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Quality Assurance Report for the 2013-14 National Rivers and Streams Assessment Fish Fillet Tissue Study

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology (4305T)
Standards and Health Protection Division
1200 Pennsylvania Avenue, NW
Washington, DC 20460

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Disclaimer

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Contact

Please address questions and comments to:

John Healey
Standards and Health Protection Division
Office of Science and Technology
Office of Water (4305T)
US Environmental Protection Agency
1200 Pennsylvania Ave, NW
Washington, DC 20460
healey.john@epa.gov

Chapter 1
Introduction

This report documents the quality of data gathered during EPA's 2013-14 National Rivers and Streams Assessment (NRSA), a probability-based survey designed to assess the condition of the nation's river and stream resources. The 2013-14 NRSA includes collection and analysis of physical, chemical, and biological indicator data that will allow a statistically valid characterization of the condition of the nation's rivers and streams. The Office of Wetlands, Oceans, and Watersheds (OWOW) within the Office of Water (OW) was responsible for the overall planning and implementation of the 2013-14 NRSA.

One component of the 2013-14 NRSA is the Fish Fillet Tissue Study, which is designed to examine national fish contamination trends in U.S. rivers. EPA's Office of Science and Technology (OST) within OW collaborated with the Office of Research and Development Western Ecology Division (ORD-WED, now called the Pacific Ecological Systems Division) in Corvallis, Oregon, to plan and implement the fish fillet tissue study under the 2013-14 NRSA. By the end of the 2014 field sampling season, whole fish composite samples (for fillet analysis) were collected from 361 sites. This report documents the quality of data gathered during the 2013-14 NRSA Fish Fillet Tissue Study.

Section 1.1 Background

Obtaining statistically representative occurrence data on multiple contaminants in fish tissue is a priority area of interest for EPA. Since 1998, OW has collaborated with ORD to conduct a series of national- and regional-scale assessments of contaminants in fish tissue through statistically based studies of U.S. lakes and rivers. These EPA studies are referred to as the National Lake Fish Tissue Study, the 2008-09 NRSA, and the Great Lakes Human Health Fish Tissue Study conducted under the 2010 National Coastal Condition Assessment. Results from the 2013-14 NRSA fish fillet tissue study generated a national baseline for fish contamination data in U.S. rivers.

Section 1.2 Study Design

OST collaborated with OWOW and with ORD-WED in Corvallis, Oregon, to plan and implement the fish fillet indicator within the framework of the 2013-14 NRSA (USEPA 2013a). Fish composite samples were collected during May through September of 2013 and 2014 at a statistical subset of approximately 360 sites in the NRSA framework (Figure 1).

The following were the key design components for the 2013-14 NRSA fish fillet tissue study:

- Sampling approximately 360 randomly selected sites during 2013 and 2014, subject to local conditions and restrictions.
- 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 leased freezer space at a commercial laboratory for storage, followed by fish sample preparation, which included collection of tissue plug samples for mercury analysis before filleting the fish, removing both fillets from each fish, homogenizing the fillet tissue composites, and preparing fillet tissue aliquots for analysis of mercury, polychlorinated biphenyls (PCBs), 13 perfluorinated compounds that are a subset of the broader group known as per- and polyfluoroalkyl substances (PFAS), and potentially for polybrominated diphenyl ethers (PBDEs).

- Analyzing all the fillet tissue samples for mercury (total) and PFAS, along with a designated subset of the fillet tissue samples (i.e., samples collected from 2013-14 NRSA sites that yielded fish samples during the 2008-09 NRSA) for PCBs. (Although aliquots were prepared for PBDE analyses, funding constraints led EPA to forego those analyses.)

