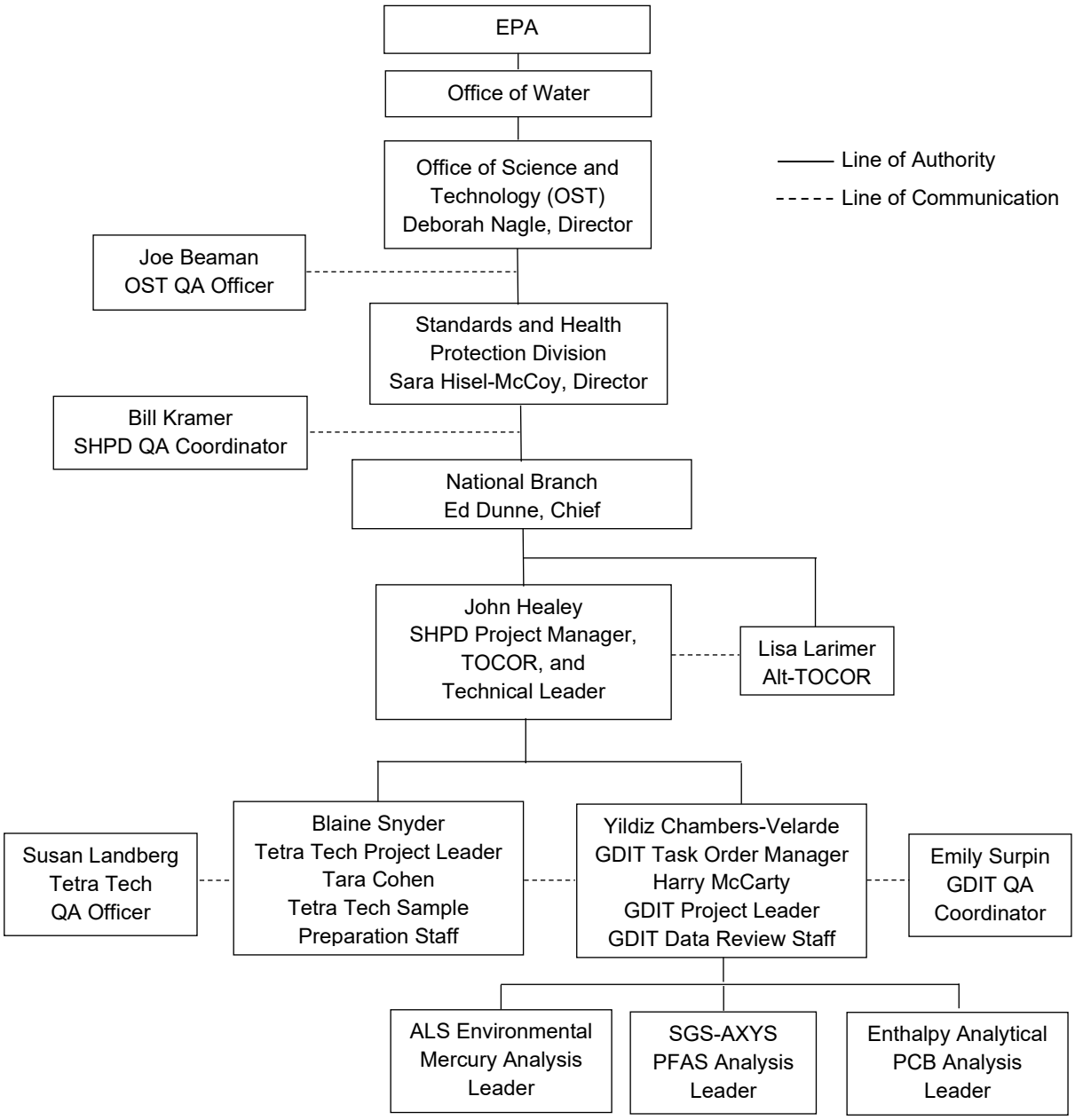

Figure 2. 2022 NLA Fish Study Project Team Organization

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/choose-fish-and-shellfish-wisely/2022-national-lakes-assessment-fish-tissue-study

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

Three separate Quality Assurance Project Plans (QAPPs) are associated with this study, with multiple revisions of each plan over the course of the study. In May 2022, OWOW developed Version 1.1 of the *National Lakes Assessment 2022 Quality Assurance Project Plan* (USEPA 2022a) that described the procedures and associated quality assurance/quality control (QA/QC) activities for collecting and shipping NLA samples of all types. It included the human health fish collection and shipping procedures that OST developed for the 2022 NLA Fish Study, 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 2022, OST developed the *Quality Assurance Project Plan for 2022 National Lake Assessment (NLA) Fish Tissue Study Sample Preparation*, that described the procedures and QA/QC activities associated with 2022 NLA Fish Study fish sample preparation and tissue homogenization effort (USEPA 2022b). OST subsequently developed Revision 1 of that QAPP in September 2023 (USEPA 2023d)

