SERDP

TECHNICAL REPORT

Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633 (Volume I): Wastewater, Surface Water, and Groundwater Matrices

Janice Willey
Naval Sea Systems Command, U.S. Navy

Adrian Hanley
U.S. Environmental Protection Agency, Office of Water

Richard Anderson
Air Force Civil Engineering Center

Andrea Leeson
SERDP and ESTCP

Timothy Thompson
Science and Engineering for the Environment, LLC

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Principal Authors

Willey, Janice	Naval Sea Systems Command, U.S. Navy
Hanley, Adrian	U.S. Environmental Protection Agency, Office of Water.
Anderson, Richard	Air Force Civil Engineering Center
Leeson, Andrea	SERDP & ESTCP
Thompson, Tim	Science and Engineering for the Environment, LLC

Other Contributing Authors

PFAS Multi-Laboratory Validation Study Data Compilation Report

Buytendyk, Allyson	Institute for Defense Analyses
Dawn Smorong	Exa Data and Mapping Services, Inc.
Denise Rivers	HydroGeoLogic ,Inc.
Harry B. McCarty	General Dynamics Information Technology, Inc.
Mirna Alpizar	General Dynamics Information Technology, Inc.

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U.S. Army Corps of Engineers
Melinda McClellan, Huntsville Environmental and Munitions Center of Expertise
Mike Malone, Huntsville Engineering and Support Center

HydroGeoLogic, Inc
Joe Skibinski
Denise Rivers
Andrea Fletcher

Exa Data and Mapping, LLC
Dawn Smorong
Peggy Myre
Michael Tweiten

Institute for Defense Analyses, Inc
Sara Runkel

Jacobs Engineering Group, Inc
Maggie Radford
Jeremy Bishop

Pyron Environmental, Inc
Mingta Lin

Noblis, Inc.
Cara Patton
Stephen Levitas

Abstract

This report is the first in a series presenting the results of a multi-laboratory validation study (MLVS) designed to validate the EPA's draft Office of Water (OW) *Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS* (EPA Method 1633). This study was conducted as a joint effort by the U.S. Department of Defense (DoD) and the Environmental Protection Agency (EPA). The MLVS objectives were as follows:

- Identify and quantify up to 40 PFAS in aqueous matrices (groundwater, surface water, landfill leachate, and wastewater), solids (soil, sediment, and biosolids), and tissues using the isotope dilution LC-MS/MS method.
- Achieve a low parts per trillion (ppt) level of quantitation (LOQ) in aqueous matrices and parts per billion (ppb) in solids and tissues.
- Produce a method that can be implemented at a typical mid-sized full-service environmental laboratory.
- Conduct single- and multi-laboratory validation studies of the draft method.

EPA Method 1633 is an interim draft method that had previously been undergone a single-laboratory validation study (*Single Laboratory Validation Study of PFAS by Isotope Dilution LC-MS/MS*). This MLVS was a follow-on to that study. Although the draft method was validated in various environmental matrices, this report only addresses the multi-laboratory study results for the aqueous matrices of wastewater, surface water, and groundwater. Additional reports will be published at a later date for all other matrices included in the scope of the method.

Note: Landfill leachate will be addressed in the next report. It is an aqueous matrix, but it has a different sample size and is usually more prone to interferences than the other aqueous matrices. Landfill leachate will have its own section in the next report. This report only addresses the aqueous matrices wastewater, surface water, and groundwater; assume only these three matrices are being discussed when aqueous results are discussed in this report.

This study was designed to evaluate the robustness of EPA Method 1633 when performed by suitable laboratories using similar instruments of different manufacturers and models, as well as provide information on the range of precision and accuracy of quantitation that is achievable by suitable laboratories. This was achieved through the evaluation of data generated from PFAS-spiked environmental samples (herein identified as study samples). A Study Plan which documented the procedures to be used throughout the entire study, including the creation and shipment of study samples, the preparation and analysis of study samples, the reporting, validation, and statistical analysis of the data generated by the study. The laboratory sample preparation and analysis procedure was EPA Method 1633 with interim quality assurance and quality control criteria included (MLVS Method, Appendix A). This study was undertaken using EPA's *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (2018) as guidance where applicable. This study was not an Alternate Test Procedure, so the guidance is not relevant for some steps. The study follows all of the steps EPA's Clean Water Act Method Program has done for previous new EPA methods.

This report, being the first in the series of MLVS reports to be published, provides information that applies to all subsequent reports in addition to this report. It provides the project background, the overall project management, data validation, and data management. This reports describes the processes for laboratory selection, selection of study sample sources, and study sample creation and delivery. In addition, it includes results from evaluation of the overall EPA Method 1633 capabilities of each laboratory. This included the evaluation of each laboratory's Standard Operating Procedure (SOP) and documentation of Initial Calibrations (ICAL), and the Initial Demonstration of Capabilities (IDOC), method detection limit (MDL) determination, and verification of their aqueous sample limit of quantitation (LOQ) for aqueous sample matrices. Subsequent reports will present results for soils and sediments, fish and shellfish tissue, biosolids, and landfill leachate (and the Initial Demonstration of Capabilities (IDOCs) and Method Detection Limits (MDLs) relevant to those matrices).

The objective of the study was to demonstrate the efficacy of the method using PFAS-spiked environmental samples. Extracts for aqueous matrices were prepared via solid-phase extraction (SPE) followed by carbon clean-up. Analyte concentrations were determined using either an isotope dilution or extracted internal standard (EIS) quantification schemes; both of which utilize isotopically labeled compounds that are added to the samples prior to extraction. Injection internal standards (IISs), referred to as non-extracted internal standards (NISs) in EPA Method 1633, were also used to determine EIS compound recoveries and provide a general indicator of overall analytical quality. The method includes 40 target analytes, 24 EIS compounds, and 7 NIS compounds. The isotope dilution and EIS compound quantification schemes correct the analyte results for the measured recovery. NIS are used to calculate EIS recovery, but do not affect the reported concentration of the forty target analytes. Analytes were quantified and reported as their acid form.

Ten laboratories participated in the Study: eight commercial laboratories and two state laboratories. All laboratories were required to complete an ICAL and IDOC study. Upon successful completion, unspiked, and PFAS-spiked wastewater, surface water, and groundwater samples were sent sequentially to each of the laboratories. Seven wastewater sample series were analyzed, each consisting of an unspiked sample, three replicate low spiked samples, and three replicate high spiked samples, for a total of 49 analyses. For surface water and ground water, three individual samples for each matrix were analyzed as unspiked, low spiked and high spiked samples (21 total analyses per matrix).

All data packages were reviewed for completeness and compliance with the requirements of the MLVS Method (Appendix A), and the Study Data Validation Guidelines (DVGs) (Attachment 5 to the Study Plan). While not explicitly cited in the Study Plan, the validation procedure also utilized the *Data Validation Guidelines Module 6: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-24*" (DoD 2022). During the validation process two laboratories were determined to be out of compliance with the Study requirements for wastewater; those results were rejected and not included in the method evaluation. One of those laboratories was found to be out of compliance in all aqueous media; those results (including landfill leachate) were rejected and not included in the method evaluation.

No specific criteria for matrix spike recoveries were established *a priori* in the Study; a goal of the study was to evaluate what criteria might be appropriately applied. The efficacy of the matrix spike

recovery was evaluated in two ways; (1) mean matrix spike recovery of 70–130% of the spike concentration as was done for the Single Laboratory Validation Study, and (2) the target recoveries in the Method for Ongoing Precision and Recovery, and for the Low-level Ongoing Precision and Recovery of 40 – 150%.

For wastewater, one sample series was found to form a viscous precipitate that could not pass through the SPE cartridge. This was the ASTM synthetic wastewater that contained flour and fine clay silt; interestingly none of the actual wastewaters had this problem. Only six sample series were then analyzed by eight laboratories. While considerable variation was observed between the individual laboratories, as well as for the low vs high spiked samples, the average percent recoveries were between 70–130% for the six low and high spiked samples with the one exception of PFDoS (52.4%). For the low and high spiked samples, the proportion of all values that were between 70–130% of the spiked concentrations is >70% for most analytes. The exceptions to this included PFDS, PFDoS, NMeFOSAA, NEtFOSAA, and 11Cl-PF3OUdS. When evaluated against the 40 – 150% OPR criteria, >95% of all values were reported within that range.

For the three surface water sample series, over the nine laboratories, the pooled spiked target PFAS percent recovery results were between 70–130% with the exception of the following compounds: PFDoS, NMeFOSAA, NEtFOSAA, and PFMPA. However, for those compounds recoveries were generally within 40 – 150%. These findings were likely skewed by poor EIS compound recoveries for one laboratory, and anomalous high values for NMeFOSAA and NEtFOSAA at a different laboratory.

For the three groundwater sample series over the nine laboratories, the pooled spiked target PFAS percent recovery results were between 70–130% with the exception of the following compounds: PFOS, NMeFOSAA, and NEtFOSAA. One laboratory reported a concentration of PFOS that was anomalously high in a low-spiked sample, as compared to the other eight laboratories. That particular value skewed the results as the PFOS measures for the other laboratories fell within the 70–130% range.

The objectives of this MLVS were achieved; validation of EPA Method 1633 and the production of a method that can be implemented at a typical mid-sized full-service environmental laboratory. Overall, the data generated during the MLVS demonstrated that EPA Method 1633, as written, is robust enough to be performed by suitable laboratories using similar instruments of different manufacturers and models. The results generated by participating laboratories in this study routinely met the requirements stated in the method for:

- Mass calibration and mass calibration verification,
- Initial calibration and calibration verification,
- Determination of MDLs and LOQs,
- Initial Performance Recovery,
- Preparatory batch QC samples (MB, OPR, LLOPR), and
- Quantitative and qualitative analyte identification criteria.

The suitability of EPA Method 1633 to detect and quantify the 40 target analytes in groundwater, surface water, and wastewater samples was successfully demonstrated through the analysis of spiked groundwater, surface water, and wastewater samples. Method Blank results demonstrated that any bias associated with background contamination introduced during sample preparation was negligible. The IPR, OPR, and LLOPR recoveries and the EIS and NIS compound recoveries associated with study samples were used to derive QC acceptance criteria for inclusion in the finalized method.

EXECUTIVE SUMMARY

E.S.1 INTRODUCTION

This report is the first in a series presenting the results of a multi-laboratory validation study (MLVS) designed to validate the Environmental Protection Agency's (EPA) draft Office of Water (OW) *Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS* (EPA Method 1633). This project was designed to validate EPA Method 1633 and were undertaken through the U.S. Department of Defense (DoD) Strategic Environmental Research and Development Program (SERDP).

The MLVS was undertaken cooperatively as the MLVS Team, which included SERDP/ Environmental Security Technology Certification Program (ESTCP); the U.S. Army Corps of Engineers (USACE); EPA's Offices of Water, of Land and Emergency Management, of Research and Development; the U.S. Navy; and the U.S. Air Force. SERDP/ESTCP, the USACE, EPA OW, the U.S. Navy, and the U.S. Air Force approved and are co-signers to the Study Plan developed for the project.

E.S.2 OBJECTIVES

This study was designed to evaluate the robustness of EPA Method 1633 when performed by suitable laboratories using similar instruments of different manufacturers and models, as well as provide information on the range of precision and accuracy of quantitation that is achievable by suitable laboratories.

The focus of the MLVS was to generate the necessary data to document the precision and accuracy and overall performance of the analytical method for quantitation of PFAS in environmental matrices. The primary objectives of this MLVS were to:

- Obtain data from matrices that are representative of the method's intended use.
- Obtain data from laboratories that are representative of those likely to use the method, but that were not directly involved in its development.
- Obtain feedback from laboratory users on the specifics of the draft method.
- Use study data to characterize performance of the method.
- Develop statistically derived QC acceptance criteria that will reflect method performance capabilities in real-world situations.

The Study was then formulated to provide the data and results necessary to update and finalize the draft EPA Method 1633.

E.S.3 METHOD DESCRIPTION

A Study Plan for the MLVS was developed by the Team based on the outcome of a *Single Laboratory Validation Study* (SLVS). A standard operating procedure was developed, tested on eight environmental matrices in the SLVS, which was completed with a report published in January 2022. That report, *Single Laboratory Validation Study of PFAS by Isotope Dilution LC-MS/MS*, resulted in the EPA OW publishing the draft EPA Method 1633 in September 2021, with updates of the method in June 2022, and December 2022. The overall method validation was undertaken using EPA's *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (2018) as guidance where applicable.

The Draft EPA Method 1633 includes sample preparation and sample analysis procedures that are applicable to a variety of environmental matrices. This report focuses solely on wastewater, surface water, and groundwater. Subsequent reports will address soil, sediment, tissue, biosolids, and landfill leachate. The aqueous matrices were prepared via solid-phase extraction (SPE) and carbon clean-up processes. The method utilized liquid chromatography–tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode to evaluate quantification and confirmation (where applicable) of ions of each of 40 PFAS target analytes. Analyte concentrations were determined using either an isotope dilution or extracted internal standard (EIS) quantification scheme; both utilized isotopically labeled compounds that were added to the samples prior to extraction. At the time of validation, only 24 isotopically labeled analogs of the 40 target analytes were commercially available, and therefore only 24 target analytes could be quantified using isotope dilution quantitation. All other analytes were quantified using EIS compound quantitation with these isotopically labeled analogs. Recovery of both quantification schemes corrects the analyte results. Analytes were quantified and reported as their acid form.

Seven non-extracted internal standards (NIS)[1] were used to determine EIS recoveries and provide a general indicator of overall analytical quality. A list of the 40 target analytes, 24 EIS compounds, and seven NIS compounds are provided in the Report.

E.S.4 TECHNICAL APPROACH

The analytical method for this study was the one validated and included in the report, *Single Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS* (SERDP 2020 and 2021), and defined in the August 2021 draft of EPA 1633. Refinements to that method were made based on interactions and feedback from the 10 laboratories that participated in this MLVS. Updates reflecting those changes were released by EPA as a Revised Errata Sheet in February 2022, and in the 2nd Draft Method 1633 in June 2022.

[1] NIS were referred to in the SLVS Report as Injected Internal Standards (IIS). EPA used the *NIS* in the draft EPA Method 1633; NIS is adopted for this MLVS report.

Funding for this project was provided by SERDP/ESTCP to the USACE, which in turn contracted with HydroGeoLogic, Inc. (HGL) to serve as the Oversight Contractor for the project. HGL sought out labs to participate in the Study; ten laboratories (eight commercial contract laboratories and two state laboratories) agreed to participate. All ten laboratories conducted analyses of the aqueous matrices in this report (wastewater, surface water, and groundwater). For the purposes of this study, the laboratories were randomly assigned identification numbers, which were used to maintain the anonymity of the results.

The laboratories were contracted to HGL, which also managed the contracting for sample spiking and shipment and received all data packages from the laboratories. HGL also contracted a commercial vendor, Wellington Laboratories, LLC (Wellington), to provide analytical standard mixtures and individual, high-concentration PFAS analytical standards as defined by the MLVS Team to the laboratories participating in the study. Another commercial vendor, Waters ERA, which specializes in proficiency testing samples, prepared and shipped the Study Samples using "real-world" environmental sample matrices. The draft method used in the SLVS stemmed from an SOP developed by SGS AXYS, who was contracted to conduct the baseline (i.e., background) measures of PFAS in the test environmental matrices and serve as the method consultant to the MLVS.

This MLVS was conducted in specific phases. The procedures for these phased studies are given in the Study Plan and are briefly summarized below.

- Initial Calibration (ICAL), Initial Demonstration of Capability (IDOC), Method Detection Limit (MDL) study, and Limit of Detection (LOD), and Limits of Quantification (LOQ) verifications.
- Method evaluation in wastewaters, surface water, and groundwater matrices.
- Method evaluation in soils and sediment.
- Method evaluation in tissue (fish and shellfish).
- Method evaluation in biosolids and landfill leachate.

In order to expedite EPA's release of a non-draft version of EPA Method 1633 for aqueous matrices, this report focuses on the wastewater, surface water and groundwater matrices. The soil, sediment, landfill leachate, and tissue matrices will be addressed in subsequent reports.

Prior to undertaking the analysis of PFAS-spiked matrices, each laboratory was required to conduct ICAL, IDOC, MDL, LOD and LOQ verification testing. The Study procured and provided each laboratory with the target analyte, EIS compound, and NIS compound standards they would use for the MLVS. By providing the standards to all the laboratories, the variability in the study results that would have resulted from having each laboratory prepare all the standards from neat materials was reduced. The standards provided by the DoD were used by the laboratories to create all of the calibration, calibration verification, and spiking solutions they used in the MLVS.

The MLVS was designed to provide a test of the method by analyses of real-world environmental matrices, including wastewaters, groundwater, surface water (fresh and marine), soil/sediment (fresh and marine), fish and clam tissues, landfill leachate, and biosolids. To obtain a wide diversity and sufficient quantity of matrices and samples, SERDP and EPA coordinated with municipal, state, and EPA Regional contacts to obtain sufficient volumes/mass used in the study.

Seven wastewater, three surface water, and three groundwater samples were used for this phase of the Study. EPA's OW arranged for representative wastewaters to be shipped to Waters ERA in Golden, CO. Six of the wastewater matrices included effluents from a publicly owned treatment works, and wastewaters from specific industrial discharges. A substitute wastewater was also included in the samples and was prepared as specified in ASTM International Reference D5905-98, *Standard Practice for the Preparation of Substitute Wastewater*.

Three surface water samples were collected for this study: freshwater samples from Lake Harsha, OH and Burley Creek, WA, and a saltwater sample from Sequim Bay, WA. Three groundwater samples were collected and contributed to the study. Two samples were collected by the USACE from active PFAS-investigation sites in Kansas and Colorado, and one sample collected by the EPA from an active PFAS-investigation site in the southwest.

The MLVS was designed so that for each sample there was an unspiked (or "native") sample, three replicates at a low spike concentration, and three replicates at a high spike concentration. Spike concentrations were determined based upon the background level of PFAS measured in the wastewater samples. Samples were prepared at Waters ERA, assigned sample identification that were blind to the testing laboratories, frozen, and shipped under chain of custody to each laboratory. Testing was done sequentially; wastewater, followed by surface water and then groundwater.

Procedures were established in the Study Plan for data management (project and analytical data), data validation after receipt of the laboratory packages, and compilation of a validated Project Database from the individual validated electronic data deliverables (EDD) for each of the laboratories. All data packages and the accompanying Electronic Data Deliverable (EDD) were reviewed for completeness and compliance with the requirements of the Method and the Study Data Validation Guidelines (DVGs) developed in the Study Plan. The validation procedure generally followed the *Data Validation Guidelines Module 6: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-24* (DoD 2022).

Once all data were validated, and the final EDDs were approved by the MLVS Team, the data were considered complete and ready to initiate the statistical analyses. An export was prepared from the project database for each individual matrix (WW, SW, GW), which underwent additional review by the MLVS Team. Once complete, the data were provided to the Institute for Defense Analysis (IDA) to conduct the supporting statistical analyses based upon the procedures described in EPA's *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (2018). The analyses produced by IDA underwent further quality assurance review by the MLVS Team. In addition, the EPA also undertook parallel statistical analyses to support finalization of the method.

E.S.5 ICAL AND IDOC FINDINGS

Initial Calibration

Each laboratory calibrated their LC-MS/MS instrument using a series of calibration standards for the target PFAS and EIS compounds. The calibration range was to be as similar as possible to the calibration standards listed in the MLVS Method. A minimum of six contiguous calibrations standards were required for a valid analysis when using a linear calibration model, with at least

five of the six calibration standards being within the quantitation range (e.g., from the LOQ to the highest calibration standard). If a second-order calibration model was used at one laboratory, a minimum of seven calibration standards was required, with at least six of the seven calibration standards within the quantitation range. The number of calibration standards used by each laboratory for each target analyte ranged from six to nine. Three calibrations were required to be submitted by each laboratory prior to receiving study samples. During the validation process, it was discovered that one laboratory incorrectly spiked their ICAL standards. Since the laboratory was unable to rectify this error in a timely manner, no data from the laboratory were included in the statistical analysis of the ICAL.

Response Ratios (RR) and Response Factors (RF) were determined from the three calibrations run for the nine accepted laboratory ICAL data sets. For the target PFAS the RR and RF values varied across all nine laboratories (interlaboratory) but were consistent within each laboratory (intra-laboratory). For the target analytes quantified by isotope dilution, the mean RR values within each calibration ranged from 0.303 to 21.24. Over 96% (625 out of 648) of the RR values were below 5.0. The target compounds showed RF values that were not greatly different from the RR values. Over 85% of the results (371 out of 432) of the RF values were below 5.0. Relative Standard Deviations (RSD) calculated on the RR and RF data were < 20%. EPA methods that employ the RSD as a linearity metric generally specify QC limits on the order of 15–25%.

A similar examination of the calibration data was performed for the 24 labeled (EIS) compounds. The mean RF_s values ranged from 0.0159 to 68.5 across all the calibrations. High RF_s values were observed for $^{13}C_8$-PFOA in two laboratories, while the remaining seven laboratories had RF_s values from 0.8992 to 9.6059. All of the other EIS compounds had mean RFs below 5 across all of the laboratories. Across all 27 calibrations, the RSDs for the labeled compounds in each laboratory were below 20% with the exception of five values reported by Laboratory 3. In these cases, Laboratory 3 opted to employ the relative standard error (RSE) as the metric for the calibration fit. In both instances, the %RSE was below 20%.

These study data shows that the commonly used linearity metric of RSD or RSE ≤ 20% can be appropriate for the target and EIS compounds in this procedure.

Initial Demonstration of Capabilities

The laboratories next submitted documentation of an IDOC that consisted of an MDL determination, an IPR study, and the limit of quantitation verification (LOQVER). All IDOC samples were created using the Wellington standard mixtures provided by the MLVS.

Aqueous Method Detection Limits

MDLs for all 40 target analytes were determined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results (EPA 2017). The procedure consists of determination of the MDL based on method blanks (called MDL_b), and determination of the MDL based on spiked samples (called MDL_s). Both MDL_b and MDL_s are determined in a reference matrix, in this case PFAS-free reagent water, using at least seven replicates prepared and analyzed on three non-consecutive days.

A total of four MDL_b values were reported by two laboratories: no other laboratory reported an MDL_b value for those same analytes. Of those MDL_b values, only one laboratory for one analyte (6:2FTS), was greater than the calculated MDL_s value and was therefore used as the final MDL value. Through these MDL data and the routine method blank results generated during the course of the validation study, the study demonstrated that background levels in typical laboratories are not a limiting factor in the application of this method, but that some laboratories had better control of background levels than others.

Initial Precision and Recovery

For the IPR studies four aliquots of 0.5 L of PFAS-free reagent water were spiked with all 40 target analytes such that the final concentration of each PFAS in the IPR was greater than or equal to the LOQ and less than or equal to the midpoint of the laboratory's calibration. These spiked aliquots of PFAS-free reagent water were prepared and analyzed in exactly the same manner as study samples, per EPA Method 1633.

A total of 36 IPRs from nine laboratories were included in the statistical analysis, which evaluated the mean percent recovery, standard deviations, and relative standard deviation (RSD) of recoveries the target, EIS compound and NIS compounds. Of the 1,440 target analyte results reported from IPRs, four target analytes exceeded the target analyte criteria (40–150%), resulting in an exceedance rate of 0.28%. Of the 864 EIS compound results reported from IPRs, four recoveries exceeded the EIS compound criteria (20–150%), resulting in a failure rate of 0.46%. All 36 IPRs met the study IPR NIS criteria (>30% recovery).

Aqueous Limits of Quantitation Verification Analyses

A process for determining the LOQ was not mandated by the Study Plan, therefore each laboratory used their in-house procedures for establishing their LOQs. The Study Plan did, however, dictate a procedure for verification of their established LOQs. The Study Plan required laboratories to analyze a limit of quantitation verification sample (LOQVER) in order to verify their stated LOQs. A single aliquot of 0.5 L of PFAS-free reagent water was spiked with all 40 target analytes such that the final concentration of each PFAS in the LOQVER was one and two times the laboratory's LOQ and analyzed per EPA Method 1633. While laboratories were required to prepare and analyze only one LOQVER per the Study Plan, some laboratories chose to prepare and analyze as many as seven. All data from nine laboratories submitted were included in the statistical analysis.

A total of 18 LOQVERs were included in the statistical analysis. Of the 720 target analyte results reported from LOQVERs, three target analyte recoveries exceeded the target analyte criteria (40–150%), resulting in an exceedance rate of 0.42%. Of the 432 EIS compound results reported from LOQVERs, one exceeded the EIS compound criteria (20–150%), resulting in a failure rate of 0.23%. All 18 LOQVERs met the study NIS compound target acceptance criteria (>30% recovery).

E.S. 6 WASTEWATER PERFORMANCE EVALUATION

The results demonstrated the efficacy of EPA Method 1633 to accurately report PFAS concentrations in real-world wastewater samples. Seven individual wastewater sample series were sent frozen to the participating laboratories. Each sample series included an unspiked sample, three

replicate low spiked samples and three replicate high spiked samples, for a total of 49 analyses. During the validation process two laboratories were determined to be out of compliance with the Study requirements with respect to the concentration of EIS compound spiked into each wastewater sample; those results were rejected and not included in the method evaluation. For wastewater, one sample series was found to form a viscous precipitate that could not pass through the SPE cartridge. That sample series was excluded from further analyses, resulting in six sample series analyzed by eight laboratories.

For the combined (pooled) results of all samples and all laboratories, the mean target analyte recoveries (39 out of 40) fell between 72.3% and 128%, the exception being PFDoS (52.4%). Considerable variation was observed between individual laboratories average results for the low- and high-spiked samples, with greater interlaboratory variability observed in the low-spiked samples. For the low- and high-spiked samples, the proportion of all values that were between 70–130% of the spiked concentrations was >70% for most analytes, i.e., 70% of all values reported by the eight laboratories for the 40 PFAS were between 70 – 130%. The exceptions to this included PFDS, PFDoS, NMeFOSAA, NEtFOSAA, and 11Cl-PF3OUdS. When evaluated against the 40 – 150% OPR criteria, >95% of all values were reported within that range, with the exceptions of PFDoS, NMeFOSAA and NEtFOSAA.

EIS compound target percent recoveries were also variable amongst the eight laboratories; and likely contributed to the variability observed in the reported target compounds. For the low- and high-spiked samples, the proportion of all values that were between 20–150% of the spiked concentrations was >70% for all target analytes, with the exception of $^{13}C_2$-4:2FTS (55%). Data from one laboratory accounted for all of the 4:2FTS exceedances. The proportion of all values that were between 20–150% of the spiked concentrations was >90% for all EIS compounds, with the exception of $^{13}C_4$-PFBA (82.4%), $^{13}C_2$-4:2FTS (54.8%), $^{13}C_2$-6:2FTS (78.6%), $^{13}C_2$-8:2FTS (74.7%), and D_9-NEtFOSA (86.9%).

E.S. 7 SURFACE WATER PERFORMANCE EVALUATION

Similar to the findings observed in the wastewater samples, the pooled spiked target analyte percent recovery results were between 70–130% with the exception of the following compounds: PFDoS (63%), NMeFOSAA (144%), NEtFOSAA (151%), and PFMPA (68%). Low EIS compound recoveries in one laboratory, and anomalous high values for NMeFOSAA and NEtFOSAA reported by a second laboratory likely skewed these results. Never-the-less, these values were within the target range of 40 – 150%.

Evaluating the results for the individual laboratories, the proportion of all individual values that were between 40–150% of the spiked concentrations is >70% for all target analytes. For individual laboratories, exceptions included NMeFOSAA and NEtFOSAA. Evaluating the individual laboratories for reported values between 70–130% of the spiked concentrations was >70% for most analytes. The exceptions to this included PFDS, PFDoS, NMeFOSAA, NEtFOSAA, 11Cl-PF3OUdS, NEtFOSE, PFMPA, and 11Cl-PF3OUdS.

Reported EIS compound recoveries in surface waters were more consistent than observed in the wastewater analyses. For the low- and high-spiked samples, the proportion of all values that were between 20–150% of the spiked concentrations was >90% for all target analytes, with the

exception of $^{13}C_4$-PFBA where of the 182 reported EIS compound values, 59 values (32.4%.) were reported below 20% recovery.

E.S. 8 GROUNDWATER PERFORMANCE EVALUATION

EPA Method 1633 was demonstrated to adequately measure PFAS concentrations in real-world groundwater samples. The following limitations are noted. The pooled spiked target PFAS percent recovery results were between 70–130% with the exception of the following compounds: PFOS (157%), NMeFOSAA (153%), and NEtFOSAA (157%). High PFOS recoveries were reported by one laboratory for the low-spiked samples, which skewed the interlaboratory pooled result. PFOS measures for the other laboratories fell within the 70–130% range.

Evaluating the results across the individual laboratories, relative proportions for all laboratories > 70% of all reported values were between 70–130% of the spiked concentrations for most of the 40 analytes. For example, the relative proportion of PFOA and PFOS values occurring between 70–130% of the spiked concentration for all laboratories was >80%. The exceptions were PFDoS, NMeFOSAA, and NEtFOSAA.

For most laboratories, > 90% of all EIS compound recoveries were between 20–150% of the spiked concentrations. One laboratory had considerable issue with EIS compound recoveries which resulted in most of that laboratory's data being rejected from the statistical analyses.

E.S. 9 COMBINED AQUEOUS MEDIA PERFORMANCE EVALUATION

MLVS results demonstrated the ability of laboratories to routinely achieve the MLVS target acceptance criteria for sample preparation batch QC samples (Method Blank, OPR, and LLOPR). The concentration of each target analyte in the method blank was required to be <½ the laboratory's LOQ and < 1/10th the concentration of the target method in associated samples. The low rate of detection in method blanks demonstrated by this study, 18 out of 2,282 target analytes reported (0.79%) indicates the processes described in the method are successful in reducing the potential for bias associated with contamination. The target percent recovery for target analytes in OPRs and LLOPRs was 40–150%, for EIS compounds was 20–150%, and for NIS compounds was greater than 30%. A total of 58 OPRs were included in the statistical analysis. All 58 OPRs met the study OPR NIS criteria (>30% recovery). Of the 2,320 target analyte results reported from OPRs, two failed to meet the target analyte criteria (40–150%), resulting in a failure rate of 0.086%. All of the 57 LLOPRs included in the statistical analysis met the study LLOPR NIS compound recovery criteria (>30%). Of the 2,280 target analyte results reported from LLOPRs, seven failed to meet the target analyte criteria, resulting in a failure rate of 0.31%.

Matrix spike recoveries were statistically evaluated by Analysis of Variance (ANOVA) to test for differences among the various independent experimental factors (i.e., main effects). Main effects included the target analytes ("PFAS"), the different matrices ("Matrix"), laboratories ("Laboratory"), and spike concentrations ("Spike Concentration"). Because the final working dataset consisted of missing permutations of main effects, 1) no interaction effects were evaluated, and 2) the Least Squares Means from the ANOVA predictions are reported to more accurately reflect mean differences (i.e., marginal means that control for other model parameters). All main effects were significant with greater than 99% confidence. Specific to the PFAS main effect,

PFDoS (the largest perfluoroalkyl sulfonic acid evaluated), NMeFOSAA and NEtFOSAA were the only three target analytes with mean recoveries outside 70–130% of the target analyte spike concentration; PFDoS was observed with a low bias, whereas, both NMeFOSAA and NEtFOSAA were observed with a high bias. Mean recoveries for the Matrix, Spike Concentration, and Laboratory main effects were all much more consistent and closer to the target spike concentration (i.e., 100% recovery).

Despite statistically significant differences among the various levels of each main effect evaluated, the overall method accuracy and precision were quantified. Method accuracy was calculated as the mean percent bias (% recovery–100%) for each spike concentration and laboratory and matrix averaging over the method analytes to avoid an impracticable number of permutations. Similarly, precision was calculated as the intra-laboratory percent RSD among replicate measures of the various spiked samples Overall, the method as validated by this multi-laboratory study can be summarized to result in less than 40% error for aqueous samples.

E.S.10 CONCLUSION

The objectives of this MLVS were achieved; validation of EPA Method 1633 and the production of a method that can be implemented at a typical mid-sized full-service environmental laboratory. Overall, the data generated during the MLVS demonstrated that EPA Method 1633, as written, is robust enough to be performed by suitable laboratories using similar instruments of different manufacturers and models. The results generated by participating laboratories in this study routinely met the requirements stated in the method for:

- Mass calibration and mass calibration verification,
- Initial calibration and calibration verification,
- Determination of MDLs and LOQs,
- Initial Performance Recovery,
- Preparatory batch QC samples (MB, OPR, LLOPR), and
- Quantitative and qualitative analyte identification criteria.

The suitability of EPA Method 1633 to detect and quantify the 40 target analytes in groundwater, surface water, and wastewater samples was successfully demonstrated through the analysis of spiked groundwater, surface water, and wastewater samples. Method Blank results demonstrated that any bias associated with background contamination introduced during sample preparation was negligible. The IPR, OPR, and LLOPR recoveries and the EIS and NIS compound recoveries associated with study samples were used to derive QC acceptance criteria for inclusion in the finalized method.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFCEC	Air Force Civil Engineer Center
AFFF	aqueous film-forming foam
ANOVA	analysis of variance
ATP	alternate test procedure
CASRN	CAS registry number
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CV	calibration verification
DoD	U.S. Department of Defense
EDD	electronic data deliverable
EIS	extracted internal standard
ELAP	Environmental Laboratory Accreditation Program
EPA	U.S. Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
Exa	Exa Data & Mapping Services, Inc.
GDIT	General Dynamics Information Technology
GW	groundwater
HGL	HydroGeoLogic, Inc.
ICAL	initial calibration
ID	isotope dilution
IDA	Institute for Defense Analyses
IDOC	initial demonstration of capability
IPR	initial precision and recovery
ISC	instrument sensitivity check
LC-MS/MS	liquid chromatography–tandem mass spectrometry
LCS	laboratory control sample
LHA	lifetime health advisory
LLLCS	low-level laboratory control sample
LLOPR	low level ongoing precision and recovery
LOD	limit of detection
LOQ	limit of quantitation
LLOQ	lower limit of quantitation
LOQVER	limit of quantitation verification
m/z	mass to charge ratio
MB	method blank
MDL	method detection limit
MDL_b	MDL based on method blank

MDL_s	MDL based on spiked samples
mg/L	milligram per liter
MLVS	Multi-Laboratory Validation Study
MRM	multiple reaction monitoring
MS	matrix spike
MSD	matrix spike duplicate
NAVSEA	Naval Sea Systems Command
NIS	non-extracted internal standard
OLEM	Office of Land and Emergency Management
OPR	ongoing precision and recovery
ORD	Office of Research and Development
OW	[EPA] Office of Water
PFAS	per- and polyfluoroalkyl substances
PFAS acronyms	**see** Table 1-1
ppb	parts per billion
ppt	parts per trillion
QA	quality assurance
QC	quality control
QSM	quality systems manual
RF	response factor
RF_s	response factor of each EIS
RR	response ratio
RSD	relative standard deviation
RSE	relative standard error
SEE	Science and Engineering for the Environment, LLC
SERDP	Strategic Environmental Research and Development Program
SGS AXYS	SGS AXYS Analytical Services, Ltd. (Sidney, BC, Canada)
SLVS	Single-Laboratory Validation Study
SOP	standard operating procedure
SOW	statement of work
SPE	solid-phase extraction
SW	surface water
TDS	total dissolved solids
TSS	total suspended solids
USACE	U.S. Army Corps of Engineers
Waters ERA	ERA – A Waters Company
Wellington	Wellington Laboratories, LLC
WW	wastewater

1 INTRODUCTION

This report is the first in a series presenting the results of a multi-laboratory validation study (MLVS) designed to validate the Environmental Protection Agency's (EPA) draft Office of Water (OW) *Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS* (EPA Method 1633). A project designed to validate EPA Method 1633 was undertaken through the U.S. Department of Defense (DoD) Strategic Environmental Research and Development Program (SERDP). Conducted as a joint effort by SERDP, the DoD, and the EPA, the objectives of this project were to:

- Identify and quantify up to 40 per- and polyfluoroalkyl substances (PFAS) in aqueous matrices (groundwater, surface water, landfill leachate, and wastewater), solids (soil, sediment, and biosolids), and tissues using the isotope dilution liquid chromatography–tandem mass spectrometry (LC-MS/MS) method.
- Achieve a low parts per trillion (ppt) level of quantitation (LOQ) in aqueous matrices and parts per billion (ppb) in solids and tissues.
- Produce a method that can be implemented at a typical mid-sized full-service environmental laboratory.
- Conduct single- and multi-laboratory validation studies of the draft EPA Method 1633.

A standard operating procedure was developed, tested on eight environmental matrices and a Single-Laboratory Validation Study (SLVS) was completed with a report published in January 2022. That report, *Single Laboratory Validation Study of PFAS by Isotope Dilution LC-MS/MS*, resulted in the EPA OW publishing the draft EPA Method 1633 in September 2021, with updates of the method in June 2022, and December 2022.

The importance of the publication of the draft EPA Method 1633 (and by extension this Study) is reflected in the DoD's December 7, 2021, *Memorandum for the Update for Establishing a Constituent Methodology for the Analysis of Per- and Polyfluoroalkyl Substances in Media Other than Drinking Water*. This memorandum required that all new contracts and task orders after December 31, 2021, use draft EPA Method 1633 for the analysis for PFAS in matrices other than drinking water, using a laboratory accredited to the method/matrix/analyte by the DoD Environmental Laboratory Accreditation Program (DoD ELAP).

EPA Method 1633 is a draft method which requires a MLVS to be performed before the method can be finalized. The SLVS documents a single laboratory's ability to utilize the method to quantify the 40 target analytes down to relevant concentrations in various environmental matrices. An MLVS provides objective quality evidence (OQE) that the method can be implemented at a typical mid-sized, full-service environmental laboratory, utilizing similar, but not identical instrumentation. Although the draft EPA Method 1633 was validated in various environmental matrices, this report only addresses the multi-laboratory study results for the aqueous matrices of wastewater, surface water, and groundwater.

This study was designed to evaluate the robustness of draft EPA Method 1633 when performed by suitable laboratories using similar, but not identical, instrumentation, as well as provide information on the range of precision and accuracy of quantitation that is achievable by suitable laboratories.

This was achieved through the evaluation of data generated from PFAS-spiked environmental samples (herein identified as study samples). A Study Plan was developed that in addition to the procedures required for draft EPA Method 1633, included interim quality assurance (QA) and quality control (QC) criteria (MLVS Method, Appendix A). This study was undertaken using EPA's *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (2018) as guidance where applicable. This study was not an Alternate Test Procedure, so the guidance is not relevant for some steps. The study follows all of the steps EPA's Clean Water Act Method Program has done for previous new EPA methods.

Evaluation of the data collected, as well as consideration of feedback from the participating laboratories, is documented herein to provide the basis for revisions to draft EPA Method 1633. This report and subsequent reports, along with all pertinent MLVS documentation needed to support publication of draft EPA Method 1633 as a final method, will be provided to the EPA OW. Additionally, the information and data from this MLVS will also be submitted to the EPA Office of Land and Emergency Management (OLEM) for the future development of an EPA solid waste (SW)-846 method.

This report, being the first in the series of MLVS reports to be published, provides information that applies to all subsequent reports in addition to this report. It provides the project background, the overall project management, data validation, and data management. This report describes the processes for laboratory selection, selection of study sample sources, and study sample creation and delivery. In addition, it includes results from evaluation of the overall draft EPA Method 1633 performance of each laboratory. This included the evaluation of each laboratory's Standard Operating Procedure (SOP) and documentation of initial calibrations (ICAL), and the Initial Demonstration of Capabilities (IDOC), method detection limit (MDL) determination, and verification of their aqueous sample LOQ for aqueous sample matrices.

Upon successful demonstration of capabilities, PFAS-spiked wastewater, surface water, and groundwater samples were sent sequentially to each of the laboratories.

Note: Landfill leachate will be addressed in the next report. It is an aqueous matrix, but it has a different sample size and is usually more prone to interferences than the other aqueous matrices. Landfill leachate will have its own section in the next report. This report only addresses the aqueous matrices wastewater, surface water, and groundwater; assume only these three matrices are being discussed when aqueous results are discussed in this report.

Subsequent reports will present results for soils and sediments, fish and shellfish tissue, biosolids, and landfill leachate.

1.1 BACKGROUND

The use of man-made organofluorine chemicals, including various PFAS, fluorinated pharmaceuticals, and fluorinated pesticides, is widespread. Of this group of chemicals, PFAS are of particular concern due to their persistence in the environment. There are challenges to providing a single comprehensive definition for PFAS, but as a class, they are generally molecules with a carbon-carbon alkyl chain with multiple carbon-fluorine bonds. PFAS comprise a group of

thousands of man-made chemicals that have been in production since the 1940s and are found in a variety of consumer products such as cookware, food packaging, and water-repellent fabrics.

Voluntary efforts to phase out the eight-carbon compounds perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) began in 2000, but they are persistent in the environment and resistant to typical environmental degradation processes. As a result of that phase out of PFOA and PFOS, a large variety of other PFAS are now in common use as alternatives. Many PFAS are soluble in water and are therefore highly mobile in the environment. As a result, they are extensively distributed across all trophic levels and are found in soil, air, and groundwater at sites across the world. Some PFAS, particularly the perfluoroalkyl carboxylic acids and sulfonic acids, do not breakdown readily in the environment and are known to bioaccumulate in aquatic and terrestrial biota, with some compounds bioaccumulating more than others.

Because PFAS are ubiquitous in the environment, there is a need for a robust method that can quantify as many PFAS as practical in a variety of environmental matrices. Therefore, a method was developed and validated in a single laboratory in 2020 through an effort by a project team headed by the DoD SERDP in conjunction with EPA, led by the OW and with contributions from the EPA OLEM and Office of Research and Development (ORD).

Evidence that continued exposure to certain PFAS above specific levels may lead to adverse health effects led the EPA to publish provisional health advisories levels for drinking water for perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonate (PFOS) in 2009. In June 2022, the EPA revised their 2009 Lifetime Health Advisories (LHA), publishing interim updated in the Congressional Federal Register (40 CFR 87 Part 118) LHAs for PFOS and PFOA to a level of 0.02 parts per trillion (ppt) and 0.004 ppt, respectively, in drinking water. In addition, the EPA also issued final health advisories for GenX chemicals and PFBS of 10 ppt and 2,000 ppt, respectively.

Publication of the LHAs led to widespread public concern about PFAS fate and transport, potential deleterious effects on human health and ecological receptors, and how to manage these recalcitrant compounds. EPA recognized the need to develop analytical methods for PFAS for other matrices that are regulated under the Clean Water Act, the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), and the Toxic Substance Control Act, as well as other ongoing efforts to demonstrate potential clean-up and PFAS-containing waste disposal (EPA, 2019; 2020). In 2022 EPA proposed designation of PFOA and PFAS as hazardous substances under CERCLA (40 CFR Part 302).

The DoD has environmental management responsibilities for PFAS released to the environment associated with the use of aqueous film-forming foam (AFFF) (Leeson et al., 2020; Anderson et al., 2020). The use of AFFF has resulted in the widespread occurrence of PFAS in groundwater, drinking water, soils, sediments, receiving waters, and ecological receptors at many current and former military installations as well as more broadly throughout the community. Site characterization and clean-up of these sites is being conducted principally under CERCLA, but these characterizations are hindered by the lack of an EPA analytical method for PFAS in those matrices managed under the Superfund program. As an interim measure, the DoD ELAP provides accreditation to analytical laboratories that demonstrate competency and document conformance to the QC standard for PFAS published in the DoD and Department of Energy Consolidated Quality Systems Manual (QSM) for Environmental Laboratories, Version 5.3, Table B-15 (QSM 5.3).