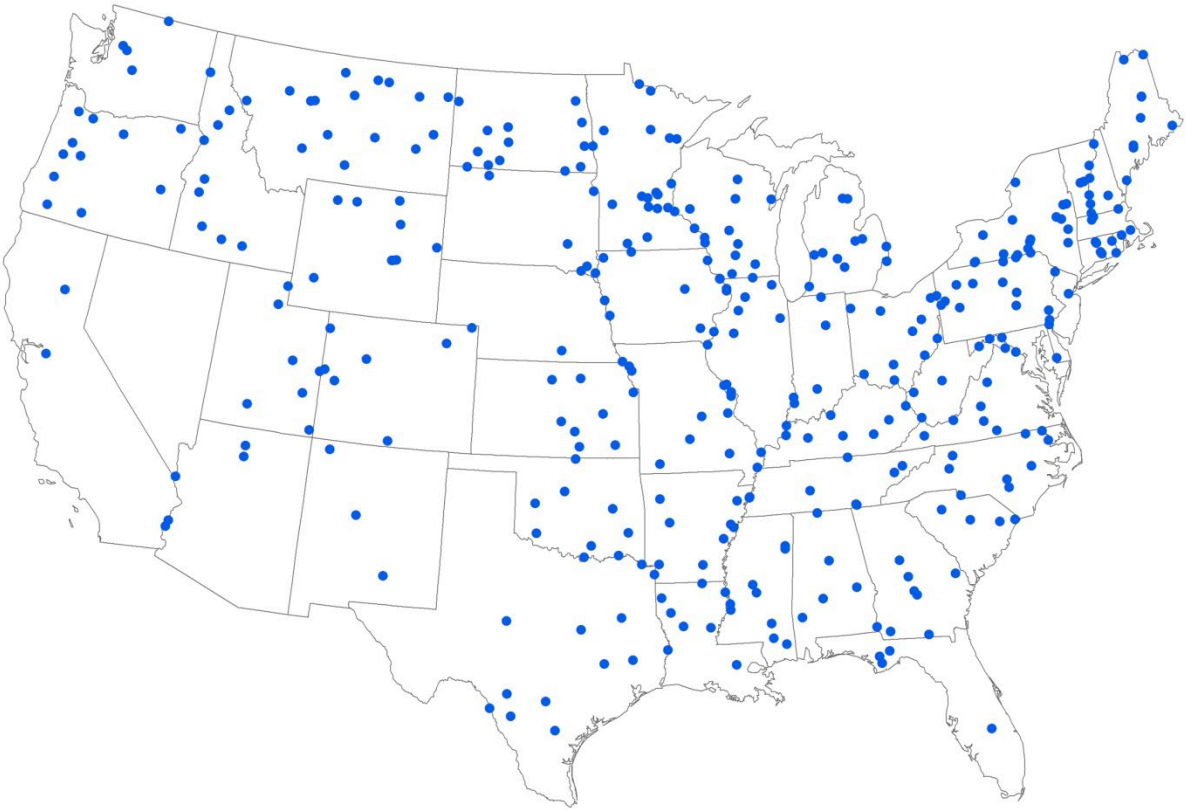


Figure 1. 2013-14 NRSA Fish Fillet Tissue Study sampling locations

EPA stored the 2013-14 NRSA whole fish samples in freezers leased by GDIT at Microbac Laboratories in Baltimore, Maryland. Microbac Laboratories also was the sample preparation laboratory preparing the homogenized fish fillet tissue samples and rinsates for analysis as outlined in the fourth bullet above. The sample preparation laboratory prepared aliquots of fillet tissue for mercury, PCBs, PFAS, PBDEs, and archive tissue samples to allow for further analysis of 2013-14 NRSA samples in the future. Commercial environmental laboratories analyzed the 2013-14 NRSA fish fillet tissue samples for mercury, PCB congeners, and PFAS, under project-specific purchase orders issued by GDIT. As noted above, the PBDE analyses were not conducted due to budget constraints. Procedures for handling and shipping homogenized fish tissue samples to Microbac and the analysis laboratories are described in Appendix B of the *Quality Assurance Project Plan for Sample Preparation for the 2013-14 National Rivers and Streams Assessment Fish Fillet Indicator* (USEPA 2013b).