In January 2023, OST developed the *Quality Assurance Project Plan for Analysis of the 2022 National Lakes Assessment Fish Fillet Samples for Mercury, Per- and Polyfluoroalkyl Substances, and Polychlorinated Biphenyls, Revision 0*, which covered the activities associated with 2022 NLA Fish Study fish sample analysis for mercury and included placeholders for the PFAS and PCB analyses (USEPA 2023a). That QAPP was revised two times to cover additional analyses of the fillet tissue samples. The first revision of this OST QAPP added PFAS analyses and was approved in May 2023 (USEPA 2023b). The second revision added PCB analyses and was approved in August 2023 (USEPA 2023c).

The OST QAPPs for the study presented performance criteria, acceptance criteria, and objectives for the preparation and analysis of mercury, PCBs, and PFAS in fish composites collected for the 2022 NLA. The QAPPs also described the methods and procedures to be followed during the study to ensure that the criteria and objectives are met. All of the OST QAPPs were 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 in early June 2022 at Tetra Tech in Owings Mills, Maryland for all laboratory staff involved with 2022 NLA Fish Study fish tissue sample preparation, to accomplish the following objectives:

- Present 2022 NLA Fish Study fish tissue preparation, homogenization and distribution procedures described in Appendix B of the *Quality Assurance Project Plan for 2022 National Lake Assessment (NLA) Fish Tissue Study Sample Preparation* (USEPA 2022b),
- Demonstrate filleting and homogenizing techniques with fish using invalid 2022 NLA Fish Study 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 sample preparation activities in the QAPP (USEPA 2022b and USEPA 2023d)
- 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 mercury rinsate samples following completion of each sample preparation batch
- Review and acceptance of mercury rinsate results by EPA before proceeding with preparation of additional samples
- Retention of the PCB and PFAS rinsates for later analyses and evaluation

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 2023a, b, and c)
- 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 method-specific 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 of method-specific isotopically labeled compounds spiked into tissue samples or of matrix spike/matrix spike duplicate samples, 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 Leader scheduled and tracked all analytical work performed by the laboratories for mercury, PCB, and PFAS analyses. The GDIT Project Leader also coordinated with staff at Tetra Tech regarding fish tissue sample shipments.

When samples were shipped to an analytical laboratory, the GDIT Project Leader contacted the designated laboratory staff by email to notify them of the forthcoming shipment(s) and requested that they contacted GDIT if the shipments did not arrive intact, as scheduled. GDIT also tracked the shipments through the carrier's web site. 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 worked 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 and immediately notified the OST 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 situations when 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.

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 (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 three analysis laboratories are described below.

Section 3.1 Preparation of Fish Tissue Samples

As noted earlier, 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 archiving, 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 the leased freezers at Microbac for long-term storage. The specific procedures for all 2022 NLA Fish Study fish sample preparation activities are described in Appendix B of the sample preparation QAPP for the study (USEPA 2022b).

Fish were filleted by qualified technicians using thoroughly cleaned 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 NLA, as in past SHPD studies, 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 prepare homogenate samples. Entire fillets (including the 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 fillet tissue aliquots according to the specifications listed in Step 18 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 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). 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 Enthalpy Analytical, in general accordance with EPA Method 1668C (USEPA 2010) 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 Enthalpy SOP deviated 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 study analytical QAPP (USEPA 2023c). 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 PFAS

By the time that the PFAS analyses for this study began, OW's Engineering and Analysis Division had issued the 3rd draft of EPA Method 1633 for PFAS analyses (USEPA 2022c). The draft method includes procedures for the analysis of aqueous, solid, biosolid, and tissue samples. Therefore, the PFAS samples were analyzed by SGS AXYS Analytical Services, Ltd. (Sidney, BC, Canada) using that 3rd draft of Method 1633. Tissue sample results were reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g).

Section 3.5 Analysis of Rinsates and Solvent Blanks

As noted in Section 2.3, Tetra Tech prepared equipment rinsate samples and solvent blanks with each batch of fish fillet tissue samples. Aqueous rinsates and aqueous solvent blanks were prepared for mercury and PFAS analyses and hexane rinsates and hexane solvent blanks were prepared for PCB analysis. As each sample preparation batch was completed, Tetra Tech shipped the mercury rinsate samples and solvent blanks to the ALS laboratory in Kelso, WA, where they were analyzed for mercury using EPA Method 245.1 (USEPA 1983) Rinsate and solvent blank results for mercury were reported in micrograms per liter (µg/L).