Commercial environmental laboratories that demonstrated competency and document conformance to QSM 5.3 were accredited for PFAS analysis in environmental matrices other than drinking water. The creation of this standard was a stop-gap approach until a suitable EPA method for PFAS analysis was available.

Recognizing this challenge and opportunity, the EPA and the DoD are collaborating on the development of an isotope dilution method for non-drinking water aqueous matrices (surface water, groundwater, wastewater influent/effluent, landfill leachate), solids (soil, sediment, biosolids), and tissues (fish and clam).

EPA Method 1633 is a draft method which requires a MLVS. The end goal of this MLVS is to use the findings generate data that EPA OW can use to develop QC acceptance criteria and to revise, as necessary, the draft EPA Method 1633, and to submit the supporting data packages to the EPA OW for consideration as a final method under the Clean Water Act. If recommended for approval, EPA will prepare a proposed rule using information provided by the MLVS Team from this study. Then, EPA will propose the method for approval through rulemaking. Assuming the proposal and public comment response are successful, the method will be promulgated and approved at 40 CFR Part 136 for NPDES monitoring.

1.2 METHOD SUMMARY

The analytical method for this study was validated and included in the report, *Single Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS* (SERDP 2020 and 2021), and defined in the August 2021 draft of EPA 1633. Refinements to that method were made based on interactions and feedback from the 10 laboratories that participated in this MLVS. Updates reflecting those changes was released by EPA as a Revised Errata Sheet in February 2022, and in the 2nd Draft Method 1633 in June 2022. The complete method used for this study is provided in Appendix A to this report.

The analytical method includes both sample preparation and sample analysis procedures that are applicable to a variety of environmental matrices. The matrices evaluated by this study included wastewater, surface water, groundwater, landfill leachate, soil, sediment, biosolids, and tissue. The aqueous matrices were prepared via solid-phase extraction (SPE) and carbon clean-up processes. Soil, sediment, biosolids, and tissue matrices were prepared via solvent extraction and SPE, followed by carbon clean-up processes. The method utilized liquid chromatography–tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode to evaluate quantification and confirmation (where applicable) of ions of each of the 40 target analytes (Table 1-1). Analyte concentrations were determined using either an isotope dilution or extracted internal standard (EIS) quantification scheme; both utilized isotopically labeled compounds that were added to the samples prior to extraction. At the time of validation, only 24 isotopically labeled analogs of the 40 target analytes were commercially available, and therefore only 24 target analytes could be quantified using isotope dilution quantitation. All other analytes were quantified using EIS quantitation with these isotopically labeled analogs. Recovery of both quantification schemes corrects the analyte results. Analytes were quantified and reported as their acid form.

Seven non-extracted internal standards (NIS)[2] were used to determine EIS recoveries and provide a general indicator of overall analytical quality. A list of the 40 target analytes, 24 EIS compounds, and seven NIS compounds is provided in Table 1-1.

1.3 SUMMARY OF THE RESULTS OF THE SINGLE-LABORATORY STUDY

The single-laboratory validation was performed by SGS AXYS Analytical Services, Ltd. (Sidney, BC, Canada) (SGS AXYS), the developer of the original laboratory SOP that was selected by the DoD and EPA workgroup and that study was deemed a success because it met EPA's three goals for the study, namely:

1. *Identify and quantify up to 40 PFAS in aqueous matrices (groundwater, surface water, landfill leachate, and wastewater), solids (soil, sediment, and biosolids), and tissues using the isotope dilution LC-MS/MS method.*

   The study generated method performance data for aqueous, solid, and tissue matrices. Of the eighty-five matrix spike samples analyzed during the single-laboratory study:

   - Eighty-two percent of the aqueous samples achieved recoveries between 70–130% (1,873 out 2280 results, or 82.1%).
   - Eighty-nine percent of the solid samples achieved recoveries between 75–130% (1,321 out of 1,480 results, or 89.3%).
   - Seventy-three percent of the fish tissue samples achieved recoveries between 70–130% (263 out of 360 results, or 73.1%).

The single-laboratory validation results demonstrated that this method can identify and quantify individual PFAS.

2. *Achieve a low ppt LOQ in aqueous matrices and ppb in solids and tissues.* The single-laboratory validation results demonstrated that this method could quantify 40 PFAS at levels between 1.6 and 40 ng/L in a 500-mL aqueous sample, between 0.2 and 5.0 ng/g in a 5-g solid sample, and between 0.5 and 12.5 ng/g in a 5-g tissue sample.

EPA's third goal for the single-laboratory study was to show that:

3. The method can be implemented at a typical mid-sized full-service environmental laboratory.

   Because all of the required instrumentation for this method has become commonplace in many full-service environmental laboratories, the results of the single-laboratory study demonstrate that this goal is achievable. The multi-laboratory validation study will determine how well a typical full-service laboratory can perform the method.

[2] NIS were referred to in the SLVS Report as Injected Internal Standards (NIS). EPA used *NIS* in the draft EPA Method 1633; NIS is adopted for this MLVS report.

Table 1-1. Names, Abbreviations, and Chemical Abstract Service Registry Numbers (CASRN) for Target PFAS, Extracted Internal Standards, and Non-extracted Internal Standards[1]

Analyte Name	Abbreviation	CASRN
Target Analytes		
Perfluoroalkyl carboxylic acids		
Perfluorobutanoic acid	PFBA	375-22-4
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTeDA	376-06-7
Perfluoroalkyl sulfonic acids		
Acid Form		
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluoropentanesulfonic acid	PFPeS	2706-91-4
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorononanesulfonic acid	PFNS	68259-12-1
Perfluorodecanesulfonic acid	PFDS	335-77-3
Perfluorododecanesulfonic acid	PFDoS	79780-39-5
Fluorotelomer sulfonic acids		
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4
Perfluorooctane sulfonamides		
Perfluorooctanesulfonamide	PFOSA	754-91-6
N-methyl perfluorooctanesulfonamide	NMeFOSA	31506-32-8
N-ethyl perfluorooctanesulfonamide	NEtFOSA	4151-50-2
Perfluorooctane sulfonamidoacetic acids		
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
Perfluorooctane sulfonamide ethanols		
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2

Table 1-1. Names, Abbreviations, and Chemical Abstract Service Registry Numbers (CASRN) for Target PFAS, Extracted Internal Standards, and Non-extracted Internal Standards[1]

Analyte Name	Abbreviation	CASRN
Per- and Polyfluoroether carboxylic acids		
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6
Ether sulfonic acids		
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OudS	763051-92-9
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Fluorotelomer carboxylic acids		
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5
2*H*,2*H*,3*H*,3*H*-Perfluorooctanoic acid	5:3FTCA	914637-49-3
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4
Extracted Internal Standard (EIS) Compounds		
Perfluoro-n-[$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	NA
Perfluoro-n-[$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	
Perfluoro-n-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	
Perfluoro-n-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	$^{13}C_2$-PFTeDA	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid	$^{13}C_3$-PFBS	
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid	$^{13}C_3$-PFHxS	
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid	$^{13}C_8$-PFOS	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	$^{13}C_8$-PFOSA	
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	D_3-NMeFOSAA	
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	D_5-NEtFOSAA	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]hexanesulfonic acid	$^{13}C_2$-4:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]octanesulfonic acid	$^{13}C_2$-6:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]decanesulfonic acid	$^{13}C_2$-8:2FTS	
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	

Table 1-1. Names, Abbreviations, and Chemical Abstract Service Registry Numbers (CASRN) for Target PFAS, Extracted Internal Standards, and Non-extracted Internal Standards[1]

Analyte Name	Abbreviation	CASRN
N-methyl-d_7-perfluorooctanesulfonamidoethanol	D_7-NMeFOSE	
N-ethyl-d_9-perfluorooctanesulfonamidoethanol	D_9-NEtFOSE	
N-methyl-d_3-perfluoro-1-octanesulfonamide	D_3-NMeFOSA	
N-ethyl-d_5-perfluoro-1-octanesulfonamide	D_5-NEtFOSA	
Non-extracted Internal Standard (NIS) Compounds		
Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid	$^{13}C_3$-PFBA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanesulfonic acid	$^{13}C_4$-PFOS	NA
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	$^{13}C_5$-PFNA	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA	
Perfluoro-1-hexane[$^{18}O_2$]sulfonic acid	$^{18}O_2$-PFHxS	

Notes:
[1] The target analyte names are for the acid and neutral forms of the analytes. See Table 8 in the draft EPA Method 1633, Analysis of PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS for the names and CASRN of the corresponding anion forms, where applicable.
CASRN = Chemical Abstracts Service Registry Number.
LC-MS/MS = liquid chromatography mass spectrometry/mass spectrometry.
NA = Not applicable; NIS and EIS compounds do not have CASRN.
PFAS = Per- and Polyfluoroalkyl Substances.

2 STUDY MANAGEMENT, OBJECTIVES, DESIGN, AND IMPLEMENTATION

The study objectives and design are described in the Study Plan *for Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS* (Study Plan), which is included as Appendix A of this report. The overall study was designed to resemble that of the *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater under EPA's Alternate Test Procedure Program* (EPA Office of Water, 2018). While this MLVS is not designed to support an alternate test procedure (ATP) application, the number of matrices and statistical analyses of the data mirror what would be required for an ATP for national use.

2.1 STUDY MANAGEMENT: PFAS METHOD VALIDATION TEAM

A joint EPA and DoD PFAS Method Validation Team was formed to oversee the PFAS analytical method development and validation. Study management was done cooperatively as the MLVS Team, which included SERDP/Environmental Security Technology Certification Program (ESTCP); the U.S. Army Corps of Engineers (USACE); EPA's Offices of Water, of Land and Emergency Management, of Research and Development; the U.S. Navy; and the U.S. Air Force. SERDP/ESTCP, the USACE, EPA OW, the U.S. Navy, and the U.S. Air Force approved and are co-signers to the *Study Plan*.

Funding for this project was provided by SERDP/ESTCP to the USACE, which in turn contracted with HydroGeoLogic, Inc. (HGL) to serve as the Oversight Contractor for the project. SERDP/ESTCP also established contracts with Science and Engineering for the Environment LLC (SEE), for program management; Exa Data & Mapping Services, Inc., (Exa) for data management; and the following firms for independent, third-party data validation: Chem Val Consulting, Inc.; Jacobs Engineering Group, Inc.; and Pyron Environmental Inc. The Institute for Defense Analyses (IDA) conducted statistical analyses on the resulting data. The funding for both the single-laboratory and the multiple-laboratory validation studies was provided by SERDP.

Ten laboratories (eight commercial contract laboratories and two state laboratories) agreed to participate in the study. The ten laboratories participating are listed in Table 2-1. All ten laboratories contributed to the analysis of the aqueous matrices in this report (wastewater, surface water, and groundwater). For the purposes of this study, the laboratories were randomly assigned numbers, which were used to maintain the anonymity of the results. Not all laboratories participated in all media; two laboratories opted out of participating in the study for landfill leachate, biosolids, and tissues, with one laboratory also opting out of the sediments (Table 2-2).

The laboratories were contracted to HGL, which also managed the contracting for sample spiking and shipment and received all data packages from the laboratories. HGL also contracted a commercial vendor, Wellington Laboratories, LLC (Wellington), to provide analytical standard mixtures and individual, high-concentration PFAS analytical standards as defined by the MLVS Team to the laboratories participating in the study. Another commercial vendor, Waters ERA, which specializes in proficiency testing samples, prepared and shipped the Study Samples using "real-world" environmental sample matrices.

The draft method used in this MLVS stemmed from an SOP developed by SGS AXYS, who was contracted to conduct the baseline (i.e., background) measures of PFAS in the test environmental matrices and serve as the method consultant to the MLVS.

2.2 STUDY OBJECTIVE AND DESIGN

The focus of the MLVS is to generate the necessary data to document the precision and accuracy and overall performance of the analytical method for quantitation of PFAS in environmental matrices. The primary objectives of this MLVS are to:

- Obtain data from matrices that are representative of the method's intended use.
- Obtain data from laboratories that are representative of those likely to use the method, but that were not directly involved in its development.
- Obtain feedback from laboratory users on the specifics of the draft method.
- Use study data to characterize performance of the method.
- Develop statistically derived QC acceptance criteria that will reflect method performance capabilities in real-world situations.

The design of the multi-laboratory study is described in a formal study plan that is included as Appendix A to this report. Briefly, the design involved:

- At least nine laboratories, with a goal of complete aqueous sample data from at least six laboratories
- Seven wastewater samples from a variety of sources plus an assortment of surface and groundwaters
- Multi-point calibration of the target analytes by each laboratory
- IDOC by each laboratory
- Determination of MDLs by each laboratory
- Analyses of matrix spike samples prepared from each of the aqueous samples.

This MLVS was conducted in specific phases. The procedures for these phased studies are given in the Study Plan in Appendix A and are briefly summarized below.

- ICAL, initial demonstration of capability, MDL study, and limit of detection and LOQ verifications.
- Method evaluation in wastewaters, surface water, and groundwater matrices.
- Method evaluation in soils and sediment.
- Method evaluation in tissue (fish and shellfish).
- Method evaluation in biosolids and landfill leachate.

In order to expedite EPA's release of a non-draft version of EPA Method 1633, this report focuses only the aqueous sample portion of the multi-laboratory study (not including landfill leachate, which has a different sample size). The solids and tissue matrices are not subject to the same requirements for analyses by methods approved at 40 CFR Part 136 as are wastewater samples and will be addressed in subsequent reports.

2.3 MATRICES AND SAMPLE SELECTION

The MLVS was designed to provide a test of the method by analyses of real-world environmental matrices, including wastewaters, groundwater, surface water (fresh and marine), soil/sediment (fresh and marine), fish and clam tissues, landfill leachate, and biosolids. To obtain a wide diversity and sufficient quantity of matrices and samples, SERDP and EPA coordinated with municipal, state, and EPA Regional contacts to obtain sufficient volumes/mass used in the study.

The list of all aqueous samples acquired for this study is found in the Study Plan (Appendix A, Attachment 2). The specific samples used are provided in Table 2-3. Samples and sources are discussed briefly below.

Seven wastewater, three surface water, and three groundwater samples were used for this phase of the Study (Table 2-3). EPA's OW arranged for representative wastewaters to be shipped to Waters ERA in Golden, CO. Six of the wastewater matrices included effluents from a publicly owned treatment works and wastewaters from specific industrial discharges. A substitute wastewater was also included in the samples and was prepared as specified in ASTM International Reference D5905-98, *Standard Practice for the Preparation of Substitute Wastewater*.

Four surface water samples were collected for this study. EPA provided two freshwater samples: one from Lake Harsha, OH, and a second collected from the St. Louis River near Duluth, MN. An additional freshwater sample was collected from Burley Creek in Washington state. A saltwater sample was collected from Sequim Bay in Washington state. Of these, the Lake Harsha, Burley Creek, and Sequim Bay waters were carried forward. Reconnaissance PFAS analysis of the sample collected from the St. Louis River reported no detectable quantities of any of the 40 target analytes; therefore, it was omitted from the study.

Three groundwater samples were collected and contributed to the study. Two samples were collected by the USACE from active PFAS-investigation sites in Kansas and Colorado, and one sample collected by the EPA from an active PFAS-investigation site in the southwest.

The MLVS was designed so that for each sample there would be a pre-spike characterization sample, an unspiked (or "native") sample, three replicates at a low spike concentration, and three replicates at a high spike concentration (Table 2-3). Each sample was assigned a matrix code (WW, SW, GW), a single letter sample identifier; the native sample was assigned the number 0, the unspiked study sample assigned the number 1, low spike replicates 2–4, and the high spike replicates 5–7.

2.4 SELECTION OF SPIKING LEVELS AND AQUEOUS MEDIA

All of the wastewaters, surface waters, and groundwaters were screened for baseline PFAS levels. In addition, all aqueous samples were measured for the following characteristics: total suspended solids (TSS), total dissolved solids (TDS), oil and grease, total petroleum hydrocarbons, calcium, sodium, pH, specific conductance, chloride, sulfate, total alkalinity (as calcium carbonate).

Waters ERA homogenized all sample matrices and shipped aliquots of composite samples collected from each to SGS AXYS for native PFAS analyses and to Eurofins-TestAmerica (ETA)-Denver for conventional physical and chemical analyses.

Results of the baseline target PFAS in the three aqueous media samples are presented in Table 2-4. At least one or more target PFAS were measured in all of the environmental samples selected for this study.

From these results, the EPA and the DoD determined appropriate low-spike, and high-spike concentrations for each target PFAS (Table 2-4). The intent was to bracket the range of PFAS concentrations observed in the test samples while keeping the concentrations within the calibration range provided in the method (Appendix A).

Conventional results are presented in Table 2-5, Table 2-6, and Table 2-7 for wastewater, surface water, and groundwater, respectively. The data were designed to match *EPA's Alternate Test Procedure Program* (USEPA 2018), where at least one of the wastewater matrix types will have one of the following characteristics, such that each criterion below is represented by at least one wastewater sample:

- TSS greater than 40 milligrams per liter (mg/L)
- TDS greater than 100 mg/L
- Oil and grease greater than 20 mg/L
- Sodium chloride greater than 120 mg/L
- Calcium carbonate greater than 140 mg/L

2.5 PREPARATION OF STUDY SAMPLES

Preparation of all selected study samples was performed by Waters ERA, and followed the general procedures documented in the Study Plan (Appendix A). Specific spiking procedures followed at Waters ERA are provided in Appendix B.

High and low spiking levels were set by the Study Quality Assurance (QA) Manager and EPA based upon review of the baseline (background) PFAS concentrations for the aqueous samples (wastewater, surface water, groundwater and landfill leachate). See Table 2-4.

Study samples of 500 mL were spiked by Waters ERA at two concentrations per analyte, using concentrated standards procured from Wellington. The bottles were inverted several times to homogenize the samples. Once the aliquots were spiked, they were sealed and segregated to a designated area of Waters ERA to prevent double spiking accidents. Samples were typically spiked during the week prior to shipping, frozen at -20º C through the weekend, and packed and shipped the following Monday.

Waters ERA issued Certificates of Spiking for all matrices and all spike samples (high and low). An example certificate is shown in Figure 2-1.

Table 2-1. Participating Laboratories

Laboratory/Supplier	Location	Role
Participating MLVS Laboratories		
Alpha Analytical [1]	Mansfield, MA	MLVS Participant Laboratory (laboratories were randomly assigned numbers 1 to 10 in the remainder of this report)
Battelle Memorial Institute	Norwell, MA	
California EPA	Pasadena, CA	
Eurofins Lancaster	Lancaster, PA	
Eurofins-TestAmerica (ETA) West Sacramento	West Sacramento, CA	
GEL Laboratories	Charleston, SC	
Pace Analytical	Baton Rouge, LA	
Maryland Department of Health	Baltimore, MD	
SGS North America	Orlando, FL	
Vista Analytical Laboratory [1]	El Dorado Hills, CA	
Ancillary Laboratories		**Role**
Waters ERA	Golden, CO	PFAS-spiked matrices and sample shipment for all aqueous, solid and tissues
SGS AXYS Analytical Services, Ltd.	Sydney, BC, Canada	Native PFAS measures for all aqueous, solid, and tissue samples
Eurofins-TestAmerica (ETA) Denver	Arvada, CO	Ancillary analytical measures for wastewater, surface water, groundwater, soils, solids, and tissue
Wellington Laboratories, LLC	Overland Park, KS	Provider of all PFAS standards for matrix spiking, calibration, as well as Extracted Internal Standards and Non-extracted Internal Standards

Notes:

1. During the MLVS Alpha Analytical was purchased by Pace Analytical. Vista Analytical Laboratory was purchased by Enthalpy Analytical.

Table 2-2. Participant Laboratory Number and Matrices Analyzed

MLVS Participant Laboratory Number	PFAS Matrix Analyses												
	Initial Calibration	Initial Dem. Capabilities			Aqueous Matrices				Solid Matrices			Tissue Matrices	
		Aqueous	Solid	Tissue	Waste water	Surface Water	Ground water	Landfill Leachate	Soil	Sediment	Biosolids	Fish	Shellfish
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	✓	✓	✓	×	✓	✓	✓	×	✓	×	×	×	×
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	✓	✓	✓	×	✓	✓	✓	×	✓	✓	×	×	×
8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
9	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
10	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Notes:

✓ indicates participated in specific media/matrices.

× indicates did not participate in specific media/matrices.

Table 2-3. Wastewater, groundwater, and surface water samples used for the low/high PFAS spikes.

Requested Name	Description	Matrix Code	Sample Identifier	Characterization Pre-Spike	MLVS Sample IDs							Sample Spike Date
					Unspiked	Low			High			
						Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	
Wastewater												
Hospital	Hospital	WW	I	WWI0	WWI1	WWI2	WWI3	WWI4	WWI5	WWI6	WWI7	4/19/2022
POTW Influent	POTW Influent	WW	J	WWJ0	WWJ1	WWJ2	WWJ3	WWJ4	WWJ5	WWJ6	WWJ7	4/19/2022
ASTM Substitute	ASTM Substitute	WW	K	WWK0	WWK1	WWK2	WWK3	WWK4	WWK5	WWK6	WWK7	4/19/2022
WW Bus Washing Station	WW Bus Wash	WW	L	WWL0	WWL1	WWL2	WWL3	WWL4	WWL5	WWL6	WWL7	4/20/2022
Playa Del Ray, CA	Plant Effluent	WW	M	WWM0	WWM1	WWM2	WWM3	WWM4	WWM5	WWM6	WWM7	4/20/2022
P&P WW	#1- 28	WW	N	WWN0	WWN1	WWN2	WWN3	WWN4	WWN5	WWN6	WWN7	4/20/2022
POTW Effluent	POTW Effluent	WW	O	WWO0	WWO1	WWO2	WWO3	WWO4	WWO5	WWO6	WWO7	4/21/2022
Groundwater												
USACE	GW #1, midwest	GW	A	GWA0	GWA1	GWA2	GWA3	GWA4	GWA5	GWA6	GWA7	5/10/2022
LRPCD	GW #2, southwest	GW	B	GWB0	GWB1	GWB2	GWB3	GWB4	GWB5	GWB6	GWB7	5/10/2022
USACE	GW Colorado #13	GW	C	GWC0	GWC1	GWC2	GWC3	GWC4	GWC5	GWC6	GWC7	5/10/2022
Surface Water												
Lake Harsha, OH	SW OH 9/10	SW	D	SWD0	SWD1	SWD2	SWD3	SWD4	SWD5	SWD6	SWD7	5/10/2022
Burley Creek, WA	Burley Creek	SW	F	SWF0	SWF1	SWF2	SWF3	SWF4	SWF5	SWF6	SWF7	5/10/2022
Sequim Bay, WA	Sequim Seawater	SW	G	SWG0	SWG1	SWG2	SWG3	SWG4	SWG5	SWG6	SWG7	5/10/2022

Table 2-4. Target Low/High PFAS Spike Concentrations and Calibration Range based on Native PFAS Analyses in Wastewater, Groundwater and Surface Water

Target PFAS	Target PFAS Spike Concentrations		Target Calibration 500-mL Sample		PFAS Target Compound Analytical Results													
					Wastewater Samples (ng/L)							Surface Water Samples (ng/L)			Groundwater Samples (ng/L)		
	Low Spike[1]	High Spike[1]	Low Cal	High Cal	WWI0	WWJ0	WWK0	WWL0	WWM0	WWN0	WWO0	SWD0	SWF0	SWG0	GWA0	GWB0 [2]	GWC0
PFBA	80	400	6.4	2000	1.93	4.83	< 1.454	10.09	9.06	2.96	7.50	2.89	< 1.449	< 1.431	10.02	**139.60**	< 1.450
PFPeA	40	200	3.2	1000	6.08	12.10	< 0.7270	31.20	10.19	3.29	19.47	1.26	< 0.7246	< 0.7156	10.53	**332.70**	< 0.7249
PFHxA	20	100	1.6	500	7.66	13.09	< 0.3635	58.50	27.20	1.62	37.50	1.00	0.72	< 0.3578	18.99	**762.70**	0.95
PFHpA	20	100	1.6	500	1.89	7.14	< 0.3635	15.99	2.99	0.96	7.89	0.81	0.40	< 0.3578	5.02	**160.20**	< 0.3624
PFOA	20	100	1.6	500	2.06	9.87	< 0.3635	5.88	9.99	1.03	9.50	1.02	0.64	< 0.3578	4.77	**688.90**	0.43
PFNA	20	100	1.6	500	0.42	3.28	< 0.3635	1.82	1.09	< 0.3689	2.66	0.49	< 0.3623	< 0.3578	< 0.3909	7.50	< 0.3624
PFDA	20	100	1.6	500	< 0.4007	0.92	< 0.3635	1.79	1.75	< 0.3689	0.65	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFUnA	20	100	1.6	500	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFDoA	20	100	1.6	500	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFTrDA	20	100	1.6	500	Q 0.4093	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFTeDA	20	100	1.6	500	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFBS	20	100	1.6	500	2.07	3.82	< 0.3635	0.99	Q 5.601	0.86	10.62	0.72	0.71	< 0.3578	38.11	**256.70**	1.63
PFPeS	20	100	1.6	500	2.26	0.51	< 0.3653	< 0.3979	< 0.3731	Q 0.4182	2.40	< 0.3697	< 0.3641	< 0.3596	37.01	**312.10**	0.44
PFHxS	20	100	1.6	500	17.01	2.86	< 0.3635	0.70	1.51	1.20	13.11	< 0.3678	0.84	< 0.3578	131.90	**3424.00**	1.92
PFHpS	20	100	1.6	500	Q 1.162	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	0.97	< 0.3678	< 0.3623	< 0.3578	3.31	47.10	< 0.3624
PFOS	20	100	1.6	500	Q 5.207	6.61	< 0.3635	1.85	2.59	3.60	32.23	0.98	< 0.3623	< 0.3578	76.49	**2314.00**	< 0.3624
PFNS	20	100	1.6	500	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFDS	20	100	1.6	500	R 0.5789	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFDoS	20	100	1.6	500	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
4:2FTS	80	240	6.4	400	< 0.4007	< 1.525	< 1.454	< 1.584	< 1.485	< 1.476	< 1.554	< 1.471	< 1.449	< 1.431	< 1.563	< 15.58	< 1.450
6:2FTS	80	240	6.4	400	< 1.444	19.68	< 1.310	5.14	9.36	< 1.330	223.90	< 1.326	< 1.306	< 1.290	< 1.409	**129.00**	< 1.307
8:2FTS	80	240	6.4	400	< 1.603	4.88	< 1.454	1.89	< 1.485	< 1.476	< 1.554	< 1.471	< 1.449	< 1.431	< 1.563	< 15.58	< 1.450
PFOSA	20	100	1.6	500	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	9.93	< 0.3624
NMeFOSA	20	100	4	500	< 0.4608	< 0.4384	< 0.4180	< 0.4553	< 0.4270	< 0.4243	< 0.4469	< 0.4230	< 0.4167	< 0.4115	< 0.4495	< 4.480	< 0.4168
NEtFOSA	20	100	4	500	< 1.002	< 0.9530	< 0.9088	< 0.9898	< 0.9282	< 0.9224	< 0.9715	< 0.9196	< 0.9058	< 0.8945	< 0.9772	< 9.739	< 0.9061

Table 2-4. Target Low/High PFAS Spike Concentrations and Calibration Range based on Native PFAS Analyses in Wastewater, Groundwater and Surface Water

Target PFAS	Target PFAS Spike Concentrations		Target Calibration 500-mL Sample		PFAS Target Compound Analytical Results													
					Wastewater Samples (ng/L)							Surface Water Samples (ng/L)			Groundwater Samples (ng/L)		
	Low Spike[1]	High Spike[1]	Low Cal	High Cal	WWI0	WWJ0	WWK0	WWL0	WWM0	WWN0	WWO0	SWD0	SWF0	SWG0	GWA0	GWB0 [2]	GWC0
NMeFOSAA	20	100	4	100	< 0.4007	1.43	< 0.3635	< 0.3959	1.04	< 0.3689	0.46	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
NEtFOSAA	20	100	1.6	500	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
NMeFOSE	160	400	16	1000	< 4.007	< 3.812	< 3.635	< 3.959	< 3.713	< 3.689	< 3.886	< 3.678	< 3.623	< 3.578	< 3.909	< 38.96	< 3.624
NEtFOSE	160	400	16	1000	< 2.997	< 2.851	< 2.719	< 2.962	< 2.777	< 2.760	< 2.907	< 2.751	< 2.710	< 2.676	< 2.924	< 29.14	< 2.711
HFPO-DA	80	240	6.4	400	< 1.523	< 1.449	< 1.381	1.88	< 1.411	< 1.402	< 1.477	< 1.398	< 1.377	< 1.360	< 1.485	< 14.80	< 1.377
ADONA	80	240	6.4	400	< 1.603	< 1.525	< 1.454	< 1.584	< 1.485	< 1.476	< 1.554	< 1.471	< 1.449	< 1.431	< 1.563	< 15.58	< 1.450
9CL-PF3ONS	80	240	6.4	400	< 1.607	< 1.529	< 1.458	< 1.588	< 1.489	< 1.479	< 1.558	< 1.475	< 1.453	< 1.435	< 1.567	< 15.62	< 1.453
11CL-PF3OudS	80	240	6.4	400	< 1.605	< 1.527	< 1.456	< 1.586	< 1.487	< 1.478	< 1.556	< 1.473	< 1.451	< 1.433	< 1.565	< 15.60	< 1.452
3:3FTCA	80	400	8	400	< 1.603	< 1.525	< 1.454	< 1.584	< 1.485	< 1.476	< 1.554	< 1.471	< 1.449	< 1.431	< 1.563	< 15.58	< 1.450
5:3FTCA	120	2000	40	2500	< 10.02	< 9.530	< 9.088	48.01	14.21	< 9.224	< 9.715	< 9.196	< 9.058	< 8.945	< 9.772	< 97.39	< 9.061
7:3FTCA	120	2000	40	2500	< 10.02	< 9.530	< 9.088	< 9.898	< 9.282	< 9.224	< 9.715	< 9.196	< 9.058	< 8.945	< 9.772	< 97.39	< 9.061
PFEESA	40	200	3.2	200	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
PFMPA	40	200	3.2	1000	< 0.8013	< 0.7624	< 0.7270	< 0.7919	< 0.7426	< 0.7379	< 0.7772	< 0.7356	< 0.7246	< 0.7156	< 0.7817	< 7.791	< 0.7249
PFMBA	40	200	3.2	1000	< 0.4007	< 0.3812	< 0.3635	< 0.3959	< 0.3713	< 0.3689	< 0.3886	< 0.3678	< 0.3623	< 0.3578	< 0.3909	< 3.896	< 0.3624
NFDHA	40	200	8	200	< 0.8013	< 0.7624	< 0.7270	< 0.7919	< 0.7426	< 0.7379	< 0.7772	< 0.7356	< 0.7246	< 0.7156	< 0.7817	< 7.791	< 0.7249

Notes:

[1] All spike concentrations are presented as acid concentrations; as final concentration in sample in ng/L.
[2] Sample GWB was diluted 10:1 before spiking due to the high native contractions of some target analytes. Specifically, those compounds in the **cells shaded and highlighted with bold font**.

Table 2-5. Results of Conventional Analyses for the Candidate Wastewater Samples

Analyte	Unit	WWH0	WWI0	WWJ0	WWK0	WWL0	WWM0	WWN0	WWO0
		Result	Result	Result	Result	Result	Result	Result	Result
HEM (Oil and Grease)	mg/L	4.6 U	8.9	4.5 U	4.6 U	4.4 U	4.3 U	4.4 U	4.2 U
SGT-HEM (Total Petroleum Hydrocarbons)	mg/L	5.8 U	5.3 U	5.7 U	5.8 U	5.5 U	5.3 U	5.5 U	5.3 U
Ammonia as N	mg/L	0.2	61	27	0.28	6.1	0.52	0.057 J	5.4
Calcium	mg/L	16	15	31	15	17	54	49	42
Sodium	mg/L	250	170	270	260	200	190	370	95
pH adj. to 25 deg C	SU	8.3	7.1	6.9	5.4	6.9	6.9	8.1	7.1
Specific Conductance	umhos/cm	1300	1400	2100	2000	1200	1700	2100	890
Chloride	mg/L	78	170	510	560	310	280	160	140
Sulfate	mg/L	290	11	49	73	7.7	130	560	130
Total Alkalinity as CaCO3	mg/L	200	410	160	5.2 J	110	74	220	59
Bicarbonate Alkalinity as CaCO3	mg/L	200	410	160	5.2 J	110	74	220	59
Carbonate Alkalinity as CaCO3	mg/L	6.4U	6.4 U	6.4 U	6.4 U	6.4 U	6.4 U	6.4 U	6.4 U
Total Dissolved Solids (TDS)	mg/L	780	590	980	1700	610	920	1400	450
Total Suspended Solids	mg/L	44	170	35	65	18	10	8	4

Notes:
J indicates value is qualitative.
U indicates analyte was not detected at a concentration that was at or above the stated concentration.

Table 2-6. Results of Conventional Analyses for the Candidate Surface Water Samples

Analyte	Unit	SWD0	SWE0	SWF0	SWG0
		Result	Result	Result	Result
HEM (Oil and Grease)	mg/L	4.2 U	4.2 U	4.3 U	4.1 U
SGT-HEM (Total Petroleum Hydrocarbons)	mg/L	5.3 U	5.2 U	5.3 U	5.1 U
Ammonia as N	mg/L	0.05 U	0.054 J	0.063 J	0.023 J
Calcium	mg/L	25	22	12	320
Sodium	mg/L	4.1J	7.5	5.7	10000
pH adj. to 25 deg C	SU	8.1	8.5	9.9	8
Specific Conductance	umhos/cm	210	240	140	48000
Chloride	mg/L	7.4	6.3	3.4	18000
Sulfate	mg/L	7.2	11	3.6 J	2400
Total Alkalinity as CaCO3	mg/L	87	100	68	120
Bicarbonate Alkalinity as CaCO3	mg/L	87	100	34	120
Carbonate Alkalinity as CaCO3	mg/L	6.4 U	6.4 U	35	6.4 U
Total Dissolved Solids (TDS)	mg/L	100	150	76	33000
Total Suspended Solids	mg/L	2.8 U	2.8 U	8.4	2.8 U

Notes:
J indicates value is qualitative.
U indicates the analyte was not detected at a concentration that was at or above the stated concentration.

Table 2-7. Results of Conventional Analyses for the Candidate Groundwater Samples

Analyte	Unit	GWA0	GWB0	GWC0
		Result	Result	Result
HEM (Oil and Grease)	mg/L	4.1 U	4.3 U	4.1 U
SGT-HEM (Total Petroleum Hydrocarbons)	mg/L	5.1 U	5.3 U	5.1 U
Ammonia as N	mg/L	0.055 J	0.15	0.031 J
Calcium	mg/L	110	400	48
Sodium	mg/L	23	670	100
pH adj. to 25 deg C	SU	8	8	8.3
Specific Conductance	umhos/cm	820	5300	820
Chloride	mg/L	15	710	23
Sulfate	mg/L	52	1900	190
Total Alkalinity as CaCO3	mg/L	410	240	180
Bicarbonate Alkalinity as CaCO3	mg/L	410	240	170
Carbonate Alkalinity as CaCO3	mg/L	6.4 U	6.4 U	6.4 U
Total Dissolved Solids (TDS)	mg/L	500	4100	520
Total Suspended Solids	mg/L	3.6 J	9.6	2.8 U

Notes:
J indicates value is qualitative.
U indicates the analyte was not detected at a concentration that was at or above the stated concentration.

ERA
A Waters Company

▪ Certificate of Spiking ▪

Hydrogeologic MLV Study Samples

ERA Project Number: 11252101

Matrix Type: Ground Water
Spike Level: High Level
Certificate Issue Date: 03-Aug-2022
Revision Number: 1.0

CERTIFICATION

Compound	Spiked Concentration[1] ng/L
PFBA	400.0
PFPEA	200.0
PFHXA	100.0
PFHPA	100.0
PFOA	100.0
PFNA	100.0
PFDA	100.0
PFUNA	100.0
PFDOA	100.0
PFTRDA	100.0
PFTEDA	100.0
PFBS	100.0
PFPES	100.0
PFHXS	100.0
PFHPS	100.0
PFOS	100.0
PFNS	100.0
PFDS	100.0
PFDOS	100.0
4:2FTS	240.0
6:2FTS	239.9
8:2FTS	240.0
PFOSA	100.0
NMeFOSA	100.0
NEtFOSA	100.0
NMeFOSAA	100.0
NEtFOSAA	100.0
NMeFOSE	400.0
NEtFOSE	400.0
HFPO-DA	240.0
ADONA	240.0
9CL-PF3ONS	240.0
11CL-PF3OUDS	240.0
3:3FTCA	400.0
5:3FTCA	2000.0
7:3FTCA	2000.0
PFEESA	200.0
PFMPA	200.0
PFMBA	200.0
NFDHA	200.0

SAMPLE-MATRIX TABLE

Lot Number:	Matrix Name:	Sampling Date[2]:	Sampling Time[2]:
GWA5 GWA6 GWA7	USACE	10-May-2022	1:00 PM
GWB5 GWB6 GWB7	LRPCD[3]	10-May-2022	1:00 PM
GWC5 GWC6 GWC7	USACE	10-May-2022	1:00 PM

16341 Table Mountain Pkwy ▪ Golden, CO 80403 ▪ T: 800.372.0122 ▪ 303.431.8454 ▪ www.eraqc.com
1 of 2

Figure 2-1. Example Groundwater Certificate of Spiking.

Samples were shipped directly from Waters ERA to each participating laboratory, in cooler boxes with frozen blue gel packs to keep the samples cool during shipping. Each laboratory received seven high-density polyethylene bottles of each of the aqueous samples: one bottle for analyses of the unspiked sample, three bottles spiked at a low spike level, and three bottles spiked at a high spike level. Any remaining sample volume was stored at Waters ERA in case they were needed at a later date. HGL tracked all sample shipments and confirmed receipt and condition with each laboratory.

One change to the preparation procedure in the Study Plan was that the laboratories were instructed ***not*** to measure the volume of the container, as required by Section 11.2.2 of the method. The instructions accompanying the samples sent by Waters ERA required the laboratories to record "500 mL" as the study volume. Per the instruction/method, the laboratories were still required to rinse the sample containers during processing.

2.5.1 Wastewater Samples

The wastewater samples prepared and shipped by Waters ERA are listed in Table 2-3. The seven parent wastewater matrices were each prepared as one unspiked, three replicates at the low spike level, and three replicate at the high spike level (Table 2-4). This resulted in 49 individual wastewater samples at each laboratory for analysis.

Of the six wastewater matrices in Table 3-2, all six met the specifications for TDS and NaCl (as conductivity), one wastewater sample (WWI) met the specification for TSS, and four wastewater samples met the specification for $CaCO_3$ (as hardness). Because none of the wastewater samples met the specification for oil and grease as received, Waters ERA was instructed to fortify study sample WWO with a combination of 15 mg/L each of hexadecane and stearic acid, which are the two compounds used in EPA Method 1664B to prepare spiked samples analyzed for hexane-extractable material (HEM, or oil and grease), to raise the final oil and grease concentration would be at or above the minimum requirement of 20 mg/L.

All of the aqueous matrices also were tested for additional water quality characteristics: alkalinity, sulfate, ammonia, pH, and separate determinations of sodium and chloride. Those results are presented in Table 3-3 but were not used by DoD and EPA is selecting the samples for inclusion in the study.

Wastewater samples were spiked on 19–21 April 2022, frozen at -20° C over the weekend, shipped on 25–26 April under chain of custody, and generally arrived within one day of shipment (all were received within two days of shipment), and below 6° C. Upon check-in, the samples were immediately stored at -20° C until preparation. The date of arrival, along with confirmation that the samples remained under that Study Plan-specified temperature of < 6° C, were confirmed during the data validation review.

A set of wastewater sample preparation guidelines accompanied each shipment to the laboratory. An example set of instructions for the wastewater samples is given in Figure 2-2.

2.5.2 Surface Water Samples

The surface water samples prepared and shipped by Waters ERA are listed in Table 2-3. The three parent surface water matrices were each prepared as one unspiked, three replicates at the low spike level, and three replicates at the high spike level (Table 2-4). This resulted in 21 individual surface water samples at each laboratory for analysis.

Surface water samples were spiked on 10 May 2022, frozen at-20° C, until shipping on 16 May under chain of custody. Spiked-surface waters and groundwaters were shipped together. Samples arrived at the laboratories on 17–18 May, with temperatures at or below 6° C. Upon check-in, the samples were immediately stored at -20° C until preparation. The date of arrival, along with confirmation that the samples remained under that Study Plan-specified temperature of < 6° C, were confirmed during the data validation review.

A second set of archived, frozen samples had to be sent to Laboratory 10 due to a bench error in EIS compound spiking levels. The frozen samples, still within the study-required holding time, were sent to Laboratory 10 on 20 July 2022, and received on 21 July 2022. The laboratory error is discussed further in Section 4.2.

2.5.3 Groundwater Samples

The groundwater samples prepared and shipped by Waters ERA are listed in Table 2-3. The three parent groundwater matrices were each prepared as one unspiked, three replicates at the low spike level, and three replicates at the high spike level (Table 2-4). This resulted in 21 individual groundwater samples at each laboratory for analysis.

Groundwater sample GWB had high background levels of PFAS (Table 2-4). The MLVS Team directed Waters ERA to dilute the parent GWB sample 10:1 prior to spiking and aliquoting.

Groundwater samples were spiked on 10 May 2022, frozen at -20° C, until shipping on 16 May under chain of custody. Samples arrived at the laboratories on 17–18 May, with temperatures at or below 6° C. Upon check-in, the samples were immediately stored at -20° C until preparation. The date of arrival, along with confirmation that the samples remained under that Study Plan-specified temperature of < 6° C, were confirmed during the data validation review.

As with the surface water, a second set of archived, frozen samples had to be sent to Laboratory 10 due to a bench error in EIS compound spiking levels. The frozen groundwater samples, still within the study-required holding time, were sent to Laboratory 10 on 20 July 2022, and received on 21 July 2022. The laboratory error is discussed further in Section 4.3.

2.6 COOLER STUDY

Prior to shipping the study samples to the participating laboratories, Waters ERA conducted a bench-scale cooler temperature study to assess the ability of the aqueous matrix samples to retain a temperature of < 6°C during the scheduled 24-hour shipping process and to measure/document sample temperatures out to 120 hours under ambient external temperature conditions. The purpose of this cooler study was to evaluate whether the pre-frozen spiked PFAS aqueous matrices would arrive at acceptable temperatures at the laboratories under ambient shipping conditions when

packed per normal Waters ERA protocol and shipped in Waters ERA coolers. Details of the study are provided in Attachment D to the *Study Plan*.