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 2013-14 NRSA project team consisted of managers, scientists, statisticians, and QA personnel in OST, and ORD-WED, along with contractors providing scientific and technical support to OST from GDIT and Tetra Tech, Inc. (Figure 2). Project team members from OST provided support for developing

and reviewing technical and program information related to all aspects of the study, including training materials, standard operating procedures, 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 NRSA fish fillet tissue study Technical Leader and OST Project Manager who provided overall direction for planning and implementation of this fillet tissue study that was conducted under the NRSA.
- Marion Kelly was the OST Quality Assurance Officer who was responsible for reviewing and approving all QAPPs that involve scientific work being conducted by OST with support from Bill Kramer, the Standards and Health Protection Division QA Coordinator.
- Blaine Snyder was the Tetra Tech Project Leader who was responsible for managing all aspects of the technical support being provided by Tetra Tech staff for the NRSA fish fillet tissue study.
- Susan Lanberg was the Tetra Tech QA Officer.
- Harry McCarty was the GDIT Project Leader who was responsible for managing all aspects of the technical support being provided by GDIT staff for the NRSA fish fillet tissue study.
- Marguerite Jones was the GDIT QA Officer.
- Tony Olsen was the Senior Statistician at ORD-WED in Corvallis, Oregon, who was supporting the NRSA fish fillet tissue study by providing technical expertise for study planning and implementation.

Three commercial laboratories analyzed the 2013-14 NRSA fish tissue samples for mercury, PCBs, and PFAS, under purchase orders issued by GDIT, as shown below and in Figure 2.

Laboratory	Analysis Type
ALS-Environmental	Mercury
Vista Analytical	PCB congeners
Test America	PFAS

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/2013-2014-national-rivers-and-streams-assessment-fish-tissue-study