Tetra Tech stored the aqueous rinsate and solvent blank samples for PFAS analyses and the hexane rinsate and solvent blanks for PCBs until after the completion of the tissue sample preparation effort. This process allowed GDIT to more effectively group the rinsates and blanks into analysis batches at each laboratory. Tetra Tech shipped the PFAS rinsates and solvent blanks to SGS-AXYS Analytical Services (Sydney, BC, Canada) after the last batch of tissue samples had been prepared. SGS-AXYS analyzed the rinsates and solvent blanks using the 3rd draft of EPA Method 1633 (USEPA 2022c) and reported the results in nanograms per liter (ng/L).

Because the PCB rinsates and solvent blanks were prepared in hexane, shipment of those samples required specialized packaging and shipping. After the last batch of tissue samples had been prepared, GDIT retrieved the PCB rinsates and solvent blanks from Tetra Tech and delivered them to a specialty packaging firm in northern Virginia that shipped the PCB rinsates and solvent blanks to shipped Enthalpy

Analytical. The PCB rinsate and solvent blank samples were analyzed using EPA Method 1668C (USEPA 2010) and reported in nanograms per liter (ng/L).

Section 3.6 Quality Control Procedures

Fish Tissue Analyses

The analytical procedures applied by the laboratories selected for analysis of 2022 NLA Fish Study fish tissue samples included the traditional EPA analytical quality control activities. In most cases, samples were grouped together in batches of 20 field samples, based on the sample preparation batches prepared by Tetra Tech. However, of the 21 batches of fish tissue samples prepared by Tetra Tech, there was one batch of 19 field samples and another of 14 field samples, and the analytical laboratories adhered to those batch sizes during the analyses.

The 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 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 and QC sample
	Non-extracted internal standards	Spiked into every field and QC sample extract
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	One per sample batch of up to 20 field samples
	Extracted internal standards, a.k.a. labeled compounds	Every field and QC sample before extraction
	Non-extracted internal standards	Spiked into every field and QC sample extract

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 were prepared as individual pairs, not in batches of up to 20 samples, and analyzed by the laboratory under contract to the sample preparation laboratory, in order to provide timely feedback of the cleanliness of the homogenization equipment. The rinsates and solvent blanks for PCBs and PFAS 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 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 and solvent blanks 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	Instrument blank	With each rinsate sample
	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
	Extracted internal standards	Added to 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, so a separate matrix spike sample was not required.

Because the rinsates for PCBs were prepared from hexane and no sample extraction was required, "matrix effects" were not possible. Therefore, a laboratory control sample and matrix spike and duplicate samples were not required for the PCB rinsate samples.

Mercury Rinsates

GDIT reviewed the results for the mercury rinsates and solvent blanks as soon as they were available from Tetra Tech's subcontracted laboratory and relayed the review findings to EPA and Tetra Tech within hours of receipt of the results. Mercury was not detected above ALS's MDL in the rinsate or solvent blank samples for 18 of the 21 sample preparation batches in the study. For the other three batches, mercury was detected in three of the rinsate samples and two of the blanks. For the two batches where mercury was reported in both samples, the concentrations of both the rinsates and blanks were 0.03 and 0.04 µg/L, respectively, versus an MDL of 0.02 µg/L. The rinsate for the third affected batch had a concentration of 0.03 µg/L, and the blank in that case was a non-detect. None of those results exceeded the 1 µg/L acceptance limit in the sample preparation QAPP (2022b) for the rinsates and solvent blanks.

The fish preparation QAPP stated that the mercury rinsates would be evaluated based on the mass of each analyte detected, and assuming that all the apparent contamination could be transferred to a nominal 410-g mass of homogenized tissue. However, in making its assessments of the rinsate results, GDIT went a step further and took a very conservative approach and assumed that the mercury in the rinsates theoretically might be transferred to the *smallest* bulk homogenized tissue sample in the sample batch (due to inadequate cleaning of the homogenization equipment). The smallest bulk tissue mass was well below the 410-g mass listed in the QAPP. GDIT compared that "worst case" estimate to the MDL for mercury in tissues. For the 18 rinsates where mercury was *not* detected, GDIT assumed that mercury *could* be present in the rinsate sample at exactly the MDL. In those cases, the "worst case" estimate was at least 6 times lower than the tissue sample MDL, and thus any contribution from the sample processing equipment would not be detectable.

For the three rinsates with results above the MDL, GDIT performed similar calculations using the smallest bulk composite tissue sample in each of those batches, as well as comparing the "worst case"

estimate against the lowest mercury concentration reported for the associated sample batch. The results of those assessments are shown in Table 3 below.