The study consisted of storing 13 frozen bottles of water identical to the bottles that were used for the study samples in pre-chilled coolers that were filled with blue gel packs in the same manner used for typical sample shipping purposes. The temperature of a bottle of water from each of the coolers was checked using an infrared temperature gun after 12, 24, 36, 48, 72, 96, and 120 hours had elapsed. A separate frozen bottle of water and cooler with blue ice was used for each time interval, such that the cooler was not opened before the temperature of the bottle was taken. The study showed that the samples remained completely frozen for at least 24 hours and were still at least half frozen for up to 120 hours, long past the anticipated time that sample might be in transit to the laboratories. Therefore, the study-specific shipping procedures using blue gel packs were determined to be appropriate for shipping the frozen study samples.

Based on the bench-scale study, Waters ERA shipped the samples directly to each participating laboratory in cooler boxes with frozen blue gel packs to maintain the samples cool during shipping. Each laboratory received seven HDPE bottles of each of the aqueous samples: one bottle for analyses of the unspiked sample, three bottles spiked at a low-spike level, and three bottles spiked at a high-spike level, although the laboratories were not informed of the actual spiking levels. Any remaining sample volume was stored at Waters ERA in case they were needed at a later date. DoD tracked all sample shipments and confirmed receipt and condition with each laboratory.

PFAS Method Validation Study:

Wastewater Sample Preparation Guidelines

Shipment Contents

- (4) 25"x15.5"x17" Styrofoam box coolers
- (7) Wastewaters Lots
- (49) 1L amber HDPE bottles
- Temperature blank
- Sample Preparation Guidelines
- Sample Chain of Custody (COC)

Sample Description

- Samples are packaged in a 1L amber HDPE bottle containing approximately 500 mL of spiked sample.
- Samples will be received at < 6°C.
- Samples are not preserved.
- Samples must be stored immediately at ≤-20°C until sample preparation.
- Each sample will contain the PFAS analytes as defined in "MLV Study Method Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS", October 2021.

Before You Begin

- Prior to preparation, samples should be allowed to equilibrate to room temperature and then analyzed as soon as possible.

Sample Instructions

1. The sample preparation procedure found in the MLV Study Method is to be followed, with one exception. Do not measure the volume of the container as required by Section 11.2.2 of the MLV Study Method. Instead, record 500 mL as the volume of sample prepared. This is the volume to be used when calculating PFAS concentrations in each sample. The container is to be rinsed as required by the MLV Study Method."

2. Report your results as ng/L and report the sample lot number that is provided on the sample container and on the COC, without any modifications, as the Sample Number (Sample NO. on the EDD).

Page 1 of 1 Version: 04

Figure 2-2. Example Wastewater Sample Preparation Guideline Form.

3 DATA MANAGEMENT, DATA VALIDATION, AND DATA RULES FOR STATISTICAL ANALYSES

Procedures were established in the Study Plan for data management (project and analytical data), data validation after receipt of the laboratory packages, and compilation of a validated Project Database from the individual validated electronic data deliverables (EDD) for each of the laboratories. The procedures for data management and data validation are described in the Study Plan (Appendix A) in Section 4.6 and detailed in the following attachments to the Study Plan.

- Attachment 3 – Electronic Data Deliverable Instructions and Format
- Attachment 4 – Data Management Plan
- Attachment 5 – Study Data Validation Guidelines.

This chapter discusses the procedures and quality assurance/quality control checks (QA/QC) for data management, validation, creation of a Project Database, and rules and procedures that governed the data used for the statistical analyses. A Data Management Report describing the established procedures is provided in Appendix C. The final data validation reports for each laboratory and each matrix are archived separate from this report. Rules established for the export of data to IDA for statistical analyses are discussed here; use of those data are presented in Appendix D (IDA Report) and the subsequent chapters of this report.

3.1 PROGRAMMATIC OVERVIEW

The data management process involved multiple levels of documentation, instructions, training, and reviews prior to and after submittal of the laboratory packages and EDDs. The general procedures, supporting documentation, corrective actions, and final documentation may be found in Table 3-1. Elements of the data management process are provided below, with additional detail found in the Data Management Report (Appendix C).

Prior to sample analyses, the laboratories participated in training and procedure reviews lead by the Study QA Manager and EPA. In addition to the Study Plan and draft EPA Method 1633, instructions were provided to the laboratories prior to receiving samples through the individual contracts, weekly laboratory training calls, the requirement to submit lab-specific standard operating procedures, matrix spike work instructions for laboratory preparation, and matrix-specific sample preparation guidelines. Each step of the process was conducted and/or reviewed by the Study QA Manager and EPA, and where needed, corrective action instructions were provided to the laboratories.

Procedures for conducting the individual matrix spikes, matrix-specific sample preparation guidelines, shipping, and chain-of-custody procedures were developed by Waters ERA with input from the Study QA Manager and the EPA. The spiking procedures were previously discussed in Section 2. The Study (through HGL) provided the PFAS standards directly to Waters ERA and to the participating laboratories. The Certificates of Analyses for those standards are included in the Study Plan (Appendix A, Attachment 6), as well as were included back with the individual laboratory packages.

The procedures for data management, data validation review, maintenance of project files, communications, development of the project database, and generating database exports for statistical analyses are discussed in the following sections.

3.2 DATA MANAGEMENT

Procedures for Data Management are detailed in the Data Management Report (Appendix C). Data Management included the processes and procedures for the transmission, tracking, verification, review, storage, and delivery of laboratory data, and the associated validation. After approval of the final data validation reports and EDDs, Data Management procedures were employed for the assembly and maintenance of the overall project database (all data, all matrices), and the subsequent export of data for statistical analyses (Table 3-1).

All raw data and reporting forms were provided electronically by the laboratories. These data packages were in a *.pdf format and contained all elements that would be required for Level IV hardcopy data package (i.e., raw data are provided, and all supporting data is present such that a 3rd party could recalculate all of the results from the raw data). EDDs were submitted in Excel format. Multiple data packages and EDDs were submitted for each phase of the study, and all of them were reviewed for completeness and data quality.

3.2.1 Initial Data Review of the Laboratory Reports and EDDs

The due dates and receipt dates for each laboratory report and EDD along with any revised submittal receipt dates were tracked by HGL. This data tracker also maintained the dates by which amended EDDs were generated, dual laboratory report completeness verifications were performed, and the date on which each laboratory report was submitted to the prescribed data validator. All laboratory analysis difficulties as well as any issues, errors, and deficiencies pertaining to the laboratory reports and EDDs were tracked in an error log with the associated resolution, date, and mechanism of communication.

On receipt of each laboratory report, an HGL Senior Chemist reviewed the report for completeness in accordance with the Phase 3 or Phase 4 checklist. Each checklist was reviewed and approved by the Study QA Manager and the EPA. The Phase 3 and Phase 4 laboratory report completeness checklists included elements required for each data package, such as a sample results summary and a transition ion summary, as stated in the Study Plan and MLVS Data Validation Procedure. Provided the first reviewer did not identify any missing elements from the laboratory report, the report was moved to a second completeness check by the HGL Program Chemist. If one or more elements were identified as erroneous or missing from a laboratory report, the issue was logged into the error tracker, and the report was returned to the laboratory for revision with a detailed description on the missing or erroneous elements. The revised report was verified by the HGL Program Chemist against the completeness checklist as well as the error tracker entry. Once a laboratory report passed the initial and secondary completeness verifications, the report was submitted to the assigned validator to initiate the data validation process. If, during the course of the data validation process, the validator identified an error or deficiency within the laboratory report, the issue was logged into the error tracker, the report was returned to the laboratory for revision with a detailed description of the issue, and the revised report was re-verified for completeness against the proper Phase checklist and the error tracker.

3.2.2 EDD Review

Upon receipt of an individual laboratory, EDD detailed checks were performed prior to providing the data to the validators. These checks were conducted in the project database using automated processes that generated error messages that were subsequently communicated back to the laboratory. Approximately sixty different checks are executed, in seven different error categories: completeness, units, formatting, nulls/placeholders, sample coding, qualifiers, and calculations. Of these many checks that were executed on each EDD, there were several errors that were found regularly. This included issues with the codes applied to describe the sample and blank entries in fields needed to ensure accurate querying of the resulting database. Details of the EDD checking procedures are included in Appendix C.

A common issue amongst all laboratories was mis-calculation or mis-reporting of the percent recovery values, in that values reported by the laboratory were not confirmed by manual recalculation based on the reported concentration/spike concentration * 100. Another issue that was frequently encountered was incorrectly applied, or missing, qualifier codes. For example: the concentration reported is greater than the MDL and less than the LOQ and not flagged with a J qualifier; the concentration is flagged with a U qualifier and the concentration reported is not equal to the MDL. It was also common to find instances where the spike concentration was not populated when expected (i.e., for method blank, ongoing precision and recovery (OPR), and low level ongoing precision and recovery (LLOPR) samples) or was populated when not expected (i.e., for test samples). There was also some confusion with communication with the laboratories on how to report NIS compounds and this often resulted in inconsistencies with the concentration, spike concentration, percent recoveries and units reported. The laboratories also commonly had problems with reporting number fields to three significant figures. Error reports were generated and sent to the laboratory with direction for changes to the EDDs. To track those EDD corrections, each version was given a number; V0 being that initially delivered; each update that was the previous version number +1 (e.g., V1, V2). Between the data package and EDD completeness reviews, and subsequent revisions required by the validators, the EPA, or the Study QA Manager, in some cases over 10 different submittals were required.

In addition to the EDD-specific QAQC procedures, there were checks that applied to all data assembled for a given matrix, including the qualifiers and comments added by the data validators, EPA, and the Study QA Manager. These procedures found issues with duplicate results reported for a sample/compound, usually due to dilutions or re-analysis, where a preferred result was not identified. This required an additional review to identify the preferred result and apply an X qualifier to the non-preferred result. X-flagged data were excluded from the statistical analysis of the data. Issues were also identified with the qualifiers applied by the data validators, EPA, and the Study QA Manager, in that J and U qualifiers applied by the laboratory were not retained in the qualifiers applied by the data validators and reviewers, as per the EDD template instructions. Finally, there were some instances where the results reported by the laboratory were directly edited by the data validators and/or reviewers instead of using the table columns designated for them.

In addition to reviewing the EDDs for correct data organization, Exa calculated matrix spike percent recovery, which accounted for the native concentration in the unspiked sample and used the concentration spiked into the test samples by ERA.

3.3 DATA VALIDATION

All data packages were reviewed for completeness and compliance with the requirements of the MLVS Method (Appendix A), and the Study Data Validation Guidelines (DVGs) (Attachment 5 to the Study Plan). While not explicitly cited in the Study Plan, the validation procedure also utilized the *Data Validation Guidelines Module 6: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-24* (DoD 2022) specifically to support the study. The guidelines are based on DoD's study-specific requirements for the EDD to be provided by each participating laboratory, as well as a PDF-format data package that includes all of the relevant instrument printouts, logs, and other raw data.

Initially, three established data validation firms were retained to conduct the reviews: ChemVal Consulting Inc (ChemVal), Jacobs Engineering Group, Inc. (Jacobs), and Pyron Environmental, Inc (Pyron). Specific laboratories were assigned to each validation firm for efficiency as well as to facilitate review of the laboratory data packages and familiarity with laboratory personnel. ChemVal provided reviews through the Initial Demonstration of Capabilities but then withdrew from the Study. Subsequently the Jacobs and Pyron teams provided all of the data validation reviews for the spiked matrix samples (5 laboratories each).

To promote a common reporting format, prior to beginning the data validation process, the three validation firms developed templates for a checklist, an outline for the data validation reports (DVRs), and a calculation confirmation spreadsheet following the MLVS DVG, and QSM Table B-24. These documents were reviewed by the Study QA Manager and EPA and were consistently used throughout the study.

The checklists and DVRs followed the multi-stage validation process defined in the DVG:

- Stage 1 examines the EDD and PDF to confirm completeness of the data (e.g., all study samples were analyzed), as well as checking on chain-of-custody forms, shipping records, holding times, etc.
- Stage 2A examines the method- and study-specific QC results, ion abundance ratios, and analyses of qualitative identification standards.
- Stage 2B involves review of the raw data covering the analytical sequence, preparation logs, mass calibration, instrument ICAL and calibration verifications, and instrument blanks.
- Stage 3 includes recalculation of study sample QC results from raw data, instrument QC recalculation, and checks on the MDL study results.
- Stage 4 involves review of the identification of each target compound detected in the study samples, and review of any manual integrations of instrument results reported by the laboratory.

The validation process was a surprisingly involved iterative process. Although clear guidance in the study method (EPA Method 1633), the requirements for reporting in the Study Plan, the specific training provided online to all participants, the EDD-reporting requirements, and the other elements listed in Table 3-1, laboratories' deliverables were inconsistent and often contained errors. This began with the ICAL studies and continued through the IDOCs and the spiked matrix samples. Some errors were omissions (e.g., missing documentation on instrument calibration), miscalculation or non-reported percent recoveries, incorrect EIS compound associations, incorrect or missing ion transition summaries; these errors were rectified by the Jacobs or Pyron communicating the deficiency or error to HGL, who would work with the laboratory to get the needed information. In most cases, the laboratory was asked to provide a completely new version of the data package and EDD (if needed).

In some cases, the laboratory errors were sufficiently significant that the data would be rejected. These included incorrect extraction volumes, failure to spike EIS compounds correctly, or pushing the spiked-matrix sample too rapidly through the SPE cartridges. Where the validation team was able to identify these problems within the sample-holding time, a new sample was quickly sent out from Waters ERA to the relevant laboratory and extracted within the holding time. In some cases, the error was identified too late, and, in those instances, the laboratory data were rejected by the validator for use in the Study. Rejected data are discussed in subsequent sections of this report.

After submittal of the DVR and EDD by the validators, there was an additional iterative process of review by the Study QA Manager and EPA. Problems were identified by the agencies and returned to the validator for additional review and correction. Each submittal was given an updated version number (V0, V1, etc.), which was tracked by Exa. The process was repeated until the Agency concerns were fully resolved.

General List of Documents comprising the final validated study report.

- PFAS Laboratory Study Completeness Checklist (HGL)
- Matrix-specific validator Checklist (Jacobs or Pyron)
- Matrix-specific validator calculation verification spreadsheet (Jacobs or Pyron)
- Matrix-Specific Data Validation Report (Jacobs or Pyron)
- Validator Electronic Data Deliverable
- Study QA Manager/EPA Review(s) and final concurrence memo
- Validator response(s) to Study QA Manager /EPA Review(s)
- Final EDD approved by Study QA Manager/EPA.

In the review of the validator-submitted EDD, EPA and Study QA Manager reviewed the validator-added flags, and either confirmed, nulled the validator-added flag, or added a different data flag after additional review of the laboratory report. The flags and the reason for the changes are fully documented in the Study QA Manager-approved EDDs, and in the Project Database.

The final validated study results comprise the documents listed in the General List of Documents, above, and are maintained in the Project record.

3.4 DATA USED IN THE STATISTICAL ANALYSES

The Statistical Data Analysis Report for Aqueous Media is Appendix D to this report. Statistical analyses of the laboratory data generally followed that listed in the EPA's *Alternate Procedures Test Procedures Program* (EPA 2018, Appendix G), where applicable, the procedures described

in the report, *Single Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS,* (SERDP and ESTCP 2021). Additional statistical analyses were conducted by the Air Forces Civil Engineering Center (AFCEC0 and EPA's contractor General Dynamics Information Technology GDIT). The AFCE and GDIT findings are reported separately in Sections 9.4 and 9.5, respectively.

Once all data has been validated, and the final EDDs were approved by Study QA Manager and EPA, the data were considered complete and ready to initiate the statistical analyses. Exa prepared an export from the project database for each individual matrix (WW, SW, GW), which underwent review by the MLVS Team. Principally, the purpose of this final review was to ensure the dataset was correct and complete, that there was a single result reported for a matrix sample/ compound pair (i.e., not duplicates), and that the matrix spike percent recovery calculations conducted by Exa were correctly reported.

During that review it became apparent that a set of rules regarding the calculation of the percent recovery in the PFAS-spiked samples was required. For most cases, Equation 1 describes how the percent recovery was calculated. The equation is based on the concentrations measured in the spiked samples, relative to the concentrations in the unspiked sample run at each lab, and the spike concentration added by Waters ERA prior to the individual matrix samples.

Equation 1. Calculation of Percent Recovery for Spiked Matrices

$$\left[\frac{\text{Final Result Spiked Sample [Analyte]} - \text{Final Result Unspiked Measured Native Sample [Analyte]}}{\text{Spike [Analyte]Added}}\right] * 100$$

Where [Analyte] is a specific PFAS target compound (e.g., PFBS, PFOA, 6:2FTS, etc.)

Additional calculation rules were developed to account for cases in which values were undetected, when the unspiked samples were excluded (X-flagged), where measured unspiked sample concentrations exceed the spike level, or where the calculation of the percent recovery resulted in a negative value. Table 3-3 shows the seven cases determined for these conditions, how the percent recovery was calculated, and whether the percent recovery result for those specific instances were excluded from the statistical analysis.

EPA and the MLVS Team reviewed one last time the application of these rules for each individual matrix. Upon approval, the final export was prepared, and the results provided to IDA and EPA for analysis.

The final data sets used for the statistical analyses by IDA, EPA, and AFCEC are in the MLVS Project electronic repository and are not included with this report.

Table 3-1. Data Management and Validation Procedures

Data Management and Validation Steps	Responsible Party	Supporting Documentation	Elements	Corrective Actions	Documentation
Pre-Analysis Procedures					
Contract and Scope Specifications	HGL	USACE Task Order/Contract, Study Plan, EPA Method 1633, Contract-specific language with USACE and each laboratory	USACE Task Order/Contract, Study Plan, EDD Instructions and Format	None	USACE/HGL Contracts and HGL/Laboratory Subcontracts
MLVS Study Team Weekly Calls	All signatories to MLVS Work Plan	Laboratory tracking sheets, data tracking sheets, communication records at HGL and Exa	Weekly calls on progress, problems, and action items to be addressed before the next call	Action items identified in meeting minutes	Call Meeting Minutes (12/9/21–ongoing)
Laboratory Training Calls	Study QA Manager, EPA, Laboratories, ERA, HGL	MLVS Study Plan MLVS Specific EPA Method 1633 (Attachment A to the Study Plan)	EPA Method 1633 - Specific training EDD Requirements Matrix-specific instructions	Interactions with laboratories contributed to modifications to EPA Method 1633.	Laboratory Call Meeting Minutes (10.25.2021–10.27.2022).
Lab-Specific Standard Operating Procedures	Study QA Manager, EPA, Laboratories, HGL	MLVS Study Plan MLVS Specific EPA Method 1633 (Attachment A to the Study Plan)	Lab-specific Standard Operating Procedures for EPA Method 1633 and MLVS	Study QA Manager/EPA Review and Modifications	Final Project SOPs in the MLVS Electronic Study Files
Matrix Spike and PFAS Standard Procedures					
Matrix Spike Work Instructions for Sample Preparation	ERA	Waters ERA *Work Instruction for the Hydrogeologic PFAS Validation Study Procedure. (V12)*	Work instructions for matrix receipt, storage and handling. Distribution of matrix to sample container. Methods for spiking, handling, packing, and shipping samples.	Study QA Manager/EPA Review and Modifications	Final Project *Work Instruction* Matrix *Certificate of Spiking* in the MLVS Electronic Study Files
Matrix Specific Sample Preparation Guidelines	ERA	Waters ERA *PFAS Method Validation Study: Sample Preparation Guidelines.*	Shipment contents, sample description, sample preparation and reporting instructions	Study QA Manager/EPA Review and Modifications	Instructions were discussed in each laboratory training call prior to shipment. Guidelines shipped in coolers for each sample. Copy retained in project files
Chain of Custody	ERA	Matrix specific COCs with sample numbers, shipment date and times	Shipment contents, sample numbers, temperature at departure	No corrective action unless sample failed to reach laboratory or temperature was above 6° C	COC receipt included in laboratory report. Copy retained in project files.
Study-supplied PFAS Standards Certificate of Analysis	Wellington Labs	MLVS Work Plan Attachment 6	Chemical, and physical properties of each standard mixture with the associated LC/MS and LC/MS/MS data, including isomer elution profiles and percent compositions.	None	Certificate of Analysis Documentation for PFAS Reference Standard Mixtures. Laboratories included CoAs in data packages submittals.

(Table 3-1 continued on next page)

Table 3-1. Data Management and Validation Procedures

Data Management and Validation Steps	Responsible Party	Supporting Documentation	Elements	Corrective Actions	Documentation
Post-Analysis Review					
Laboratory Data Package and EDD	Participating Laboratories	MLVS Study Plan MLVS Specific EPA Method 1633 (Attachment A to the Study Plan) Final laboratory-specific SOPs in the MLVS Electronic Study Files	All data and documentation, including raw data and chromatograms necessary to perform Stage 4 validation complete with recalculations. Each EDD was in accordance with the EDD guidelines provided in the MLVS Study Plan.	Corrections to EDDs and data packages were in accordance with the data validation process.	Data packages and EDDs from each laboratory; HGL completeness checklists; data validation reports and reviews; EDD error reports.
Laboratory Submittal Tracking and Completeness Review	HGL	MLVS DMP, Section 4.1.1 and 4.1.2	Ensure all mandatory elements are present in Data Packages for validation.	When errors or omissions were found, the issues were documented, the EDD and Data Package rejected. Laboratories were informed of the correction(s) required and resubmission of the data package and EDD.	Laboratory Data Report Checklist Tracking records of data packages and EDDs submitted and required edits.
		Data package and EDD Tracking Spreadsheets	Deliverable tracking for data packages and EDDs, and version control when laboratory products required revisions.		
		Data Package Completeness Review Checklist	Confirm all data for samples and QC samples reported in the Data Packages have been included and that all fields are completed		
		Issues Tracking spreadsheet	The tracker encompassed all issues brought up by the data validators, Study QA Manager/EPA reviewers, Exa EDD error checks, HGL completeness reviews, and the laboratories themselves during sample processing as a means to ensure follow up and remedy.	None	
Laboratory EDD Quality Assurance/ Quality Control Checks	Exa/HGL	MLVS Section 5.2 MLVS DMP, Section 4.1.2 MLVS QA/QC and Data Processing Procedures	Automated QA/QC checks of the EDD to ensure all required information in the DMP template guidance (Study Plan), Attachment 3), and each data field in each EDD is completed in accordance with those instructions	When errors were found, EDD was rejected, with the reasons for rejection. Laboratory notified of the issue(s) and resubmit of the data required.	EDD error summary reports and resolution. Complete amended EDDs with all fields prior to data validation

Table 3-1. Data Management and Validation Procedures

Data Management and Validation Steps	Responsible Party	Supporting Documentation	Elements	Corrective Actions	Documentation
Data Validation Reports (DVRs)	Jacobs Engineering Pyron Environmental	MLVS DMP, Section 4.1.3 MLVS Data Validation Procedures	Stage 4 validation of each data package and EDD. Includes checklists for all procedures, confirmation of EIS and NIS compound recoveries, 10% recalculation of all reported values.	When errors or omissions were found, the laboratory report and/or EDD were rejected, the laboratory notified of the issue(s) and resubmission of the data package/EDD was required.	DV Reports, Checklist, Calculation Checks, and Study QA Manager/EPA Reviews and Reponses in the MLVS Electronic Study files
QA/QC Review of DVRs and EDDs	Study QA Manager, EPA, SEE	MLVS Data Validation Procedures	Complete review of the DVR, EDD, and both laboratory and validator-added qualifiers.	When errors or omissions were found, the issues were documented in a memo to the validator, and with a resubmittal of the DVR required.	Study QA Manager/EPA approved final DVRs and amended EDDs
QA/QC Review of compiled dataset per matrix	Exa	MLVS DMP, Section 4.2.3 MLVS QA/QC and Data Processing Procedures	Automated QA/QC checks of the compiled EDDs, including data validator and Study QA Manager/EPA qualifiers, for each matrix to ensure internal consistency. Checks include correctly applied final qualifier, duplicate analysis, approval status.	When errors or omissions were found, resolution was determined with the MLVS Team and edits made in the database.	Database edits were documented within the Project Database, on the record-level.
Data qualify review of compiled dataset per matrix	Study QA Manager, EPA, SEE	This report, Attachment C	Manual, eyes-on review to identify data quality issues and anomalous results. Exa prepares various summary tables to assist with this review.	When errors or omissions were found, resolution was determined with the MLVS Team and edits made in the database.	Database edits were documented within the Project Database, on the record-level.
Statistical Analyses					
Database Output for Statistical Analyses	Exa	MLVS DMP, Section 4.2.3	DB export is automated; Exa checks for correct record count.	NA	NA
Statistical Analyses QA/QC Check of Statistical Analyses	IDA, AFCEC, GDIT Study QA Manager, EPA, AFCEC, SEE	MLVS Section 6.0. EPA (2018) *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program*	Calculate means, standard deviations, relative standard deviations for matrix spikes, OPR, LLOPR, EIS and NIS compound recoveries. First for each specific spiked media, and then for all combined aqueous media (WW, SW, GW).	Spot checks of means, standard deviations, relative standard deviations. Where errors found, identified and the statistics are re-run, with additional checks to confirmation of final values.	IDA Statistical Analysis Report Appendix B AFCEC analysis Section 9.4, this report GDIT analyses Tables 9-14 through 9-24, this report.

Notes
NA = not applicable

Table 3-2. Summary of Type and Number of Analyses Reviewed

Sample Type	Number of Laboratories	Total # Results Submitted by Laboratories[1]	Number Post-validation Results used in Statistical Analysis[2]				
			Samples	Target Analyte Results	EIS Compound Results	NIS Compound Results	Total Results Reviewed
ICAL and IDOC: Reagent Water							
MDL Study (7 method blanks [MDL_b])	9	4,906	73	2,640	1,584	472	4,696
MDL Study (7 MDL spiked samples [MDL_s])	9	4,774	64	2,560	1,538	460	4,558
Initial Precision and Recovery (IPR) Study	9	2,556	36	1,440	864	252	2,556
Method Blanks	9	2,130	30	1,200	720	210	2,130
Ongoing Precision and Recovery	9	639	9	360	216	63	639
Limit of Quantitation Verification	9	1,309	18	720	432	127	1,279
Wastewater							
Unspiked Samples	8	3,892	48	1,892	1,152	344	3,388
Low Spike	8	11,836	144	5,643	3,457	1,044	10,144
High Spike	8	11,826	144	5,713	3,456	1,046	10,215
Low-Level Ongoing Precision and Recovery	8	2,461	30	1,200	720	208	2,128
Method Blanks	8	2,390	30	1,200	720	207	2,127
Ongoing Precision and Recovery	8	2,390	30	1,200	720	207	2,127
Surface water[3]							
Unspiked Samples	9	2,065	27	1,053	647	190	1,890
Low Spike	9	6,544	81	3,205	1,941	567	5,713
High Spike	9	6,328	81	3,201	1,941	567	5,709
Low-Level Ongoing Precision and Recovery	9	1,494	19	760	456	133	1,349
Method Blanks	9	1,491	20	800	480	140	1,420
Ongoing Precision and Recovery	9	1,491	19	760	456	133	1,349

Table 3-2. Summary of Type and Number of Analyses Reviewed

Sample Type	Number of Laboratories	Total # Results Submitted by Laboratories[1]	Number Post-validation Results used in Statistical Analysis[2]				
			Samples	Target Analyte Results	EIS Compound Results	NIS Compound Results	Total Results Reviewed
Groundwater[3]							
Unspiked Samples	8	1,921	24	887	572	170	1,669
Low Spike	8	5,689	72	2,711	1,728	513	5,060
High Spike	8	6,116	72	2,719	1,731	513	5,033
Low-Level Ongoing Precision and Recovery	8	1,353	18	720	432	126	1,320
Method Blanks	8	1,350	19	760	456	134	1,340
Ongoing Precision and Recovery	8	1,421	19	760	456	134	1,350
Total Number of Results		**88,372**	**1,127**	**44,104**	**26,875**	**7,960**	**79,939**

[1]Number of results submitted by the laboratories (i.e., pre-validation).

[2]Post-validation results included in the dataset used in statistical analysis.

[3]Due to laboratories batching surface water samples and groundwater samples in the same preparation batch, results for some Method Blanks, Low-Level Ongoing Precision and Recovery and Ongoing Precision and Recovery samples have been included in both the counts for surface water and for groundwater analyses

Table 3-3. Data Rules for Calculating Percent Matrix Spike Recoveries

Case	Unspiked Sample	Spiked Sample	Calculation of MS Spike Recovery	Data for Statistical Analyses
1	detected	detected	Base case. Use Equation 1	All resultant values used
2	not detected	detected	**(**Final Result Spiked Sample [ ] / (Spike [ ] Added]**)** * 100	All resultant values used
3	not detected/X-flagged	not detected/X-flagged	when spiked sample is X or U, it is excluded, and %recovery is not calculated	No % recovery value for that sample and analyte pair
4	not detected/X-flagged	detected	**(**Final Result Spiked Sample [ ] / (Spike [ ] Added]**)** * 100	All resultant values used
5	detected/X-flagged	detected	**(**Final Result Spiked Sample [ ] / (Spike [ ] Added]**)** * 100	Values were reviewed on a case-by-case basis for inclusion or rejection.
6	detected [ ] > spike level	detected	Not calculated	No % recovery value for that sample and analyte pair
7	detected	< Unspiked [ ]	Calculated, but results in negative % recovery.	Negative % Recovery values excluded from statistical analyses

Notes:

[ ] - reported analyte concentration.

X-flagged data are excluded from calculations and excluded from statistical analyses.

4 CALIBRATION AND QUANTIFICATION: AQUEOUS MEDIA

Aqueous media sample extracts were analyzed by LC-MS/MS in MRM mode. The mass spectrometer underwent mass calibration to ensure the accuracy of the mass to charge ratio (m/z) values assigned to the instrument per the manufacturer's instructions. After the mass calibration had been verified, a multi-point ICAL was performed using quantitative standards that included 40 target analytes, 24 EIS compounds, and seven NIS compounds. Twenty-four target analytes with corresponding stable isotope analogs were quantified using isotope dilution quantitation, and 16 target analytes which did not have stable isotope analogs were quantified using an EIS quantitation approach. The NIS compound responses were used to determine the recovery of the EIS compounds. Target analytes were quantified and reported in their acid form. The calibration standards used for PFOS, PFHxS, NMeFOSAA, and NEtFOSAA included branched and linear isomers. These were the only quantitative isomeric mixtures commercially available at the time of the study. All other analytes were calibrated using standards that only included the linear isomer of the target analyte. Qualitative standards of PFOA, PFNA, PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE were analyzed after the calibration curve to identify the retention time of the branched isomers of these analytes. If a quantitative branched/linear isomeric mixture of an analyte was used for calibration standards or a qualitative branched/linear isomeric mixture of an analyte was analyzed after the calibration curve, when detected in a sample, it was included in the quantitation of that target analyte. Since the completion of this study, additional quantitative isomeric standards have become commercially available for PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE, therefore, in accordance with EPA Method 1633, these standards must be used when creating calibration standards, calibration verification standards, and spiking solutions and these five PFAS compounds were eliminated from the qualitative identification standard required by the method.

4.1 MASS CALIBRATION AND MASS CALIBRATION VERIFICATION

Each laboratory performed mass calibration and mass calibration verification in accordance with the manufacturer's instructions. Initially, the MLVS Method contained the same mass calibration and mass calibration verification requirements as version 1 of EPA Method 1633. Included were a requirement for the mass calibration to "evaluate an ion range that encompasses the ion range (Q1 and Q2 m/z) of the analytes of interest" and the mass calibration verification to include the demonstration of unit resolution of each peak of interest by the value of the peak width at half-height being within 0.5±0.1 Dalton (or amu) and the mass of each peak of interest to be within 0.1 Dalton of the expected masses. During the MLVS, it was discovered that not all manufacturer's procedures met these requirements. As a result, these requirements were stricken from subsequent versions of EPA Method 1633. The original mass drift requirement was replaced in the 2nd Draft of EPA Method 1633 with a drift requirement that was achievable by each manufacturer's mass verification requirements; a mass drift of no more than 0.2 Dalton. The resulting mass calibration and mass calibration verification requirements were met throughout the MLVS.

4.2 MULTI-POINT INITIAL CALIBRATION

To provide each laboratory with the target analyte, EIS compound, and NIS compound standards they would use for the MLVS, DoD procured sets of the standards (Study Plan, Attachment 6)

from Wellington Laboratories, a commercial standards vendor. By providing the standards to all the laboratories, the variability in the study results that would have resulted from having each laboratory prepare all the standards from neat materials was reduced. This approach also reduced the direct costs to each laboratory for their participation, allowing more laboratories to participate. It also expanded the pool of potential participants because not all commercial laboratories were willing or able to prepare standards from neat materials. The standards provided by the DoD were used by the laboratories to create all of the calibration, calibration verification, and spiking solutions they used in the MLVS.

Each laboratory calibrated their LC-MS/MS instrument using a series of calibration standards as similar as possible to the calibration standards listed in the MLVS Method. The concentrations of the study-specific calibration standards differ from those described in the method (Appendix A) to accommodate the variety of LC-MS/MS instruments employed by the laboratories in this study. As such, each laboratory used the standards provided to DoD to create a laboratory-specific set of nine calibration standards. Three calibrations were required to be submitted by each laboratory as part of Phase 3 of the Study Plan (Appendix A), prior to receiving study samples. During the validation process, it was discovered that Laboratory 8 incorrectly spiked their ICAL standards. Since the laboratory was unable to rectify this error in a timely manner, no data from Laboratory 8 were included in the statistical analysis of the ICAL.

A minimum of six contiguous calibration standards were required for a valid analysis when using a linear calibration model, with at least five of the six calibration standards being within the quantitation range (e.g., from the LOQ to the highest calibration standard). If a second-order calibration model was used, then a minimum of seven calibration standards was required, with at least six of the seven calibration standards within the quantitation range. The number of calibration standards used by each laboratory for each target analyte ranged from six to nine. The lowest concentration calibration standard had to have a signal-to-noise ratio of at least 3:1 and be at a concentration less than or equal to the LOQ. Table 4-1 provides the lowest and highest concentrations used by the remaining nine laboratories for each calibration point in these calibrations. Note that the concentrations of the EIS and NIS compounds did not vary across the calibration standards within a given laboratory. Subsequent ICALs utilized for the analysis of MLVS samples (Phase 4 of the Study Plan) were consistent within the ranges provided in Table 4-1.

The method outlines calibration and quantification of 40 PFAS by one of two approaches:

- True isotope dilution quantification: the target analyte response was compared with the response of its isotopically labeled analog. Twenty-four target analytes were quantified by isotope dilution (ID).
- EIS quantification: the target analyte response was compared with the response of the isotopically labeled analog of another target analyte that was closest in chemical structure and retention time. Sixteen target analytes were quantified by EIS.

The EIS approach was utilized for 16 target analytes due to the lack of commercially available isotopically labeled analogs of those analytes during method development and validation. If isotopically labeled analogs of these analytes become available in the future, then isotope dilution quantification would be recommended because it is more accurate.

Table 4-1. Initial Calibration Standards Concentration Ranges

Analyte	Range of Calibration Solution Concentrations (ng/mL)																	
	CS1 n = 9		CS2 n = 9		CS3 n = 9		CS4 n = 9		CS5 n = 9		CS6 n = 9		CS7 n = 9		CS8 n = 6		CS9 n = 2	
	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Target Compounds																		
PFBA	0.2	0.801	0.4	2	0.8	5.02	2	10	5	20	10	50	20	250	50	250	250	500
PFPeA	0.1	0.4	0.2	1	0.4	2.5	1	5	2.5	10	5	25	10	125	25	125	125	250
PFHxA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFHpA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFOA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFNA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFDA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFUnA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFDoA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFTrDA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFTeDA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
PFBS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	111
PFPeS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	118
PFHxS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	114
PFHpS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	119
PFOS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	116
PFNS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	120
PFDS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	121
PFDoS	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	62.5
4:2FTS	0.2	0.8	0.4	2	0.8	5	2	10	5	20	10	50	20	250	50	250	250	469
6:2FTS	0.2	0.8	0.4	2	0.8	5	2	10	5	20	10	50	20	250	50	250	250	475
8:2FTS	0.2	0.8	0.4	2	0.8	5	2	10	5	20	10	50	20	250	50	250	250	480
PFOSA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
NMeFOSA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125

Table 4-1. Initial Calibration Standards Concentration Ranges

Analyte	Range of Calibration Solution Concentrations (ng/mL)																	
	CS1 n = 9		CS2 n = 9		CS3 n = 9		CS4 n = 9		CS5 n = 9		CS6 n = 9		CS7 n = 9		CS8 n = 6		CS9 n = 2	
	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
NEtFOSA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
NMeFOSAA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
NEtFOSAA	0.05	0.2	0.1	0.5	0.2	1.25	0.5	2.5	1.25	5	2.5	12.5	5	62.5	12.5	62.5	62.5	125
NMeFOSE	0.08	2	1	5	2	12.5	5	25	12.5	50	25	125	50	625	125	625	625	1250
NEtFOSE	0.08	2	1	5	2	12.5	5	25	12.5	50	25	125	50	625	125	625	625	1250
PFMPA	0.1	0.4	0.2	1	0.4	2.5	1	5	2.5	10	5	25	10	125	25	125	125	250
PFMBA	0.1	0.4	0.2	1	0.4	2.5	1	5	2.5	10	5	25	10	125	25	125	125	250
NFDHA	0.1	0.4	0.2	1	0.4	2.5	1	5	2.5	10	5	25	10	125	25	125	125	250
HFPO-DA	0.1	0.835	0.2	2.09	0.4	5.3	1	10.6	2.5	21.2	5	52	10	264	25	250	125	500
ADONA	0.1	0.8	0.2	2	0.4	5	1	10	2.5	20	5	50	10	250	25	250	125	473
PFEESA	0.1	0.4	0.2	1	0.4	2.5	1	5	2.5	10	5	25	10	125	25	125	125	223
9Cl-PF3ONS	0.1	0.8	0.2	2	0.4	5	1	10	2.5	20	5	50	10	250	25	250	125	468
11Cl-PF3OUdS	0.1	0.8	0.2	2	0.4	5	1	10	2.5	20	5	50	10	250	25	250	125	473
3:3FTCA	0.2	1	0.4	2.5	0.8	6.26	2	12.5	5	25	10	62.5	20	312	50	312.5	250	624
5:3FTCA	1	5	2	12.5	4	31.4	10	62.7	25	125	50	315	100	1560	250	1560	1250	3120
7:3FTCA	1	5	2	12.5	4	31.4	10	62.7	25	125	50	315	100	1560	250	1560	1250	3125
Extracted Internal Standard Compounds																		
$^{13}C_4$-PFBA	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
$^{13}C_5$-PFPeA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
$^{13}C_5$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFHpA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_9$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_6$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_7$-PFUnA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25

Table 4-1. Initial Calibration Standards Concentration Ranges

Analyte	Range of Calibration Solution Concentrations (ng/mL)																	
	CS1 n = 9		CS2 n = 9		CS3 n = 9		CS4 n = 9		CS5 n = 9		CS6 n = 9		CS7 n = 9		CS8 n = 6		CS9 n = 2	
	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
$^{13}C_2$-PFDoA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFTeDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_3$-PFBS	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.33	2.5	2.33	2.5
$^{13}C_3$-PFHxS	2.37	2.5	2.37	2.5	2.37	2.5	2.37	2.5	2.37	2.5	2.37	2.5	2.37	2.5	2.37	2.5	2.37	2.5
^{13}C8-PFOS	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5
$^{13}C_2$-4:2FTS	4.69	5	4.69	5	4.69	5	4.69	5	4.69	5	4.69	5	4.69	5	4.69	5	4.69	5
$^{13}C_2$-6:2FTS	4.75	5	4.75	5	4.75	5	4.75	5	4.75	5	4.75	5	4.75	5	4.76	5	4.76	5
$^{13}C_2$-8:2FTS	4.75	5	4.75	5	4.75	5	4.75	5	4.75	5	4.75	5	4.75	5	4.8	5	4.8	5
$^{13}C_8$-PFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_5-NEtFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSAA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
D_5-NEtFOSAA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
D_7-NMeFOSE	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
D_9-NEtFOSE	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
$^{13}C_3$-HFPO-DA	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Non-extracted Internal Standard Compounds																		
$^{13}C_3$-PFBA	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
$^{13}C_2$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_5$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{18}O_2$-PFHxS	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.3	2.5	2.37	2.5	2.37	2.5
$^{13}C_4$-PFOS	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5	2.4	2.5

Source file: ICAL_calibration_V0_220907_093746.csv

Notes:

Notes to Table 4-1
[1] The number of laboratories for which an MDL value was calculated. Laboratory #8 data omitted from summary due to spiking error. No aqueous sample data from this laboratory was utilized in the MLVS statistical analysis.
L = Lowest concentration reported by laboratories
H = Highest concentration reported by laboratories

The 24 isotopically labeled analogs are added before any sample preparation steps were performed. The target analyte results are corrected for any loss or apparent gains occurring as a result of the sample preparation procedure and analytical process using the response of its associated isotopically labeled compound.

Table 4-2 provides a list of the 40 target PFAS and their associated quantification approach and associated quantification reference compound per the method (Appendix A). All laboratories utilized these associations, with one exception. Although improved accuracy can be achieved when quantitating PFTrDA using the average areas of the labeled compounds $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA, some LC-MS/MS vendor software utilized by the participating laboratories were unable to use the average of two internal standards in their calculations. As a result, most laboratories utilized one of the two EIS compounds ($^{13}C_2$-PFTeDA or $^{13}C_2$-PFDoA) instead of the average of the two EIS compounds.

Table 4-2. Quantification Reference and Calibration Approach for the Target Analytes

Target Analyte	Quantification Reference Compound (EIS)	Calibration Approach[1]
PFBA	$^{13}C_4$-PFBA	ID
PFPeA	$^{13}C_5$-PFPeA	ID
PFHxA	$^{13}C_5$-PFHxA	ID
PFHpA	$^{13}C_4$-PFHpA	ID
PFOA	$^{13}C_8$-PFOA	ID
PFNA	$^{13}C_9$-PFNA	ID
PFDA	$^{13}C_6$-PFDA	ID
PFUnA	$^{13}C_7$-PFUnA	ID
PFDoA	$^{13}C_2$-PFDoA	ID
PFTrDA[2]	avg.$^{13}C_2$-PFTeDA and$^{13}C_2$-PFDoA	EIS
PFTeDA	$^{13}C_2$-PFTeDA	ID
PFBS	$^{13}C_3$-PFBS	ID
PFPeS	$^{13}C_3$-PFHxS	EIS
PFHxS	$^{13}C_3$-PFHxS	ID
PFHpS	$^{13}C_8$-PFOS	EIS
PFOS	$^{13}C_8$-PFOS	ID
PFNS	$^{13}C_8$-PFOS	EIS
PFDS	$^{13}C_8$-PFOS	EIS
PFDoS	$^{13}C_8$-PFOS	EIS
4:2FTS	$^{13}C_2$-4:2FTS	ID
6:2FTS	$^{13}C_2$-6:2FTS	ID
8:2FTS	$^{13}C_2$-8:2FTS	ID

Table 4-2. Quantification Reference and Calibration Approach for the Target Analytes

Target Analyte	Quantification Reference Compound (EIS)	Calibration Approach[1]
PFOSA	$^{13}C_8$-PFOSA	ID
NMeFOSA	D_3-NMeFOSA	ID
NEtFOSA	D_5-NEtFOSA	ID
NMeFOSAA	D_3-NMeFOSAA	ID
NEtFOSAA	D_5-N-EtFOSAA	ID
NMeFOSE	D_7-NMeFOSE	ID
NEtFOSE	D_9-NEtFOSE	ID
HFPO-DA	$^{13}C_3$-HFPO-DA	ID
ADONA	$^{13}C_3$-HFPO-DA	EIS
PFMPA	$^{13}C_5$-PFPeA	EIS
PFMBA	$^{13}C_5$-PFPeA	EIS
NFDHA	$^{13}C_5$-PFHxA	EIS
9Cl-PF3ONS	$^{13}C_3$-HFPO-DA	EIS
11Cl-PF3OUdS	$^{13}C_3$-HFPO-DA	EIS
PFEESA	$^{13}C_5$-PFHxA	EIS
3:3FTCA	$^{13}C_5$-PFPeA	EIS
5:3FTCA	$^{13}C_5$-PFHxA	EIS
7:3FTCA	$^{13}C_5$-PFHxA	EIS

Source file: MLVS Study Plan for EPA Method 1633 in Appendix A.