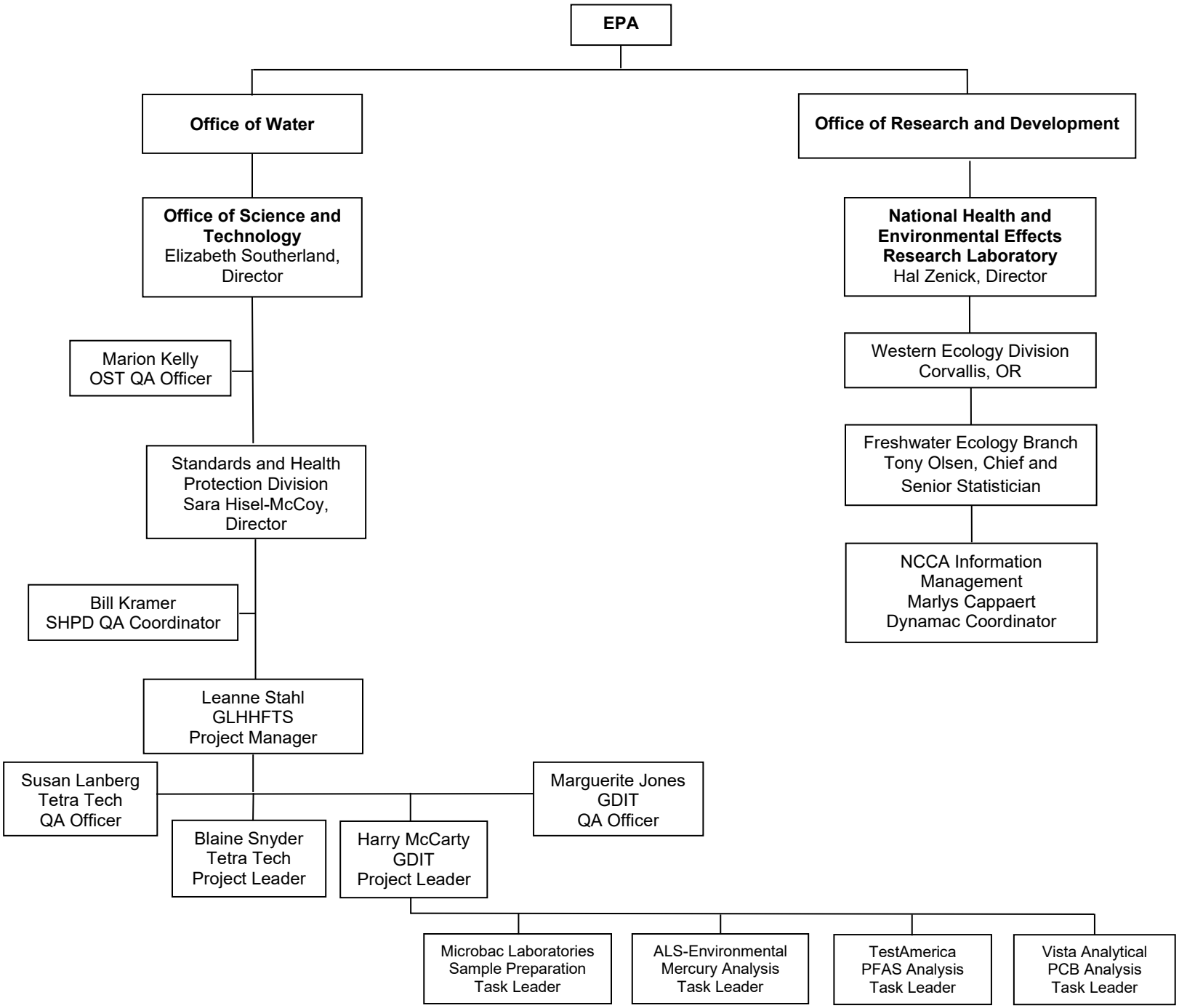


Figure 2. NRSA project team organization

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 2013, OWOW developed the *National Rivers and Streams Assessment 2013-2014: Quality Assurance Project Plan* (USEPA 2013a) that contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for the NRSA, and is based on the guidelines developed and followed in the Western Environmental Monitoring and Assessment Program (EMAP).

In 2013, OST developed the Quality Assurance Project Plan for Sample Preparation for the 2013-14 National Rivers and Streams Assessment Fish Fillet Indicator that described the procedures for preparing composite fish tissue samples (USEPA 2013b). In 2014, the first revision to the OST QAPP was released to include the requirements for mercury analysis (USEPA 2014a). The second revision to the QAPP was issued later in 2014 and added requirement for PCB analysis (USEPA 2014b). Revision 3 (USEPA 2015) added the details of the PFAS analysis of the fillet tissue samples.

Section 2.2 Training

Fish Tissue Sample Preparation

Specialized training was provided for laboratory technicians who prepared fish tissue fillets, homogenates, and rinsates for the study. This training was conducted at Microbac in Baltimore, Maryland, on July 16, 2013, for all laboratory staff involved with 2013-2014 NRSA fish tissue sample preparation, to accomplish the following objectives:

- present NRSA fish tissue preparation, homogenization and distribution procedures described in Appendix B to the QAPP,
- demonstrate filleting and homogenizing techniques with fish from invalid 2013-2014 NRSA 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 2013b)
- 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 and solvent blanks with each batch fish fillet tissue samples prepared
- Requirement for analyses of the rinsate samples for mercury, selected PCB and PBDE congeners, and PFAS
- Review and acceptance of mercury and PCB rinsate results by EPA before proceeding with preparation of additional samples (PFAS rinsates were analyzed later in the study)

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 2013b, 2014a, 2014b, and 2015)
- 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 isotopically labeled compounds spiked into field-based tissue samples, matrix spike (MS) samples, laboratory duplicate samples, 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, and PFAS 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, GDIT 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 three 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 2013-2014 NRSA fish sample preparation activities are described in Appendix B of the QAPP for the study (USEPA 2013b).

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. 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. Homogeneity was confirmed by conducting triplicate analyses of the lipid content in one of every twenty samples. The collective weight of the homogenized tissue from each sample was recorded to the nearest gram (wet weight) after processing. Microbac prepared fillet tissue aliquots according to the specifications listed in Steps 18 to 28 of the fish sample preparation procedures in Appendix B of the 2013-14 NRSA fish fillet sample preparation QAPP.

Section 3.2 Analysis of Fish Tissue Samples for Mercury

Fish tissue samples were prepared and analyzed by ALS-Environmental (Kelso, WA), using Procedure I from "Appendix to Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation" from Revision B of Method 1631 (1631B) for sample preparation (USEPA 2001), and Revision E of Method 1631 (1631E) for the analysis of mercury in fish tissue samples (USEPA 2002). The laboratory utilized approximately 0.5 g of tissue for the analysis. The sample was digested with a combination of nitric and sulfuric acids. The mercury in the sample was oxidized with bromine monochloride (BrCl) and analyzed by cold-vapor atomic fluorescence spectrometry. Tissue sample results were reported based on the wet weight of the tissue sample, in nanograms per gram (ng/g).