Table 3. Assessment of Mercury Rinsates

Batch	Rinsate Result (µg/L)	Rinsate Volume (L)	Total Mass (ng)	Smallest Homogenate (g)	Worst Case Concentration (ng/g)	Nominal Tissue MDL (ng/g)	Lowest Result in Batch (ng/g)
14	0.03	0.25	7.5	61.0	0.123	0.08	15.6
16	0.04	0.25	10	156.5	0.064	0.08	18.3
17	0.03	0.25	7.5	118.0	0.064	0.08	6.4

The "worst case" estimates for the rinsates from Batches 16 and 17 in Table 3 would be theoretical tissue sample concentrations *below* the nominal tissue MDL and thus not detectable. The "worst case" estimate for the rinsate from Batch 14 is slightly above the nominal tissue sample MDL and thus would have been detectable. However, the composite sample in Batch 14 with the smallest homogenate mass (61.0 g) also was the tissue sample with the lowest reported mercury result for any sample in that batch. The reported mercury concentration in that sample, 15.6 ng/g, was more than 125 times higher than the "worst case" estimate, and any mercury from the homogenization equipment would have contributed less than 0.8% of the reported sample result.

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.

PCB Rinsates

As noted earlier, the PCB rinsate and solvent blank samples were analyzed after the end of the preparation of all the fish samples and thus were not used to determine if Tetra Tech could proceed with preparing additional batches of fish. As a result, the fish preparation QAPP did not contain acceptance criteria for either the PCB rinsate or solvent blank samples. The analytical QAPP included the QC operations from the PCB method and their respective acceptance criteria, but did not establish limits on the concentrations of the PCB congeners, in part because, as noted above, the rinsates and solvents blanks were not being used to determine if Tetra Tech could proceed with preparing additional batches of fish.

Therefore, GDIT evaluated the PCB rinsates and solvent blanks using the same approach that was used in several earlier SHPD studies, including the 2020 Great Lakes Human Health Fish Fillet Tissue Study. In those earlier studies where the results from the PCB rinsates *were* used to determine if Tetra Tech could proceed with preparing additional batches of fish, the studies set the acceptance limit at 0.5 ng/mL of any congener in the rinsates or solvent blanks.

GDIT's assessment of the PCB rinsates and solvent blanks was complicated by the fact that some of the containers had leaked at some point during storage at Tetra Tech or during the transfer to GDIT for specialty packaging and shipment. GDIT noticed the problem when the samples were put into GDIT's refrigerated storage. Some of the lids were loose on arrival and the bubblewrap bags smelled of hexane, the solvent used for the rinsates, and the ink on some of the jar labels was smeared; however there was no other sign of liquid solvent in the bags that would suggest that the leaks occurred during the transfer. In total, 19 of the 42 containers were affected to varying degrees. GDIT advised the PCB laboratory of the situation and worked with them to develop an appropriate analysis scheme for these samples. The laboratory measured the solvent volume in each container and reported the results in "picograms per liter." GDIT subsequently used the data on the volume of rinsate or solvent blank in the container and converted the concentration in pg/L to the concentration in ng/mL that would have been present in the

original 100-mL volume of the rinsate or solvent blank. GDIT compared those results to the acceptance limit of 0.5 ng/mL.

The sample container for the Batch 6 solvent blank was completely dry by the time it arrived at the laboratory and no analysis of that sample was possible. In addition, the sample for the Batch 8 Rinsate was lost during sample processing.

The remaining 40 rinsate and solvent blank samples were analyzed in two separate analysis batches and there were large numbers of "hits" in most of the samples. This was largely due to the extreme sensitivity of Method 1668C and the prevalence of PCBs in all laboratory environments, despite typical precautions at both the fish sample preparation laboratory and the analysis laboratory. For example, there were as many as 25 PCB congeners reported in the various method blanks prepared and analyzed by the analytical laboratory. However, all were reported at very low concentrations and many of those congeners were not reported in the associated rinsates or solvent blanks.

In other cases, the results for the paired rinsates and solvent blanks had very similar results for some congeners, indicating that the PCBs in the rinsates often did not come from the fish processing equipment, but were present in the original solvent.

Overall, 105 congeners were reported across the 20 rinsates and 20 solvent blanks. However, even the highest of those results, 0.0825 ng/mL for PCB-153 in the Batch 1 Rinsate sample, was more than 60 times *lower* than the 0.5 ng/mL acceptance limit, and most of the other PCB results were much lower than that highest result. Therefore, GDIT concluded that *none* of the PCB rinsates represented any risk to the tissue sample PCB results for the 413 fish tissue samples.

PFAS Rinsates

As noted earlier, the PFAS rinsate and solvent blank samples were analyzed after the end of the preparation of all the fish samples and thus were not used to determine if Tetra Tech could proceed with preparing additional batches of fish. GDIT examined all 42 of the PFAS rinsates and blanks. Of the 40 PFAS target analytes in Method 1633, only five were ever reported in these samples, and only once each for those five analytes (5/1680 = 0.3% occurrence).