Notes:

[1] Isotope dilution (ID) and extracted internal standard (EIS).

[2] In some instances, laboratories utilized either $^{13}C_2$-PFTeDA or $^{13}C_2$-PFDoA, not the average of these two EIS for quantitation.

In addition to the EIS compounds added before sample preparation, another seven isotopically labeled analogs were added after extraction: the NIS compounds. These seven NIS compounds were used to calculate the recoveries of the 24 EIS compounds. Table 4-3 provides a list of the 24 EIS compounds and their associated NIS compound that were utilized by all laboratories participating in the MLVS.

Table 4-3. EIS Compounds and Their Associated NIS Compounds

EIS Compound	Associated NIS Compound
$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	$^{13}C_2$-PFHxA
$^{13}C_8$-PFOA	$^{13}C_4$-PFOA
$^{13}C_9$-PFNA	$^{13}C_5$-PFNA
$^{13}C_6$-PFDA	$^{13}C_2$-PFDA
$^{13}C_7$-PFUnA	$^{13}C_2$-PFDA
$^{13}C_2$-PFDoA	$^{13}C_2$-PFDA
$^{13}C_2$-PFTeDA	$^{13}C_2$-PFDA
$^{13}C_3$-PFBS	$^{18}O_2$-PFHxS
$^{13}C_3$-PFHxS	$^{18}O_2$-PFHxS
$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	$^{18}O_2$-PFHxS
$^{13}C_2$-6:2FTS	$^{18}O_2$-PFHxS
$^{13}C_2$-8:2FTS	$^{18}O_2$-PFHxS
$^{13}C_8$-PFOSA	$^{13}C_4$-PFOS
D_3-NMeFOSA	$^{13}C_4$-PFOS
D_5-NEtFOSA	$^{13}C_4$-PFOS
D_3-NMeFOSAA	$^{13}C_4$-PFOS
D_5-NEtFOSAA	$^{13}C_4$-PFOS
D_7-NMeFOSE	$^{13}C_4$-PFOS
D_9-NEtFOSE	$^{13}C_4$-PFOS
$^{13}C_3$-HFPO-DA	$^{13}C_2$-PFHxA

Source file: MLVS Study Plan for EPA Method 1633 in Appendix A.

4.2.1 Response Ratios and Response Factors

The response ratio (RR) for each method analyte calibrated by isotope dilution was calculated according to the equation below, separately for each of the calibration standards, using the areas of the quantification ion masses shown in Table 4-4. RR was used for the 24 target analytes quantified by isotope dilution.

$$RR = \frac{Area_n \, M_{EIS}}{Area_{EIS} \, M_n}$$

where

$Area_n$ = The measured area of the quantification ion mass for the target analyte
$Area_{EIS}$ = The measured area at the quantification ion mass for the corresponding EIS
M_{EIS} = The mass of the EIS compound in the calibration standard
M_n = The mass of the target analyte in the calibration standard

The response factor (RF_n) for each target analyte calibrated by EIS was calculated according to the equation below, separately for each of the calibration standards, using the areas of the quantification ion masses shown in Table 4-4. RF_n was used for the 16 target analytes quantified by EIS.

$$\text{RFn} = \frac{Area_n\, M_{EIS}}{Area_{EIS}\, M_n}$$

where

$Area_n$ = The measured area of the quantification ion mass for the target analyte
$Area_{EIS}$ = The measured area at the quantification ion mass for the EIS compound
M_{EIS} = The mass of the EIS compound in the calibration standard
M_n = The mass of the target analyte in the calibration standard

The response factor of each EIS compound (RF_s) was calculated for each calibration standard using the equation below according to the equation below, separately for each of the calibration standards, using the areas of the quantification ion masses shown in Table 4-4. RF_s was used for the 24 EIS compounds quantified by NIS compound.

$$RF_s = \frac{Area_{EIS}\, M_{NIS}}{Area_{NIS}\, M_{EIS}}$$

where

$Area_{EIS}$ = The measured area of the quantification ion mass for the EIS compound
$Area_{NIS}$ = The measured area of the quantification ion mass for the NIS compound
M_{NIS} = The mass of the NIS compound in the calibration standard
M_{EIS} = The mass of the EIS compound

Table 4-4. Target Analyte Ions Monitored, Extracted Internal Standards, and Non-extracted Internal Standards Used for Quantification

Abbreviation	Example Retention Time	Parent Ion Mass	Quant Ion Mass	Confirm Ion Mass	Typical Ion Ratio
Target Analytes					
PFBA	1.96	212.8	168.9	NA	NA
PFPeA	4.18	263	219	68.9	NA
PFHxA	4.81	313	269	118.9	13
PFHpA	5.32	363.1	319	169	3.5
PFOA	6.16	413	369	169	3
PFNA	6.99	463	419	219	4.9
PFDA	7.47	512.9	469	219	5.5
PFUnA	7.81	563.1	519	269.1	6.9
PFDoA	8.13	613.1	569	319	10
PFTrDA[2]	8.53	663	619	168.9	6.7
PFTeDA	8.96	713.1	669	168.9	6
PFBS	4.79	298.7	79.9	98.8	2.1
PFPeS	5.38	349.1	79.9	98.9	1.8
PFHxS	6.31	398.7	79.9	98.9	1.9
PFHpS	7.11	449	79.9	98.8	1.7
PFOS	7.59	498.9	79.9	98.8	2.3
PFNS	7.92	548.8	79.9	98.8	1.9
PFDS	8.28	599	79.9	98.8	1.9
PFDoS	9.14	699.1	79.9	98.8	1.9
4:2FTS	4.67	327.1	307	80.9	1.7
6:2FTS	5.81	427.1	407	80.9	1.9
8:2FTS	7.28	527.1	507	80.8	3
PFOSA	8.41	498.1	77.9	478	47
NMeFOSA	9.7	511.9	219	169	0.66
NEtFOSA	9.94	526	219	169	0.63
NMeFOSAA	7.51	570.1	419	483	2
NEtFOSAA	7.65	584.2	419.1	526	1.2
NMeFOSE	9.57	616.1	58.9	NA	NA
NEtFOSE	9.85	630	58.9	NA	NA
HFPO-DA	4.97	284.9	168.9	184.9	1.95
ADONA	5.79	376.9	250.9	84.8	2.8
9Cl-PF3ONS	7.82	530.8	351	532.8→353.0	3.2

Table 4-4. Target Analyte Ions Monitored, Extracted Internal Standards, and Non-extracted Internal Standards Used for Quantification

Abbreviation	Example Retention Time	Parent Ion Mass	Quant Ion Mass	Confirm Ion Mass	Typical Ion Ratio
11Cl-PF3OUdS	8.62	630.9	450.9	632.9→452.9	3
PFEESA	5.08	314.8	134.9	82.9	9.22
PFMPA	3.21	229	84.9	NA	NA
PFMBA	4.53	279	85.1	NA	NA
NFDHA	4.84	295	201	84.9	1.46
3:3FTCA	3.89	241	177	117	1.7
5:3FTCA	5.14	341	237.1	217	1.16
7:3FTCA	6.76	441	316.9	336.9	0.69
Extracted Internal Standard Compounds					
$^{13}C_4$-PFBA	1.95	216.8	171.9	NA	---
$^{13}C_5$-PFPeA	4.18	268.3	223	NA	---
$^{13}C_5$-PFHxA	4.8	318	273	120.3	---
$^{13}C_4$-PFHpA	5.32	367.1	322	NA	---
$^{13}C_8$-PFOA	6.16	421.1	376	NA	---
$^{13}C_9$-PFNA	6.99	472.1	427	NA	---
$^{13}C_6$-PFDA	7.47	519.1	474.1	NA	---
$^{13}C_7$-PFUnA	7.81	570	525.1	NA	---
$^{13}C_2$-PFDoA	8.13	615.1	570	NA	---
$^{13}C_2$-PFTeDA	8.96	715.2	670	NA	---
$^{13}C_3$-PFBS	4.78	302.1	79.9	98.9	---
$^{13}C_3$-PFHxS	6.3	402.1	79.9	98.8	---
$^{13}C_8$-PFOS	7.59	507.1	79.9	98.9	---
$^{13}C_2$-4:2FTS	4.67	329.1	80.9	309	---
$^{13}C_2$-6:2FTS	5.82	429.1	80.9	409	---
$^{13}C_2$-8:2FTS	7.28	529.1	80.9	509	---
$^{13}C_8$-PFOSA	8.41	506.1	77.8	NA	---
D_3-NMeFOSA	9.7	515	219	NA	---
D_5-NEtFOSA	9.94	531.1	219	NA	---
D_3-NMeFOSAA	7.51	573.2	419	NA	---
D_5-NEtFOSAA	7.65	589.2	419	NA	---
D_7-NMeFOSE	9.56	623.2	58.9	NA	---
D_9-NEtFOSE	9.83	639.2	58.9	NA	---
$^{13}C_3$-HFPO-DA	4.97	286.9	168.9	184.9	---

Table 4-4. Target Analyte Ions Monitored, Extracted Internal Standards, and Non-extracted Internal Standards Used for Quantification

Abbreviation	Example Retention Time	Parent Ion Mass	Quant Ion Mass	Confirm Ion Mass	Typical Ion Ratio
Non-extracted Internal Standard Compounds					
$^{13}C_3$-PFBA	1.95	216	172	NA	---
$^{13}C_2$-PFHxA	4.8	315.1	270	119.4	---
$^{13}C_4$-PFOA	6.16	417.1	172	NA	---
$^{13}C_5$-PFNA	6.99	468	423	NA	---
$^{13}C_2$-PFDA	7.47	515.1	470.1	NA	---
$^{18}O_2$-PFHxS	6.3	403	83.9	NA	---
$^{13}C_4$-PFOS	7.59	502.8	79.9	98.9	---

Source file: MLVS Study Plan for EPA Method 1633 in Appendix A.
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4.2.2 Ion Mass and Ion Ratio

The equations above for RR and RF are based on the area of the most intense response of the two ions produced by the parent ion after fragmentation. For the purposes of the method, the "quantification ion" is the ion with the most intense response and the "confirmation ion" is the ion with the next most intense response. Some target analytes do not produce a second ion during fragmentation, or the signal is so low as not to be reliable. In those cases, only the "quantification ion" is used for the calculations above.

Each laboratory operated their LC-MS/MS under the mass spectrometer negative electrospray ionization conditions as stated in the Study Plan (Appendix A). MRM mode was utilized to monitor the ion masses. Table 4-4 presents the ions monitored for the target compounds, labeled compounds, and NIS as well as their typical retention times.

Laboratory 7 found that a different ion mass produced stronger responses than the one identified as the quantitative ion mass in Table 4-4 for one target analyte. On their instrument system, the confirmation ion mass (336.9) provided a more stable and stronger response than the quantitative ion mass (316.9) for 7:3FTCA, therefore they utilized ion mass of m/z 336.9 as the quantitative ion mass and ion mass of m/z 316.9. In addition, there were five other target analytes (NEtFOSAA, PFTeDA, PFTrDA, PFEESA, NFDHA) that this laboratory identified confirmation ion masses that provided a stronger more stable response than the confirmation ion masses listed in Table 4-4. In these five instances, the laboratory also monitored the method-specified confirmation ion mass as well but used their optimized more robust confirmation ion mass to evaluate the ion ratio. Because they could demonstrate improved performance, these deviations were permitted. This is a method modification that would normally be allowed for a Clean Water Act Method. 40 CFR 136.6(b)(4)(xx) states an allowed modification is: "Changes in equipment operating parameters such as the monitoring wavelength of a colorimeter or the reaction time and temperature as needed to achieve the chemical reactions defined in the unmodified CWA method." This would qualify as an equipment operating parameter.

Some laboratories participating in the MLVS did not monitor confirmation ions for some EIS and NIS compounds. Given that the method does not require the evaluation of the ion ratio of EIS and NIS compounds, monitoring a confirmation ion for these compounds provides little to no value, and this deviation was permitted for this study. Eliminating the monitoring of these ion masses, in theory, could result in better sensitivity relative to the response of the quantitation ion masses since the more masses monitored in a monitoring window, the lower the sensitivity.

Qualitative evaluation of target analyte detections included the evaluation of the ion ratio, the ratio of the quantitative ion mass to the confirmation ion mass. An example of ion ratios observed are provided in Table 4-4. While a confirmation transition for PFPeA (263 → 68.9) can be detected, during both the SLVS and MLVS it was found to be of inadequate S/N ratio. Therefore, the ion ratio of PFPeA was not evaluated during this study. Other target analytes that did not have a suitable confirmation transition (not detected or inadequate S/N) include PFBA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA.

The ion ratios of all analytes in a sample were evaluated against the expected ion ratio of the analyte, as determined in the mid-point ICAL standard analyte ratio or the initial daily calibration verification (CV), depending on the sample concentration. If the sample concentration was greater than the LOQ, it was evaluated against the mid-point ICAL standard analyte ratio. If the sample concentration was less than the LOQ, it was evaluated against the initial daily CV analyte ratio. If the ion ratio of an analyte was not within 50-150% expected ion ratio, there was a higher degree of uncertainty associated with the reported target analyte concentration, resulting in an estimated value. In these instances, the laboratory qualified the target analyte sample result with an "I" qualifier.

4.2.3 Calibration Linearity and Stability

Prior to the analysis of study samples, each laboratory was required to submit documentation for three ICALs that they had performed in accordance with the method (Appendix A). All 10 participating laboratories submitted the required ICAL documentation; however, as stated earlier, Laboratory 8 was eliminated from the statistical analysis due to an error they made when creating their ICAL standards. As a result, no data for the MLVS from Laboratory 8 is included in this report.

All RRs and RFs were calculated consistently throughout the data set, except for the case of PFTrDA. As stated previously, some laboratories utilized the average of the responses of $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA, while others utilized the response of solely $^{13}C_2$-PFTeDA or $^{13}C_2$-PFDoA when determining the RFs of the calibration standards for PFTrDA.

Table 4-5 includes the RR and RF values corresponding to the three ICALs submitted by the nine-remaining laboratories in Phase 3 of the Study Plan (Appendix A).

Table 4-5. Summary of Response Ratios or Response Factors the Three Calibrations Run for All Laboratories.

Analyte	Response Ratio or Response Factor			Relative Standard Deviation (%)			
	Minimum	Median	Maximum	Minimum	Median	Maximum	Pooled
Target Compounds							
PFBA	0.303	0.832	1.23	2.2	6.60	14.40	7.72
PFPeA	0.749	0.894	9.55	1.71	6.84	12.80	7.86
PFHxA	0.527	0.919	1.11	2.44	7.07	14.90	8.83
PFHpA	0.662	1.02	1.31	3.64	7.60	14.10	8.74
PFOA	0.511	0.917	1.31	3.32	8.21	15.10	8.88
PFNA	0.657	0.84	1.08	3.09	10.50	15.90	10.30
PFDA	0.761	1.02	2.2	2.85	8.63	16.20	9.90
PFUnA	0.547	0.795	1.09	3.3	9.34	15.70	9.87
PFDoA	0.758	0.938	1.12	3.9	8.79	17.70	9.59
PFTrDA	0.707	1.03	3.57	4.64	8.39	17.30	9.22
PFTeDA	0.717	1.3	3.84	3.4	8.25	18.90	9.68
PFBS	0.801	0.909	1.22	2.47	7.36	14.30	8.55
PFPeS	0.534	0.976	3.52	3.4	7.94	18.60	9.28
PFHxS	0.621	1.03	1.33	3.28	7.22	17.50	8.93
PFHpS	0.705	1.25	3.49	2.5	9.21	15.60	9.62
PFOS	0.661	1.12	5.26	1.83	8.65	15.70	9.86
PFNS	0.536	1.06	3.19	1.38	7.60	15.00	8.48
PFDS	0.538	0.963	3.83	2.99	7.90	16.70	8.54
PFDoS	0.431	0.715	4.6	4.13	7.06	14.40	7.78
4:2FTS	0.363	2.54	9.38	2.57	8.90	18.80	10.90
6:2FTS	1.19	2.41	6.58	2.4	11.10	19.60	11.70
8:2FTS	0.718	3.08	10.5	4.14	11.80	19.60	12.70
PFOSA	0.547	0.911	1.36	2.08	5.46	12.90	7.40
NMeFOSA	0.4	0.943	1.15	2.62	6.60	12.60	7.98
NEtFOSA	0.471	1.02	1.63	2.35	5.60	15.10	7.31
NMeFOSAA	0.299	0.81	1.32	3.45	9.90	15.00	10.00
NEtFOSAA	0.584	0.823	1.13	2.91	7.90	15.50	8.70
NMeFOSE	0.838	1.09	4.29	2.3	5.70	13.10	7.83
NEtFOSE	0.781	1.08	2.94	3	6.05	15.20	7.66
PFMPA	0.0397	0.522	5.12	1.93	5.35	16.10	8.30
PFMBA	0.406	0.701	14.3	1.6	5.82	20.80	8.55
NFDHA	0.0174	0.091	0.408	3.7	11.00	26.10	13.80
HFPO-DA	0.725	0.912	21.2	2.8	9.43	17.60	10.10

Table 4-5. Summary of Response Ratios or Response Factors the Three Calibrations Run for All Laboratories.

Analyte	Response Ratio or Response Factor			Relative Standard Deviation (%)			
	Minimum	Median	Maximum	Minimum	Median	Maximum	Pooled
ADONA	2.35	8.95	37.9	3.82	9.90	17.80	10.80
PFEESA	0.809	2.79	5.42	1.9	6.98	13.60	8.06
9Cl-PF3ONS	2.06	5.91	41.8	1.81	8.51	16.80	10.00
11Cl-PF3OUdS	2.4	6.36	27.8	4.1	9.65	15.60	9.91
3:3FTCA	0.0208	0.076	0.678	3.61	6.70	16.90	9.15
5:3FTCA	0.0549	0.146	0.665	2.39	6.40	15.00	8.63
7:3FTCA	0.0637	0.0999	0.685	2.6	7.42	26.50	11.30
Extracted Internal Standard Compounds							
13C4-PFBA	0.864	1.09	1.21	0.532	2.17	10.80	4.11
13C5-PFPeA	0.0688	0.775	1.33	1.65	4.70	11.80	6.46
13C5-PFHxA	0.796	1.04	1.49	1.46	4.30	12.30	5.96
13C4-PFHpA	0.541	1.03	1.87	1.3	4.89	19.60	7.66
13C8-PFOA	0.899	7.51	68.5	1.42	6.14	17.30	8.35
13C9-PFNA	0.863	0.983	1.28	2.9	6.91	17.00	8.87
13C6-PFDA	0.327	1.05	1.37	3.4	6.00	14.70	8.33
13C7-PFUnA	0.878	1.14	1.61	3.1	8.20	15.80	9.48
13C2-PFDoA	0.709	0.976	1.22	2.92	7.36	20.40	9.05
13C2-PFTeDA	0.377	0.828	1.32	3.07	6.40	34.10	12.00
13C3-PFBS	0.859	1.44	4.77	2.83	6.34	19.80	8.37
13C3-PFHxS	1.04	1.15	4.48	0.959	5.29	14.20	7.51
13C8-PFOS	0.224	0.965	1.38	2.9	5.40	11.70	6.71
13C2-4:2FTS	0.0492	0.208	0.943	2.26	9.80	16.00	10.60
13C2-6:2FTS	0.0281	0.167	0.424	2.16	10.60	18.90	11.30
13C2-8:2FTS	0.0159	0.179	0.41	2.66	11.00	19.80	12.10
13C8-PFOSA	1.16	1.83	4.67	1.81	5.92	10.90	6.64
D3-NMeFOSA	0.198	0.756	1.58	3.19	8.80	16.60	9.40
D5-NEtFOSA	0.174	0.85	1.61	2.51	7.50	14.20	8.49
D3-NMeFOSAA	0.119	1.21	2.34	2.77	7.19	22.60	10.70
D5-NEtFOSAA	0.118	0.668	2.08	3.47	8.10	11.70	8.11
D7-NMeFOSE	0.0224	0.81	2.63	3.8	7.19	16.50	9.25
D9-NEtFOSE	0.0456	0.601	3.17	4.91	7.88	14.40	8.85
13C3-HFPO-DA	0.0345	0.339	1.01	3.06	8.20	13.90	8.99

Source file: AverageRF_ICAL_results_V4_23051

Notes to Table 4-5:
Minimum Ave RR or RF - The minimum average RR or RF from 27 calibrations with three calibrations for each of the nine laboratories.
Median Ave RR or RF - The median average RR or RF from 27 calibrations with three calibrations for each of the nine laboratories.
Maximum Ave RR or RF - The maximum average RR or RF from 27 calibrations with three calibrations for each of the nine laboratories.
Minimum RSD (%) - The minimum percent relative standard deviation (RSD) of the average RR or RF from the 27 calibrations with three calibration for each of the nine laboratories.
Median RSD (%) - The median percent relative standard deviation (RSD) of the average RR or RF from 27 calibrations with three calibration for each of the nine laboratories.
Maximum RSD (%) - The maximum percent relative standard deviation (RSD) of the average RR or RF from 27 calibrations with three calibrations for each of the nine laboratories.
Pooled RSD (%) - The pooled percent RSD calculated as a weighted mean using the RSDs from the average RRs or RFs from the 27 calibrations. Equation from Pure Appl. Chem., 1981, 53 (9), 1805-1826.

The RR and RF values varied across all nine laboratories, but within each laboratory, the values were generally quite consistent. For the target analytes quantified by isotope dilution, the mean RR values within each calibration ranged from 0.303 to 21.24. Over 96% (625 out of 648) of the RR values were below 5.0. The remaining 23 values which had mean RR values greater than 5.0 were observed for PFPeA, PFOS, HFPO-DA, 4:2FTS, 6:2FTS, and 8:2FTS. HFPO-DA had the highest mean RR by far, far higher than the second highest RR of 10.5 (8:2FTS). The maximum RR value of 21.24 for HFPO-DA was observed on Laboratory 2 and that laboratory had consistently high RRs for that compound. For that analyte, the remaining eight laboratories had RR values that ranged from 0.725 to 1.158; therefore, this one laboratory result should most likely be considered an outlier. The higher RR values tended to occur consistently in several calibrations from a given laboratory for each analyte, suggesting that those high values are not a pervasive concern and not issue of a random variation in the response in a single standard among the calibration points. This observation is supported by the fact that the relative standard deviation (RSD) values for those analytes in the laboratories with the higher-than-expected response ratios are not noticeably different from the RSDs for other analytes in that calibration, nor from the RSDs for those analytes in other laboratories. Whatever may be responsible for the higher response ratios for those six analytes in certain calibrations, it is occurring consistently across all calibration standards, such that the calibration still meets the linearity criterion in the draft procedure.

The ranges of mean RF values generally tend to have higher upper limits than the ranges of the mean RR values, which is expected, because these congeners are not calibrated using isotope dilution, whereas the native analyte and its label have identical structures and fragmentation patterns. However, for this method, most target compounds showed RF values that were not greatly different from the RR values. Over 85% of the results (371 out of 432) of the RF values were below 5.0. The highest RR values were observed for ADONA, 9Cl-P3ONS, and 11Cl-PF3OUdS, where over 50% of the laboratories had values well above 5. PFEESA, PFMPA, and PFMBA had one laboratory each where the RF value was above 5.

The MLVS Method required the linearity of the instrument calibration to be evaluated using one of two approaches.

- The RSD of the RR or RF values of the six ICAL standards for each native compound and isotopically labeled compound must be ≤ 20% to establish instrument linearity, or
- The relative standard error (RSE) of the six ICAL standards for each native compound and isotopically labeled compound must be ≤ 20% to establish instrument linearity.

Table 4-5 also contains the range of RSD values for all 27 calibrations, which is a measure of the variability in the actual RR or RF values for the analyte in each ICAL. The RSD is used as a metric of linearity and assumes that the calibration relationship can be represented by a straight line that runs through the origin. EPA methods that employ the RSD as a linearity metric generally specify QC limits on the order of 15–25%. The lower RSD values in Table 4-5 are all below 5%. The upper RSD values are below 20% for all the analytes, with the exception of six values, all reported by Laboratory 3. In these cases, Laboratory 3 opted to employ the relative standard error (RSE) as the metric for the calibration fit. EPA methods that employ the RSE as a linearity metric generally specify QC limits on the order of 15–25%. In each of these cases, the RSE was <20%. In addition, although having RSD values meeting the RSD requirements, some of the laboratories opted to employ the relative standard error (RSE) as the metric for the calibration fit, which is an option within the MLVS method.

Overall, the study data demonstrate that calibration standards specified in the draft procedure exhibit excellent linearity for the target analytes. Moreover, the commonly used linearity metric of RSD ≤ 20% can be appropriate for the target analytes in this procedure.

A similar examination of the calibration data was performed for the 24 labeled compounds and the data also are summarized in Table 4-5. The mean RF_s values ranged from 0.0159 to 68.5 across all the calibrations. High RF_s values were observed for $^{13}C_8$-PFOA in Laboratory 6, with a mean RFs of 68.5 and Laboratory 10 at 31.98, while the remaining seven laboratories had RF_s values from 0.8992 to 9.6059. All of the other EIS compounds had mean RFs below 5 across all of the laboratories. Across all 27 calibrations, the RSDs for the labeled compounds in each laboratory were below 20% with the exception of five values reported by Laboratory 3. In these cases, Laboratory 3 opted to employ the relative standard error (RSE) as the metric for the calibration fit. In both instances, the %RSE was below 20%.

The results for the initial calibration are further demonstrated visually in Figures 4-1 and 4-2. Figure 4-1 shows box and whisker plots of the ICAL RSDs by analyte and laboratory (9 laboratories and three calibrations for each analyte) for both target compound recoveries, and EIS compound recoveries. The pooled data across the laboratories demonstrates a relatively tight grouping of the median values. In addition, the RSD values were below 20% with the following exceptions: NFDHA (34.1%), 7:3 FTCA (28%), and PFMBA (20.8%). Figure 4-1 suggests that Laboratory 3 and had outliers for the EIS compounds as noted previously Laboratory 3 reported RSEs as the metric for the calibration fit. For those compounds, the %RSE was below 20%.

Figure 4-2 shows a Z-score heat map plot that describes the relationship of the average response factor value, for each calibration value reported by a lab, compared to the mean of all the values for each analyte (i.e., the mean down a column in the plot). Numerically, a Z-score is a measure

of how many standard deviations below or above the population mean (in this case n=27) a value (that is, the average response factor value reported by a laboratory for an analyte) is. A Z-score of zero indicates that the data point's (i.e., the average response factor value reported by a laboratory for one calibration) score is identical to the mean score for an analyte. Figure 4-2 further corroborates that across all the laboratories the RFs were generally within a factor of two standard deviations of the inter-laboratory mean.

4.3 QUALITATIVE STANDARDS

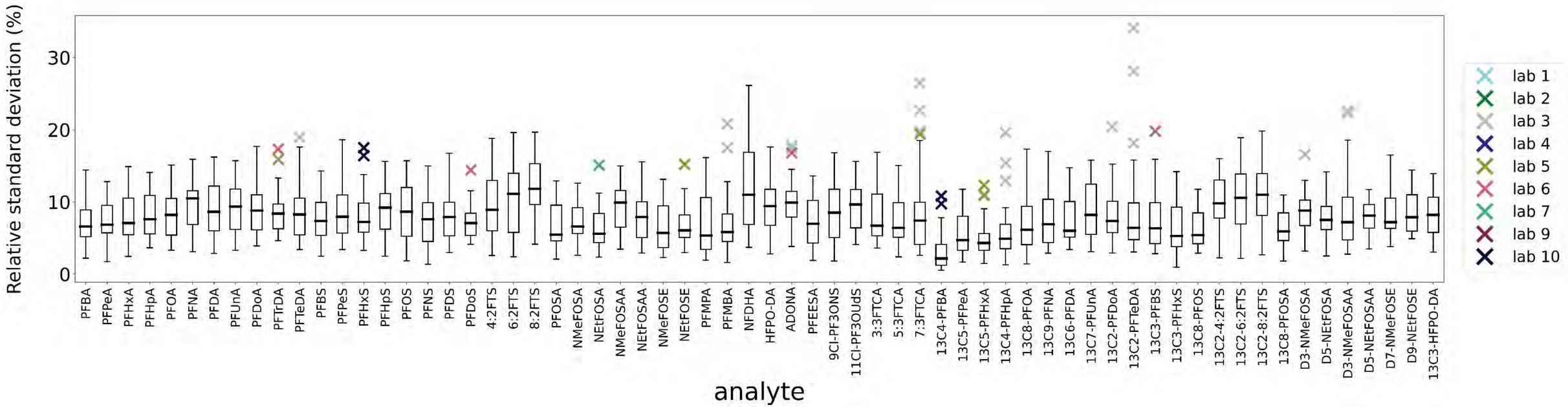
For this study, qualitative standards that included the branched and linear isomers of PFOA, PFNA, PFOSA, NMeFOSA, NEtFOSA, NEtFOSE, and NMeFOSE were required to be analyzed with every batch, prior to sample analyses, to determine the retention time of the branched isomers or isomeric groups of these analytes. The aim was to identify branched isomers in study samples through comparison of retention times with these standards; the peak response of the branched isomers was included in the quantitation of the analyte. When the branched isomers were determined to be present in study samples, the peak response in the study sample was included with the peak response of the linear isomer for the quantitation of the target analyte. Only in these instances and when branched isomers were determined to be present through comparison with quantitative standards used for calibration that contained branched isomers (PFOS, PFHxS, NMeFOSAA, and NEtFOSAA) were branched isomers included in the quantitation of target analytes. At the time of the study, qualitative standards were commercially available for only the seven target analytes listed above. Since the completion of this study, quantitative standards have become commercially available for five of these seven target analytes (PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE). Per the EPA Method 1633, these five isomeric mixtures are required to be used to create calibration, calibration verification, and QC sample spiking solutions.

4.4 CALIBRATION VERIFICATION

The CV standards reported by each laboratory were created using the Wellington standard mixtures provided by the MLVS. CVs were analyzed daily, prior to analysis of samples, after every 10 study samples or less, and at the end of each analytical sequence. The concentration of the CV was approximately the mid-level of the calibration curve used by each laboratory. Target analytes and EIS compounds were required to recover within ±30% of their true value. Data submitted from all laboratories met this criteria with only a few exceptions. There were only three instances of CV standards failing to meet this criteria that affected the data that was reported; one was associated with wastewater samples, the other two, groundwater samples (Table 4-6). In all three instances, the upper limit of the acceptance criteria was exceeded, indicating the concentration reported for these target analytes in the samples that were bracketed by these CVs are potentially biased high. Per the Study Plan, the concentration detected in these samples was retained and qualified with a “J+” qualifier. No sample results were eliminated from the study due to CV failures. The low CV failure rate documented by this study indicates the MLVS CV % recovery criteria is routinely achievable.

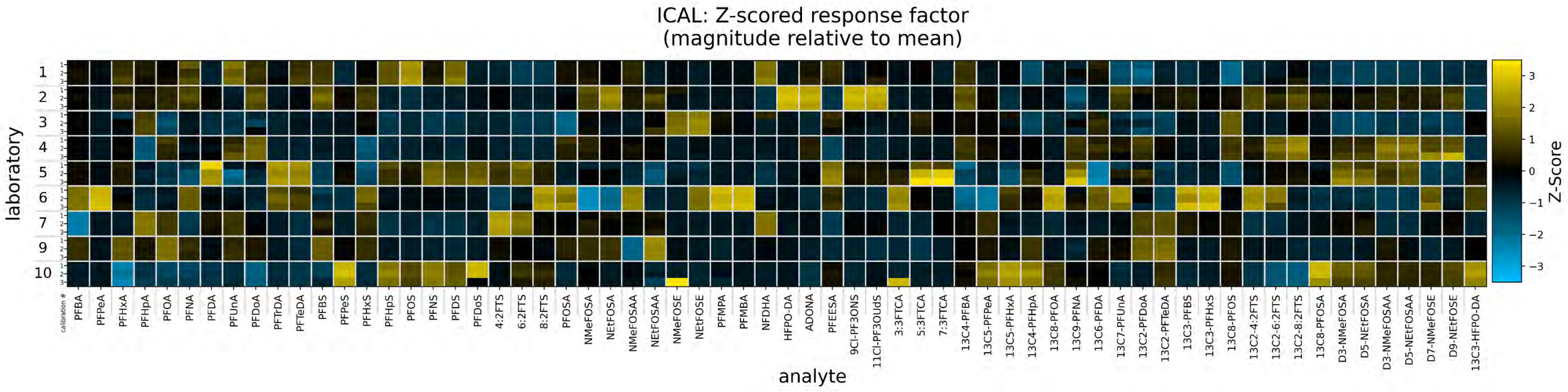
4.5 INSTRUMENT SENSITIVITY CHECK

Each laboratory created instrument sensitivity check (ISC) standards using the Wellington standard mixtures provided for the MLVS. The ISC standard was required to contain the target analytes at the concentration equal to the laboratory's LOQ concentrations, and be analyzed daily, prior to sample analysis, to verify the sensitivity of the instrument. All laboratories met this criteria with the exception of Laboratory #1. The concentration of the ISCs associated with groundwater sample analysis were at a concentration that was 2.5 times their LOQ and for those associated with surface water sample analysis were 1.25 times their LOQ. No sample results were eliminated from the study due to this nonconformance. Target analytes and EIS compounds were required to recover within ±30% of their true value. Data submitted from all laboratories met this criteria with only a few exceptions. There were only seven instances of ISC standards failing to meet this criterion that affected the data that was reported (Table 4-7). All of these failures were associated with wastewater sample analyses. Per the Study Plan, samples that were bracketed by ISC standards whose % recoveries exceeded the acceptance criteria were retained and qualified with a "J+" qualifier and samples that were bracketed by ISC standards whose % recoveries fell below the lower limit of the acceptance criteria were retained and qualified with a "J-" qualifier. No sample results were eliminated from the study due to ISC failures. The low ISC failure rate documented by this study indicates the ISC % recovery acceptance criteria required by this study is routinely achievable.



File: WW_ ICAL_Boxplot_V4

Figure 4-1. Initial Calibration Relative Standard Deviations Results by Analyte by Laboratory.
Nine laboratories x three calibrations for each analyte. Figure includes both target compound recoveries, and EIS compound recoveries.



File: WW_ ICAL_RF_Heatmap_V4_230519

Figure 4-2. Initial Calibration Z-score response factor by Analyte by Laboratory.

Nine laboratories x three calibrations for each analyte. Figure includes both target compound recoveries, and EIS compound recoveries.

Blue shades indicate a laboratory's reported value is below the mean for an analyte, yellow shades depict a value is above the mean and black or dark shades represent the value is identical or close to the mean score

Table 4-6. Summary of Instances of CV Recoveries Outside of MLVS Acceptance Criteria Range.

Laboratory #	Matrix	Target Analyte	Target Analyte % Recovery	Number of Affected Samples
3	WW	NFDHA	146	6
3	GW	7:3FTCA	141.4	8
3	GW	7:3FTCA	133.6	7

Source file: Chapter 4 ICAL Tables 05262023 taken from the data validation reports.

Table 4-7. Summary of Instances of ISC Recoveries Outside of MLVS Acceptance Criteria

Laboratory	Matrix	Target Analyte	Target Analyte % Recovery	Number of Affected Samples
3	WW	PFTeDA	131.0	11
		NFDHA	137.2	12
3	WW	NMEFOSAA	27.2	7
3	WW	NMEFOSAA	36.8	14
3	WW	NMEFOSAA	40.4	7
		PFNA	56	
4	WW	PFBA	67.8	13
4	WW	PFBA	55.4	1
		NFDHA	134.6	
4	WW	NFDHA	136.1	6

Source file: Chapter 4 ICAL Tables 05262023 taken from the data validation reports.

5 INITIAL DEMONSTRATION OF CAPABILITIES

In addition to performing a minimum of three initial multi-point calibrations, laboratories submitted documentation of an IDOC that was compliant with requirements of Phase 3 of the Study Plan (Appendix A). The IDOC consisted of the initial precision and recovery (IPR) study, MDL determination, and the limit of quantitation verification (LOQVER). All IDOC samples were created using the Wellington standard mixtures provided for the MLVS. The IDOC was performed in accordance with the requirements of EPA Method 1633. During validation of the IDOC submittals, it was determined that Laboratory 8 made the same spiking error that occurred with their ICAL submittal. Due to these errors, data from Laboratory 8 was omitted from the statistical analysis of the data generated in the MLVS in its entirety. In addition, it was discovered that Laboratory 3 incorrectly spiked their first set of IDOC samples. The laboratory was required to completely rerun the IDOC study, which after final validation was determined to be acceptable and included in the statistical analyses.

5.1 AQUEOUS METHOD DETECTION LIMITS

As part of Phase 3 of the MLVS, each laboratory was required to determine the MDLs for all 40 PFAS target analytes. MDLs were determined using the revised MDL procedure promulgated by EPA in 2017. The revised procedure defines the MDL as:

> *"… the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results."*

The procedure consists of two parts: determination of the MDL based on method blanks (called MDL_b), and determination of the MDL based on spiked samples (called MDL_s). Both MDL_b and MDL_s are determined in a reference matrix, in this case reagent water, using at least seven replicates prepared and analyzed on three non-consecutive days.

The MDL_b is calculated as:

$$MDL_b = \overline{X} + t_{(n-1,\ 1-\alpha=0.99)} S_b$$

where:

$\overline{X}$ = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom

S_b = sample standard deviation of the replicate method blank sample analyses

Note: The equation above is used when all the method blanks for an individual analyte give numerical results. If some (but not all) of the method blank results give numerical results, then the MDL_b is set equal to the highest method blank result.

The MDL_s is calculated as:

$$MDL_s = t_{(n-1,\ 1-\alpha=0.99)} S_s$$

where:

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = Student's *t*-value appropriate for a single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom

S_s = sample standard deviation of the replicate spiked sample analyses

PFAS-free reagent water was the reference media used to prepare the seven MDL method blank replicates. Each was spiked with the 24 EIS and seven NIS compounds to create seven MDL method blanks. Seven MDL spiked replicates were prepared in the same manner as the MDL method blanks except the 40 target analytes were also added to each MDL spike replicate. All MDL method blanks and MDL spiked samples were prepared per EPA Method 1633 (Appendix A), in at least three batches on three separate calendar dates and analyzed on three separate calendar dates. The EIS and NIS compounds were spiked at the same concentrations as in the ICAL standards. The MDL value based on method blanks (MDL_b) and spiked samples (MDL_S) were calculated by each laboratory following data review, and an initial MDL was determined as the higher of these two values. Table 5-1 provides a summary of the MDL values.

The preliminary acceptance criterion for EIS compound recovery stated in the Study Plan was 50–200% recovery. All EIS compounds met this criterion for all analyses.

Of the three MDL_b values reported by Laboratory 3 and the one MDL_b by Laboratory 7 (Table 5-2), all of them were exclusive to that laboratory (in other words, no other laboratory reported an MDL_b value for those four analytes). Only one MDL_b value for one analyte (6:2FTS), by one laboratory, Laboratory 3, was greater than the calculated MDL_s value, and was therefore used as the final MDL value.

Through these MDL data and the routine method blank results generated during the course of the validation study, the study demonstrated that background levels in typical laboratories are not a limiting factor in the application of this method, but that some laboratories had better control of background levels than others.

5.2 INITIAL PRECISION AND RECOVERY (IPR) RESULTS

IPR studies were performed in aqueous matrices. Four aliquots of 0.5 L of PFAS-free reagent water were spiked with all 40 target analytes such that the final concentration of each PFAS in the IPR was greater than or equal to the LOQ and less than or equal to the midpoint of the laboratory's calibration. These spiked aliquot of PFAS-free reagent water were prepared and analyzed in exactly the same manner as study samples, per EPA Method 1633 (Appendix A).

A total of 36 IPRs were included in the statistical analysis. The mean percent recovery, standard deviations, and RSD of recoveries is presented in Table 5-3. All 36 IPRs met the study IPR NIS criteria (>30% recovery). Of the 1,440 target analyte results reported from IPRs, four target analyte recoveries exceeded the target analyte criteria (40–150%), resulting in an exceedance rate of

0.28%. The lowest reported percent recovery was 66%, reported by Laboratory 6 for 7:3FTCA in a single IPR sample. The four exceedances were reported by Laboratory 5 and affected three out of the four IPR samples reported. The exceedances were associated with four target analytes: PFHpA (152%), PFUnA (165%), PFOA (154%), and NFDHA (164%). Of the 864 EIS compound results reported from IPRs, four exceeded the EIS compound acceptance criteria (20–150%), resulting in a failure rate of 0.46%. All exceedances were reported by Laboratory 5 and were associated with three of the four IPRs they reported. The exceedances were associated with two EIS compounds; $^{13}C_2$-PFBS (155%, 170%, and 174%) and $^{13}C_2$-4:2FTS (164%).

Most of the highest target analyte recoveries were associated with Laboratories 5 and 10 (Figure 5-1) and are predominantly associated with perfluorocarboxylic acids. Results for PFPeA, PFHxA, and PFTeDA associated with Laboratory 6 were routinely lower than all other laboratory results reported. None of these results can be explained by their EIS compound recoveries since they were not statistically different than those from the other laboratories.