Section 3.3 Analysis of Fish Tissue Samples for PCBs

The PCB samples were prepared and analyzed by Vista Analytical Laboratory (El Dorado Hills, CA), in general accordance with EPA Method 1668C (USEPA 2010a) and as detailed in the laboratory's SOP. The samples were analyzed for all 209 PCB congeners and reported as either individual congeners or coeluting groups of congeners. The Vista Analytical Laboratory SOP deviated from the published EPA method in several aspects, including:

- Section 7.6.4: Vista used sodium sulfate as the reference matrix for QC samples associated with tissue analyses rather than vegetable oil because they have not found a source of vegetable oil that did not have traces of PCBs in it.
- Section 12.5: Vista used sodium hydroxide to adjust the pH of the solution in the back-extraction procedure, rather than potassium hydroxide.
- Sections 7.10.1 and 15.4.2.1: Vista used a CS-3 (mid-level calibration) standard that contains all 209 of the PCB congeners, rather than the subset of congeners listed in the method. Therefore, they do not run a separate standard containing all 209 congeners during the calibration verification process in Section 15.4.2.1.
- Table 3: Vista added 44 ^{13}C-labeled compounds to each sample, five more than the 39 labeled compounds specified in the method, and monitored the recoveries of all of these standards in each sample.

The entire list of modifications is presented in detail in the 2013-14 NRSA sample analysis 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.

This laboratory utilized approximately 10 g of tissue for the analysis. The samples were extracted with methylene chloride analyzed by high resolution gas chromatography-mass spectrometry. 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

At the time of this study, there were no formal analytical methods from EPA or any voluntary consensus standard bodies for the PFAS analyses of tissue samples. Therefore, fish tissue samples were analyzed by the TestAmerica (Sacramento, CA) using procedures developed, tested, and documented in that laboratory. The SOPs for those procedures are considered proprietary by the laboratory. However, the SOPs were 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 12 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 with tandem mass spectrometry (HPLC-MS/MS).

The concentration of each PFAS was determined using the responses from the $^{13}C_{12}$-labeled standards added prior to sample extraction. (Because a labeled standard for PFBS is not commercially available, this target analyte is quantified using the response for ^{18}O-labeled PFHxS, a closely related compound.), 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 is equivalent to the units of nanograms per gram (ng/g).

Section 3.5 Analysis of Rinsates and Solvent Blanks

As noted in Section 2.3, Microbac prepared equipment rinsates and solvent blanks with each batch of fish fillet tissue samples. Rinsates and solvent blanks were prepared for mercury, PCBs, and PFAS. Microbac analyzed the aqueous rinsates and solvent blanks for mercury using EPA Method 245.1, a cold-vapor atomic absorption procedure applicable to water samples (USEPA 1983). Rinsate results for mercury were reported based on the volume of the sample, in micrograms (µg) per liter.

The rinsates and solvent blanks for PCBs were analyzed by Cape Fear Analytical, under subcontract to Microbac Laboratories, during the course of the homogenization of the fish tissue samples. Cape Fear analyzed these samples using EPA Method 1668A (USEPA, 1999) and results were reported in absolute mass units of picograms (pg). Vista Analytical analyzed the rinsates and solvent blanks for PBDEs under subcontract to Microbac during the course of the homogenization of the fish tissue samples, using EPA Method 1614A and results were reported in pg/L.

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. The aqueous rinsates and solvent blanks for PFAS were analyzed using a procedure based on EPA Method 537 from the Office of Groundwater and Drinking Water (USEPA 2009). A 250-mL aliquot of the aqueous rinsate sample or solvent blank was spiked with the labeled standards and acidified with dilute hydrochloric acid (HCl) to a pH < 2. That diluted extract was processed by solid-phase extraction (SPE), in a similar manner as the tissue samples. The PFAS were eluted from the SPE cartridge and the eluant is spiked with additional labeled recovery standards and analyzed by HPLC-MS/MS. The results were reported in nanograms (ng) per liter.