The only rinsate with any detectable PFAS was that for Batch 2 of the fish tissue samples. That rinsate contained four PFAS analytes: PFPeA, PFHxA, PFHpA, and 5:3 FTCA. PFPeA and PFHxA were reported at concentrations well above the PFAS laboratory's MDLs for aqueous samples, while PFHpA and 5:3 FTCA were reported at concentrations above their MDLs, but below or almost equal to the laboratory's quantitation limits. The fact that these four analytes were not reported in the other 20 rinsate samples indicates that their presence is likely the result of some sporadic contamination, and not a systematic problem with the fish tissue preparation procedures.

PFBA was only reported in the Batch 10 solvent blank, not in the associated rinsate sample from that batch, nor in any tissue sample in Batch 10. PFBA was only detected in 3 of the 413 fish samples in the study.

GDIT evaluated the PFAS rinsate results in the same manner used for the mercury rinsates. Table 4 presents the PFAS rinsate and solvent blank results and their implications for the fish tissue sample results.

Table 4. Assessment of PFAS Rinsate and Solvent Blank Results							
Sample	**Analyte**	**Result (ng/L)**	**Total Mass (ng)**	**Smallest Homogenate (g)**	**Worst Case Concentration (ng/g)**	**Nominal Tissue MDL (ng/g)**	**Detectable?**
Batch 2 rinsate	PFPeA	18.9	4.574	76.0	0.06	0.08	**No**
Batch 2 rinsate	PFHxA	13.8	3.340	76.0	0.04	0.18	**No**
Batch 2 rinsate	PFHpA	2.70	0.653	76.0	0.01	0.08	**No**
Batch 2 rinsate	5:3 FTCA	83.0	20.09	76.0	0.26	3.14	**No**
Batch 10 blank	PFBA	9.76	2.303	47.5	0.05	1.60	**No**

The smallest mass of homogenized tissue for any of the composite fish samples in Batch 2 was 76.0 g, so even if all the mass of each analyte in the rinsate was transferred to that tissue sample, the "worst case" concentration in Table 5 was still below the detection limit for the analyte in a tissue sample. Hence, those four rinsate contaminants would not be detectable in any sample in Batch 2. And in fact, none of those four analytes were reported in any of the 20 tissue samples in Batch 2. Further, even across all 413 fish tissue samples, PFPeA and 5:3 FTCA were reported only once, in one sample from Site NLA22_ID-10163. PFHpA was reported at low levels in five samples, and PFHxA was not reported in any sample from the study.

The smallest mass of homogenized tissue for any of the composite fish samples in Batch 10 was 47.5 g and the potential "worst case" contribution of PFBA from the solvent blank was 33 times lower than the tissue sample MDL. The fact that PFBA was not detected in the actual rinsate sample for Batch 10 further supports the conclusion that this analyte is not a concern for the Batch 10 samples.

Overall, the rinsate and solvent blank results for mercury, PCBs, and PFAS 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 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 errors, the laboratory manager verified that the final package was complete and compliant with the contract, then 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 called "SCC Codes" 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 databases. Table 5 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 5. Individual SCC Codes Applied to the 2022 NLA Fish Study Results		
SCC Code	**Comments**	**Implication**
B, RMAX	Blank Contamination, Result is a Maximum Value	Blank contamination was observed and the target chemical 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 is Not Affected	Blank contamination was present but was not considered to adversely impact the sample result. The presence of the chemical 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 chemical 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 chemical 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 chemical 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 chemical 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.
HLBL, RNAF	High Labeled Compound Recovery, Result is Not Affected	The labeled analog of the target chemical 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 chemical was not detected in a field sample, there is no concern and the RNAF is added to the HLBL flag.
HLCS, J	High Lab Control Sample Recovery, Estimated	The recovery in the LCS was high. Detected analytes also are considered estimated values.
HLCS, RNAF	High Lab Control Sample Recovery, Result is Not Affected	The recovery in the LCS was high, but the chemical was not detected in the associated fillet tissue sample, so there was no high bias concern 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 a chemical, all the detected results for that chemical in any of the samples in the batch with the duplicate sample were qualified as estimated values.
HVER, RNAF	High CALVER, Result is Not Affected	The results for the calibration verification associated with the chemical were above the acceptance limit, suggesting a possible high bias. If the chemical was not detected in a field sample, there is no concern and the RNAF is added to the HVER flag
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 5. Individual SCC Codes Applied to the 2022 NLA Fish Study 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 chemical was recovered below acceptance criteria, suggesting the possible presence of matrix interferences or incomplete recovery of both the labeled compound and target chemical during the extract cleanup processes used in the analytical procedure. The use of isotope dilution quantitation automatically corrects the results for the target chemical, even when the labeled compound recovery is below expectations. This flag is applied when the chemical associated with the labeled analog is not detected in the sample.
LLBL, J	Low Labeled Compound Recovery, Estimated	The labeled analog of the target chemical was recovered below acceptance criteria, suggesting the possible presence of matrix interferences or incomplete recovery of both the labeled compound and target chemical during the extract cleanup processes used in the analytical procedure. The use of isotope dilution quantitation automatically corrects the results for the target chemical, even when the labeled compound recovery is below expectations. When the chemical associated with the labeled analog is detected in the sample, the result is also flagged as an estimated value.
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 chemical. When low LCS recovery was observed for a chemical, the results for that chemical were qualified in all of the samples in the batch with the LCS.
LLCS, J	Low LCS result, Estimated	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 chemical. When low LCS recovery was observed for a chemical, the results for that chemical were qualified in all of the samples in the batch with the LCS.
LVER	Low CALVER	The results for the calibration verification associated with the chemical were below the acceptance limit, suggesting a possible low bias.
NQ	Not Quantified	The chemical could not be quantified by isotope dilution and was reported as a non-detect at the MDL.
PIO, J	Peak Interference Observed, Estimated	An interference was observed in the peak for the analyte and therefore the result is flagged as an estimated value.