Table 5-1. Aqueous Method Detection Limit Study Results

Target Analyte	Number of Labs[1]	Max MDL_s[2]	Max MDL_b[3]	Minimum Concentration of MDL (ng/L)[4]	Maximum Concentration of MDL (ng/L)[5]	# Labs Using MDL_b as Final MDL[6]	Pooled MDL[7]
PFBA	9	1.91	U	0.542	1.91	0	0.789
PFPeA	9	1.07	U	0.249	1.07	0	0.537
PFHxA	9	1.45	U	0.0767	1.45	0	0.463
PFHpA	9	1.06	U	0.158	1.06	0	0.372
PFOA	9	1.69	U	0.136	1.69	0	0.542
PFNA	9	1.06	U	0.167	1.06	0	0.451
PFDA	9	1.36	U	0.181	1.36	0	0.522
PFUnA	9	0.927	U	0.181	0.927	0	0.451
PFDoA	9	0.829	U	0.15	0.829	0	0.397
PFTrDA	9	0.974	U	0.196	0.974	0	0.46
PFTeDA	9	1.59	U	0.168	1.59	0	0.49
PFBS	9	1.08	U	0.105	1.08	0	0.374
PFPeS	9	1.32	U	0.116	1.32	0	0.503
PFHxS	9	1.44	U	0.129	1.44	0	0.535
PFHpS	9	2.99	U	0.111	2.99	0	0.498
PFOS	9	1.69	0.38	0.255	1.69	0	0.629
PFNS	9	1.36	U	0.218	1.36	0	0.472
PFDS	9	6.59	U	0.153	6.59	0	0.601
PFDoS	9	1.43	U	0.105	1.43	0	0.596
4:2FTS	9	4.57	U	0.634	4.57	0	1.69
6:2FTS	9	7.89	2.16	0.947	7.89	1	2.45
8:2FTS	9	7.46	U	0.548	7.46	0	2.5
PFOSA	9	0.72	U	0.154	0.72	0	0.315
NMeFOSA	9	0.967	U	0.153	0.967	0	0.426

Table 5-1. Aqueous Method Detection Limit Study Results

Target Analyte	Number of Labs[1]	Max MDL_s[2]	Max MDL_b[3]	Minimum Concentration of MDL (ng/L)[4]	Maximum Concentration of MDL (ng/L)[5]	# Labs Using MDL_b as Final MDL[6]	Pooled MDL[7]
NEtFOSA	9	1.11	U	0.107	1.11	0	0.446
NMeFOSAA	9	2.28	U	0.183	2.28	0	0.683
NEtFOSAA	9	1.33	U	0.281	1.33	0	0.586
NMeFOSE	9	10.9	U	1.51	10.9	0	3.81
NEtFOSE	9	12.8	U	1.55	12.8	0	4.84
PFMPA	9	1.1	U	0.318	1.1	0	0.514
PFMBA	9	1.14	U	0.296	1.14	0	0.504
NFDHA	8	2.91	U	0.494	2.91	0	1.17
HFPO-DA	9	4.15	U	0.342	4.15	0	1.46
ADONA	9	4.09	U	0.573	4.09	0	1.41
PFEESA	9	2.25	U	0.226	2.25	0	0.746
9Cl-PF3ONS	9	4.17	U	0.737	4.17	0	1.38
11Cl-PF3OUdS	9	5.01	U	0.815	5.01	0	1.67
3:3FTCA	9	6.64	U	0.858	6.64	0	2.47
5:3FTCA	9	29.2	U	1.92	29.2	0	9.59
7:3FTCA	9	25.3	U	2.49	25.3	0	8.71

Source: MDL_results_V1_230503_215159.csv; updated 5/31/2023

Notes:
[1] The number of laboratories for which an MDL value was calculated. Laboratory #8 data omitted from summary due to spiking error. No aqueous sample data from this laboratory was utilized in the MLVS statistical analysis.
[2] The maximum MDLs value across individual spiked samples.
[3] The maximum MDL_b value across individual spiked samples. "U" indicates analyte was not detected.
[4] The minimum MDL across the values calculated for each laboratory.
[5] The maximum MDL across the values calculated for each laboratory.
[6] The number of laboratories for which the MDL_b value was the final MDL value.
[7] Pooled MDL using the individual laboratory MDL values calculated. Equation from EPA 821-B-18-001 page G-22.

Table 5-2. Frequency of Detection in Aqueous MDL_b by Laboratory

# MDL_b Detections	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 10
	0	0	3	0	0	0	1	0	0

Table 5-3. Aqueous IPR Results

Analyte	Number of Labs[1]	Number of Results[2]	Mean % Recovery[3]	Pooled Between-Lab std. dev. (s_b)[4]	Pooled Within-Lab std. dev. (s_w)[5]	Pooled Between- and Within-Lab std. dev. (s_c)[6]	RSD (s_w)[7]
Target Analytes							
PFBA	9	36	104	11.4	3.49	12	3.35
PFPeA	9	36	103	12.1	3.63	12.8	3.53
PFHxA	9	36	105	14.6	7.13	15.4	6.8
PFHpA	9	36	107	14.1	7.77	14.8	7.28
PFOA	9	36	108	14.8	8.82	15.6	8.19
PFNA	9	36	104	10.6	7.28	11.1	6.99
PFDA	9	36	99.8	11.7	8.68	12.4	8.7
PFUnA	9	36	105	14	11.7	14.7	11.1
PFDoA	9	36	104	8.85	5.06	9.32	4.84
PFTrDA	9	36	98.3	11.7	9.52	12.4	9.69
PFTeDA	9	36	103	14.5	6.37	15.3	6.21
PFBS	9	36	103	10.6	5.55	11.1	5.37
PFPeS	9	36	107	12.3	9.41	13	8.82
PFHxS	9	36	102	8.38	5.61	8.84	5.48
PFHpS	9	36	105	13.4	6.71	14.2	6.37
PFOS	9	36	105	10.4	7.89	11	7.53
PFNS	9	36	101	7.89	7.45	8.31	7.39
PFDS	9	36	100	9.49	7.72	10	7.68
PFDoS	9	36	95.4	11	9.24	11.6	9.68
4:2FTS	9	36	106	6.82	9.11	7.19	8.6
6:2FTS	9	36	104	11.4	9.91	12	9.49
8:2FTS	9	36	109	7.95	10.5	8.38	9.69
PFOSA	9	36	104	11.1	3.7	11.7	3.55
NMeFOSA	9	36	102	7.57	6.27	7.98	6.16
NEtFOSA	9	36	99	7.77	4.44	8.19	4.49
NMeFOSAA	9	36	104	8.79	7.63	9.26	7.31
NEtFOSAA	9	36	105	10.5	7.61	11.1	7.23
NMeFOSE	9	36	99.6	5.12	4.02	5.4	4.04
NEtFOSE	9	36	98.4	5.12	4.3	5.4	4.37
PFMPA	9	36	98	8.17	5.58	8.61	5.7
PFMBA	9	36	98.1	8.47	4.06	8.93	4.14
NFDHA	9	36	106	13.6	12.2	14.3	11.5
HFPO-DA	9	36	101	8.92	5.78	9.41	5.75
ADONA	9	36	104	7.01	5.28	7.39	5.06
PFEESA	9	36	104	11.3	6.14	11.9	5.89

Table 5-3. Aqueous IPR Results

Analyte	Number of Labs[1]	Number of Results[2]	Mean % Recovery[3]	Pooled Between-Lab std. dev. (s_b)[4]	Pooled Within-Lab std. dev. (s_w)[5]	Pooled Between- and Within-Lab std. dev. (s_c)[6]	RSD (s_w)[7]
9Cl-PF3ONS	9	36	104	8.02	7.85	8.45	7.56
11Cl-PF3OUdS	9	36	99.9	10.5	7.77	11.1	7.77
3:3FTCA	9	36	97.4	5.51	6.76	5.81	6.94
5:3FTCA	9	36	101	7.92	6.84	8.35	6.79
7:3FTCA	9	36	95	10.2	6.54	10.8	6.89
EIS Compounds							
$^{13}C_4$-PFBA	9	36	86	8.25	4.82	8.69	5.61
$^{13}C_5$-PFPeA	9	36	92.5	12.9	4.96	13.6	5.36
$^{13}C_5$-PFHxA	9	36	87.7	11	6.31	11.6	7.19
$^{13}C_4$-PFHpA	9	36	84.1	13.8	5.77	14.6	6.86
$^{13}C_8$-PFOA	9	36	87.2	8.89	7.49	9.37	8.59
$^{13}C_9$-PFNA	9	36	87.2	9.52	7.88	10	9.04
$^{13}C_6$-PFDA	9	36	91	12.4	8.89	13	9.77
$^{13}C_7$-PFUnA	9	36	88.6	9.7	7.3	10.2	8.23
$^{13}C_2$-PFDoA	9	36	82.7	11.4	6.31	12	7.64
$^{13}C_2$-PFTeDA	9	36	83.1	16.8	9.4	17.7	11.3
$^{13}C_3$-PFBS	9	36	96	22.8	13.1	24	13.6
$^{13}C_3$-PFHxS	9	36	87.9	12.2	4.85	12.9	5.51
$^{13}C_8$-PFOS	9	36	87	10.6	5.87	11.1	6.75
$^{13}C_2$-4:2FTS	9	36	95.9	16.8	12.5	17.8	13.1
$^{13}C_2$-6:2FTS	9	36	92.8	11.1	12.5	11.7	13.5
$^{13}C_2$-8:2FTS	9	36	98.1	14.2	12.2	14.9	12.4
$^{13}C_8$-PFOSA	9	36	84.2	17.2	7.2	18.1	8.56
D_3-NMeFOSA	9	36	69.9	22.5	12	23.7	17.2
D_5-NEtFOSA	9	36	69.1	19.7	11.8	20.8	17.1
D_3-NMeFOSAA	9	36	88.9	10.6	10	11.2	11.3
D_5-NEtFOSAA	9	36	91.3	15.7	9.83	16.6	10.8
D_7-NMeFOSE	9	36	73.5	13.6	12.1	14.4	16.4
D_9-NEtFOSE	9	36	72.3	12.7	12.1	13.4	16.8
$^{13}C_3$-HFPO-DA	9	36	84.9	9.21	7	9.71	8.24

Source: IPR_results_V1_230503_215140.csv; updated 5/31/2023

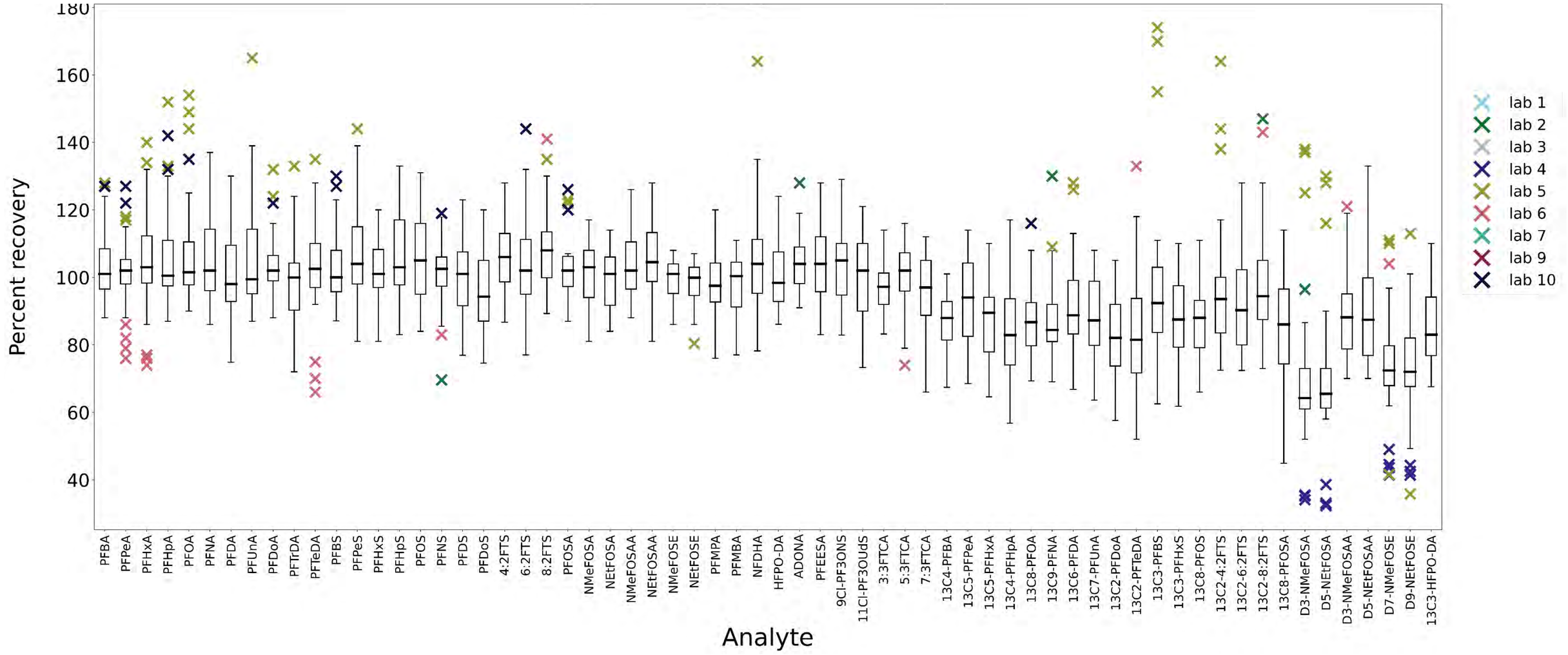
Notes Table 5-3:
[1] The number of laboratories reporting initial precision recovery (IPR) results. Laboratory #8 data omitted from summary due to spiking error. No aqueous sample data from this laboratory was utilized in the MLVS statistical analysis.
[2] The number of individual IPR results across all laboratories included in the calculations.
[3] The mean percent recovery across all of the IPR individual samples across all laboratories for the given analyte.
[4] The pooled between-laboratories standard deviation of the percent recovery. Equation from EPA 821-B-18-001 page G-25.
[5] The pooled within-laboratory standard deviation. Equation from EPA 821-B-18-001 page G-25.
[6] The combined within and between laboratory standard deviations. Equation from EPA 821-B-18-001 page G-25.
[7] The pooled within-laboratory relative standard deviation (RSD, (sw/(mean % recovery) *100).

5.3 AQUEOUS LIMIT OF QUANTITATION VERIFICATION ANALYSES

Since an LLOPR is not included in EPA IDOC requirements, the Study Plan required laboratories to analyze an LOQVER sample in order to verify their stated LOQs. A single aliquot of 0.5 L of PFAS-free reagent water was spiked with all 40 target analytes such that the final concentration of each PFAS in the LOQVER was one and two times the LOQ. This spiked aliquot of PFAS-free reagent water was prepared and analyzed in exactly the same manner as study samples, per EPA Method 1633 (Appendix A). While laboratories were required to prepare and analyze only one LOQVER per the Study Plan, some laboratories chose to prepare and analyze as many as seven. All data submitted was included in the statistical analysis.

A total of 18 LOQVERs were included in the statistical analysis (Table 5-4). All 18 LOQVERs met the study NIS target acceptance criteria (>30% recovery). Of the 720 target analyte results reported from LOQVERs, three target analytes' recoveries exceeded the target analyte criteria (40–150%), resulting in an exceedance rate of 0.42%. The lowest reported percent recovery was 53%, reported by Laboratory 2 for NFDHA. The three exceedances were reported by Laboratory 5: PFHpA (154%), PFNA (163%), and PFBA (186%). Of the 432 EIS compound results reported from LOQVERs, one exceeded the EIS compound acceptance criteria (20–150%), resulting in a failure rate of 0.23%. The exceedance was reported by Laboratory 5 for $^{13}C_2$-PFBS (156%). The concentration range of the verified LOQs is provided in Table 5-5.

Most of the highest target analyte recoveries were associated with Laboratory 5 (Figure 5-2) and are predominantly associated with perfluorocarboxylic acids, which is consistent with the IPR results. Table 5-5 provides the range of LOQs the laboratories used to report groundwater, surface water, and wastewater samples in this study. Concentrations are based on a sample volume of 500 mL; LOQs that were elevated due to extract dilutions prior to analysis were omitted from the summary



File: RW_IPR_Boxplot_V1_230505

Figure 5-1. Initial Precision and Recovery (IPR) Results by Analyte by Laboratory.
Figure includes both target compound recoveries, and EIS compound recoveries.

. Table 5-4. Aqueous LOQVER Summary

Target Analyte	Number of Laboratories[1]	Number of Results[2]	Minimum Concentration (ng/L)[3]	Maximum Concentration (ng/L)[4]	Minimum Percent Recovery[5]	Maximum Percent Recovery[6]
Target Analyte						
PFBA	9	18	3.47	29.7	83.3	186
PFPeA	9	18	1.72	13.7	86	135
PFHxA	9	18	0.901	7.25	90.1	116
PFHpA	9	18	0.914	7.25	90	154
PFOA	9	18	0.949	8.08	94.9	148
PFNA	9	18	0.891	6.88	85	163
PFDA	9	18	0.829	7.72	82.9	134
PFUnA	9	18	0.821	7.09	75.6	127
PFDoA	9	18	0.919	6.89	81.2	141
PFTrDA	9	18	0.803	6.92	76.2	124
PFTeDA	9	18	0.879	7.41	87.6	133
PFBS	9	18	0.812	6.47	83	117
PFPeS	9	18	0.85	7.8	79.3	131
PFHxS	9	18	0.845	7.17	65.1	123
PFHpS	9	18	0.894	6.93	77.9	149
PFOS	9	18	0.959	7.04	58.8	132
PFNS	9	18	0.924	6.61	66.6	117
PFDS	9	18	0.731	7.18	62.6	118
PFDoS	9	18	0.627	5.79	60.7	115
4:2FTS	9	18	3.22	29.5	85.9	130
6:2FTS	9	18	3.69	28.7	70	121
8:2FTS	9	18	3.66	32.9	64.2	136
PFOSA	9	18	0.893	6.6	85.3	131
NMeFOSA	9	18	0.605	7.11	60.5	128
NEtFOSA	9	18	0.677	7.24	67.7	132
NMeFOSAA	9	18	0.787	7.59	75	119
NEtFOSAA	9	18	0.997	8.36	76.9	131
NMeFOSE	9	18	8.97	67.9	85.8	115
NEtFOSE	9	18	10.2	68.9	86.5	108
PFMPA	9	18	1.65	13	82.5	112
PFMBA	9	18	1.67	12.7	83.5	108
NFDHA	9	18	1.73	14.1	53.3	150
HFPO-DA	9	18	3.73	33.2	88.4	130

. **Table 5-4. Aqueous LOQVER Summary**

Target Analyte	Number of Laboratories[1]	Number of Results[2]	Minimum Concentration (ng/L)[3]	Maximum Concentration (ng/L)[4]	Minimum Percent Recovery[5]	Maximum Percent Recovery[6]
ADONA	9	18	3.33	31	75.1	128
PFEESA	9	18	1.47	12.2	74.4	109
9Cl-PF3ONS	9	18	3.13	29.9	79.7	125
11Cl-PF3OUdS	9	18	2.88	28.5	73.5	118
3:3FTCA	9	18	5.09	34.3	89	110
5:3FTCA	9	18	23.5	166	88.2	120
7:3FTCA	9	18	17.5	176	70	118

Source: LOQVER_results_V1_230503_215921.csv; updated 5/31/2023

Notes:

[1] The number of laboratories reporting limit of quantitation verification (LOQVER) results. Laboratory 8 data was omitted from summary due to spiking error. No aqueous sample data from this laboratory was utilized in the MLVS statistical analysis.

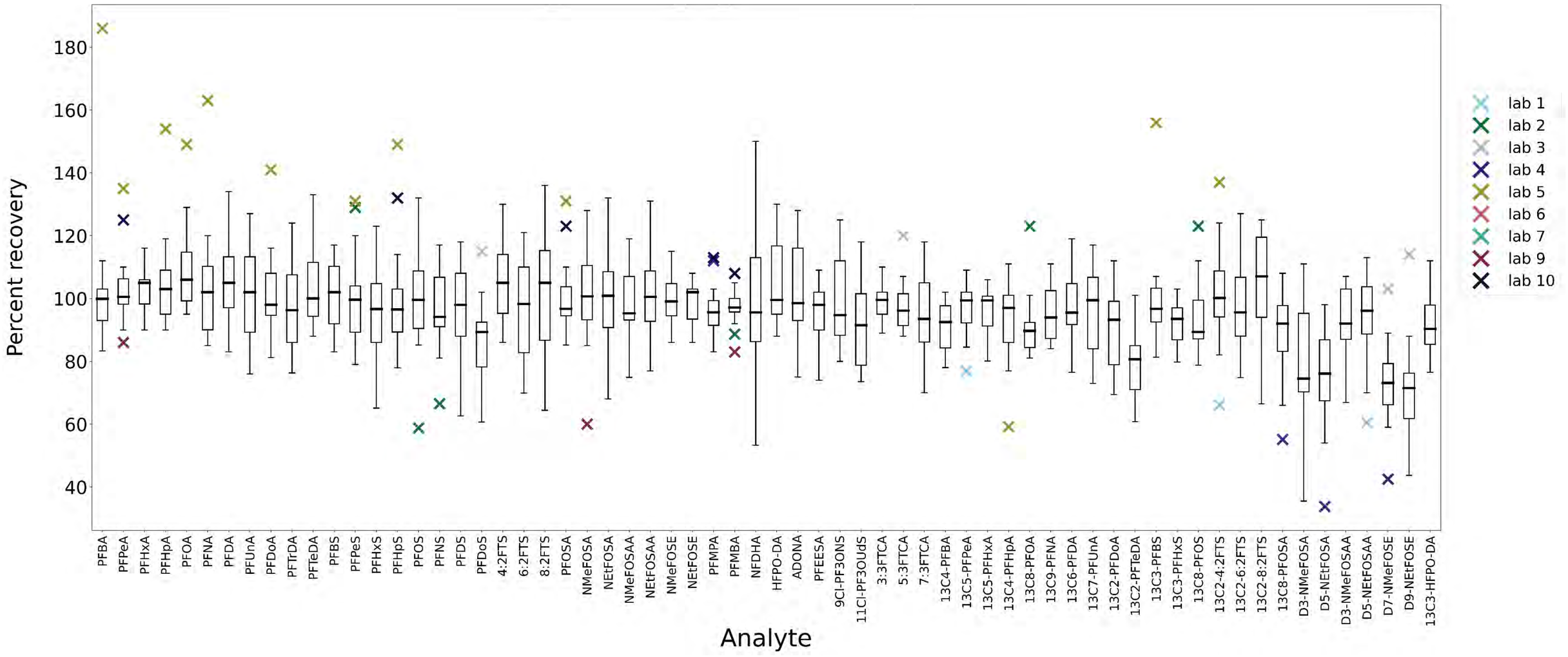
[2] The total number of results reported across all laboratories.

[3] The minimum concentration measured across all laboratories.

[4] The maximum concentration measured across all laboratories.

[5] The minimum percent recovery across all laboratories.

[6] The maximum percent recovery across all laboratories.



Source file: WW RW_LOQVER_Boxplot_V1_230505

Figure 5-2. Limit of Quantitation Verification (LOQVER) Results by Analyte by Laboratory.
Figure includes both target compound recoveries, and EIS compound recoveries.

Table 5-5. Summary of Verified LOQs

Target Analyte	Number of Laboratories[1]	LOQ Minimum Concentration (ng/L) [2]	LOQ Maximum Concentration (ng/L) [2]
PFBA	9	4	16
PFPeA	9	2	8
PFHxA	9	1	4
PFHpA	9	1	4
PFOA	9	1	4
PFNA	9	1	4
PFDA	9	1	4
PFUnA	9	1	4
PFDoA	9	1	4
PFTrDA	9	1	4
PFTeDA	9	1	4
PFBS	9	1	3.55
PFPeS	9	1	3.76
PFHxS	9	1	3.66
PFHpS	9	1	3.81
PFOS	9	1	3.75
PFNS	9	1	3.85
PFDS	9	1	3.86
PFDoS	9	1	3.88
4:2FTS	9	4	15
6:2FTS	9	4	15.2
8:2FTS	9	4	15.4
PFOSA	9	1	4
NMeFOSA	9	1	4
NEtFOSA	9	1	4
NMeFOSAA	9	1	4
NEtFOSAA	9	1	4
NMeFOSE	9	10	40
NEtFOSE	9	10	40
PFMPA	9	2	8
PFMBA	9	2	8
NFDHA	9	2	8
HFPO-DA	9	4	16
ADONA	9	4	15.1
PFEESA	9	2	7.12
9Cl-PF3ONS	9	4	15
11Cl-PF3OUdS	9	4	15.1

Table 5-5. Summary of Verified LOQs

Target Analyte	Number of Laboratories[1]	LOQ Minimum Concentration (ng/L) [2]	LOQ Maximum Concentration (ng/L) [2]
3:3FTCA	9	5	20
5:3FTCA	9	25	100
7:3FTCA	9	25	100

Source: Chapter 5 IDC 06142023 Exa compilation from database

Notes:
[1] Laboratory 8 data omitted from summary due to spiking error. No aqueous sample data from this laboratory was utilized in the MLVS statistical analysis.
[2] Concentrations based on a sample volume of 500 mL.

6 WASTEWATER

A total of 49 study samples were created and shipped to each participating laboratory as described in Section 2 of this report. All wastewater study samples were prepared and analyzed by each laboratory as required by EPA Method 1633, with the exception of one sample series. The sample series associated with the ASTM Substitute, WWK1 through WWK7, was unable to be prepared by the laboratories. When the samples were thawed, the sample became viscus and a precipitate formed. Following the method, laboratories attempted to utilize multiple SPE cartridges; however, they were still unable to extract the entire volume of each of the samples in this series. As a result, there are no data for this series of sample matrices, leaving six sample matrix series to be included in the statistical analysis of wastewater samples. The problem is likely due to the significant amount of flour used to create synthetic wastewater. None of the diverse real wastewaters exhibited this problem. Due to EIS compound spiking errors that affected all of wastewater analyses, all wastewater data from Laboratories 8 and 10 was omitted from the statistical analysis. These two laboratories did not follow EPA Method 1633 and spiked the EIS compounds at levels comparable to the MDL for many analytes. The accuracy and precision of the data from these laboratories were noticeably worse than from the other laboratories. Data were reported and validated in accordance with the requirements of the Study Plan. The rules used for omission of individual analyte results are presented in Section 3 of this report.

6.1 NATIVE PFAS CONCENTRATIONS IN WASTEWATER

Each laboratory received and analyzed a single sample of each wastewater sample (Table 2-3). The concentrations detected in this sample were considered the background or "native" concentration for each of the environmental matrices for each laboratory. The native concentrations measured in wastewater samples by each laboratory are presented in Appendix E, Table E-1, which also includes the results for the initial reconnaissance survey that are presented in Table 2-4, for comparison.

Table 6-1 summarizes the detections reported by each laboratory for the six wastewater samples, as well as the results from the reconnaissance analysis.

Table 6-1. Summary of Target Analytes Detected in Unspiked Wastewater Samples in ng/L

Target Analyte	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Recon
Total # Analytes Reported across all six samples	64	67	64	58	68	45	60	60	67

Of the 40 PFAS target analytes in the draft EPA Method 1633, thirteen were not detected in any of the six wastewater samples by any of the eight laboratories that completed the wastewater portion of the validation study, nor by the reconnaissance laboratory. Those 13 analytes were:

PFNS, PFDS, PFDoS, 4:2FTS, NEtFOSA, NMeFOSE, NEtFOSE, ADONA, 9Cl-PF3ONS, 11Cl-PF3OUdS, PFEESA, PFMBA, and NFDHA. PFOA was detected in every unspiked sample by every laboratory (Appendix E, Table E-2).

Although there was considerable overall agreement across all of the laboratories, the results for the unspiked samples did vary across both the samples and the laboratories. Across the six wastewater samples, 12 to 18 of the target analytes were reported by at least one of the eight laboratories that completed the aqueous portion of the study, as show in Table 6-2.

Table 6-2. Numbers of Detected Analytes by Wastewater Sample

Wastewater Sample	Total Number of Analytes Detected
WWI – Hospital	12
WWJ – POTW Influent	17
WWL – Bus Washing Station	17
WWM – POTW Effluent	18
WWN – Pulp and Paper Effluent	10
WWO – POTW Effluent	16

Source file: WW_Unspiked_summary_20230410.xls

As shown in Table 6-1, seven of the laboratories reported between 45 and 68 “hits” across the six wastewater samples. Laboratory 6 reported only 45 hits, while all other laboratories reported a similar number of hits (58 to 67). Laboratory 6 was often the only laboratory that did not report an analyte that was detected in all of the other laboratories, e.g., PFBA, PFBS, PFPeA, PFPeS, PFHpA, and PFDA. In contrast, the reconnaissance laboratory reported those analytes in all of the samples, except for PFBS in Sample WWJ. Given that four of those six analytes are the shortest chain analytes in the method and extraction efficiency is known to be affected by chain length, the results from Laboratory 6 may reflect differences in their SPE technique compared to that of the other laboratories.

Laboratory 5 had the highest number of “hits” across all six samples, 68, and tended to be the only laboratory to report certain analytes in some samples, including PFOSA in five of the samples. In the case of PFOSA, this discrepancy could be attributed to contamination issues, given that PFOSA was detected in all three of the method blanks associated with the wastewater samples. In other cases, it was the only laboratory to report an analyte in one sample, generally at low concentration.

The results for the target analytes in the unspiked sample were not used to develop EPA Method 1633 performance data, but they do illustrate general agreement across most of the laboratories in individual samples. In contrast, the results for the EIS compounds in these unspiked samples were used in the evaluation of EIS compound recoveries, as described in Section 9.

Some of the differences in the results across the laboratories may be caused by the fact that one cannot perfectly homogenize every sample. Many of the analytes are not completely water soluble and are known surfactants, so some or all of the analyte partition to suspended particulates in the

wastewater matrix and the walls of the container. The wastewater samples distributed for this study were thoroughly mixed, but it is impossible to perfectly distribute the amount of particulates present in hundreds of 500-mL samples, nor is it possible to make sure each piece of particulate contains a similar concentration of PFAS analyte. Even minor differences in the amounts of suspended particulates across the replicate samples from any of the bulk wastewater matrices can cause a difference of several ng/L. Many of the unspiked samples contained very low quantities of some PFAS analytes, so the variance in detection thresholds between laboratories can also cause different detection rates.

6.2 WASTEWATER MATRIX SPIKE RESULTS

The spiked wastewater samples were evaluated to demonstrate the precision and accuracy of EPA Method 1633 on real-world samples. Isotope dilution methods typically do not include the use of matrix spike (MS) and matrix spike duplicate (MSD) samples as part of the sample preparation batch QC elements. An objective of this study was to demonstrate performance of the method in real-world samples that contain target analytes.

As detailed in Section 2 of this report, the matrix spike samples created by Waters ERA were prepared and analyzed by each laboratory in accordance with EPA Method 1633. Preparation methods for the PFAS-spiked matrices are in Appendix B. Seven individual wastewater matrices were analyzed for an unspiked sample, three low spiked samples and three high spiked samples. While 49 samples were sent to the laboratories, as noted previously samples for series WWK, the ASTM prepared wastewater samples, were excluded from the analyses. A total of 36 matrix spikes were analyzed per laboratory.

The methods used by IDA to calculate the percent recoveries, within-laboratory standard deviation, within and between laboratory standard deviation and within-laboratory relative standard deviations followed the ATP-prescribed methods (EPA 2018); these are presented in Appendix D. The rules applied to the wastewater data set for statistical analyses were described in Section 3.4. The results of the determination of the matrix spike recoveries for the individual samples are presented in Appendix G.

Initial evaluation of the matrix spike recovery results indicated the results for one sample (WWO4) from one laboratory (Laboratory 3), were significantly different than the results for that sample that were reported by all other laboratories. The recoveries of these analytes ranged from 250–1833%, while EIS and NIS compound recoveries met the MLVS target criteria. The cause of the high target analyte recoveries could not be determined, but possible causes could include an error during sample preparation at Waters ERA. As a result, all sample results for target analytes, EIS compounds, and NIS compounds for WWO4 from Laboratory #3 were omitted from any further evaluation of the matrix spike results.

The compiled PFAS-spiked wastewater sample results from all eight laboratories are shown in Table 6-3. There were differences observed both in the recoveries by individual laboratories (Appendix E, Table E-3 and E-4) and in the comparison of low-spiked sample and high-spiked sample recoveries (Figures 6-1 and 6-2, respectively). Variability between the laboratories is more evident in the low spike samples than in the high-spiked samples. One would expect that the variability in background levels of PFAS in the original samples would have a greater effect on the low-spiked samples than the high-spiked samples. The mean percent recovery of the low-

spiked samples for each target analyte fell between 52.2% (PFDoS) and 145% (NMeFOSAA) (Table 6-3). For the low spiked samples this is particularly evident for the FOSA and FOSE-target analytes (e.g., PFOSA, NMeFOSA, NEtFOSE), and the FTCA-target analytes (Figure 6-1). This may be in part due to the variability observed in the EIS compound recoveries (Figure 6-1 and discussed further below). That variability is also reflected in combined laboratory results in Table 6-3. The table shows relatively high variability between laboratory standard deviations (s_b) and within laboratory standard deviations (s_w) for PFOSA, NMeFOSAA, NEtFOSAA, as well as for ADONA 9Cl-PF3ONS, 11Cl-PF3OUdS, 5:3 FTCA, and 7:3 FTCA. The mean % recoveries of 92.5% (37 out of 40) of the target analytes fell between 78.8 % and 114%, the exceptions being PFDoS, NMeFOSAA, and NEtFOSAA.

The mean percent recoveries of the high-spiked samples fell between 52.6% (PFDoS) and 111% (NMeFOSAA) (Table 6-3). The variability in results was generally much tighter across the laboratories, with a couple of exceptions. Laboratory 6 had higher recovery for PFBS and 4:2 FTS than the other seven laboratories. Table 6-3 shows relatively low between-laboratory standard deviations (s_b) and low within-laboratory standard deviations (s_w), with the exception of PFBA and 4:2FTS. The results for PFBS and 4:2FTS associated with sample WWJ7 were identified as the cause of the excessively high recoveries (599% and 1238%, respectively). During review, it was determined that concentrations of these analytes were calculated using the incorrect peak areas for their associated EIS compounds ($^{13}C_3$-PFBS and $^{13}C_2$-4:2FTS). A shoulder off of the actual peak for the EIS compound response was used instead of the actual EIS compound peak itself. The laboratory later corrected this; however, only the retention time summary and percent recovery summaries in data package were updated. This error was caught during the review of the statistical analysis of the MS data. Consequently, these data points should not be viewed as an indication of EPA Method 1633 performance and have been eliminated from further discussion. The mean % recovery of 97.5% (39 out of 40) of the target analytes fell between 71.3–111%, the exception being PFDoS.

The combined low/high spiked sample statistical results are also shown in Table 6-3. The mean percent recoveries were between 52.4% (PFDoS) and 128% (NMeFOSAA), both of which fall within the targeted recovery range (40–150%). The mean % recovery of 97.5% of the target analytes (39 out of 40) fell between 72.3% and 128%, the exception being PFDoS.

Results comparing the different wastewaters using the pooled laboratory results are given in Table 6-4. Generally, the mean % recoveries were similar for all target PFAS across the six wastewater samples. The range of recoveries observed for specific samples were higher for WWI (Hospital wastewater) and WWJ (POTW influent). For WWI this included NMeFOSAA (78.1–535%), NEtFOSAA (74–400%), ADONA (65.4–446.4%), 5:3 FTCA (77.5–302.5%), and 7:3 FTCA (63.9–301.7%). For WWJ this list includes PFBS (71.7–599%), 4:2 FTS (64.2–1237.5%), NMeFOSAA (85.5–396.1), and NEtFOSAA (88.8–368.5%).

Table 6-3. Pooled Laboratory PFAS-Spiked Wastewater Samples Results. Low-spiked, high-spiked, and combined low/high spiked samples.

Analyte	Number of Labs	Low-spiked Samples					High-spiked Samples					Combined Low/High Spiked Samples				
		Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)
PFBA	8	134	98.8	10.8	9.0	9.1	134	99.2	9.23	8.3	8.4	268	99	9.79	8.85	8.94
PFPeA	8	130	101	9.58	16.0	15.9	144	96.5	10.9	9.0	9.4	274	98.5	8.7	13.9	14.1
PFHxA	8	60	102	9.82	16.0	15.7	144	94.7	6.79	9.8	10.3	204	96.8	4.66	13.2	13.6
PFHpA	8	119	93.6	7.69	11.5	12.3	144	93.0	7.82	8.8	9.5	263	93.3	7.25	10.3	11.1
PFOA	8	143	98.2	11.5	13.3	13.5	144	98.5	9.77	10.2	10.4	287	98.3	10.3	12	12.2
PFNA	8	143	95.8	10.1	10.2	10.7	144	94.9	9.4	10.7	11.3	287	95.4	9.47	10.6	11.1
PFDA	8	143	96.4	9.76	10.3	10.7	144	94.6	9.54	10.1	10.7	287	95.5	9.34	10.4	10.9
PFUnA	8	143	92.2	8.32	11.7	12.7	144	91.1	7.27	11.3	12.4	287	91.7	7.14	11.7	12.8
PFDoA	8	141	86.7	7.87	13.6	15.7	143	86.4	9.45	12.6	14.6	284	86.5	8.45	13.1	15.1
PFTrDA	8	140	78.8	12.4	17.4	22.1	143	78.5	11.5	16.9	21.5	283	78.7	11.5	17.2	21.9
PFTeDA	8	134	96.0	13.6	17.8	18.5	140	98.0	18.7	18.3	18.7	274	97	15.9	18.2	18.7
PFBS	8	143	99.0	9.81	14.8	15.0	144	99.1	8.26	44.0	44.4	287	99	8.62	32.5	32.8
PFPeS	8	143	96.6	6.59	10.0	10.4	144	94.8	8.72	9.5	10.0	287	95.7	7.37	9.96	10.4
PFHxS	8	140	94.6	8.82	12.8	13.5	144	94.0	8.40	9.2	9.8	284	94.3	8.24	11.3	12
PFHpS	8	143	102	8.67	13.1	12.9	144	99.3	9.66	13.7	13.8	287	100	8.95	13.5	13.4
PFOS	8	120	96.4	7.74	7.9	8.2	144	95.1	8.59	10.0	10.5	264	95.7	8.05	9.13	9.54
PFNS	8	143	81.6	9.53	11.7	14.4	144	83.8	7.19	11.6	13.8	287	82.7	8.11	11.8	14.2
PFDS	8	143	68.5	12.3	18.0	26.3	144	71.3	10.1	17.4	24.5	287	69.9	10.7	17.8	25.5
PFDoS	8	138	52.2	16.9	16.4	31.4	144	52.6	15.5	17.0	32.3	282	52.4	16	16.6	31.6
4:2FTS	8	143	95.8	7.68	9.6	10.0	144	105	27.7	95.1	90.8	287	100	14.3	68.2	68
6:2FTS	8	120	101	7.69	13.5	13.4	123	99.2	7.46	14.8	15.0	243	100	7.46	14	14
8:2FTS	8	143	104	11.9	11.2	10.8	144	105	9.80	11.6	11.1	287	104	10.7	11.5	11
PFOSA	8	138	114	38.7	89.0	78.2	140	99.1	9.91	16.6	16.7	278	106	23.3	65.3	61.4
NMeFOSA	8	138	87.9	5.63	15.3	17.4	141	88.0	7.47	16.3	18.6	279	88	6.34	15.7	17.9
NEtFOSA	8	136	85.6	7.95	15.0	17.5	140	87.5	9.25	16.6	19.0	276	86.6	8.46	15.7	18.2
NMeFOSAA	8	131	145	32.2	52.7	36.4	132	111	16.4	16.3	14.7	263	128	23.6	43	33.6
NEtFOSAA	8	140	140	25.7	55.3	39.5	143	108	9.95	19.6	18.2	283	124	17.3	44.6	36
NMeFOSE	8	134	81.5	4.90	15.3	18.8	141	84.3	7.96	13.1	15.5	275	82.9	6.49	14.2	17.1
NEtFOSE	8	131	80.6	9.74	15.5	19.2	137	83.5	9.15	13.4	16.1	268	82.1	9.06	14.3	17.4
PFMPA	8	142	81.3	16.1	20.3	25.0	144	79.9	15.4	21.4	26.8	286	80.6	15.6	20.7	25.7
PFMBA	8	142	104	18.0	12.5	12.0	144	104	18.3	14.0	13.5	286	104	17.9	13.4	12.9
NFDHA	8	143	91.6	18.4	16.6	18.1	144	88.4	10.1	17.6	19.9	287	90	14	17.6	19.6
HFPO-DA	8	143	105	20.5	16.8	16.1	144	101	15.9	12.0	11.8	287	103	18.1	14.8	14.4
ADONA	8	143	110	24.6	32.6	29.6	144	105	21.4	14.0	13.3	287	108	22.6	25.2	23.4

Table 6-3. Pooled Laboratory PFAS-Spiked Wastewater Samples Results. Low-spiked, high-spiked, and combined low/high spiked samples.

Analyte	Number of Labs	Low-spiked Samples					High-spiked Samples					Combined Low/High Spiked Samples				
		Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)
PFEESA	8	143	94.5	10.4	11.7	12.3	144	91.5	7.39	10.2	11.1	287	93	8.37	11.4	12.3
9Cl-PF3ONS	8	143	100	22.0	34.2	34.1	144	99.2	17.9	19.5	19.6	287	99.8	19.2	28	28
11Cl-PF3OUdS	8	141	72.2	16.9	30.2	41.8	144	72.3	14.1	22.9	31.7	285	72.3	14.8	26.8	37.1
3:3FTCA	8	142	89.5	11.7	19.3	21.6	144	91.6	10.6	21.3	23.2	286	90.5	10.9	20.2	22.3
5:3FTCA	8	143	101	10.2	27.1	27.0	144	96.9	11.3	14.3	14.7	287	98.7	9.99	21.8	22.1
7:3FTCA	8	143	99.3	15.2	34.3	34.6	144	98.4	10	22.7	23.1	287	98.8	11.4	29.2	29.6

File: Matrix_compiled_results_V4_230406_211329.csv

Notes:

Number of Labs - The number of laboratories reporting matrix spiked sample results.

Number of Results - The total number of matrix sample results categorized as low spike concentration (indicated in Row 1) that do not have a U flag.

Mean % Recovery - The mean percent recovery for spiked samples across all laboratories.

s_b - The pooled between-laboratory standard deviation of the percent recovery for spiked samples (low, high, or combined as applicable). Equation from EPA 821-B-18-001 page G-25.

s_w - The pooled within-laboratory standard deviation of the percent recovery for spiked samples (low, high, or Combined as applicable). Equation from EPA 821-B-18-001 page G-25.

RSD - The pooled within-laboratory relative standard deviation for spiked samples (RSD = s_w / (mean % recovery) *100).