Section 3.6 Quality Control Procedures

Fish Tissue Analyses

The analytical procedures applied by the laboratories designated for analysis of 2013-2014 NRSA 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
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

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 analyses for mercury, PCBs, and PBDEs were prepared and analyzed individually, not in batches of up to 20, in order to provide timely feedback of the cleanliness of the homogenization equipment. (The rinsates and solvent blanks for PFAS 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 and PBDEs were prepared in 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 samples are described in Table 2.

Table 2. Quality Control Activities for Analysis of Rinsates		
Analyte Type	**Quality Control Sample**	**Frequency**
Mercury	Instrument blank	With each rinsate sample
	Laboratory control sample	With each rinsate sample
PCBs	Instrument blank	With each rinsate sample
	Labeled compounds	Added to every rinsate sample
PBDEs	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
	Labeled compound recovery	Every rinsate sample

Because the mercury rinsates and the PFAS rinsates were prepared in reagent water, there was little chance of a "matrix effect" and the laboratory control sample, which was also prepared in reagent water,

provided sufficient information on the performance of the method and the laboratory in reagent water, so a separate matrix spike sample was not required.

Because the rinsates for PCBs and PBDEs 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.

GDIT reviewed the results for the mercury, PCB, and PBDE rinsates as soon as they were available from Microbac and its subcontracted laboratories and relayed the review findings to EPA within hours of receipt of the results. Mercury was never detected above the 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 rinsates and solvent blanks. Because the PCB rinsates and blanks were analyzed using the very sensitive procedures in EPA Method 1668C (USEPA 2010a), each of the 10 of the PCB congeners of interest were detected sporadically among the 18 pairs of rinsates and solvent blanks. The amounts reported in the rinsates and solvent blanks generally were hundreds to thousands of times below the concentration that might be detected in a tissue sample.

Given the use of EPA Method 1614A (USEPA 2010b), the PBDE rinsates and solvent blanks exhibited similar sporadic occurrences of the 8 congeners of interest. The amounts reported in the rinsates and solvent blanks generally were tens to thousands of times below the concentration that might be detected in a tissue sample. In the worst case, one PBDE congener was detected at 7 times below the concentration that might have been detected in a tissue sample. However, as noted earlier, the tissue samples ultimately were not analyzed for PBDEs.

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. The only PFAS detected in any of the rinsates and solvent blanks was PFBA, which was reported in 6 of the 18 rinsates and 7 of the solvent blanks. Using the highest PFBA in any of those 6 rinsates and the lowest total sample mass for any of the study samples, the worst case PFBA rinsate concentration was still 8.5 times lower than what could have been detected in a tissue sample. Perhaps more importantly, PFBA was not detected in that smallest sample, nor was that sample in the preparation batch with the highest rinsate results for PFBA. 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.

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

Chapter 4
Data Quality Assessment

Section 4.1 Data Review

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

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

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

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

GDIT 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 3 contains the individual data qualifiers that were applied to results from the study and provides an explanation of the implications of each qualifier for the use of the data.

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

Table 3. Individual SCC Codes Applied to the 2013-2014 NRSA 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 is 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.
HICAL	High Initial Calibration RSD	The relative standard deviation (RSD) used to assess the linearity of the calibration did not meet the acceptance criterion in the respective method for a specific target analyte, suggesting that there may be slightly greater uncertainty in the reported result for that analyte. If the analyte was not detected in the sample, this flag appears on its own. If the analyte was detected, then the "J" flag is added to the HICAL flag to indicate the increased uncertainty.
HLBL	High Labeled Compound Recovery	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.
HLBL, J	High Labeled Compound Recovery, Estimated	The labeled analog of the target analyte was recovered above acceptance criteria, suggesting the possible presence of matrix interferences. Isolated instances of high recovery are not uncommon, and patterns across multiple samples are more of a concern.
HLBL, RNAF	High Labeled Compound Recovery, Result is 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.
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.
HVER	High CALVER	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, 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 is Not Affected	The results for the calibration verification associated with the analyte were above the acceptance limit, suggesting a possible high bias, but the analyte was not detected in the associated tissue sample, so there is no high bias concern and the RNAF flag is applied.