Section 4.2 Analysis of Method Blanks

Method 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 5) 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 4.2.3.

4.2.1 Method Blanks for Mercury Analysis

Mercury was only detected above the QC acceptance limit of 0.4 nanograms (ng) in any of the three method blanks associated with two batches of samples, affecting 8.23% of the 413 study samples (one batch of 20 samples and a second batch of only 14 samples). However, the amount of mercury reported in the associated samples was well over 10 times the levels in those method blanks, so the sample results were not affected by the blank contamination. As shown in Figure 3, 91.77% of the sample results had no blank qualifier at all.

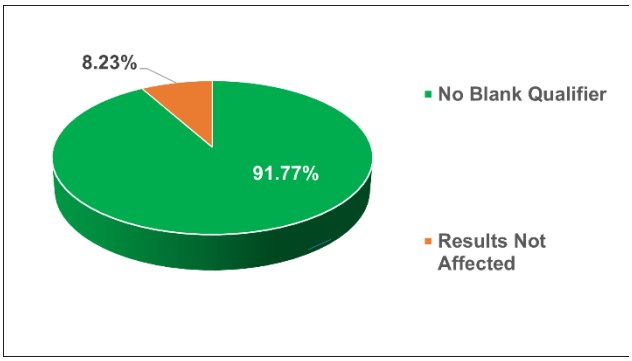

Figure 3. Impacts of Method Blank Contamination on Mercury Results

4.2.2 Method Blanks for PCB Analysis

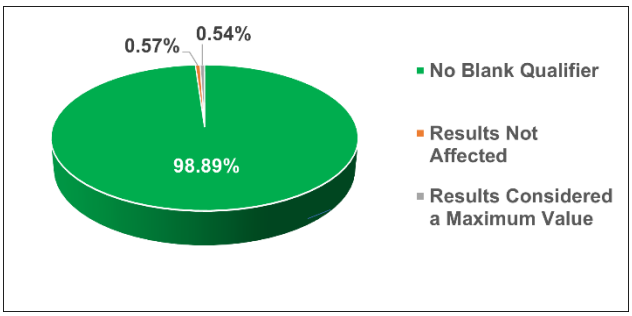

Figure 4. Impacts of Method Blank Contamination on PCB Results

The PCB method blanks associated with the analytical batches showed minimal contamination, and as shown in Figure 4, 98.89% of the PCB results had no method blank qualifier. Another 0.57% of the results were not affected because the sample concentration was more than 10 times the level observed in the blank, and for another 0.54% the data, reviewers judged that the sample result was 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.

4.2.3 Method Blanks for PFAS Analysis

The method blanks associated with the analytical batches exhibited even less PFAS contamination than was observed for the PCB samples. Overall, 99.95% of the PFAS results were not affected by the blank contamination, either because the analytes were not detected in the blanks or samples (99.88%) or because the sample concentration was more than 10 times the level observed in the blank (0.07%). For 0.01% of these results, the data reviewers judged that the sample result was likely a maximum value (RMAX) because there was some chance that the sample result was inflated by the background contamination from the laboratory that is evident in the blank. Another 0.03% of the sample results were changed to non-detects (RNON) due sample results being less than five times the blank value. Given the low percentage of results that were affected by method blank issues, a pie chart has not been included in this section because the tiny slivers of affected results would not be visible.

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 analytical QAPP for the study (USEPA 2023a) 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 PFAS LCS Results

There were a few data quality issues with the LCS results for the PFAS analyses, as shown in Figure 5. Of the 413 samples, 92.71% of the results were not affected by LCS issues, either because they were not qualified (85.83%) or because the analyte was not detected in the sample and thus the high LCS recovery had no affect (6.88%). Only 0.02% of the results (4 samples) were qualified due to a high LCS recovery that might reflect a high bias in the results for the detected analyte, while 7.27% were qualified due to low LCS recovery that might reflect a low bias in the results.