Table 6-4. PFAS-Spiked Wastewater Samples Results By Individual Wastewater Sample

Analyte	Number of Labs	WWI - *Hospital*			WWJ - *POTW Influent*			WWL - *Bus Washing Station*			WWM - *POTW Effluent*			WWN - *Pulp and Paper Effluent*			WWO - *POTW Effluent*		
		Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery
PFBA	8	42	99.2	51.4–131.4	48	99.3	77.2–114.7	48	101.0	80.8–125	48	99.8	74.6–112.7	35	99.7	71.4–132.5	47	94.7	22.5–113.2
PFPeA	8	47	98.7	74.5–139.9	48	100.0	71–123.5	36	96.6	64.5–120.5	48	99.3	78.0–134.0	48	104.0	73.5–195.0	47	91.9	20.5–114.2
PFHxA	8	48	99.0	74–149	36	97.3	77–121	24	94.6	67.5–115.4	24	93.8	74.7–110.0	48	100.0	77.5–170.5	24	90.1	32.2–113.1
PFHpA	8	48	95.6	75.1–129	48	94.9	74.6–114.8	24	89.4	65.4–106.5	48	92.4	67.7–104.5	48	95.9	72.5–149.5	47	89.4	21.1–115.7
PFOA	8	48	99.1	58.6–127.8	48	99.7	71.5–136	48	97.4	68.8–138.5	48	99.8	68.5–138.0	48	97.9	63.0–152.5	47	96.3	21.6–127.5
PFNA	8	48	97.3	70.5–141.5	48	96.0	70.5–121.4	48	94.4	70.4–130.5	48	97.8	70.0–141.2	48	95.7	70.0–139.5	47	90.9	19.5–113.2
PFDA	8	48	95.7	59.7–122	48	96.2	77–129.1	48	96.3	61.5–117	48	96.5	62.5–137.2	48	95.9	70.5–169.5	47	92.2	22.7–113.3
PFUnA	8	48	89.5	55.8–135	48	92.9	65.2–128	48	93.5	64.1–120.3	48	91.0	69.0–123.5	48	94.0	69.0–148.0	47	89.1	27.0–124.0
PFDoA	8	48	84.8	54.6–112	48	89.5	71.2–120	48	87.8	69.1–113	46	85.9	60.9–103.0	48	92.2	64.3–131.0	46	78.7	28.5–140.0
PFTrDA	8	48	73.2	32.6–102.5	47	82.5	26–124.5	48	78.0	23.8–113	46	81.1	51.0–105.5	48	86.0	57.0–144.0	46	71.0	15.6–144.0
PFTeDA	8	45	104.0	59–184	45	97.7	61.3–171	46	100.0	62–180.5	46	97.3	60.0–127.0	47	101.0	65.5–132.5	45	81.8	18.0–157.0
PFBS	8	48	95.1	75.4–129.2	48	112.0	71.7–599	48	101.0	74.3–137.5	48	99.4	74.5–132.0	48	95.8	69.8–144.5	47	91.4	18.5–121.0
PFPeS	8	48	94.7	59.9–129.6	48	97.4	61.1–122	48	92.4	60.2–106	48	99.3	68.3–123.8	48	97.0	74.9–147.0	47	93.2	22.4–116.3
PFHxS	8	45	96.7	48.8–140.3	48	95.0	71–112.5	48	95.9	72.5–118.9	48	94.5	73.3–112.4	48	92.9	74.1–139.6	47	90.9	19.4–130.8
PFHpS	8	48	102.0	70.1–154	48	103.0	86–156	48	98.9	64–137	48	104.0	81.0–173.0	48	98.8	74.4–139.5	47	96.9	22.3–143.3
PFOS	8	48	95.3	67.9–121.5	48	97.1	67.7–122.4	48	94.6	70.9–112.6	48	96.7	70.6–134.0	48	95.5	68.1–143.7	24	94.3	25.3–115.7
PFNS	8	48	75.9	54.7–98.6	48	83.1	44.5–105.9	48	83.9	51.5–109.9	48	83.1	31.8–110.0	48	91.7	61.4–133.7	47	78.3	19.4–101.0
PFDS	8	48	54.3	19.9–92.2	48	69.3	14.7–112	48	73.6	17–114.6	48	71.9	8.8–96.0	48	85.9	39.4–125.3	47	64.2	17.3–96.4
PFDoS	8	47	34.9	8.5–67.7	47	54.0	3–106.5	47	63.9	1.9–113.1	46	52.8	27.0–79.4	48	59.0	11.7–87.6	47	49.7	5.3–96.9
4:2FTS	8	48	98.8	63.6–197.1	48	120.0	64.2 – 1237	48	93.8	51.2–125.3	48	96.4	81.8–112.9	48	98.6	75.2–150.4	47	93.7	34–140.4
6:2FTS	8	48	98.8	45.3–189.9	48	101.0	62.8–125.7	48	98.7	57.3–143.8	48	104.0	62.2–152.8	48	97.6	46.7–127.5	3	99.8	96.7–104.6
8:2FTS	8	48	101.0	67.5–136.8	48	104.0	60.1–149.3	48	107.0	75.3–140.5	48	108.0	85.8–151.8	48	103.0	62.6–132.5	47	101.0	47.9–130.0
PFOSA	8	48	112.0	77.3–183.5	48	92.3	73.5–115.5	42	98.5	68–115	46	102.0	77.7–190.0	48	145.0	78.8–770	46	86.9	25.8–116.0
NMeFOSA	8	48	84.1	50.5–108.5	47	86.4	49.8–106	46	91.7	74.2–122	46	86.4	68.1–106.0	47	93.3	73.5–122.5	45	85.9	32.8–212.0
NEtFOSA	8	46	83.9	59.6–106	47	87.1	57.5–108.5	45	92.1	70.9–124	46	82.8	69.0–96.7	47	91.2	75.5–125.0	45	82.4	23.4–193.0
NMeFOSAA	8	48	147.0	78.1–535	48	132.0	85.5–396.1	42	111.0	76.4–166	41	132.0	86.3–181.1	42	152.0	97.6–213.0	42	91.3	52.0–119.0
NEtFOSAA	8	48	122.0	74–400	47	135.0	88.8–368.5	48	108.0	79.6–150.5	46	122.0	97.8–177.0	48	165.0	100.0–381.5	46	89.9	30.6–124.0
NMeFOSE	8	45	78.9	46.3–103.2	46	84.1	54.7–105.5	46	93.1	73.1–113.1	46	81.3	65.8–114.2	47	76.0	47.1–95	45	84.2	34.2–134.8
NEtFOSE	8	42	82.5	54.2–103.8	45	80.4	53.1–103	45	91.3	65.8–110	46	79.4	63.7–100.0	46	77.7	55.2–107.2	44	81.4	21.9–136.5
PFMPA	8	47	70.8	20.2–116.8	48	95.8	60–118	48	86.4	28.7–112.8	48	91.2	56.5–108.5	48	57.2	15.2–102.0	47	81.8	20.9–107.0
PFMBA	8	47	107.0	71.5–138	48	100.0	64–139.5	48	103.0	48.8–175	48	101.0	63.5–131.0	48	116.0	59.5–156.0	47	96.7	24.5–133.0
NFDHA	8	48	89.1	25.8–155	48	89.3	54.5–151.8	48	79.6	37.5–152.5	48	95.4	63.0–155.0	48	96.3	71.8–133.8	47	90.2	19.6–131.2
HFPO-DA	8	48	108.0	76.2–227.5	48	104.0	75.4–152.5	48	110.0	73.0–228.8	48	99.8	70.0–124.3	48	100.0	77.9–128.8	47	96.3	35.6–132.5
ADONA	8	48	118.0	65.4–446.4	48	104.0	63.1–152.1	48	117.0	53.4–228.2	48	101.0	64.2–127.2	48	108.0	59.2–145.9	47	97.6	35.1–132.2
PFEESA	8	48	94.8	60.3–125.2	48	92.1	64.3–119	48	89.6	44.8–114.5	48	92.4	76.8–114.7	48	97.0	78.5–159.6	47	91.9	21.4–113.7
9Cl-PF3ONS	8	48	107.0	61–447.1	48	96.1	28.5–137.5	48	107.0	35.5–145.7	48	89.6	9.9–120.8	48	109.0	44.0–168.3	47	89.9	21.1–115.6

Table 6-4. PFAS-Spiked Wastewater Samples Results By Individual Wastewater Sample

Analyte	Number of Labs	WWI - *Hospital*			WWJ - *POTW Influent*			WWL - *Bus Washing Station*			WWM - *POTW Effluent*			WWN - *Pulp and Paper Effluent*			WWO - *POTW Effluent*		
		Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery
11Cl-PF3OUdS	8	48	59.5	9.3–313	48	69.2	5.0–105.8	48	80.7	3.7–127.2	46	69.0	38–103.8	48	93.7	21.3–141.2	47	61.1	5.7–105.4
3:3FTCA	8	47	93.5	64.5–119.4	48	92.9	68.0–114.9	48	106.0	58.8–173.2	48	93.7	70.8–116	48	69.9	35.2–116.0	47	87.7	20.6–109.5
5:3FTCA	8	48	107.0	77.5–302.5	48	90.5	64.0–115.8	48	108.0	63–165	48	94.7	66.2–115	48	98.6	77.9–253.3	47	94.2	6.3–133.8
7:3FTCA	8	48	103.0	63.9–301.7	48	84.8	24.9–115.8	48	113.0	66.6–178.3	48	92.9	24.6–128.3	48	98.0	54.0–257.5	47	101.0	6.6–255.8

File: Matrix_sample_results_V4_230406_211329.csv

Notes:

Number of Labs - The number of laboratories reporting matrix spiked sample results.

Number of Results - The total number of results for the WWI2-7 samples (indicated in Row 1) that do not have a U flag.

Mean % Recovery - The mean percent recovery for WWI2-7 samples across all laboratories.

Range % Recovery - The minimum to maximum percent recovery for samples across a sample set (e.g., WWI2 through WWI7) across all laboratories.

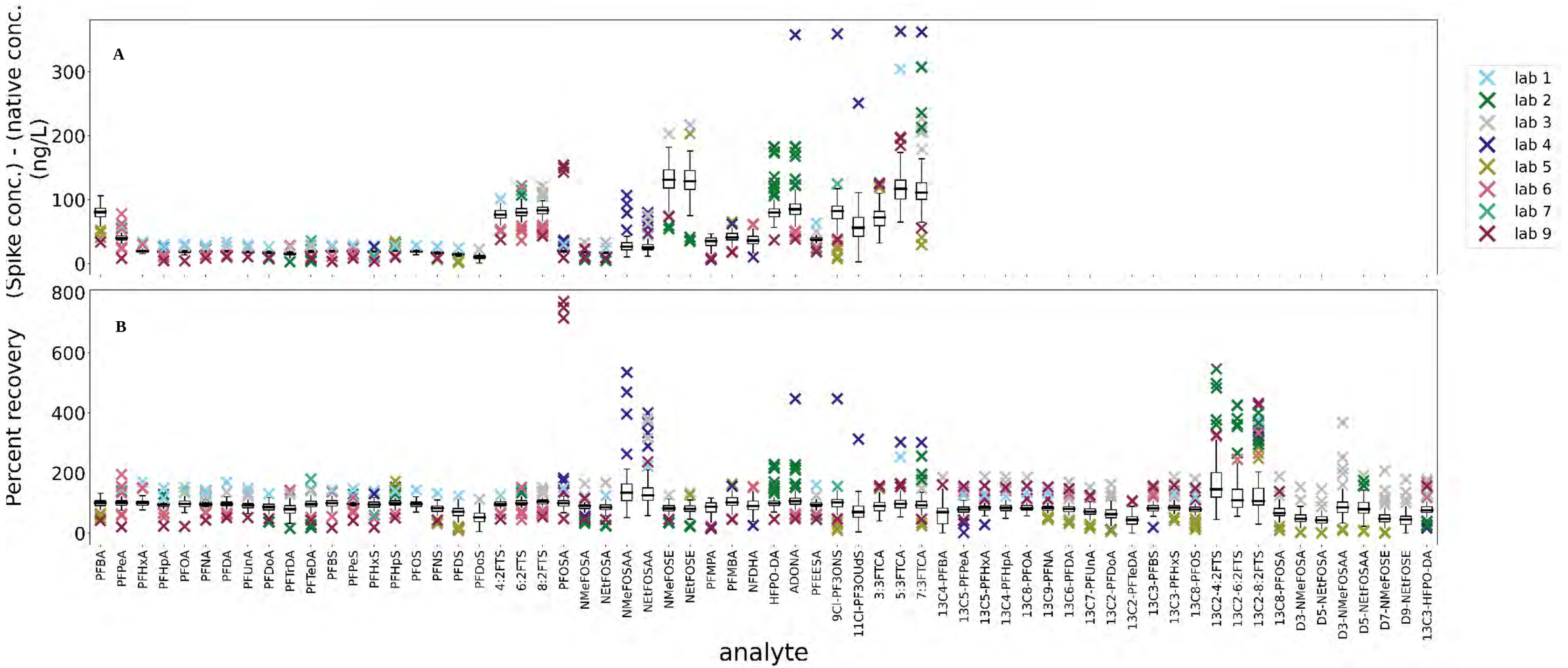


Figure 6-1. Wastewater low spike results by analyte by laboratory.

File: WW_DBexporet_V7_20230328.vsv

(A) Spiked concentration minus the laboratory-reported native concentration. (B) Calculated percent recovery.

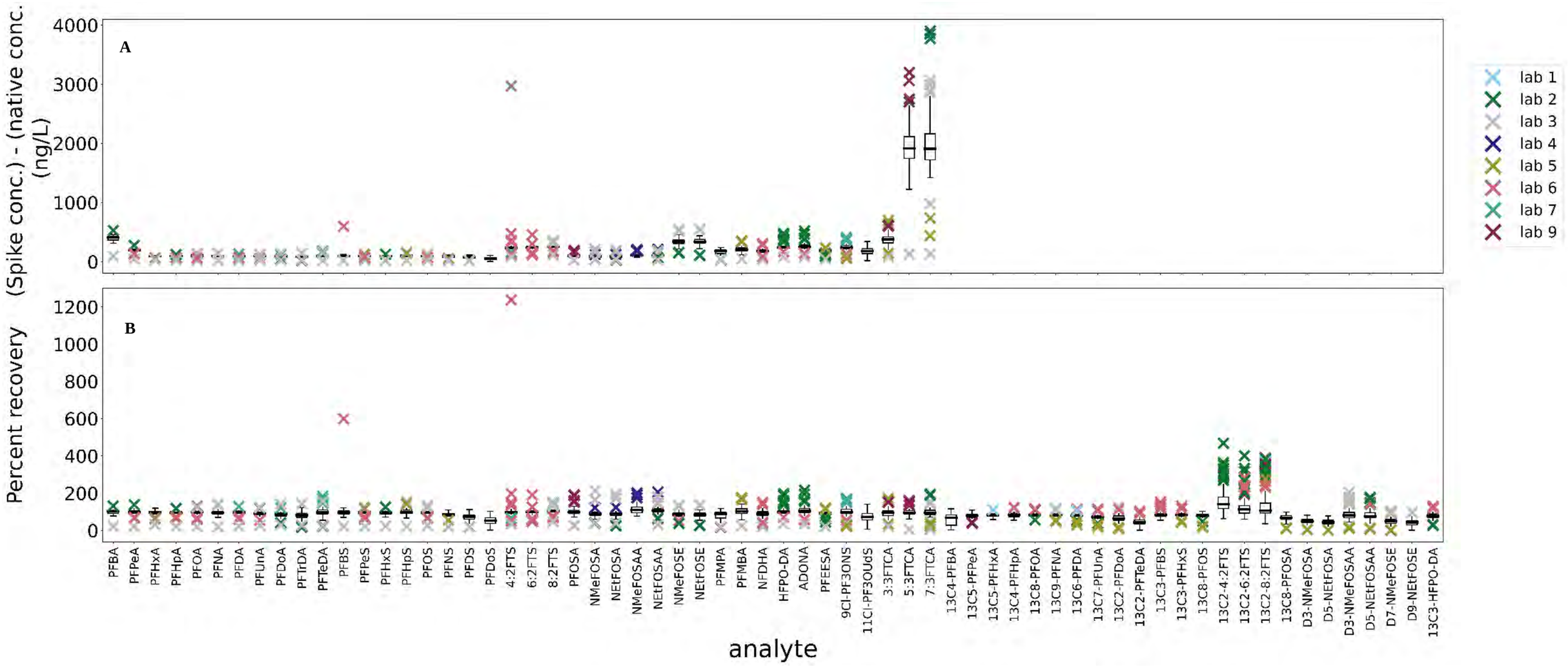


Figure 6-2. Wastewater high spike results by analyte by laboratory.

File: WW_DBexporet_V7_20230328.vsv

Spiked concentration minus the laboratory-reported native concentration. (B) Calculated percent recovery.
Figure includes both target compound recoveries, and EIS compound recoveries.

Note: The high spike mass results for 5:3 FTCA and 7:3 FTCA appear higher as these were spiked at 2,000 ng/L, as opposed 100 ng/L for the remaining PFAS

6.3 WASTEWATER EXTRACTED INTERNAL STANDARD RESULTS

Per EPA Method 1633, EIS compounds were spiked into each sample prior to preparation. The amount of each EIS compound added to each sample varied slightly, depending on the target analyte and laboratory. The range of the EIS compound concentrations used by the laboratories is presented in Table 6-5. Since concentration levels between laboratories are not significantly different from one another, any interlaboratory variability observed in their recoveries cannot be attributed to concentration differences.

Table 6-5. Range of Concentration of EIS Compounds Used by All Laboratories

EIS Compound	Minimum Concentration (ng/L)	Maximum Concentration (ng/L)
$^{13}C_4$-PFBA	80	100
$^{13}C_5$-PFPeA	40	50
$^{13}C_5$-PFHxA	20	25
$^{13}C_4$-PFHpA	20	25
$^{13}C_8$-PFOA	20	25
$^{13}C_9$-PFNA	10	12.5
$^{13}C_6$-PFDA	10	12.5
$^{13}C_7$-PFUnA	10	12.5
$^{13}C_2$-PFDoA	10	12.5
$^{13}C_2$-PFTeDA	10	12.5
$^{13}C_3$-PFBS	18.6	25
$^{13}C_3$-PFHxS	19	25
$^{13}C_8$-PFOS	19.2	25
$^{13}C_2$-4:2FTS	37.5	50
$^{13}C_2$-6:2FTS	38	50
$^{13}C_2$-8:2FTS	38.4	50
$^{13}C_8$-PFOSA	20	25
D_3-NMeFOSA	20	25
D_5-NEtFOSA	20	25
D_3-NMeFOSAA	40	50
D_5-NEtFOSAA	40	50
D_7-NMeFOSE	200	250
D_9-NEtFOSE	200	250
$^{13}C_3$-HFPO-DA	80	100
$^{13}C_4$-PFBA	80	100

Source file: Chapter 6 WW Tables 04252023

The MLVS Method did not prescribe definitive acceptance criteria for EIS compound recoveries; however, it did provide target acceptance criteria. The target percent recovery for EIS compounds was 20–150%. These target criteria were based on the results from the SLVS. Since the statistical evaluation from the MLVS will be the basis for the acceptance criteria included in future versions of EPA Method 1633, each laboratory was instructed to follow their routine corrective action process when the target criteria were not met. This includes reanalysis and dilution. If the reanalysis or dilution met the target criteria, the reanalysis was reported, otherwise, the first analysis was reported. By doing so, results that were extremely biased due to events such as a mis-injection or carryover, were eliminated from the statistical analysis.

The combined results for the minimum, maximum, and average percent recovery are given in Table 6-6. Supporting individual laboratory results may be found in Appendix E, Table E-5. The average recovery for each EIS compound over all laboratories fell between 20–150%, except for $^{13}C_2$-4:2FTS. The range of recoveries by individual laboratories was considerable, ranging from 0.1–599%. Figures 6-1 and 6-2 show that greatest variability in EIS compound recoveries for all laboratories were for the three FTS EIS compounds: $^{13}C_2$-4:2FTS, $^{13}C_2$-6:2FTS, and $^{13}C_2$-8:2FTS. While all EIS compound data were retained to evaluate the EIS performance, the only target analyte data retained for statistical evaluation is where the associated EIS compounds was ≥ 10%.

The pooled-laboratory statistical analysis results for EIS compounds are given in Table 6-7 and shown on Figures 6-1 and 6-2. The lowest mean percent recovery was for $^{13}C_2$-PFTeDA (44%) and the highest was for $^{13}C_2$-8:2FTS (144%). The greatest level of variation between and within laboratories was for the trio of C_2-labelled FTS compounds: $^{13}C_2$-4:2, $^{13}C_2$-6:2, and $^{13}C_2$-8:2 FTS.

6.4 WASTEWATER SUMMARY

The MLVS results demonstrate the efficacy of EPA Method 1633 to accurately report PFAS concentrations in real-world wastewater samples. The pooled (low spike/high spiked samples) average percent recoveries as shown in Table 6-8 were between 70–130% with one exception of PFDoS (52.4%). As noted above, for the low-spiked samples, for some laboratories and for some specific wastewaters, there was considerable variation for PFBS, NMeFOSAA, NEtFOSAA ADONA, 4:2 FTS, 5:3FTCA, and 7:3FTCA.

Tables 6-8 and 6-9 provides a summary of the relative proportions for all laboratories that fell between the study target analyte target percent recovery acceptance criteria used to evaluate the OPR and LLOPR (40–150%). For the low- and high-spiked samples, the proportion of all values that were between 40–150% of the spiked concentrations is >70% for all target analytes, with the exception of NMeFOSAA (low spike, 62%). For the low- and high-spiked samples, the proportion of all values that were between 70–130% of the spiked concentrations is >70% for most analytes. The exceptions to this were PFDS, PFDoS, NMeFOSAA, NEtFOSAA, and 11Cl-PF3OUdS.

Table 6-10 provides a summary of the relative proportions for all laboratories that fell between the study EIS compound target percent recovery acceptance criteria. For the low- and high-spiked samples, the proportion of all values that were between 20–150% of the spiked concentrations is >70% for all target analytes, with the exception of $^{13}C_2$-4:2FTS (55%). Data from Laboratory 5 accounted for all of the 4:2FTS exceedances. The proportion of all values that were between 20–

150% of the spiked concentrations is >90% for all EIS compounds, with the exceptions of $^{13}C_4$-PFBA (82.4%), $^{13}C_2$-4:2FTS (54.8%), $^{13}C_2$-6:2FTS (78.6%), $^{13}C_2$-8:2FTS (74.7%), and D_9-NEtFOSA (86.9%).

Table 6-6. Summary of EIS Compound percent recovery in wastewater for all laboratories

EIS Compound	n	% Recovery		
		Min	Max	Mean
$^{13}C_4$-PFBA	336	1.0	291	59.1
$^{13}C_5$-PFPeA	336	2.1	244	79.1
$^{13}C_5$-PFHxA	336	28.3	306	84.9
$^{13}C_4$-PFHpA	336	31.1	350	86.1
$^{13}C_8$-PFOA	337	25.9	268	85.2
$^{13}C_9$-PFNA	336	12.2	266	83.9
$^{13}C_6$-PFDA	336	2.7	302	80.9
$^{13}C_7$-PFUnA	336	0.6	278	72.5
$^{13}C_2$-PFDoA	336	0.6	282	65.9
$^{13}C_2$-PFTeDA	336	0.1	130	44.0
$^{13}C_3$-PFBS	336	19.0	273	85.7
$^{13}C_3$-PFHxS	336	11.8	337	85.8
$^{13}C_8$-PFOS	336	0.3	288	78.2
$^{13}C_2$-4:2FTS	336	45.7	550	167.1
$^{13}C_2$-6:2FTS	336	18.6	426	126.3
$^{13}C_2$-8:2FTS	336	2.5	441	143.7
$^{13}C_8$-PFOSA	336	0.1	282	69.5
D_3-NMeFOSA	336	1.4	225	49.7
D_5-NEtFOSA	336	0.9	220	45.0
D_3-NMeFOSAA	336	1.1	599	89.6
D_5-NEtFOSAA	336	0.8	279	84.6
D_7-NMeFOSE	336	0.1	299	50.4
D_9-NEtFOSE	336	0.2	289	44.7
$^{13}C_3$-HFPO-DA	336	17.7	305	79.7

Source File: Chapter 6 WW Tables 04252023

Notes:
Includes all laboratories except Laboratories 8 and 10.
n = number of results for unspiked and matrix spiked samples only (excludes QA/QC samples).

Table 6-7. Statistical Evaluation of EIS Compound Results Associated with Wastewater Samples

EIS Compound	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)
$^{13}C_4$-PFBA	8	336	59.1	17.3	30.3	51.3
$^{13}C_5$-PFPeA	8	336	79.1	8.74	18.2	23
$^{13}C_5$-PFHxA	8	336	84.8	8	17.2	20.3
$^{13}C_4$-PFHpA	8	336	86.1	12.5	18.6	21.6
$^{13}C_8$-PFOA	8	337	85.2	6.64	16.2	19
$^{13}C_9$-PFNA	8	336	83.9	6.9	15.9	18.9
$^{13}C_6$-PFDA	8	336	80.9	8.51	17.9	22.2
$^{13}C_7$-PFUnA	8	336	72.5	8.26	18.9	26
$^{13}C_2$-PFDoA	8	336	65.9	13.2	21.9	33.2
$^{13}C_2$-PFTeDA	8	336	44	11.6	16.7	37.9
$^{13}C_3$-PFBS	8	336	85.7	13.3	16.4	19.2
$^{13}C_3$-PFHxS	8	336	85.7	10.2	18.9	22.1
$^{13}C_8$-PFOS	8	336	78.1	10.6	17.3	22.1
$^{13}C_2$-4:2FTS	8	336	167	64.8	50.1	30
$^{13}C_2$-6:2FTS	8	336	126	38.2	48	38
$^{13}C_2$-8:2FTS	8	336	144	38	83.4	58
$^{13}C_8$-PFOSA	8	336	69.5	13.5	18.1	26.1
D_3-NMeFOSA	8	336	49.7	12.1	16.5	33.2
D_5-NEtFOSA	8	336	45	13	16.3	36.2
D_3-NMeFOSAA	8	336	89.6	25.6	37.6	42
D_5-NEtFOSAA	8	336	84.6	19.5	25.9	30.6
D_7-NMeFOSE	8	336	50.4	17.8	21.8	43.1
D_9-NEtFOSE	8	336	44.7	17.5	20.7	46.3
$^{13}C_3$-HFPO-DA	8	336	79.7	18.4	18.6	23.4

Source File: Matrix_EIS_results_V4_230406_212819.csv

Notes:
Number of Labs - The number of laboratories reporting matrix (native & spiked) results.
Number of Results - The total number of matrix results that do not have a U flag.
Mean % Recovery - The mean percent recovery across all of the EIS compound individual samples across all laboratories for the given analyte.
s_b - The pooled between-laboratory standard deviation. Equation from EPA 821-B-18-001page G-25.
s_w - The pooled within-laboratory standard deviation. Equation from EPA 821-B-18-001page G-25.
RSD - The pooled within-laboratory relative standard deviation (RSD, (s_w / (mean % recovery) *100).

Table 6-8. Proportion of wastewater matrix spike %recovery results for target analytes within ranges (low-spiked samples).

Analyte	Low-Spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBA	134	0	3.0	96.3	0.7	0	0
PFPeA	130	0.8	2.3	93.1	2.3	1.5	0
PFHxA	60	0	0	95.0	1.7	3.3	0
PFHpA	119	0.8	2.5	95.8	0.8	0	0
PFOA	143	0.7	1.4	93.7	3.5	0.7	0
PFNA	143	0	1.4	95.8	2.8	0	0
PFDA	143	0	2.1	96.5	0.7	0.7	0
PFUnA	143	0	3.5	95.1	1.4	0	0
PFDoA	141	2.1	6.4	90.8	0.7	0	0
PFTrDA	140	3.6	25.0	70.0	1.4	0	0
PFTeDA	134	2.2	5.2	88.1	3.7	0.7	0
PFBS	143	0.7	2.1	91.6	5.6	0	0
PFPeS	143	0	2.1	97.2	0.7	0	0
PFHxS	140	0.7	3.6	93.6	2.1	0	0
PFHpS	143	0	1.4	94.4	2.8	1.4	0
PFOS	120	0	0.8	98.3	0.8	0	0
PFNS	143	1.4	18.9	79.0	0.7	0	0
PFDS	143	10.5	35.7	53.8	0	0	0
PFDoS	138	28.3	50.7	21.0	0	0	0
4:2FTS	143	0	4.2	95.8	0	0	0
6:2FTS	120	0	2.5	93.3	3.3	0.8	0
8:2FTS	143	0	4.9	89.5	4.9	0.7	0
PFOSA	138	0	1.4	92.0	2.2	2.2	2.2
NMeFOSA	138	1.4	7.2	90.6	0	0.7	0
NEtFOSA	136	2.2	7.4	89.7	0	0.7	0
NMeFOSAA	131	0	1.5	42.0	18.3	31.3	6.9
NEtFOSAA	140	0	2.1	55.0	16.4	16.4	10
NMeFOSE	134	2.2	14.2	83.6	0	0	0
NEtFOSE	131	2.3	16.8	80.2	0.8	0	0
PFMPA	142	10.6	13.4	76.1	0	0	0
PFMBA	142	0	6.3	83.8	7.7	2.1	0
NFDHA	143	1.4	13.3	79.7	2.8	2.8	0
HFPO-DA	143	0	0.7	92.3	2.1	2.1	2.8

Table 6-8. Proportion of wastewater matrix spike %recovery results for target analytes within ranges (low-spiked samples).

Analyte	Low-Spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
ADONA	143	0	7.0	81.8	5.6	2.1	3.5
PFEESA	143	0	7.0	92.3	0	0.7	0
9Cl-PF3ONS	143	2.8	12.6	73.4	9.8	0.7	0.7
11Cl-PF3OUdS	141	13.5	35.5	48.9	1.4	0	0.7
3:3FTCA	142	0	18.3	76.8	2.8	2.1	0
5:3FTCA	143	0	3.5	88.8	4.2	2.1	1.4
7:3FTCA	143	2.1	7.0	82.5	2.1	4.2	2.1

Data Source: Exa WW_TRG_PCT_REC_summary_20230510.xlsx

Table 6-9. Proportion of wastewater matrix spike %recovery results for target analytes within ranges (high-spiked samples).

Analyte	High-Spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBA	134	0.7	0	98.5	0.7	0	0
PFPeA	144	0.7	2.1	96.5	0.7	0	0
PFHxA	144	0.7	1.4	97.9	0	0	0
PFHpA	144	0.7	2.1	97.2	0	0	0
PFOA	144	0.7	2.1	96.5	0.7	0	0
PFNA	144	0.7	0	97.9	1.4	0	0
PFDA	144	0.7	3.5	95.8	0	0	0
PFUnA	144	0.7	2.1	97.2	0	0	0
PFDoA	143	1.4	9.1	88.8	0.7	0	0
PFTrDA	143	4.9	23.1	71.3	0.7	0	0
PFTeDA	140	2.9	7.1	82.9	4.3	2.9	0
PFBS	144	0.7	0.7	97.9	0	0	0.7
PFPeS	144	0.7	4.9	94.4	0	0	0
PFHxS	144	0.7	0	99.3	0	0	0
PFHpS	144	0.7	0.7	93.1	4.9	0.7	0
PFOS	144	0.7	4.2	94.4	0.7	0	0
PFNS	144	0.7	13.2	86.1	0	0	0
PFDS	144	9.7	29.9	60.4	0	0	0
PFDoS	144	26.4	50.0	23.6	0	0	0
4:2FTS	144	0.7	2.1	93.8	1.4	1.4	0.7
6:2FTS	123	0	5.7	93.5	0	0.8	0
8:2FTS	144	0	1.4	92.4	6.2	0	0
PFOSA	140	0.7	0	95.0	1.4	2.9	0
NMeFOSA	141	2.1	3.5	92.9	0	0.7	0.7
NEtFOSA	140	2.9	7.1	88.6	0	1.4	0
NMeFOSAA	132	0	0	84.1	12.9	2.3	0.8
NEtFOSAA	143	0.7	2.8	87.4	4.9	3.5	0.7
NMeFOSE	141	2.1	10.6	86.5	0.7	0	0
NEtFOSE	137	2.2	12.4	83.9	1.5	0	0
PFMPA	144	14.6	11.1	74.3	0	0	0
PFMBA	144	0.7	6.9	81.9	8.3	2.1	0
NFDHA	144	2.8	10.4	85.4	0.7	0.7	0
HFPO-DA	144	0.7	0	93.1	2.8	3.5	0

Table 6-9. Proportion of wastewater matrix spike %recovery results for target analytes within ranges (high-spiked samples).

Analyte	High-Spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
ADONA	144	0.7	8.3	81.2	6.2	2.8	0.7
PFEESA	144	0.7	3.5	95.8	0	0	0
9Cl-PF3ONS	144	2.8	10.4	77.1	6.9	2.8	0
11Cl-PF3OUdS	144	10.4	35.4	53.5	0.7	0	0
3:3FTCA	144	1.4	16.0	77.8	2.1	2.8	0
5:3FTCA	144	0.7	1.4	94.4	2.1	1.4	0
7:3FTCA	144	2.1	0.7	88.9	5.6	2.8	0

Data Source: Exa WW_TRG_PCT_REC_summary_20230510.xlsx

Table 6-10. Proportion of wastewater matrix %recovery results for EIS compounds within ranges.

EIS Compound	All Laboratories Proportion % Recovery					
	n	<10%	≥10% to <20%	≥20% to <150%	≥150% to <200%	≥200%
$^{13}C_4$-PFBA	336	6.5	9.5	82.7	0.9	0.3
$^{13}C_5$-PFPeA	336	0.3	0	98.8	0.6	0.3
$^{13}C_5$-PFHxA	336	0	0	98.8	0.9	0.3
$^{13}C_4$-PFHpA	336	0	0	98.5	1.2	0.3
$^{13}C_8$-PFOA	337	0	0	98.8	0.9	0.3
$^{13}C_9$-PFNA	336	0	0.3	98.8	0.6	0.3
$^{13}C_6$-PFDA	336	0.3	0	99.1	0.3	0.3
$^{13}C_7$-PFUnA	336	0.3	0.6	98.5	0.3	0.3
$^{13}C_2$-PFDoA	336	1.2	1.8	96.4	0.3	0.3
$^{13}C_2$-PFTeDA	336	4.2	5.7	90.2	0	0
$^{13}C_3$-PFBS	336	0	0.3	98.2	1.2	0.3
$^{13}C_3$-PFHxS	336	0	0.3	98.5	0.9	0.3
$^{13}C_8$-PFOS	336	0.3	0.6	98.2	0.6	0.3
$^{13}C_2$-4:2FTS	336	0	0	54.8	23.2	22
$^{13}C_2$-6:2FTS	336	0	0.3	78.6	12.2	8.9
$^{13}C_2$-8:2FTS	336	0.3	0	74.7	8	17
$^{13}C_8$-PFOSA	336	1.5	0.6	97.3	0.3	0.3
D_3-NMeFOSA	336	2.7	2.1	94.6	0.3	0.3
D_5-NEtFOSA	336	3.6	2.7	93.5	0	0.3
D_3-NMeFOSAA	336	0.6	1.2	93.8	3.0	1.5
D_5-NEtFOSAA	336	1.5	0.6	94.9	2.7	0.3
D_7-NMeFOSE	336	4.5	3.3	91.7	0	0.6
D_9-NEtFOSE	336	6.5	6.0	86.9	0.3	0.3
$^{13}C_3$-HFPO-DA	336	0	0.3	97.9	1.5	0.3

Source File: Exa WW EIS_PCT_REC_summary_20230510.xls

7 SURFACE WATER

A total of 21 study samples were shipped to each participating laboratory, as described in Section 2 of this report. This included a single unspiked sample, triplicate low- and triplicate high-spiked samples, in two freshwater samples and one saltwater sample. All surface water samples were prepared and analyzed by EPA Method 1633. Due to errors in the EIS compound spiking that affected all of the surface water analyses, the data for Laboratory 8 were omitted from the statistical analyses. Data were reported and validated in accordance with the requirements of the Study Plan. The rules for use/omission of individual analyte results are presented in Section 3 of this report.

7.1 NATIVE PFAS CONCENTRATIONS IN SURFACE WATER

The background native concentrations first measured prior to setting the spiking concentrations were done by SGS AXYS and are listed in Table 2-3. The concentrations measured by the nine individual laboratories are given in Table 7-1, which also includes the original background concentration for comparison. The minimum and maximum results for each individual surface water sample is presented Appendix Table F-1.

Of the 40 PFAS analytes, only 11 were detected in the baseline samples, including PFOA by at least one laboratory. Of the three baseline samples, the marine water SG1 had the lowest number of PFAS analytes detected (Table 7-2). The results for all laboratories, including the reconnaissance survey, were in general agreement, and for the detected compounds agreed within a factor of 2. Some differences between the number of detected PFAS in the surface water samples were due in part to differences in the detection limits. For example, Laboratory 9 detected 22 PFAS, because it had lower detection limits (evidenced by "U" flagged values) than Laboratory 2, which detected only seven PFAS.

7.2 SURFACE WATER MATRIX SPIKE RESULTS

The compiled (all laboratory) PFAS-spiked surface water samples are given in Table 7-3, organized as low spiked samples, high spiked samples, and the combined low- and high-spiked sample results. Results are also shown in Figures 7-1 and 7-2. Supporting individual laboratory spike recovery data are given in Appendix F, Tables F-2 (low spike) and F-3 (high spike).

The trends in spiked results were similar to that observed for wastewater, with some exceptions. The low spike mean percent recovery for each target analyte fell between 60–182% (PFDoS and NEtFOSAA, respectively) (Table 7-3). For the high-spiked samples, the range was 66–120% (PFDoS and NEtFOSAA, respectively), with the combined low/high spiked data from 63–151% (PFDoS and NEtFOSAA, respectively). Variability, as indicated by the pooled between-laboratory standard deviation (s_b) and the pooled within-laboratory standard deviation (s_w) was greater in the low-spiked samples than in the high-spiked samples. This was particularly evident for PFOS, PFHxS, NMeFOSAA, and NEtFOSA (Figures 7-1 and 7-2). One would expect that the variability in background levels of PFAS in the original samples would have a greater effect on the low-spiked samples than the high-spiked samples.

The variability observed for NMeFOSAA and NEtFOSAA in the low-spiked samples were also observed in the high-spiked samples (Table 7-3), albeit the s_w and RSD of the high-spiked samples were approximately half that estimated for low-spiked samples. Much of the observed variability can be attributed to the results from Laboratory 10, with Laboratory 1 contributing (Figure 7-1 and 7-2). The figures, supported by the data in Appendix Table F-2 show that the percent recoveries for Laboratory 10 for these two PFAS approached 700% in the low spiked samples, and 300% in the high spiked samples.

The results observed in the low-spiked samples for PFOS and PFHxS from Laboratory 1 are anomalous (>1,000% recovery). The MLVS team recalculated the reported concentrations from the laboratory data package and went back to Laboratory 1 and inquired if an error in reporting had occurred. The Study Team calculations confirmed the measures, and Laboratory 1 was unable to identify any procedural or reporting errors. The data are thus reported "as is" here, and clearly contribute to the higher overall variability.

Comparison of the results for the two freshwater samples and the one marine sample is shown in Table 7-5. Generally, the mean % recoveries were similar for all compounds across all three samples. What is different is that in SWD1, the Lake Harsha freshwater sample, for some PFAS there was a high degree of variability compared to what was observed in the Burley Creek (SWF) or Sequim Bay saltwater (SWG) samples. For example, for SWD1, PFBA ranged from 0.9–117% recovery. PFPeA ranged from 4.4–124%, PFNA from 4.1–124%, and 7:3 FTCA from 6.8–115%. For the same compounds, the range of measured responses was much tighter (e.g., 75–120% for PFBA) for the other two surface water samples. The low-end reported values occurred across different laboratories. One possible explanation is that the baseline concentrations in the Harsha freshwater sample may have had higher concentrations of PFAS analytes, which caused greater variability. The elevated recoveries for NMeFOSAA and NEtFOSAA by Laboratory 10 noted above were in the SWD samples.

Table 7-1. Summary of Target Analytes Detected in Unspiked Surface Water Samples in ng/L.

Analyte	Number of Labs	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		Lab 9		Lab 10			SGS-AXYS Baseline
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual		
SWD1 - Lake Harsha OH Freshwater																					
PFBA	9	0.941	U	4.05	J	2.56	J	1.31	U	2.23	J	0.597	U	6.5	J	2.99	J	2.53	J		2.89
PFPeA	9	0.552	U	1.7	JI	1.12	J	0.306	U	1.26	J	0.563	U	1	J	1.1	IJ	0.549	U		1.26
PFHxA	9	1.65	J	2.02	J	1.04	JI	1.32	J	1.02	J	0.412	U	1.1	J	1.16		0.298	U		1.00
PFHpA	9	0.849	J	1.66	J	0.8	J	0.788	JI	0.845	J	0.173	U	0.66	J	0.785	J	1.02	J		0.81
PFOA	9	1.89	J	--	X	1.2	J	1.12	J	1.17	J	1.28	J	1	J	1.18		1.33	J		1.02
PFNA	9	0.657	U	--	X	1.28	J	0.332	J	0.792	U	0.25	U	0.61	U	0.331	J	0.565	J		0.49
PFBS	9	0.736	J	1.18	JI	1.36	J	0.94	J	0.348	U	0.177	U	0.78	J	0.879	J	1.22	JI		0.72
PFHxS	9	0.393	U	--	X	0.464	U	0.789	U	0.625	U	0.291	U	0.7	U	0.363	J	0.567	U		< 0.3678
PFOS	9	0.978	J	--	X	1.28	J	1.7	U	1.43	JI	0.96	J	0.54	U	0.977	J	0.415	U		0.98
6:2FTS	9	1.07	U	--	X	2.82	U	1.6	U	2.39	J	1.48	U	3.5	U	0.945	U	2.36	UJ		< 1.326
PFOSA	9	0.346	U	--	X	0.416	U	0.565	U	0.198	U	0.188	U	0.67	U	11.1		0.212	U		< 0.3678
SWF1 - Burley Creek WA Freshwater																					
PFBA	9	0.941	U	1.93	J	1.04	U	--	X	1.47	J	--	X	1.9	U	--	X	0.952	U		< 1.449
PFPeA	9	0.552	U	1.08	U	0.768	U	0.306	U	0.772	U	0.563	U	0.94	U	0.726	J	0.549	U		< 0.7246
PFHxA	9	0.454	U	1.45	U	0.8	J	0.768	J	0.604	J	0.412	U	0.67	J	0.704	J	0.298	U		0.72
PFHpA	9	0.501	U	1.06	U	0.4	J	0.382	J	0.76	U	0.173	U	0.44	U	0.415	J	0.519	U		0.40
PFOA	9	1.5	J	2.93	J	0.88	J	0.924	J	1.09	J	0.29	U	0.74	J	0.932	J	1.09	J		0.64
PFBS	9	0.801	J	1.08	U	1.04	JI	0.628	U	0.717	J	0.177	U	0.66	J	0.928	J	0.292	JI		0.71
PFPeS	9	0.425	J	1.31	U	0.272	U	0.502	JI	0.729	U	0.129	U	1.1	U	0.399	IJ	0.468	U		< 0.3641
PFHxS	9	0.835	J	1.43	U	1.04	J	0.968	J	0.679	J	0.784	J	0.79	J	0.912	J	0.824	J		0.84
PFOS	9	0.441	U	1.68	U	0.64	J	1.7	U	0.486	U	0.248	U	0.54	U	0.563	J	0.415	U		< 0.3623
PFOSA	9	0.346	U	0.724	U	0.416	U	0.565	U	0.198	U	0.188	U	0.67	U	12.3		0.212	U		< 0.3623
NEtFOSAA	9	0.554	U	2.26	U	0.88	J	0.61	U	0.531	U	0.571	U	1.3	U	0.283	U	0.693	U		< 0.3623
SWG1 - Sequim Bay WA Saltwater																					
PFHxA	9	0.454	U	1.45	U	0.472	U	0.455	U	0.509	J	0.412	U	0.39	U	0.493	J	0.298	U		< 0.3578
PFOA	9	0.367	U	1.78	U	0.696	U	0.651	U	0.427	U	0.29	U	0.46	U	0.189	J	0.634	U		< 0.3578
PFHxS	9	0.393	U	1.43	U	0.384	U	0.789	U	0.625	U	0.291	U	0.7	U	0.189	J	0.567	U		< 0.3578
PFOSA	9	0.346	U	0.724	U	0.432	U	0.565	U	0.198	U	0.188	U	0.67	U	13.6		0.212	U		< 0.3578
Total # Analytes Reported Across All samples		9		7		15		8		13		3		10		22		8			13

Table 7-2. Numbers of Detected Analytes in Unspiked Surface Water Sample.