Table 3. Individual SCC Codes Applied to the 2013-2014 NRSA Results		
SCC Code	**Comments**	**Implication**
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.
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. When low LCS recovery was observed for an analyte, the results for that analyte 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 analyte were below the acceptance limit, suggesting a possible low bias.
LVER, J	Low CALVER, Estimated	The results for the calibration verification associated with the analyte were below the acceptance limit, suggesting a possible low bias. Detected analytes are considered estimates, and the J flag is applied.

Section 4.2 Analysis of Blanks

Blanks are used to verify the absence of contamination that may occur at any point in the measurement process. The data reviewers evaluated each sample result in comparison to the result for that analyte in the method blank prepared in the same extraction batch. For those analytes reported as present in the method blank, the data reviewers applied the 5x and 10x rules (described in the first three SCC codes of Table 3) to determine the potential impact of the blank contamination on the study results. The impacts of blank contamination are discussed separately for each analyte class in Sections 4.2.1 to 4.2.3.

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

As shown in Figure 3, more than 99% of the PCB results were not affected by blank contamination, either because the analyte was not detected in the blank (97.97%) or because the concentration in the sample was more than 10 times the level observed in the blank (1.22%). For 0.22% 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 contam-ination from the laboratory that is evident in the blank. Only 0.59% of the PCB results were changed to non-detects (RNON) because of concerns about blank contamination.

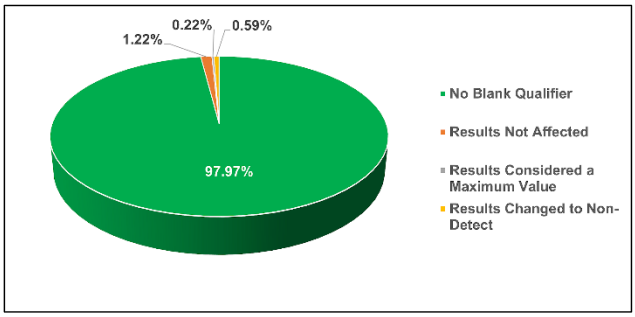
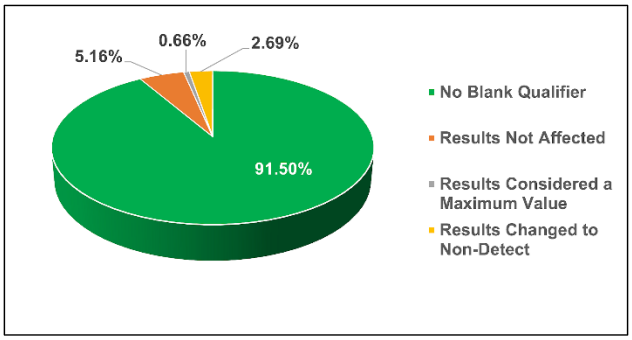


Figure 3. Impacts of Blank Contamination on the PCB Results

4.2.3 Blanks for PFAS Analysis



Overall, there were few data quality issues with the blanks from the PFAS analyses. As shown in Figure 4, more than 96% of the PFAS results were not affected by blank contamination, either because the analyte was not detected in the blank (91.50%) or because the concentration in the sample was more than 10 times the level observed in the blank (5.16%). For 0.66% 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 2.69% of the PFAS results were changed to non-detects (RNON) because of concerns about blank contamination.

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 4.3.3.

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.

4.3.4 PFAS LCS Results

There were very few data quality issues with the LCS results for the PFAS analyses, with 99.98% of the PFAS results not affected by LCS issues. Given that only 0.02% of the PFAS results were qualified because of a low LCS result that might reflect a low bias in the results, a pie chart has not been included in this section because the tiny sliver of affected results would not be visible. The 0.02% represented the result for one PFAS analyte in one sample.

Section 4.4 Analysis of Matrix Spike 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 laboratory duplicate sample is a second aliquot of one field sample that is prepared and analyzed to provide information on the precision of the analytical method. Laboratory duplicate samples are routinely used for analytes such as metals that are expected to be found in most or all samples. However, other types of analytes, particularly organic contaminants, are not detected as frequently in field samples, and the analysis of an unspiked duplicate sample often will not yield useful data on analytical precision when both the original sample and the duplicate are reported as "not detected." Therefore, EPA methods for organic contaminants often require that a second spiked aliquot of the sample matrix be prepared as a matrix spike duplicate (MSD). By spiking the analytes into both the MS and MSD aliquots, there is a greater chance of generating useful data on method and laboratory precision.