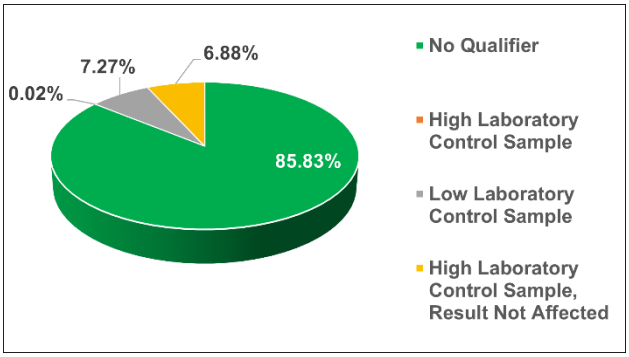

Figure 5. Impacts of LCS Recoveries on PFAS Results

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

A matrix spike (MS) sample 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. By analyzing a second spiked sample, called a matrix spike duplicate (MSD), the laboratory can provide data on the precision of the method by comparing the recoveries of the MS and MSD pairs. For the mercury analyses, the analytical QAPP for the study (USEPA 2023a) required that the mercury laboratory prepare two MS/MSD pairs for each batch of 20 field samples analyzed, as shown in Table 6.

In contrast, the EPA methods used for the PCBs and PFAS, spike isotopically labeled compounds into every sample and the recoveries of those labeled compounds provide sample-specific data on method performance, as opposed to the batch-specific data generated from matrix spike samples, so MS/MSD samples are not required in the PCB and PFAS methods. To provide precision data for or those analyses, the analytical QAPPs for the study (USEPA 2023b and 2023c) required that the laboratories performing analyses of fish tissue samples prepare and analyze one duplicate unspiked sample with each batch of field samples, as shown in Table 6.

Table 6. 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
PFAS			X

The data reviewers evaluated the results for each MS, MSD, and laboratory duplicate sample. The impacts are discussed separately for each analyte class in Sections 4.4.1 to 4.4.3.

4.4.1 Mercury Matrix Spike and Matrix Spike Duplicate Sample Results

All of the matrix spike and matrix spike duplicate sample results associated with each batch of samples analyzed for mercury met the QC acceptance limit. Therefore, no data qualifiers for recovery or precision 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 99.99% of the PCB results not affected by duplicate issues. There were only 4 results with high relative percent difference (RPD) values. Given that only 0.01% 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.

4.4.3 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.

Section 4.5 Labeled Compounds

The EPA methods for PCBs and PFAS 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" or "extracted internal standards" and often incorporate multiple atoms of naturally occurring, but less common isotopes such as ^{13}C, ^{18}O, ^{37}Cl, or Deuterium (^{2}H). 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 during the analysis.

The PCB laboratory added known amounts of 44 ^{13}C-labeled PCB congeners to each sample before extraction. The PFAS laboratory added known amounts of 18 ^{13}C-labeled PFAS and 6 Deuterium-labeled PFAS to each sample before extraction.

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 a 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. 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. No labeled compounds are applicable to the mercury analyses.

The analytical QAPPs for the study (USEPA 2023a and 2023b) includes acceptance criteria for the recoveries of the labeled compounds for the PCB and PFAS analyses. The impacts of labeled compound results are discussed separately for each analyte class in Sections 4.5.1 and 4.5.2.

4.5.1 PCB Labeled Compound Recoveries

The labeled compounds recoveries associated with each batch of samples analyzed for PCBs met the QC acceptance limits for 99.98% the individual PCB results. High labeled compound recoveries were reported for 11 results, with seven of those high recoveries (0.01%) associated with analytes that were not detected, thus those high labeled compound recoveries did not affect those seven sample results. The remaining four instances of high labeled compound recovery (0.006%) were for analytes that were reported in the sample, and thus might have a slight bias. Those four results were qualified as estimated values. Given these miniscule percentages, no pie chart has been included in this report.

4.5.2 PFAS Labeled Compound Recoveries

Some labeled compounds in the PFAS analyses had high recoveries and some had low recoveries. As shown in Figure 6, 99.30% of sample results were not affected by the recoveries of the labeled compounds, either due to the compounds not being outside the acceptance limits (98.41%), or because the associated native compounds were not detected and therefore the results were not affected (0.89%). Only 0.12% of sample results with high labeled compounds recoveries were for analytes that were detected, and those values were qualified as estimated values. A mere 0.10% of samples with low labeled compound recoveries were associated with detected results, which were qualified as estimated values. The remaining 0.49% of the results were analytes that were not detected and associated with low labeled compound recoveries.