Surface Water Sample	Total Number of Analytes Detected by at least One Laboratory
SWD1 - Lake Harsha OH Freshwater	11
SWF1 - Burley Creek WA Freshwater	11
SWG1 - Sequim Bay WA Saltwater	4

Table 7-3. Pooled Laboratory PFAS-Spiked Surface Water Samples Results. Low-spiked, high-spiked, and combined low/high spiked samples.

Analyte	Number of Labs	Low spiked Samples					High spiked Samples					Combined Low/High Spiked Samples				
		Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within- Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD of s_w
PFBA	9	69	92.1	11.7	14.6	15.8	65	98.2	6.53	4.7	4.8	134	95.1	8.24	12.2	12.8
PFPeA	9	81	94.8	9.24	10.4	11.0	81	97.3	6.07	5.3	5.5	162	96	7.15	8.7	9.06
PFHxA	9	81	98.5	12.1	31.8	32.3	81	96	4.72	7.1	7.3	162	97.2	6.87	23.2	23.8
PFHpA	9	81	93.8	9.96	6.6	7.1	81	97.5	7.57	4.5	4.6	162	95.6	8.55	6.21	6.5
PFOA	9	78	100	14.5	33.7	33.6	80	101	8.67	5.8	5.7	158	101	9.33	24.5	24.3
PFNA	9	81	94.8	10.9	12.5	13.2	80	98.1	4.76	7.5	7.7	161	96.5	7.16	11	11.4
PFDA	9	80	98.9	12.3	11.5	11.6	80	101	8.46	8.6	8.5	160	99.8	10.1	10.3	10.4
PFUnA	9	79	96.1	10.7	10.4	10.8	80	96	7.88	8.9	9.3	159	96	7.94	10.6	11
PFDoA	9	79	87.8	7.21	11.5	13.1	80	88.7	6.66	10.9	12.3	159	88.2	6.66	11	12.5
PFTrDA	9	78	84.1	8.9	11.0	13.1	80	85.3	8.88	8.9	10.4	158	84.7	8.49	10.1	11.9
PFTeDA	9	78	85.1	8.5	7.9	9.3	80	90.1	4.2	9.7	10.8	158	87.6	4.95	9.99	11.4
PFBS	9	81	92.8	10.3	10.5	11.4	81	95.7	8.27	6.3	6.6	162	94.3	8	9.74	10.3
PFPeS	9	81	95.5	10.6	10.7	11.2	80	98.4	5.61	5.8	5.9	161	96.9	6.44	10.1	10.4
PFHxS	9	81	110	**49.7**	**151.0**	**138.0**	80	94.9	3.86	5.1	5.3	161	102	25	107	104
PFHpS	9	77	105	21.1	22.6	21.5	80	105	11.3	11.5	10.9	157	105	15.8	18.4	17.5
PFOS	9	80	112	**37.9**	**112.0**	**100.0**	80	101	7.35	8.8	8.7	160	107	20	79.6	74.6
PFNS	9	79	84.9	6.57	11.4	13.4	80	90.8	6.99	6.8	7.5	159	87.9	4.32	10.8	12.3
PFDS	9	76	73.3	8.99	13.2	18.1	80	79.3	11.4	7.0	8.8	156	76.4	9.19	11.3	14.8
PFDoS	9	78	60.1	11.6	10.2	17.0	80	65.7	12.3	9.4	14.2	158	62.9	11.4	10.6	16.8
4:2FTS	9	80	92.5	6.59	9.5	10.2	81	95.9	10.2	10.4	10.8	161	94.3	8.08	10.2	10.8
6:2FTS	9	81	101	9.9	14.0	13.8	80	105	7.55	13.5	12.8	161	103	8.34	13.8	13.3
8:2FTS	9	80	108	13.8	13.3	12.4	80	106	8.27	8.4	7.9	160	107	10.4	11.7	11
PFOSA	9	80	104	14.4	19.5	18.7	80	101	8.52	8.0	7.9	160	103	10.6	15.4	15
NMeFOSA	9	79	91.6	6.73	10.7	11.7	80	94.4	6.22	8.4	8.9	159	93	5.78	9.89	10.6
NEtFOSA	9	79	88.2	7.25	10.5	12.0	80	91.1	4.69	9.6	10.5	159	89.7	5.3	10.3	11.5
NMeFOSAA	9	80	171	**77.2**	**89.5**	**52.3**	80	117	**23**	**28.5**	**24.3**	160	144	**49.9**	**74.9**	**52**
NEtFOSAA	9	79	182	**72.7**	**127.0**	**69.6**	80	120	**25.4**	**36.6**	**30.5**	159	151	**48.8**	**98.7**	**65.4**
NMeFOSE	9	76	79.7	9.2	13.2	16.5	80	84.3	6.46	9.5	11.2	156	82.1	7	11.8	14.4
NEtFOSE	9	72	75.1	9.68	15.3	20.3	80	82.4	10.4	11.1	13.4	152	78.9	9.65	13.5	17.1
PFMPA	9	80	67.1	22.1	18.8	28.0	81	68.3	22.1	19.8	29.0	161	67.7	22	18.9	27.9
PFMBA	9	80	100	9.67	9.8	9.8	81	103	10.1	9.7	9.4	161	102	9.45	9.95	9.77
NFDHA	9	80	94.6	9.3	11.0	11.6	81	94	6.26	9.7	10.4	161	94.3	7.16	10.6	11.2
HFPO-DA	9	80	99	10.8	7.5	7.6	81	101	7.7	6.7	6.7	161	100	8.76	7.68	7.68
ADONA	9	80	100	12.3	7.5	7.5	81	99.4	12	11.4	11.5	161	99.9	11.8	9.81	9.81

Table 7-3. Pooled Laboratory PFAS-Spiked Surface Water Samples Results. Low-spiked, high-spiked, and combined low/high spiked samples.

Analyte	Number of Labs	Low spiked Samples					High spiked Samples					Combined Low/High Spiked Samples				
		Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within- Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD of s_w
PFEESA	9	80	96.8	8.92	5.9	6.1	81	93.7	6.56	8.5	9.0	161	95.2	7.01	7.99	8.39
9Cl-PF3ONS	9	79	91.2	11	15.2	16.6	80	94.2	13.6	9.4	10.0	159	92.7	11.9	12.8	13.8
11Cl-PF3OUdS	9	78	70.3	20.4	12.1	17.1	80	73.6	19.7	9.3	12.6	158	72	19.9	10.8	15.1
3:3FTCA	9	80	73.6	13.1	14.8	20.1	81	77.5	13	15.5	20.0	161	75.6	12.3	15.5	20.5
5:3FTCA	9	80	88.6	7.75	8.2	9.3	81	90.9	7.94	10.0	11.1	161	89.7	7.33	9.38	10.5
7:3FTCA	9	80	78.9	8.34	12.5	15.9	81	86.7	8.4	20.0	23.0	161	82.8	7.62	17	20.5

Output File Name: Matrix_compiled_results_V0_230411_080232.csv

Notes:
Number of Labs - The number of laboratories reporting matrix spiked sample results.
Number of Results - The total number of matrix sample results categorized as low/high spike concentration that do not have a U flag.
Mean % Recovery - The mean percent recovery for low/high spiked samples across all laboratories.
s_b - The pooled between-laboratory standard deviation of the percent recovery for low spiked samples. Equation from EPA 821-B-18-001 page G-25.
s_w - The pooled within-laboratory standard deviation of the percent recovery for low spiked samples. Equation from EPA 821-B-18-001 page G-25.
RSD - The pooled within-laboratory relative standard deviation for low/high spiked samples (RSD, (sw / (mean % recovery) *100)).

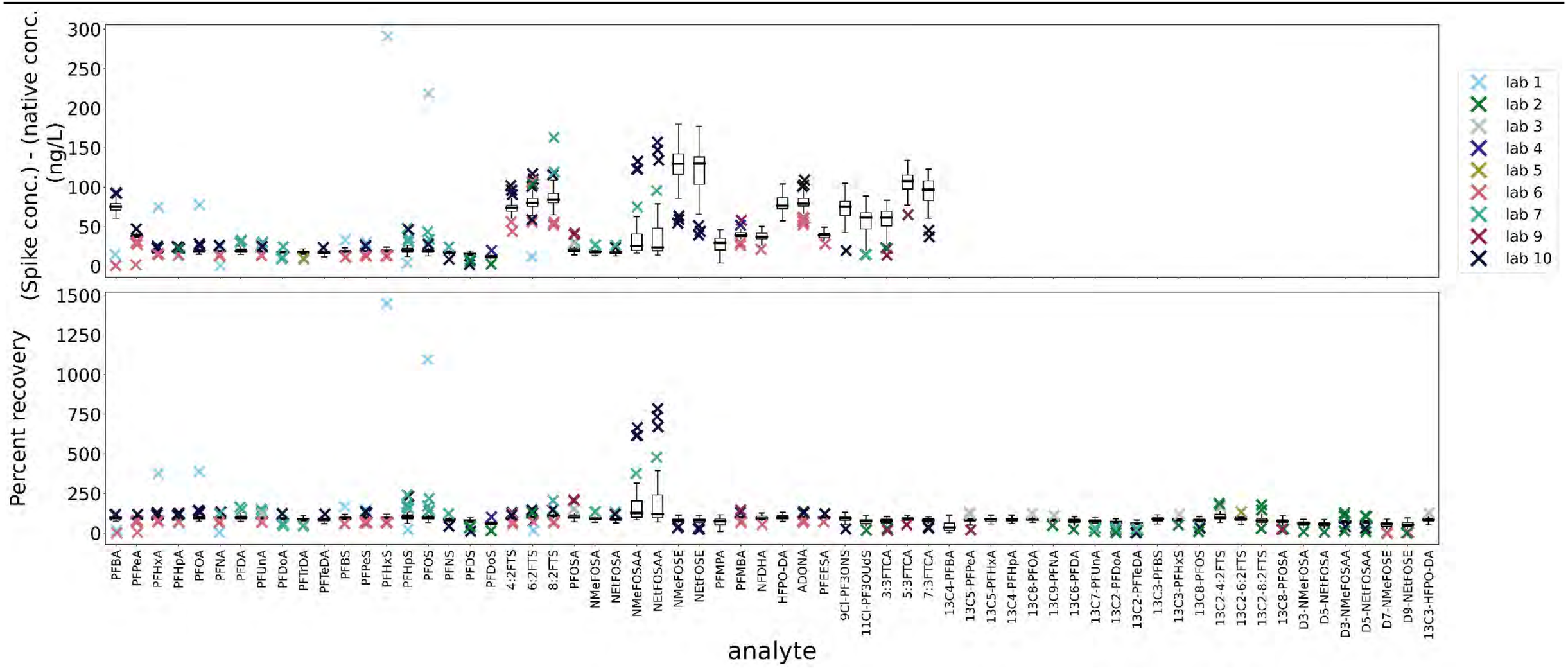


Figure 7-1. Surface water low spike results by analyte by laboratory.

File: SW_LowSpike_Boxplot_V0_230413

(A) Spiked concentration minus the laboratory-reported native concentration. (B) Calculated percent recovery. Figure includes both target compound recoveries, and EIS compound recoveries.

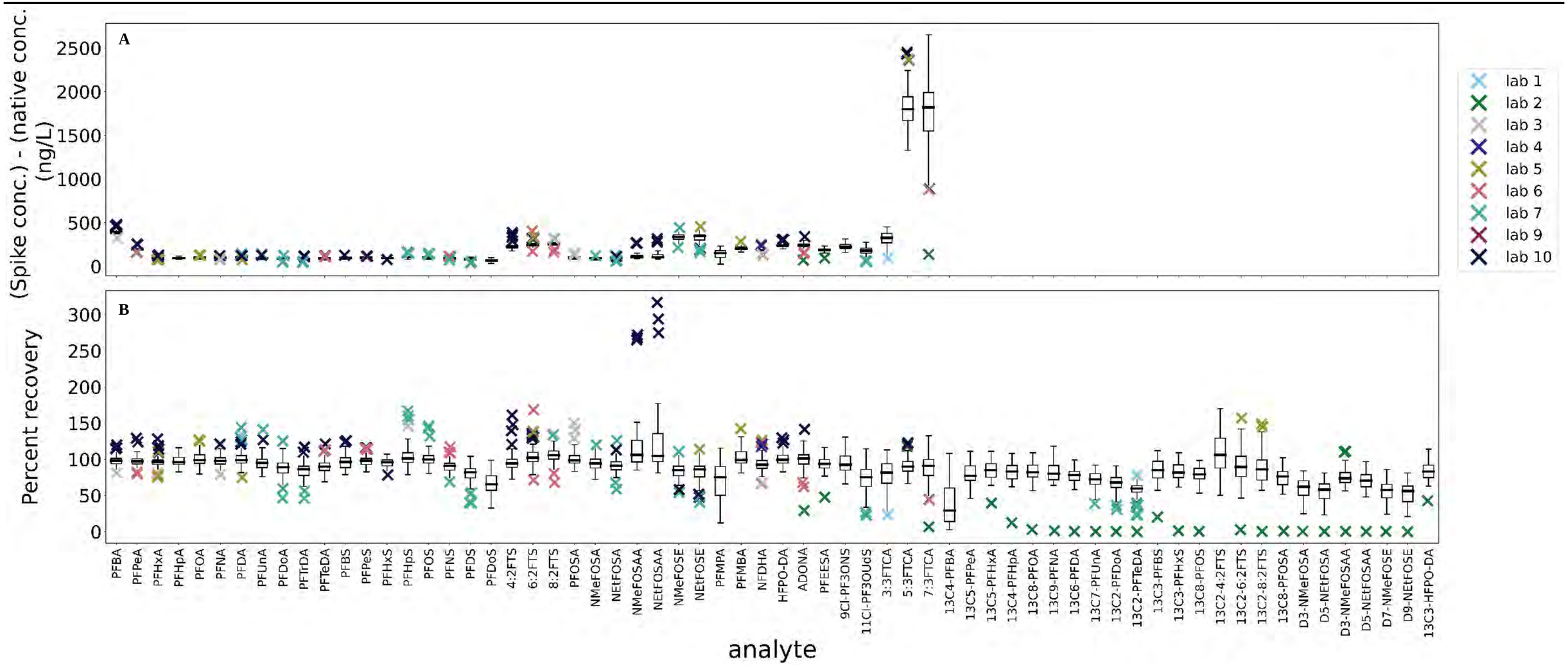


Figure 7-2. Surface water high spike results by analyte by laboratory.

(A) Spiked concentration minus the laboratory-reported native concentration. (B) Calculated percent recovery. Figure includes both target compound recoveries, and EIS compound recoveries.

Note: The high spike mass results for 5:3FTCA and 7:3FTCA appear higher as these were spiked at 2,000 ng/L, as opposed 100 ng/L for the remaining PFAS

7.3 SURFACE WATER EXTRACTED INTERNAL STANDARD RESULTS

The limits for EIS compounds defined by the MLVS Method were 20–150%. The combined results for the minimum, maximum, and average percent recovery is given in Table 7-6. Supporting individual laboratory results are in Appendix Table F-4. The combined average for all laboratories fell between 41–105%, which is within the limits set for the Study. The range of values by individual laboratories ranges from 0.01–187%. On closer examination, the lowest EIS compound recoveries were reported by Laboratory 2. This was evident in figures where for the low-spiked samples (Figure 7-1), the EIS compound recoveries were below the median, and in the high-spiked samples the recoveries were less than 5%. In Table 7-4, below, are the minimum values reported by Laboratory 2. These poor EIS compound recoveries not only impacted the evaluation of EIS compound limits but caused much of the Laboratory 2 target compound data to be rejected (X-flagged).

Table 7-4. Minimum EIS Compound Recovery Values Reported by Laboratory 2 in Surface Water.

$^{13}C_4$-PFBA	$^{13}C_8$-PFOA	$^{13}C_9$-PFNA	$^{13}C_6$-PFDA	$^{13}C_7$-PFUnA	$^{13}C_2$-PFDoA
6.16	2.37	0.35	0.35	0.01	0.02
$^{13}C_2$-PFTeDA	$^{13}C_3$-PFHxS	$^{13}C_8$-PFOS	$^{13}C_2$-6:2FTS	$^{13}C_2$-8:2FTS	$^{13}C_8$-PFOSA
0.02	0.75	0.04	2.61	0.11	0.35
D_3-NMeFOSA	D_5-NEtFOSA	D_3-NMeFOSAA	D_5-NEtFOSAA	D_7-NMeFOSE	D_9-NEtFOSE
0.11	0.03	0.01	0.04	0.01	0.01

The pooled-laboratory statistical analysis results for EIS compounds is in Table 7-7. The lowest mean percent recovery was for $^{13}C_4$-PFBA (40.7%) and the highest for $^{13}C_2$-6:2FTS (89.2%). Notwithstanding the low EIS compound recoveries of Laboratory 2, the pooled between-laboratory (s_b) and within-laboratory standard deviations (s_w) are relatively narrow across all compounds.

EIS compound recoveries across all laboratories over the individual surface water samples are presented in Appendix Table F-4. There were no apparent difference in the EIS compound recoveries for the individual samples.

Table 7-5. PFAS-Spiked Sample Results By Individual Surface Water Sample.

Analyte	Number of Labs	SWD Lake Harsha Freshwater			SWF Burley Creek Freshwater			SWG Sequim Bay Saltwater		
		Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery
PFBA	9	53	94.2	0.9–116.6	30	96.8	78.7–116.3	51	95.0	75.8–119.8
PFPeA	9	54	96.5	4.4–124	54	96.8	69.2–129	54	94.8	73.5–118
PFHxA	9	54	99.8	76.5–373.8	54	97.5	72–128	54	94.4	74–115
PFHpA	9	54	95.5	65.3–121.4	54	95.4	71.5–121.5	54	96.1	74–124
PFOA	9	50	106.0	75.1–388	54	98.1	75–142	54	99.3	75.5–125
PFNA	9	53	95.3	4.1–125	54	96.1	63–121	54	97.9	68.5–133
PFDA	9	52	102.0	73.5–163.5	54	101.0	75.5–149.5	54	96.1	74.7–124.5
PFUnA	9	52	97.3	68–151	53	99.5	69–141	54	91.4	68.5–116
PFDoA	9	52	87.9	61.9–123.5	53	92.7	72.5–125	54	84.2	46.6–104
PFTrDA	9	52	82.5	62.4–106.5	53	88.2	49.6–117	53	83.2	43.5–107
PFTeDA	9	52	83.9	68.8–104	53	91.6	65.5–121	53	87.4	59–109
PFBS	9	54	96.3	57.5–166.3	54	92.0	59–114	54	94.5	68.5–116.5
PFPeS	9	53	97.6	62.4–147	54	96.9	63.9–135.6	54	96.3	64.4–124.8
PFHxS	9	53	120.0	66.2 – 1448	54	93.8	63.3–113.1	54	93.3	76.1–107
PFHpS	9	50	104.0	20.8–239.5	53	104.0	74–179.5	54	107.0	70–231.5
PFOS	9	53	121.0	71.2–1095	53	102.0	77–169.5	54	97.8	65–139
PFNS	9	52	88.6	62.4–121.8	53	92.0	67.8–110.9	54	83.3	42.8–117
PFDS	9	49	75.8	48.9–94.7	53	80.1	52.2–101	54	73.3	10.9–104
PFDoS	9	52	56.0	31.2–82.7	53	62.7	36.2–86.7	53	69.8	14.6–100
4:2FTS	9	53	95.2	55.5–147.9	54	96.4	72.5–161.2	54	91.1	72.5–120.6
6:2FTS	9	53	104.0	15.4–168.4	54	104.0	78.8–137.5	54	102.0	69.2–138.8
8:2FTS	9	52	110.0	65.2–204.5	54	106.0	80.4–136.8	54	104.0	67.9–134.3
PFOSA	9	52	107.0	78.5–153	54	104.0	71.5–211	54	97.0	77–200.5
NMeFOSA	9	52	90.0	68–132.5	53	99.0	75–135.5	54	89.9	68.5–113

Table 7-5. PFAS-Spiked Sample Results By Individual Surface Water Sample.

Analyte	Number of Labs	SWD Lake Harsha Freshwater			SWF Burley Creek Freshwater			SWG Sequim Bay Saltwater		
		Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery	Number of Results	Mean % Recovery	Range % Recovery
NEtFOSA	9	52	87.6	65–114	53	98.1	69–133	54	83.4	58.6–101
NMeFOSAA	9	52	205.0	101–665	54	129.0	92.5–304.5	54	101.0	83–218
NEtFOSAA	9	52	241.0	110–785	53	120.0	83.9–244.5	54	94.7	70.5–154
NMeFOSE	9	51	73.8	34.1–104.5	52	86.3	56–112.5	53	85.9	53.5–110.6
NEtFOSE	9	48	66.7	24.8–105.6	51	85.2	59.8–110.5	53	83.9	40.5–114
PFMPA	9	53	76.5	36–114.5	54	48.6	10.2–91.8	54	78.3	19.9–115.5
PFMBA	9	53	100.0	73.8–126.5	54	108.0	76.5–145.5	54	97.2	65.3–130
NFDHA	9	53	93.5	53.8–121	54	93.4	63.5–126.5	54	96.0	70.8–125.7
HFPO-DA	9	53	102.0	71.9–130	54	98.7	78.1–121.2	54	99.2	76.8–127.1
ADONA	9	53	101.0	29.4–135.9	54	99.4	69.7–123.3	54	99.7	62.1–141.2
PFEESA	9	53	93.7	47.8–114.5	54	95.6	76.5–116.5	54	96.4	78–122.4
9Cl-PF3ONS	9	52	95.5	66.5–130.8	53	97.1	69.7–130.8	54	85.7	24.9–117.5
11Cl-PF3OUdS	9	52	71.7	25.7–112.5	53	75.2	25.3–109.6	53	69.2	18.6–114.6
3:3FTCA	9	53	84.3	44–112.8	54	61.9	17.5–96.2	54	80.7	28.1–102
5:3FTCA	9	53	94.3	72.1–121.5	54	92.1	64.2–122.5	54	82.8	54.2–100.8
7:3FTCA	9	53	90.0	6.8–115.5	54	91.0	65.5–132.5	54	67.6	30.8–91.7

Output File Name: Matrix_sample_results_V0_230411_080232.csv

Notes:

Number of Labs - The number of laboratories reporting matrix spiked sample results.

Number of Results - The total number of results for the SWD2-7 samples that do not have a U flag.

Mean % Recovery - The mean percent recovery for SWD2-7 samples across all laboratories.

Range % Recovery - The minimum to maximum percent recovery for SWD2-7 samples across all laboratories.

Table 7-6. Summary of EIS Compound percent recovery in Surface Water samples for all laboratories.

EIS Compound	All Labs Combined % Recovery			
	n	Min	Max	Mean
$^{13}C_4$-PFBA	182	1.7	113	40.7
$^{13}C_5$-PFPeA	189	19	132	81.5
$^{13}C_5$-PFHxA	189	39.5	112	84.8
$^{13}C_4$-PFHpA	189	12.3	112	82.7
$^{13}C_8$-PFOA	189	2.37	124	83.6
$^{13}C_9$-PFNA	189	0.35	118	80.9
$^{13}C_6$-PFDA	189	0.35	103	76.1
$^{13}C_7$-PFUnA	189	0.01	97.9	71
$^{13}C_2$-PFDoA	189	0.02	91.2	65
$^{13}C_2$-PFTeDA	189	0.02	81.4	56.5
$^{13}C_3$-PFBS	189	17.7	115	85.8
$^{13}C_3$-PFHxS	189	0.75	117	83.3
$^{13}C_8$-PFOS	189	0.04	105	76.9
$^{13}C_2$-4:2FTS	189	45.4	187	105
$^{13}C_2$-6:2FTS	189	2.61	157	89.2
$^{13}C_2$-8:2FTS	189	0.11	178	84.3
$^{13}C_8$-PFOSA	189	0.35	111	73.6
D_3-NMeFOSA	189	0.11	85.6	58.8
D_5-NEtFOSA	189	0.03	85	54.5
D_3-NMeFOSAA	189	0.01	125	73.6
D_5-NEtFOSAA	189	0.04	108	69.1
D_7-NMeFOSE	189	0.01	87	54.2
D_9-NEtFOSE	189	0.01	95.8	51.7
$^{13}C_3$-HFPO-DA	189	39.3	127	83.1

Data Source: EXA file Chapter 7 Surface Water Tables 04262023

Notes:
Includes all laboratories except Laboratory 8.
Results shown for EIS compound run with Target Analytes (excludes QA/QC samples).
final qualifier = X excluded.

Table 7-7. EIS Compound Results associated with Surface Water Samples.

Analyte	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD of s_w
$^{13}C_4$-PFBA	9	182	40.7	29.6	12.5	30.7
$^{13}C_5$-PFPeA	9	189	81.6	11.1	11	13.5
$^{13}C_5$-PFHxA	9	189	84.8	9.75	8.44	9.95
$^{13}C_4$-PFHpA	9	189	82.7	10.2	10	12.1
$^{13}C_8$-PFOA	9	189	83.6	8.15	11.8	14.2
$^{13}C_9$-PFNA	9	189	80.9	7.42	12.2	15.1
$^{13}C_6$-PFDA	9	189	76.1	5.94	13.1	17.3
$^{13}C_7$-PFUnA	9	189	71	9.32	13.5	19
$^{13}C_2$-PFDoA	9	189	65	10.3	12.6	19.4
$^{13}C_2$-PFTeDA	9	189	56.5	8.94	12.2	21.6
$^{13}C_3$-PFBS	9	189	85.9	11.8	10.8	12.5
$^{13}C_3$-PFHxS	9	189	83.2	9.31	11.8	14.2
$^{13}C_8$-PFOS	9	189	76.8	6.77	14.1	18.4
$^{13}C_2$-4:2FTS	9	189	105	20.2	20.6	19.6
$^{13}C_2$-6:2FTS	9	189	89.2	11.9	16.9	18.9
$^{13}C_2$-8:2FTS	9	189	84.3	13.2	21.2	25.2
$^{13}C_8$-PFOSA	9	189	73.5	8.08	13.8	18.8
D_3-NMeFOSA	9	189	58.8	10.6	11.9	20.2
D_5-NEtFOSA	9	189	54.5	10.7	11.5	21.1
D_3-NMeFOSAA	9	189	73.7	6.52	14.9	20.2
D_5-NEtFOSAA	9	189	69.1	7.17	14.1	20.4
D_7-NMeFOSE	9	189	54.2	11.6	13.9	25.6
D_9-NEtFOSE	9	189	51.7	11.4	15.2	29.5
$^{13}C_3$-HFPO-DA	9	189	83.1	10.1	8.9	10.7

Data Source: EXA file Chapter 7 Surface Water Tables 04262023

Notes:

Number of Labs - The number of laboratories reporting matrix (native & spiked) results.

Number of Results - The total number of matrix results that do not have a U flag.

Mean % Recovery - The mean percent recovery across all of the EIS compound individual samples across all laboratories for the given analyte.

s_b - The pooled between-laboratory standard deviation. Equation from EPA 821-B-18-001page G-25.

s_w - The pooled within-laboratory standard deviation. Equation from EPA 821-B-18-001page G-25.

RSD - The pooled within-laboratory relative standard deviation (RSD, (s_w / (mean % recovery) *100).

7.4 SURFACE WATER SUMMARY

The MLVS results demonstrate the ability of EPA Method 1633 to adequately measure PFAS concentrations in real-world surface water samples, with certain limitations. The pooled spiked target PFAS percent recovery results in Table 7-3 were between 70–130% with the exception of the following compounds: PFDoS (63%), NMeFOSAA (144%), NEtFOSAA (151%), and PFMPA (68%). As noted above, the low EIS compound recoveries for Laboratory 2, and the anomalous high values for NMeFOSAA and NEtFOSAA by Laboratory 10 may have skewed these results.

Tables 7-8 and 7-9 provides a summary of the relative proportions for all laboratories that fell between the study target analyte target percent recovery acceptance criteria used to evaluate the OPR and LLOPR (40–150%). For the low- and high-spiked samples, the proportion of all values that were between 40–150% of the spiked concentrations is >70% for all target analytes, with the exception of NMeFOSAA (low spike, 57.5%) and NEtFOSAA (low spike, 59.5%). For the low- and high-spiked samples, the proportion of all values that were between 70–130% of the spiked concentrations is >70% for most analytes. The exceptions to this included PFDS (low spike), PFDoS (low and high spike), NMeFOSAA (low spike), NEtFOSAA (low spike), and 11Cl-PF3OUdS, NEtFOSE (low spike), PFMPA (low and high spike), and 11Cl-PF3OUdS (low and high spike). Percentages of exceedances were fairly consistent across the laboratories with the exception of that of 11Cl-PF3OUdS. With the exception of Laboratory 7 (low spike 88.9%, high spike, 66.7%), laboratories had low percentages of exceedances of the 40% limit for both the low spike (0–12.5%) and high spike (0%).

Table 7-10 provides a summary of the relative proportions for all laboratories that met the study EIS compound target percent recovery acceptance criteria. For the low- and high-spiked samples, the proportion of all values that were between 20–150% of the spiked concentrations is >90% for all target analytes, with the exception of $^{13}C_4$-PFBA (67.6%).

Table 7-8. Proportion of surface water matrix spike %recovery results for target analytes within ranges (low-spiked samples).

Analyte	Low-spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBA	69	2.9	0	97.1	0	0	0
PFPeA	81	1.2	1.2	97.5	0	0	0
PFHxA	81	0	0	98.8	0	0	1.2
PFHpA	81	0	1.2	98.8	0	0	0
PFOA	78	0	0	96.2	2.6	0	1.3
PFNA	81	1.2	2.5	95.1	1.2	0	0
PFDA	80	0	0	97.5	1.2	1.2	0
PFUnA	79	0	3.8	93.7	1.3	1.3	0
PFDoA	79	0	6.3	93.7	0	0	0
PFTrDA	78	0	14.1	85.9	0	0	0
PFTeDA	78	0	9.0	91.0	0	0	0

Table 7-8. Proportion of surface water matrix spike %recovery results for target analytes within ranges (low-spiked samples).

Analyte	Low-spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBS	81	0	4.9	93.8	0	1.2	0
PFPeS	81	0	4.9	91.4	3.7	0	0
PFHxS	81	0	4.9	93.8	0	0	1.2
PFHpS	77	1.3	1.3	89.6	1.3	3.9	2.6
PFOS	80	0	1.2	91.2	3.8	1.2	2.5
PFNS	79	0	10.1	89.9	0	0	0
PFDS	76	3.9	27.6	68.4	0	0	0
PFDoS	78	11.5	64.1	24.4	0	0	0
4:2FTS	80	0	1.2	98.8	0	0	0
6:2FTS	81	1.2	1.2	92.6	4.9	0	0
8:2FTS	80	0	3.8	88.8	6.2	0	1.2
PFOSA	80	0	0	91.2	5.0	1.2	2.5
NMeFOSA	79	0	2.5	94.9	2.5	0	0
NEtFOSA	79	0	6.3	92.4	1.3	0	0
NMeFOSAA	80	0	0	50.0	7.5	15.0	27.5
NEtFOSAA	79	0	0	57.0	2.5	6.3	34.2
NMeFOSE	76	3.9	15.8	80.3	0	0	0
NEtFOSE	72	4.2	27.8	68.1	0	0	0
PFMPA	80	17.5	28.7	53.8	0	0	0
PFMBA	80	0	1.2	96.2	2.5	0	0
NFDHA	80	0	2.5	97.5	0	0	0
HFPO-DA	80	0	0	98.8	1.2	0	0
ADONA	80	0	2.5	96.2	1.2	0	0
PFEESA	80	0	0	100.0	0	0	0
9Cl-PF3ONS	79	1.3	11.4	86.1	1.3	0	0
11Cl-PF3OUdS	78	12.8	28.2	59.0	0	0	0
3:3FTCA	80	6.2	23.8	70.0	0	0	0
5:3FTCA	80	0	3.8	96.2	0	0	0
7:3FTCA	80	2.5	23.8	73.8	0	0	0

Data Source: EXA file Chapter 7 Surface Water Tables 20230511

Table 7-9. Proportion of surface water matrix spike %recovery results for target analytes within ranges (high-spiked samples).

Analyte	High-spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBA	65	0	0	100	0	0	0
PFPeA	81	0	0	100	0	0	0
PFHxA	81	0	0	100	0	0	0
PFHpA	81	0	0	100	0	0	0
PFOA	80	0	0	100	0	0	0
PFNA	80	0	0	100	0	0	0
PFDA	80	0	0	96.2	3.8	0	0
PFUnA	80	0	0	98.8	1.2	0	0
PFDoA	80	0	6.2	93.8	0	0	0
PFTrDA	80	0	6.2	93.8	0	0	0
PFTeDA	80	0	1.2	98.8	0	0	0
PFBS	81	0	0	100	0	0	0
PFPeS	80	0	0	100	0	0	0
PFHxS	80	0	0	100	0	0	0
PFHpS	80	0	0	95	1.2	3.8	0
PFOS	80	0	0	96.2	3.8	0	0
PFNS	80	0	1.2	98.8	0	0	0
PFDS	80	2.5	15.0	82.5	0	0	0
PFDoS	80	3.8	60.0	36.2	0	0	0
4:2FTS	81	0	0	96.3	2.5	1.2	0
6:2FTS	80	0	0	91.2	7.5	1.2	0
8:2FTS	80	0	1.2	96.2	2.5	0	0
PFOSA	80	0	0	97.5	1.2	1.2	0
NMeFOSA	80	0	0	100.0	0	0	0
NEtFOSA	80	0	2.5	97.5	0	0	0
NMeFOSAA	80	0	0	78.8	16.2	1.2	3.8
NEtFOSAA	80	0	0	70.0	17.5	8.8	3.8
NMeFOSE	80	0	8.8	91.2	0	0	0
NEtFOSE	80	0	18.8	81.2	0	0	0
PFMPA	81	24.7	14.8	60.5	0	0	0
PFMBA	81	0	0	95.1	4.9	0	0
NFDHA	81	0	2.5	97.5	0	0	0
HFPO-DA	81	0	0	100	0	0	0
ADONA	81	1.2	2.5	95.1	1.2	0	0
PFEESA	81	0	1.2	98.8	0	0	0
9Cl-PF3ONS	80	0	7.5	91.2	1.2	0	0

Table 7-9. Proportion of surface water matrix spike %recovery results for target analytes within ranges (high-spiked samples).

Analyte	High-spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
11Cl-PF3OUdS	80	7.5	35.0	57.5	0	0	0
3:3FTCA	81	3.7	24.7	71.6	0	0	0
5:3FTCA	81	0	4.9	95.1	0	0	0
7:3FTCA	81	1.2	18.5	77.8	2.5	0	0

Data Source: EXA file Chapter 7 Surface Water Tables 20230511

Table 7-10. Proportion of surface water matrix %recovery results for EIS compounds within ranges.

EIS Compound	All Laboratories Proportion % Recovery					
	n	<10%	≥10% to <20%	≥20% to <150%	≥150% to <200%	≥200%
$^{13}C_4$-PFBA	182	14.3	18.1	67.6	0	0
$^{13}C_5$-PFPeA	189	0	0.5	99.5	0	0
$^{13}C_5$-PFHxA	189	0	0	100.0	0	0
$^{13}C_4$-PFHpA	189	0	1.1	98.9	0	0
$^{13}C_8$-PFOA	189	1.1	0	98.9	0	0
$^{13}C_9$-PFNA	189	1.1	0	98.9	0	0
$^{13}C_6$-PFDA	189	1.1	0	98.9	0	0
$^{13}C_7$-PFUnA	189	1.6	0	98.4	0	0
$^{13}C_2$-PFDoA	189	1.6	0.5	97.9	0	0
$^{13}C_2$-PFTeDA	189	2.1	0.5	97.4	0	0
$^{13}C_3$-PFBS	189	0	0.5	99.5	0	0
$^{13}C_3$-PFHxS	189	1.1	0	98.9	0	0
$^{13}C_8$-PFOS	189	1.6	0	98.4	0	0
$^{13}C_2$-4:2FTS	189	0	0	90.5	9.5	0
$^{13}C_2$-6:2FTS	189	1.1	0	98.4	0.5	0
$^{13}C_2$-8:2FTS	189	1.1	0	98.4	0.5	0
$^{13}C_8$-PFOSA	189	1.1	0	98.9	0	0
D_3-NMeFOSA	189	1.6	0	98.4	0	0
D_5-NEtFOSA	189	1.6	0	98.4	0	0
D_3-NMeFOSAA	189	1.1	0.5	98.4	0	0
D_5-NEtFOSAA	189	1.6	0	98.4	0	0
D_7-NMeFOSE	189	3.2	0.5	96.3	0	0
D_9-NEtFOSE	189	3.7	1.6	94.7	0	0
$^{13}C_3$-HFPO-DA	189	0	0	100.0	0	0

Source file: SW EIS_PCT_REC_20230511.xls

8 GROUNDWATER

Twenty-one individual groundwater samples were sent to each of the 10 participating laboratories (See Section 2.0 for details). Due to an EIS compound spiking error that affected all of the groundwater sample results from Laboratory 8, all data from this laboratory were omitted from the statistical analysis. Laboratory 9 stated in their groundwater data package submittal that a sample preparation error occurred that affected all but GWA6 and the method blank, LLOPR, and OPR with which it was associated. During the extraction process, these samples were loaded onto the SPE too quickly. As a result, the contact time the sample had with the SPE media was insufficient for analyte retention, as indicated by the extremely low EIS compound recoveries (generally 0 - 10%). Since the cause of these failures was human error, all groundwater data from Laboratory 9 was omitted from the statistical analysis. The unspiked sample, GWB1 that Laboratory 1 received appears to have been spiked before shipping as every one of the 40 target analytes were detected at concentrations which coincide with the concentrations of the low level spikes. Because of this error, GWB1 through GWB7 reported by Laboratory 1 were omitted from the groundwater statistical analysis. Data was reported and validated in accordance with the requirements of the Study Plan. The rules used for omission of individual analyte results are presented in Section 3 of this report.

8.1 NATIVE PFAS CONCENTRATIONS IN GROUNDWATER

Each laboratory received and analyzed a single unspiked sample of the three groundwater samples (Table 2-3). The detected background, or "native" concentrations for the three groundwater samples are given in Table 8-1. The results from the reconnaissance analyses (SGS AXYS) that were also reported by at least one of the study laboratories are also included in Table 8-1. The complete results for all laboratories for the three native groundwater samples is provided in Appendix G.

The reconnaissance results for GWB are of the sample prior to the dilution implemented for the creation of study samples that were shipped to the participating laboratories. As noted in Section 2, the reconnaissance testing results for GWB indicated a ten-fold dilution was needed in order to bring native concentrations to a level where, when adding a high concentration spike, the resulting concentrations would remain within the quantitation range of most laboratories. As will be discussed later in this section, concentrations of PFOS and PFHxS even after a $1/10^{th}$ dilution were still too high.

Of the 40 PFAS target analytes in the draft method, only 16 were detected in the baseline samples by the participating laboratories. The list includes PFBA, PFPeA, PFHxA, PFHpA PFOA, PFNA. PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFOSA, NEtFOSA, NEtFOSAA, NMeFOSA, and 6:2FTS. Evident in Table 8-1 is that the unspiked results were generally in good agreement between the eight laboratories, as well as with the PFAS measured in the reconnaissance samples. Of additional note is that the results for GWB1, the $1/10^{th}$ dilution of the reconnaissance values are also in good agreement, within a factor of two. The number of detected analytes by groundwater sample is shown in Table 8-2.

Table 8-1. Summary of Target Analytes Detected in Unspiked Groundwater Samples (ng/L)

Analyte	Number of Labs	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		Lab 10		SGS-AXYS Baseline	
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	*Native*	*1/10 Dilution*
GWA1 - USACE GW#1 Midwest																			
PFBA	8	10.3		12.6	J	10.0		6.29	J	9.26		12.1		9.7		11.4		10.02	---
PFPeA	8	11.4		12.7		11.8		11.7		10.7		16.6		10.0		9.3		10.53	---
PFHxA	8	22.9		24.1		20.0		22.0		25.0		24.0		19.8		22.6		18.99	---
PFHpA	8	6.59		6.39		6.08		5.86		5.75		7.09		5.5		8.31		5.02	---
PFOA	8	5.55		5.09		4.72		5.6		6.36		5.64		4.2		6.79		4.77	---
PFNA	8	0.657	U	1.06	U	0.504	U	0.29	U	1.24	J	0.25	U	0.61	U	0.493	U	< 0.39	---
PFBS	8	41.9		48.0		39.3		47.9		36.6		43.1		45.6		58.7		38.11	---
PFPeS	8	33.4		35.2		30.1		34.1		27.1		36.3		32.2		47.1		37.01	---
PFHxS	8	139.0		139		123		138		98.9		126		126		131		131.90	---
PFHpS	8	2.96		3.06	U	4.32		2.97		3.31		5.44		4.7		3.17		3.31	---
PFOS	8	78.8		78.5		70.6		62.3		75.8		80.0		78.4		0.415	U	76.49	---
NMeFOSA	8	0.453	U	0.822	J	0.696	U	1.21	U	0.341	U	0.199	U	0.64	U	0.35	U	< 0.45	---
GWB1 - LRPCD GW#2 Southwest																			
PFBA	8	--	X	17		15.1		14.2		13.7		16.2		13.3		16.6		139.6	13.96
PFPeA	8	--	X	37.7		35.8		33.6		31.9		39.6		33.4		36.7		332.7	33.27
PFHxA	8	--	X	95.5		73.4		87.2		85.8		93.8		85.6		108		762.7	76.27
PFHpA	8	--	X	15.3		14.1		14.1		11.3		15.9		12.5		14		160.2	16.02
PFOA	8	--	X	83.0		75.8		76.2		78.3		90.1		76.3		96		688.9	68.89
PFNA	8	--	X	1.06	U	1.12	J	0.72	J	0.792	U	0.922	J	0.61	U	0.849	J	7.5	0.75
PFBS	8	--	X	37.4		31.3		36.6		27.6		29.5		34		34.5		256.70	25.67
PFPeS	8	--	X	33.5		30.6		29.6		26.4		32.7		31.4		35.3		312.10	31.21
PFHxS	8	--	X	369		322		324		244		316		341		308		3424	342.4
PFHpS	8	--	X	5.65		6.4		4.29		4.22		8.77		6.9		4.48		47.10	4.71
PFOS	8	--	X	246		197		200		198		246		240		212		2314	231.4
6:2FTS	8	--	X	13.8	J	10.9		13.4		37.3		9.94		11		12		129	12.9
GWC1 - USACE GW #13																			
PFPeA	8	0.552	U	1.39	JI	0.856	U	0.533	JI	0.772	U	0.563	U	0.94	U	0.549	U	< 0.72	---
PFHxA	8	1.22	J	--	X	0.88	JI	1.0	J	0.944	JI	0.412	U	0.63	J	0.298	U	0.95	---
PFOA	8	1.15	J	--	X	0.696	U	0.651	U	0.547	J	0.29	U	0.46	U	1.03	J	0.43	---
PFBS	8	1.57	J	--	X	1.92		1.53	J	1.36	J	2.04		1.5	J	2.46		1.63	---
PFPeS	8	0.361	J	--	X	0.56	J	0.422	J	0.729	U	0.542	J	1.1	U	0.72	JI	0.44	---
PFHxS	8	1.62	J	--	X	2.4		1.67	J	1.61	J	1.83		1.3	J	1.81	J	1.92	---
PFOS	8	0.441	U	--	X	0.728	U	1.7	U	0.631	J	0.548	J	0.54	U	0.415	U	< 0.36	---
6:2FTS	8	1.07	U	--	X	2.16	U	1.6	U	3.02	BJ+	1.48	U	3.5	U	2.36	U	< 1.3	---
PFOSA	8	0.346	U	--	X	0.432	U	1.58	JI	2.17		0.188	U	0.67	U	0.212	U	< 0.36	---
NEtFOSA	8	0.365	U	--	X	0.736	U	1.07	J	0.521	U	0.0998	U	0.62	U	0.273	U	< 0.91	---
NEtFOSAA	8	0.554	U	--	X	0.88	J	0.61	U	0.531	U	0.571	U	1.3	U	0.693	U	< 0.36	---

Table 8-1. Summary of Target Analytes Detected in Unspiked Groundwater Samples (ng/L)

Analyte	Number of Labs	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		Lab 10		SGS-AXYS Baseline
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	
Total # Analytes Reported Across All samples		10		11		11		13		13		11		11		10		11

Notes:
J indicates an estimated value
X indicates results could not be reported due to spiking error.