Alternatively, some EPA methods such as those used for the PCBs, and the procedure used for the PFAS, call for spiking 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. For those methods, only a laboratory duplicate sample per batch is analyzed. 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 by comparing the results of the original analysis of the sample and the analysis of the laboratory duplicate sample.

The analytical QAPP for the study (USEPA 2015) required that the laboratories performing analyses of fish tissue samples prepare and analyze MS and/or duplicate samples with each batch of field samples as follows.

Table 4. Matrix Spike and Duplicate Sample Requirements by Analysis Type

Analysis Type	Matrix Spike	Duplicate
Mercury	X	X
PCBs		X
PFAS		X

The data reviewers evaluated the results for each MS 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 Duplicate Sample Results

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 approximately 99.98% of the PCB results not affected by duplicate issues. Given that only 0.02% 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 not be visible.

4.4.3 PFAS Duplicate Sample Results

The PFAS laboratory duplicate analyses exhibited excellent precision, with approximately 99.85% of the PFAS results not affected by duplicate issues. Given that only 0.15% 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 not be visible.

Section 4.5 Labeled Compounds

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

The labeled compounds in such methods serve two functions. First, their responses can be used to quantify the responses for the unlabeled target analytes in each sample through the technique known as isotope dilution. Secondly, the measured recovery of each labeled compound provide 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.

The PCB laboratory added known amounts of 44 ^{13}C-labeled PCBs to each sample before extraction. The PFAS laboratory added known amounts of 11 ^{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 labeled compounds. The impacts of the labeled compound results are discussed separately for each analyte class in Sections 4.5.1 and 4.5.2.

No labeled compounds were employed for the mercury analyses.

4.5.1 PCB Labeled Compound Recoveries

Virtually all (99.87%) of the labeled compound recoveries for the PCB samples met the QC acceptance limits. Given that only 0.13% 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 PFAS Labeled Compound Recoveries

Over 99% of the labeled compound recoveries for the PFAS samples met the QC acceptance limits. As shown in Figure 5, only 0.33% of the results were affected by low labeled compound recoveries, and another 0.44% (after rounding) of the results exhibited high labeled compound recoveries. Of that 0.44%, 0.17% of the high labeled compound recoveries were associated with analytes that were not detected in the samples, such that the high labeled compound recovery did not affect the result for the target analyte. A pie chart has not been included in this section because the slivers of affected results would not be visible.

Section 4.6 Ion Abundance Ratios

The instruments used for PCBs and PFAS analyses monitor 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 fraction 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 ratio

(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%).

There were no IAR issues with any of the PCB or PFAS results in the study.

Section 4.7 Other QC parameters

As evidenced by the list of individual SCC data qualifier codes in Table 3, the data review effort identified instances where the calibration linearity for the PCB analyses and the calibration verifications for both the PCB and PFAS analyses did not always meet the acceptance criteria (see Table 3). However, in all of these instances, the frequencies were very low. For example, 0.08% of the PCB calibration verification results were outside of the acceptance criterion, as were 0.09% of the PFAS calibration verification results. Given these very low occurrences, pie charts have not been included in this section because the tiny slivers of affected results would not be visible.

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 three types of analyses (mercury, PCBs and PFAS) yields a total of 173 measured results for each sample (based on 159 results that cover all 209 PCB congeners). For the 361 field samples collected for the 2013-2014 NRSA, the total number of sample/analyte combinations would be 62,453. However, not all of the 361 samples were intended for PCB analyses. Rather, EPA decided to only analyze 224 samples for the PCBs because it has PCB data for samples from those sites from the 2008-09 NRSA. Further, the small specimens collected by some of the sampling teams did not provided enough tissue mass for all of the analyses and as a result, only 352 samples were analyzed for PFAS. Therefore, based on the analysis of 361 samples for mercury, 352 samples for PFAS, and 224 samples for PCBs, the total number of sample/analyte combinations was 40,553.

Despite the data quality concerns outlined in this report, all of the available and intended 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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