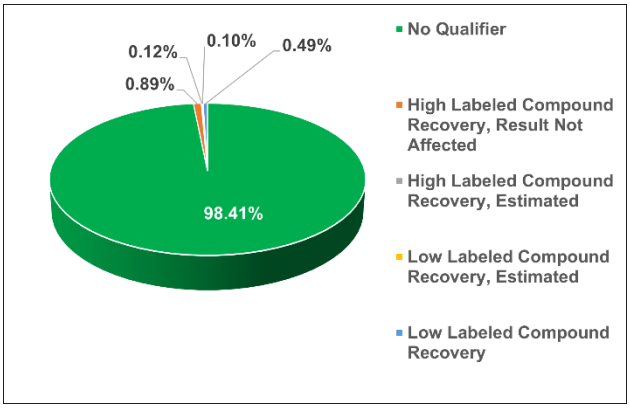

Figure 6. Impacts of Labeled Compound Recoveries on PFAS Results

Further, in two samples, the labeled compound D_7-N-MeFOSE could not be detected, resulting in the laboratory's inability to quantify the target analyte N-MeFOSE in those two samples by isotope dilution. Thus, 0.012% of the study results were flagged "NQ," for "not quantifiable," and those two results were omitted in Figure 6 because the sliver they represent would not be visible.

Section 4.6 Ion Abundance Ratio

The methods for PCBs and PFAS utilize a 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 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 (IAR) 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% for the PCBs).

In some cases, the observed IAR may fall outside of the consensus-based acceptance limits. 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 IAR suggests an interference with the ion in the pair for the target analyte with the smaller mass, while a

lower-than-expected IAR 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 PFAS 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 IAR issue are reviewed in more depth. If all the other identification criteria in the method are met, the results are reported for the analyte with the appropriate qualifier flag. The impacts of IAR concerns are discussed separately for the PCBs and PFAS 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 PFAS Ion Abundance Ratios

Overall, 99.44% of the PFAS results were not qualified due to ion abundance ratio concerns. Approximately 0.54% of the results were found to have higher-than-expected ion abundance ratios and 0.01% had lower-than-expected ion abundance ratios. Each such value is considered an estimated value. Given that a low percentage of results were affected by labeled compound recoveries, a pie chart has not been included in this section because the tiny slivers of affected results would not be visible.

Section 4.7 Certified Reference Material for Mercury

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 materials 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. The results for the CRM used in the analyses for mercury are discussed below

As part of the mercury analyses, the laboratory analyzed an aliquot of "TORT-3," a Certified Reference Material from the National Research Council (NRC) of Canada, which is a frozen tissue homogenate of lobster hepatopancreas. The NRC certificate of analysis provides "certified concentration values" for trace metals, with one of them being mercury.

The results from the analysis of TORT-3 associated with each batch of field samples in this study were compared to the reference value for mercury. All of the CRM results associated with each batch of samples analyzed for mercury met the QC acceptance limits. Therefore, no CRM qualifiers were applied to the mercury results for the study.

Section 4.8 Other QC parameters

The data review effort identified instances where the calibration verifications (CALVERs) for the PFAS analyses did not always meet the acceptance criteria in the method (see SCC codes on Table 5). As shown in Figure 7, the overall frequencies were low, with only 2.83% of the sample results associated with calibration verifications falling outside of the acceptance criteria. Of those results, 1.81% were samples where the CALVER results were above the limit, but the analyte was not detected in the sample, so the result was not affected. Another 1.02% were results associated with a low CALVER result where the sample results were considered estimated due to the CALVER failure.

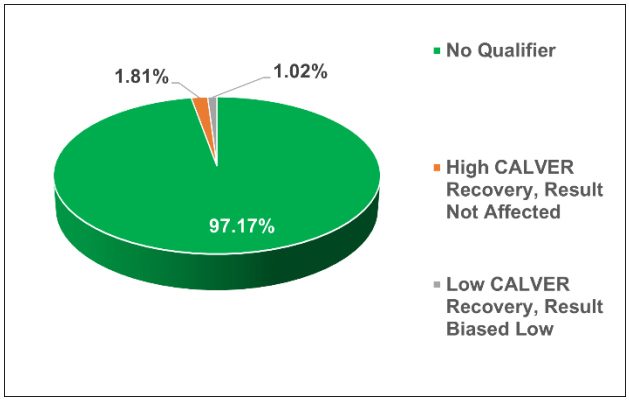

Figure 7. Impacts of CALVER Recoveries on PFAS Results

Section 4.9 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 analytical QAPP in this study (USEPA 2023a) was to obtain valid measurements from 95% of the samples analyzed.

For multi-analyte methodologies, analytical completeness is best calculated based on 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 three types of analyses (mercury, PCBs, and PFAS) yields a total of 209 measured results for each sample (based on 168 results that cover all 209 PCB congeners, but not including the calculated "total PCBs" results). For the 413 samples collected for the 2022 NLA, the total number of sample/analyte combinations is 86,317.

Despite the data quality concerns outlined in this report, all 413 samples were successfully analyzed for all the target analytes and none of the other results were excluded from consideration based on data quality concerns. Therefore, analytical completeness was 100%, and OST met its completeness goal.

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