Table 8-2. Numbers of Detected Analytes by Groundwater Sample

Groundwater Sample	Total Number of Analytes Detected
GWA1 - USACE GW#1 Midwest	13
GWB1 - LRPCD GW#2 Southwest	12
GWC1 - USACE GW #13	11

Notes:
Number of analytes detected by at least one laboratory

8.2 GROUNDWATER MATRIX SPIKE RESULTS

The compiled (all laboratory) PFAS-spiked groundwater samples are presented in Table 8-3 organized as low-spiked samples, high-spiked samples, and the combined low- and high-spiked sample results. Individual laboratory results are found in Appendix tables G-3 and G-4. Results are also shown in Figures 8-1 and 8-2.

The overall trends observed for the wastewater and surface water analyses are similar to the PFAS-spiked groundwater samples. For the low-spiked samples the mean percent recovery was 74–157% (PFDoS and NEtFOSAA, respectively), the high-spiked samples from 72–114% (PFDoS and PFHpS, respectively), and the combined mean percent recovery from 73–134% (PFDoS and NEtFOSAA, respectively) (Table 8-3). The observed range is nearly identical to the range of recoveries in wastewater (52.4–128%, PFDoS, NMeFOSAA) and surface water (63–151%, PFDoS, NEtFOSAA). The pooled between-laboratory standard deviation (s_b) and the pooled within-laboratory standard deviation (s_w) were relatively narrow for all PFAS in the low-spiked and high-spiked samples, with the exceptions of PFOS, NMeFOSAA, and NEtFOSAA (Table 8-3). Figure 8-1 shows that for the low-spiked samples, with the exception of Laboratory 10, the results for PFOS are clustered around the median between 89–189% recovery. Laboratory 10 reported several measures up to 575% recovery. The same may be observed for Laboratory 10 for NMeFOSAA and NEtFOSAA. Laboratory 10 also had the highest percent recoveries for several compounds including PFHpS, PFOS, NMeFOSAA and NEtFOSAA in the high-spiked samples (Figure 8-2). Individual laboratory recovery ranges may be found in Appendix G tables G4-G5.

There are no discernible differences in pooled matrix spike recoveries across the three groundwater samples for all the targeted compounds, with the exception of PFOS, NMeFOSAA and NEtFOSAA. (Table 8-4). For PFOS, the range of laboratory-measured recoveries were high in sample GWA, ranging from 30.5–575%. Percent recoveries for PFOS (and PFHxS) could not be determined for groundwater sample GWB (discussed below). PFOS in sample GWC across all laboratories had a narrow range at 88.3–189.5%. Similarly, for NMeFOSAA the recoveries were high in GWA (88.6–339%), acceptable in GWB (82.5–137%), and again high in GWC (93.8–570%). The same is observed for NEtFOSAA: GWA (79–262%), GWB (86.5–136.5%), and GWC (93.7–655%).

PFOS and PFHxS recoveries could not be calculated for the low- or high-spiked GWB samples. Discussed in Section 2, sample GWB had elevated levels of PFHxS and PFOS (Table 8-1): 3,424 and 2,314 ng/L, respectively. The sample was diluted by $1/10^{th}$ and the reported results for all laboratories were between 70–110% of the estimated diluted concentration. The actual measured values for PFOS and PFHxS in the unspiked and spiked GWB samples are shown in Table 8-5. For the low-spiked samples, the added 20.1 ng/L of PFOS and PFHxS was too low to effectively measure the addition. For example, PFOS in Laboratories 2 and 6 was reported lower than the unspiked samples. For PFHxS the same is true for Laboratories 2, 5, and 10. Recoveries for the high-spiked samples are reported in Table 8-5.

Table 8-3. Pooled Laboratory PFAS-Spiked Groundwater Samples Results. Low-spiked, High-spiked and combined low/high spiked samples.

Analyte	Number of Labs	Low-spiked Samples					High-spiked Samples					Combined Low/High Spiked Samples				
		Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between Lab std. dev. (s_b)	Pooled Within Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)
PFBA	8	67	99.3	7.6	3.4	3.5	66	99.8	6.35	3.4	3.4	133	99.6	6.8	3.69	3.71
PFPeA	8	69	103	7.61	8.7	8.5	69	100	5.07	5.8	5.7	138	102	5.84	7.74	7.62
PFHxA	8	30	97.4	7.7	8.7	8.9	66	95.9	7.88	9.8	10.2	96	96.4	7.51	9.54	9.9
PFHpA	8	69	101	13.5	11.6	11.5	69	99.4	7.94	8.0	8.0	138	100	10.5	10.2	10.2
PFOA	8	45	103	8.8	9.3	9.1	66	103	10.2	9.9	9.7	111	103	8.18	10.5	10.3
PFNA	8	67	99.5	7.77	8.7	8.7	68	97.8	8.61	8.3	8.5	135	98.6	7.95	8.51	8.62
PFDA	8	67	102	7.73	11.9	11.6	68	102	9.74	10.6	10.4	135	102	8.47	11.1	10.9
PFUnA	8	67	101	8.41	9.6	9.5	68	99.4	8.59	8.9	9.0	135	100	7.89	9.56	9.52
PFDoA	8	67	96.7	7.91	8.8	9.1	67	94.5	6.65	7.1	7.5	134	95.6	7.18	7.94	8.3
PFTrDA	8	67	90.9	9.96	11.0	12.1	65	90.8	8.91	12.0	13.2	132	90.9	9.14	11.2	12.3
PFTeDA	8	66	90.7	9.93	11.4	12.5	65	90.2	5.82	9.0	9.9	131	90.4	7.89	10.2	11.2
PFBS	8	24	99.4	12.5	6.0	6.0	69	95	9.16	7.9	8.3	93	96.1	9.63	8.02	8.34
PFPeS	8	23	101	10.1	9.3	9.1	68	101	10.2	8.2	8.2	91	101	9.47	8.83	8.74
PFHxS	8	23	96.2	9.03	8.5	8.8	27	96.2	6.12	7.2	7.5	50	96.2	5.19	9	9.36
PFHpS	8	67	113	20.1	18.8	16.7	68	114	18.3	21.5	18.9	135	113	18.3	20.4	18
PFOS	7	24	157	**86**	**91.9**	**58.4**	47	108	26.4	26.1	24.1	71	125	**52**	**68**	**54.5**
PFNS	8	65	93.3	7.67	12.8	13.7	68	90.7	4.5	14.6	16.1	133	92	4.49	13.7	14.9
PFDS	8	65	84.3	11.2	13.6	16.2	68	82.3	5.15	18.9	23.0	133	83.3	6.18	16.6	20
PFDoS	8	66	74.3	14.2	11.3	15.2	66	72.1	7.44	14.1	19.6	132	73.2	10.5	13	17.8
4:2FTS	8	69	97.8	6.85	8.3	8.5	69	94.1	3.55	7.9	8.4	138	95.9	4.34	8.65	9.02
6:2FTS	8	68	102	18	13.5	13.2	69	102	8.66	13.0	12.7	137	102	12.9	14	13.7
8:2FTS	8	65	109	5.19	12.3	11.2	65	108	8.15	11.9	11.0	130	109	6.56	11.9	10.9
PFOSA	8	65	106	10.2	11.2	10.6	68	103	7.85	10.2	9.9	133	104	8.39	11	10.6
NMeFOSA	8	65	94.1	3.21	8.7	9.2	68	93.6	5.21	7.3	7.8	133	93.9	3.07	8.31	8.86
NEtFOSA	8	65	89.8	5.3	7.6	8.4	67	88.6	5.15	6.8	7.6	132	89.2	4.98	7.13	7.99
NMeFOSAA	8	67	153	**73.1**	**73.8**	**48.2**	67	109	13.6	14.2	13.1	134	131	**42.8**	**63.2**	**48.3**
NEtFOSAA	8	65	157	**64.4**	**80.4**	**51.1**	64	110	10.8	16.9	15.3	129	134	**36.5**	**66.5**	**49.7**
NMeFOSE	8	64	88	8.77	9.0	10.3	62	89.6	6.49	6.5	7.2	126	88.8	7.37	7.99	9
NEtFOSE	8	64	84.1	11.1	10.4	12.4	61	85.8	10.6	7.5	8.7	125	85	10.7	9.11	10.7
PFMPA	8	69	94.5	13.1	12.5	13.3	69	91.3	15	13.4	14.7	138	92.9	13.6	13.2	14.2
PFMBA	8	69	104	9.74	7.7	7.4	69	104	11.9	6.5	6.2	138	104	10.6	7.21	6.95
NFDHA	8	69	100	7.67	11.3	11.3	69	95.9	5.28	8.6	9.0	138	98.1	5.7	10.5	10.7
HFPO-DA	8	69	104	11.2	8.7	8.4	69	102	7.41	8.8	8.7	138	103	8.57	9.43	9.16

Table 8-3. Pooled Laboratory PFAS-Spiked Groundwater Samples Results. Low-spiked, High-spiked and combined low/high spiked samples.

Analyte	Number of Labs	Low-spiked Samples					High-spiked Samples					Combined Low/High Spiked Samples				
		Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between Lab std. dev. (s_b)	Pooled Within Lab std. dev. (s_w)	RSD (s_w)	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)
ADONA	8	69	101	9.34	12.0	11.9	69	99.5	12.6	10.4	10.5	138	100	10.7	11.3	11.3
PFEESA	8	69	98.7	9.38	10.5	10.6	69	93.7	5.07	8.4	9.0	138	96.2	5.65	10.8	11.3
9Cl-PF3ONS	8	67	98.6	11.9	12.5	12.7	68	92.0	12.3	18.9	20.6	135	95.3	10.9	16.6	17.4
11Cl-PF3OUdS	8	67	82.3	23.1	13.6	16.5	67	78.6	20.9	17.7	22.6	134	80.4	21.2	16.2	20.2
3:3FTCA	8	69	89.1	10.5	8.1	9.1	69	91.5	11.5	9.2	10.0	138	90.3	10.8	8.74	9.68
5:3FTCA	8	69	94.0	6.39	7.6	8.1	69	95.2	6.77	6.8	7.1	138	94.6	5.92	7.53	7.96
7:3FTCA	8	68	87.9	4.67	12.9	14.6	69	93.5	4.99	12.2	13.0	137	90.7	3.22	12.9	14.3

Source file: Matrix_compiled_results_V0_230421_153930.csv

Notes:
Shaded cells indicate high variability.
Number of Labs - The number of laboratories reporting matrix spiked sample results.
Number of Results - The total number of matrix sample results categorized as low/high spike concentration that do not have a U flag.
Mean % Recovery - The mean percent recovery for low/high spiked samples across all laboratories.
s_b - The pooled between-laboratory standard deviation of the percent recovery for low spiked samples. Equation from EPA 821-B-18-001 page G-25.
s_w - The pooled within-laboratory standard deviation of the percent recovery for low spiked samples. Equation from EPA 821-B-18-001 page G-25.
RSD - The pooled within-laboratory relative standard deviation for low /high spiked samples (RSD, (sw / (mean % recovery) *100).

Table 8-4. PFAS-Spiked Sample Results By Individual Groundwater Sample

Analyte	GWA				GWB				GWC			
	Number of Labs	Number of Results	Mean % Recovery	Range % Recovery	Number of Labs	Number of Results	Mean % Recovery	Range % Recovery	Number of Labs	Number of Results	Mean % Recovery	Range % Recovery
PFBA	8	44	99.4	83.7-123.2	7	42	99.6	83.6-115.5	8	47	99.7	84.5-118.4
PFPeA	8	48	101	74.8-141.2	7	42	102	76.7-125.5	8	48	102	88.2-122
PFHxA	8	30	94.7	70-123.4	6	18	95.5	53.2-128.2	8	48	97.8	78.3-116.5
PFHpA	8	48	95.6	68.2-120	7	42	102	78.5-160.5	8	48	103	85-155
PFOA	8	48	103	83.7-144	7	21	103	76.2-144.7	7	42	102	87.1-135.5
PFNA	8	47	96.7	74.8-124	7	41	97.6	79.1-122.4	8	47	101	72.8-132
PFDA	8	47	100	64.7-122	7	41	103	72-144	8	47	104	80.8-166.5
PFUnA	8	47	98.0	74.1-119	7	41	101	74-143	8	47	102	81.5-130
PFDoA	8	47	94.4	54.0-116.5	7	40	98.8	76.5-117	8	47	94.2	69-120.5
PFTrDA	8	46	87.0	44.0-114.5	7	40	91.4	61.5-118	8	46	94.3	62.7-168
PFTeDA	8	45	87.8	39.5-107	7	40	89.1	56-110.5	8	46	94.2	67.5-115.5
PFBS	8	24	92.7	72.9-114.1	7	21	96.5	74.5-120.5	8	48	97.7	77.7-124.7
PFPeS	8	23	99.2	67.7-131.9	7	21	104	84.3-126.5	8	47	100	81.4-132.6
PFHxS	1	3	109	98.1-123.1	0	0	-	-	8	47	95.4	74.6-116.6
PFHpS	8	47	112	85.2-185.8	7	41	115	88.1-197	8	47	114	83.5-242
PFOS	8	26	**159**	**30.5-575**	0	0	-	-	8	45	105	88.3-189.5
PFNS	8	47	89.2	36.1-125.2	7	41	94.8	38.6-139.6	8	45	92.3	30.9-131
PFDS	8	47	79.8	10.2-110.6	7	41	86.4	3.9-114.6	8	45	84	8.5-123
PFDoS	8	45	69.7	41.7-107.5	7	40	75.5	23.1-107	8	47	74.6	2.5-103.5
4:2FTS	8	48	97.1	85.0-123.3	7	42	96.3	71.3-125.3	8	48	94.5	79.6-129.2
6:2FTS	8	48	99.0	30.0-140.1	7	42	101	64.8-139.7	8	47	106	88.9-178.8
8:2FTS	8	47	105	86.7-128	7	41	110	87-162.9	7	42	112	81.7-154.3
PFOSA	8	47	102	85-135.5	7	41	101	83.5-129	8	45	109	79.6-156
NMeFOSA	8	47	91.7	61.0-108.0	7	41	96	80.6-116	8	45	94.1	76-124
NEtFOSA	8	47	87.7	47-104.5	7	40	91.4	77-104	8	45	88.8	61.2-112
NMeFOSAA	8	47	**125**	**88.6-339**	7	40	103	82.5-137	8	47	**160**	**93.8-570**
NEtFOSAA	8	47	**117**	**79-262**	7	40	104	86.5-136.5	7	42	**181**	**93.7-655**
NMeFOSE	8	45	88.6	54.4-103.1	7	40	91.7	73.8-110.6	7	41	86.2	50.9-118.8
NEtFOSE	8	44	85.6	43.5-116.9	7	40	88.3	59.8-123.8	7	41	81	49-112.8
PFMPA	8	48	88.8	25.7-125.7	7	42	97.2	73-124.8	8	48	93.2	34.8-130
PFMBA	8	48	105	85.0-139.0	7	42	102	76.5-134.5	8	48	104	86-131
NFDHA	8	48	98.8	75.5-119.3	7	42	99.5	57.8-121.8	8	48	96.1	69-128.2
HFPO-DA	8	48	103	84.5-135	7	42	101	74.2-132.5	8	48	105	88.9-147.5
ADONA	8	48	98.5	52.5-132.2	7	42	102	63.7-144.2	8	48	101	56.9-130.4
PFEESA	8	48	96.4	61-117.2	7	42	96.8	54.4-122.4	8	48	95.6	45.6-121.2
9Cl-PF3ONS	8	47	94.0	19.5-142.0	7	41	95.8	32.2-137.5	8	47	96	6.4-130

Table 8-4. PFAS-Spiked Sample Results By Individual Groundwater Sample

Analyte	GWA				GWB				GWC			
	Number of Labs	Number of Results	Mean % Recovery	Range % Recovery	Number of Labs	Number of Results	Mean % Recovery	Range % Recovery	Number of Labs	Number of Results	Mean % Recovery	Range % Recovery
11Cl-PF3OUdS	8	47	76.1	1.2-123.3	7	41	81.6	0.5-130.9	8	46	83.7	30.7-136.7
3:3FTCA	8	48	87.5	35.0-120.8	7	42	92.2	74.2-105.6	8	48	91.4	46.8-131
5:3FTCA	8	48	95.0	79.5-121.5	7	42	98.2	81.5-124.2	8	48	91.1	76.5-106.7
7:3FTCA	8	48	92.1	16.4-110.0	7	42	93.5	21.1-124	8	47	86.9	60.4-108

Notes:
Shaded cells indicate high variability.
Number of Labs - The number of laboratories with GWA2-7 samples results.
Number of Results - The total number of results for the GWA2-7 samples that do not have a U flag.
Mean % Recovery - The mean percent recovery for GWA2-7 samples across all laboratories.
Min % Recovery - The minimum percent recovery for GWA2-7 samples across all laboratories.
Max % Recovery - The maximum percent recovery for GWA2-7 samples across all laboratories.

Table 8-5. Concentrations for PFOS and PFHxA for GWB Sample Series (ng/L)

Concentrations of PFOS

Sample ID	Spike Category	Spike Level (ng/L)[1]	Laboratory Number													
			2		3		4		5		6		7		10	
			Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual
GWB1	Unspiked	0	246		197		200		198		246		240		212	
GWB2	Low	20.1	169		231		220		238		211		393		249	
GWB3	Low	20.1	172		236		235		231		255		287		262	
GWB4	Low	20.1	--	X	229		212		244		218		400		251	
GWB5	High	100	379		282		308		275		313		349		343	
GWB6	High	100	413		319		312		326		349		429		317	
GWB7	High	100	393		310		311		324		385		485		340	

Concentrations of PFHxS

Sample ID	Spike Category	Spike Level (ng/L)[1]	Laboratory Number													
			2		3		4		5		6		7		10	
			Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual
GWB1	Unspiked	0	369		322		324		244		316		341		308	
GWB2	Low	20.1	326		337		378		280		334		372		304	
GWB3	Low	20.1	305		334		398		268		299		354		336	
GWB4	Low	20.1	--	X	322		312		319		351		385		355	
GWB5	High	100	500		401		463		358		366		403		369	
GWB6	High	100	487		442		467		407		363		495		354	
GWB7	High	100	455		443		450		413		397		496		478	

Notes:
[1] All spike concentrations are presented as acid concentrations; as final concentration in sample in ng/L.
For Laboratory 2 the PFOS and PFHxS values for sample GWB4 were rejected due to poor EIS compound recovery (<10%)

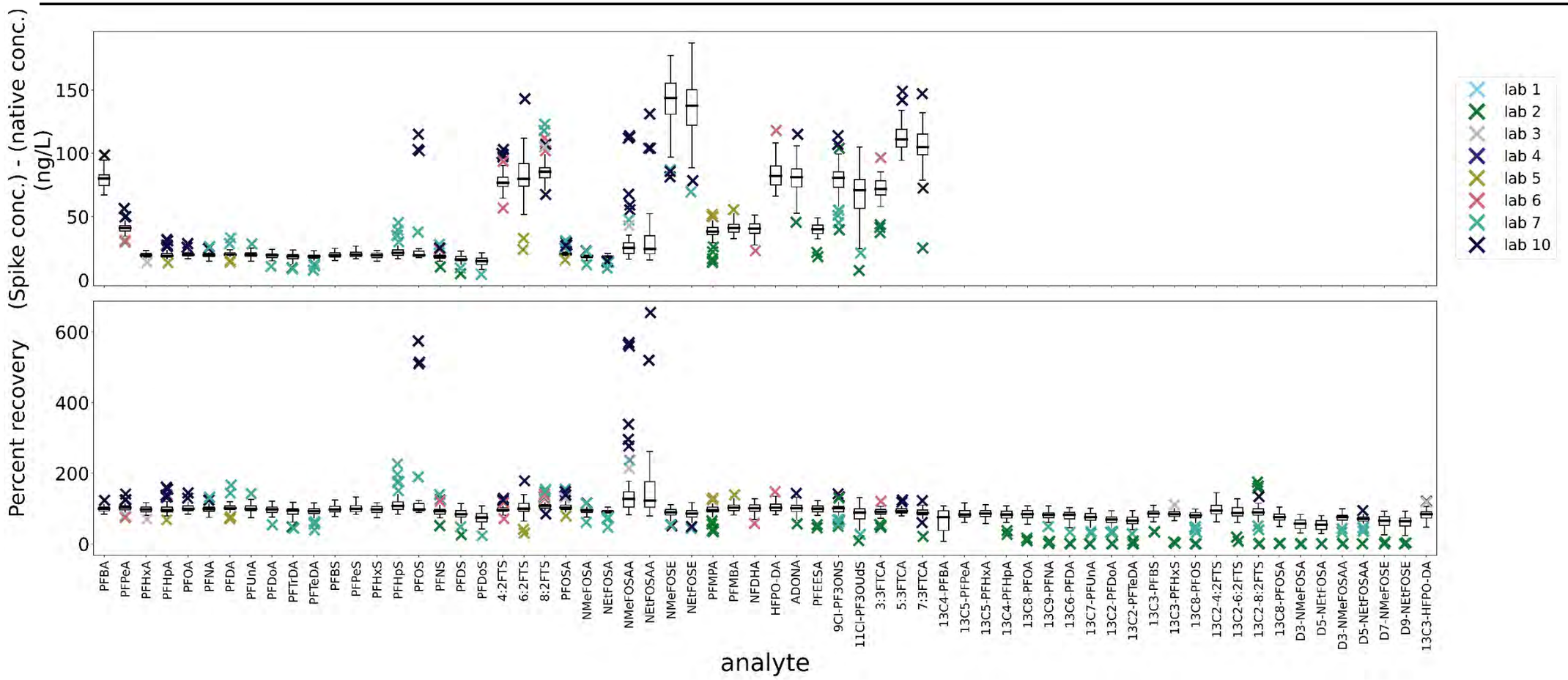


Figure 8-1. Groundwater low spike results by analyte by laboratory.
(B) Spiked concentration minus the laboratory-reported native concentration. (B) Calculated percent recovery. Figure includes both target compound recoveries, and EIS compound recoveries.

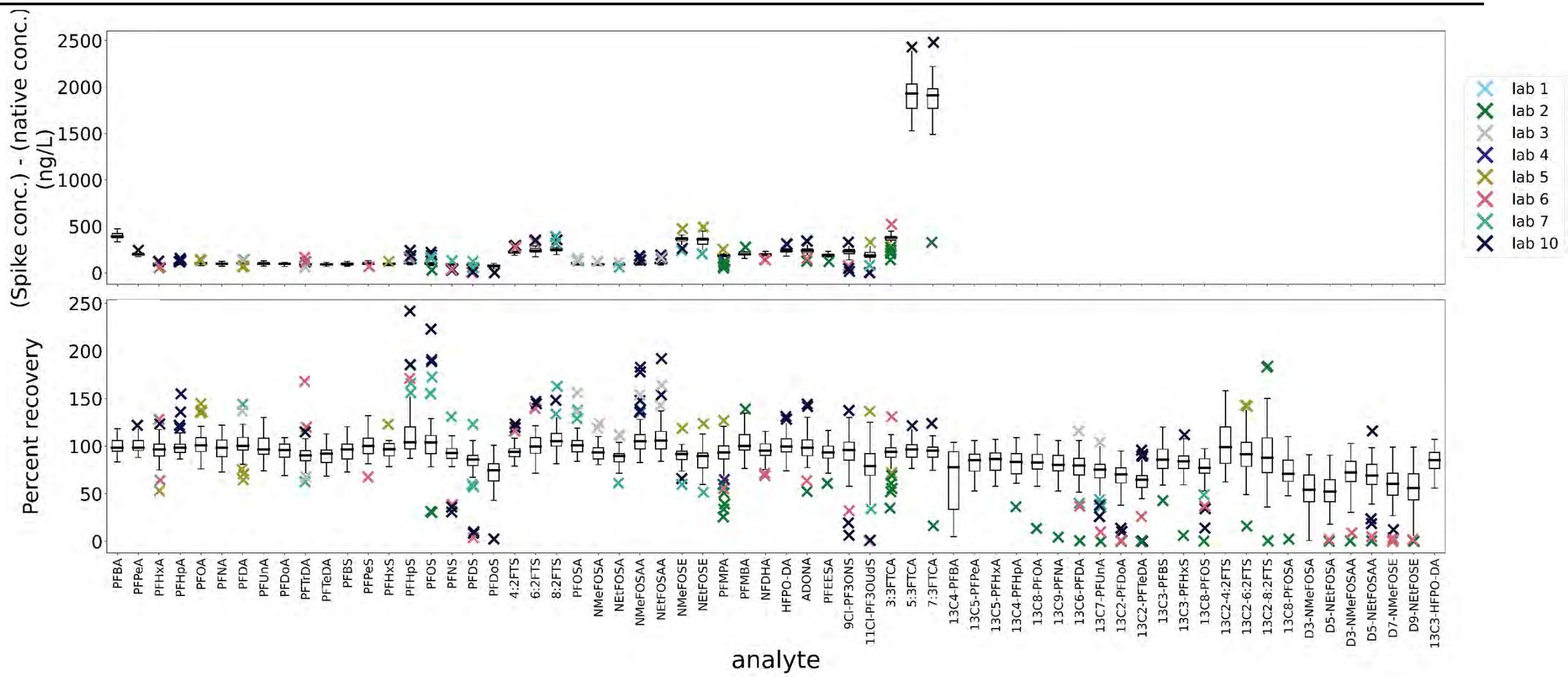


Figure 8-2. Groundwater high spike results by analyte by laboratory.
Spiked concentration minus the laboratory-reported native concentration. (B) Calculated percent recovery.
Figure includes both target compound recoveries, and EIS compound recoveries.

Note: The high spike mass results for 5:3 FTCA and 7:3 FTCA appear higher as these were spiked at 2,000 ng/L, as opposed 100 ng/L for the remaining PFAS

8.3 GROUNDWATER EXTRACTED INTERNAL STANDARD RESULTS

A summary of the EIS compound spike concentrations by laboratory is given in Table 8-6. These spike levels are similar to those for surface water (Table 7-6). Although there were instructions to the contrary in EPA Method 1633, several laboratories ran the surface water and groundwater samples as a single batch. The pooled EIS compound percent recoveries are listed in Table 8-7 and shown on Figures 8-1 and 8-2. Individual laboratory EIS compound recoveries are found in Appendix G, Table G5.

The target recovery limits for EIS compounds defined in the MLVS Method were 20–150%. The combined average for all laboratories fell between 52–98.5%, which is within the limits set for the Study. There was a considerable range of recoveries by individual laboratories ranging from a minimum of 0.3% to a maximum of 242%. As observed for the surface waters, the lowest EIS compound recoveries were from Laboratory 2 (Figures 8-1 through 8-3). This was evident in figures for the low and high spike samples (Figures 8-1 and 8-2) where the EIS compound recoveries were below the median, and in many cases less than 5%. This trend is especially evident for the combined EIS compound data for the unspiked and spiked samples (Figure 8-3). Due to poor EIS compound recoveries (<5%) much of the target compound measures in the spiked samples data from Laboratory 2 were rejected.

The pooled-laboratory statistical analysis results for EIS compounds is in Table 8-7. The lowest mean percent recovery was for D_5-NEtFOSA (52.1%) and the highest for $^{13}C_2$-6:2FTS (98.5%). Notwithstanding the low EIS compound recoveries of Laboratory 2, the pooled between-laboratory (s_b) and within-laboratory standard deviations (s_w) are narrow across all compounds.

Table 8-6. Summary of groundwater EIS compound spike concentrations.

EIS Compound	Spike Concentration (ng/L) - individual laboratories							
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 10
$^{13}C_4$-PFBA	100	80	80	80	100	80	100	100
$^{13}C_5$-PFPeA	50	40	40	40	50	40	50	50
$^{13}C_5$-PFHxA	25	20	20	20	25	20	25	25
$^{13}C_4$-PFHpA	25	20	20	20	25	20	25	25
$^{13}C_8$-PFOA	25	20	20	20	25	20	25	25
$^{13}C_9$-PFNA	12.5	10	10	10	12.5	10	12.5	12.5
$^{13}C_6$-PFDA	12.5	10	10	10	12.5	10	12.5	12.5
$^{13}C_7$-PFUnA	12.5	10	10	10	12.5	10	12.5	12.5
$^{13}C_2$-PFDoA	12.5	10	10	10	12.5	10	12.5	12.5
$^{13}C_2$-PFTeDA	12.5	10	10	10	12.5	10	12.5	12.5
$^{13}C_3$-PFBS	23.3	18.6	18.6	18.6	23.3	18.6	23.3	23.3
$^{13}C_3$-PFHxS	23.7	19	19	19	23.7	19	23.7	23.7
$^{13}C_8$-PFOS	23.9	19.2	19.2	19.2	24	19.2	24	24
$^{13}C_2$-4:2FTS	46.7	37.5	37.5	37.5	46.9	37.5	47	46.9
$^{13}C_2$-6:2FTS	47.5	38	38	38	47.6	38	47.6	47.6
$^{13}C_2$-8:2FTS	47.9	38.4	38.4	38.4	48	38.4	48	48
$^{13}C_8$-PFOSA	25	20	20	20	25	20	25	25
D_3-NMeFOSA	25	20	20	20	25	20	25	25
D_5-NEtFOSA	25	20	20	20	25	20	25	25
D_3-NMeFOSAA	50	40	40	40	50	40	50	50
D_5-NEtFOSAA	50	40	40	40	50	40	50	50
D_7-NMeFOSE	250	200	200	200	250	200	250	250
D_9-NEtFOSE	250	200	200	200	250	200	250	250
$^{13}C_3$-HFPO-DA	100	80	80	80	100	80	100	100

Table 8-7. EIS Compound Results associated with Groundwater Samples

EIS Compound	Number of Labs	Number of Results	Percent Recovery			Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	RSD (s_w)
			Min	Max	Mean			
$^{13}C_4$-PFBA	8	168	4.73	108	65.9	30.8	11.0	16.6
$^{13}C_5$-PFPeA	8	168	34.1	116	83.6	11.4	7.65	9.15
$^{13}C_5$-PFHxA	8	168	4.55	111	84.3	9.83	9.11	10.8
$^{13}C_4$-PFHpA	8	168	0.832	110	81.5	10.1	11.4	14.0
$^{13}C_8$-PFOA	8	168	0.0857	112	82.5	10.0	13.0	15.8
$^{13}C_9$-PFNA	8	168	0.157	110	80.6	8.71	14.4	17.9
$^{13}C_6$-PFDA	8	167	0.133	116	78	9.71	14.8	19.0
$^{13}C_7$-PFUnA	8	167	0.0556	104	72.5	11.4	15.6	21.5
$^{13}C_2$-PFDoA	8	167	0.0292	94.9	66.5	11.7	14.8	22.2
$^{13}C_2$-PFTeDA	8	167	0.003	95.9	62.2	8.92	15.8	25.4
$^{13}C_3$-PFBS	8	168	1.47	120	85.2	10.9	11.9	14.0
$^{13}C_3$-PFHxS	8	171	0.0497	112	83	8.6	13.1	15.8
$^{13}C_8$-PFOS	8	168	0.00469	101	76.2	10.0	16.1	21.1
$^{13}C_2$-4:2FTS	8	168	5.22	158	98.5	16.4	17.4	17.6
$^{13}C_2$-6:2FTS	8	168	0.112	143	89.7	13.3	17.6	19.6
$^{13}C_2$-8:2FTS	8	168	0.0158	242	90.3	15.6	28.2	31.2
$^{13}C_8$-PFOSA	8	168	0.0465	110	74.4	7.74	15.5	20.9
D_3-NMeFOSA	8	168	0.143	90.7	55.3	11.6	13.8	25.0
D_5-NEtFOSA	8	168	0.0502	90.6	52.1	11.5	13.3	25.5
D_3-NMeFOSAA	8	168	0.0681	103	72	9.57	16.0	22.3
D_5-NEtFOSAA	8	168	0.0885	116	68.3	10.1	15.6	22.9
D_7-NMeFOSE	8	168	0.016	99	59.2	13.1	17.0	28.8
D_9-NEtFOSE	8	168	0.0149	98.9	57.3	12.5	18.2	31.7
$^{13}C_3$-HFPO-DA	8	168	5.91	120	83.5	10.4	10.3	12.3

Notes:
Includes all laboratories except Laboratories 8 and 9.
Data excluded where final qualifier = X; Data for Matrix-spiked samples only. EIS compound for QC samples excluded.

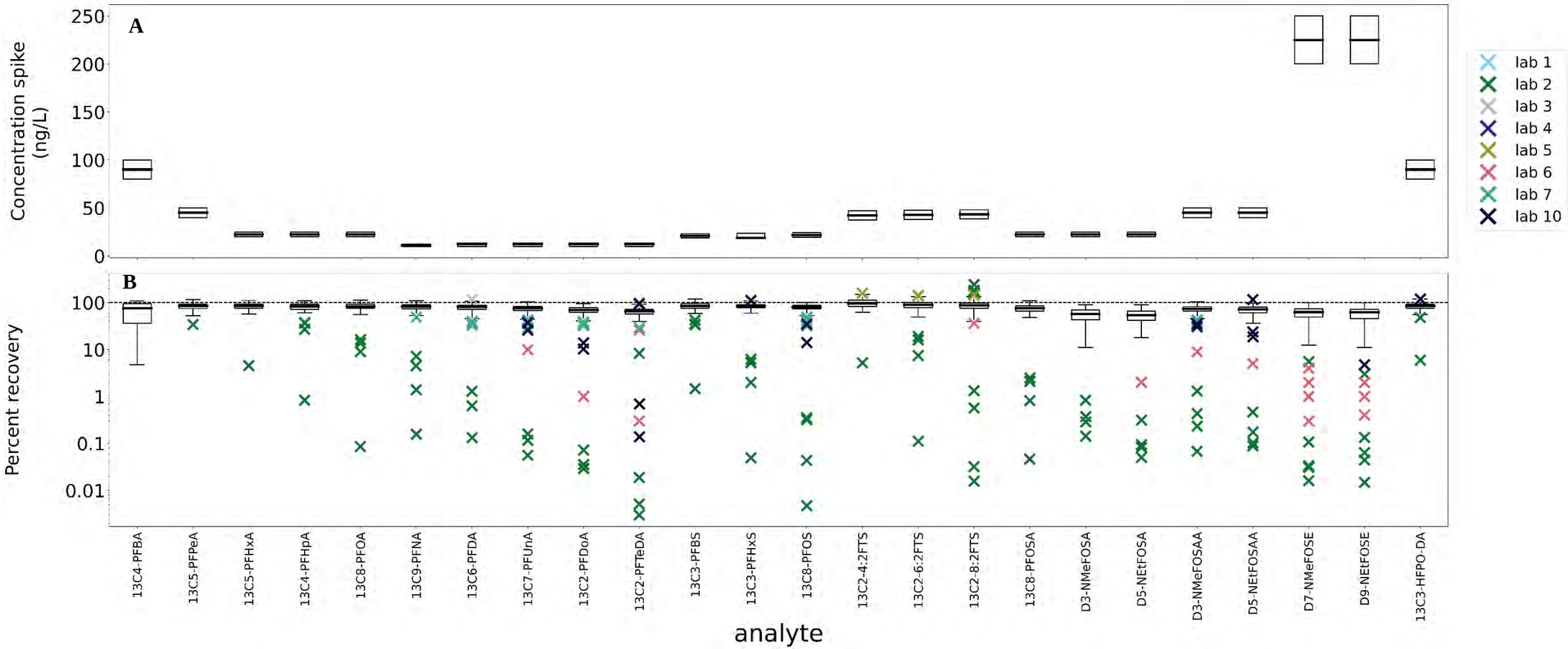


Figure 8-3. Groundwater EIS compound results by compound by laboratory.
(A) Spiked Concentration. (B) Calculated percent recovery.
Figure includes all EIS compound data for unspiked and spiked samples.

8.4 GROUNDWATER SUMMARY

The efficacy of EPA Method 1633 to adequately measure PFAS concentrations in real-world groundwater samples is demonstrated with these results. The following limitations are noted. The pooled spiked target PFAS percent recovery results in Table 8-3 were between 70–130% with the exception of the following compounds: PFOS (157%), NMeFOSAA (153%), and NEtFOSAA (157%). As noted above, the high percent recoveries for PFOS observed in the low-spiked samples skewed the results. PFOS measures for the other laboratories fell within the 70–130% range.

Tables 8-8 and 8-9 provide a summary of the relative proportions for all laboratories that fell between 70–130% percent recovery in the low- and high-spiked samples. For the low-spiked samples, the proportion of all values that were between 70–130% of the spiked concentrations is >70% for most of the 40 analytes. For example, for PFOA and PFOS, the relative proportion of values occurring between 70–130% of the spiked concentration for all laboratories is >80%. The exceptions to this included PFDoS (64%), NMeFOSAA (57%), and NEtFOSAA (55%). For the high-spiked samples, only PFDoS had a lower proportion recovery across all laboratories, but here the relative proportions of recovery were 98.5% across a range of 40–130%.

Table 8-10 provides a summary of the relative proportions for all laboratories that met the study EIS compound target percent recovery acceptance criteria. For the low- and high-spiked samples, the proportion of all values that were between 20–150% of the spiked concentrations is >90%. As shown in Figure 8-3, at least one laboratory had problems with EIS compound recoveries, which resulted in most of that laboratory's data being rejected from the matrix spike analysis.

Table 8-8. Proportion of groundwater matrix spike %recovery results for target analytes within ranges (low-spike samples)

Analyte	Low-Spike Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBA	67	0	0	100	0	0	0
PFPeA	69	0	0	98.6	1.4	0	0
PFHxA	30	0	0	100	0	0	0
PFHpA	69	0	1.4	88.4	7.2	2.9	0
PFOA	45	0	0	97.8	2.2	0	0
PFNA	67	0	0	98.5	1.5	0	0
PFDA	67	0	0	97.0	1.5	1.5	0
PFUnA	67	0	0	98.5	1.5	0	0
PFDoA	67	0	1.5	98.5	0	0	0
PFTrDA	67	0	6.0	94.0	0	0	0
PFTeDA	66	1.5	9.1	89.4	0	0	0
PFBS	24	0	0	100	0	0	0
PFPeS	23	0	0	95.7	4.3	0	0
PFHxS	23	0	0	100	0	0	0
PFHpS	67	0	0	86.6	4.5	7.5	1.5
PFOS	24	0	0	83.3	0	4.2	12.5
PFNS	65	0	1.5	96.9	1.5	0	0
PFDS	65	1.5	10.8	87.7	0	0	0
PFDoS	66	1.5	34.8	**63.6**	0	0	0
4:2FTS	69	0	0	100	0	0	0
6:2FTS	68	1.5	4.4	85.3	7.4	1.5	0
8:2FTS	65	0	0	90.8	7.7	1.5	0
PFOSA	65	0	0	90.8	7.7	1.5	0
NMeFOSA	65	0	1.5	98.5	0	0	0
NEtFOSA	65	0	1.5	98.5	0	0	0
NMeFOSAA	67	0	0	**56.7**	23.9	7.5	11.9
NEtFOSAA	65	0	0	**55.4**	6.2	23.1	15.4
NMeFOSE	64	0	6.2	93.8	0	0	0
NEtFOSE	64	0	15.6	84.4	0	0	0
PFMPA	69	2.9	4.3	91.3	1.4	0	0
PFMBA	69	0	0	97.1	2.9	0	0
NFDHA	69	0	2.9	97.1	0	0	0
HFPO-DA	69	0	0	94.2	5.8	0	0
ADONA	69	0	4.3	91.3	4.3	0	0
PFEESA	69	0	2.9	97.1	0	0	0
9Cl-PF3ONS	67	0	9.0	88.1	3	0	0

Table 8-8. Proportion of groundwater matrix spike %recovery results for target analytes within ranges (low-spike samples)

Analyte	Low-Spike Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
11Cl-PF3OUdS	67	10.4	13.4	74.6	1.5	0	0
3:3FTCA	69	0	4.3	95.7	0	0	0
5:3FTCA	69	0	0	100	0	0	0
7:3FTCA	68	1.5	2.9	95.6	0	0	0

Source file: Exa GW_TRG_PCT_REC_summary_20230512.xls

Table 8-9. Proportion of groundwater matrix spike %recovery results for target analytes within ranges (high-spike samples).

Analyte	High-Spike Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBA	66	0	0	100	0	0	0
PFPeA	69	0	0	100	0	0	0
PFHxA	66	0	3	97	0	0	0
PFHpA	69	0	0	97.1	1.4	1.4	0
PFOA	66	0	0	93.9	6.1	0	0
PFNA	68	0	0	100	0	0	0
PFDA	68	0	1.5	95.6	2.9	0	0
PFUnA	68	0	0	98.5	1.5	0	0
PFDoA	67	0	1.5	98.5	0	0	0
PFTrDA	65	0	3.1	95.4	0	1.5	0
PFTeDA	65	0	3.1	96.9	0	0	0
PFBS	69	0	0	100	0	0	0
PFPeS	68	0	1.5	95.6	2.9	0	0
PFHxS	27	0	0	100	0	0	0
PFHpS	68	0	0	82.4	7.4	8.8	1.5
PFOS	47	4.3	0	83	0	10.6	2.1
PFNS	68	4.4	0	94.1	1.5	0	0
PFDS	68	4.4	5.9	89.7	0	0	0
PFDoS	66	1.5	40.9	57.6	0	0	0
4:2FTS	69	0	0	100	0	0	0
6:2FTS	69	0	0	94.2	5.8	0	0
8:2FTS	65	0	0	92.3	6.2	1.5	0
PFOSA	68	0	0	95.6	2.9	1.5	0
NMeFOSA	68	0	0	100	0	0	0
NEtFOSA	67	0	1.5	98.5	0	0	0

Table 8-9. Proportion of groundwater matrix spike %recovery results for target analytes within ranges (high-spike samples).

Analyte	High-Spike Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
NMeFOSAA	67	0	0	89.6	6	4.5	0
NEtFOSAA	64	0	0	87.5	6.2	6.2	0
NMeFOSE	62	0	3.2	96.8	0	0	0
NEtFOSE	61	0	14.8	85.2	0	0	0
PFMPA	69	4.3	7.2	88.4	0	0	0
PFMBA	69	0	0	95.7	4.3	0	0
NFDHA	69	0	1.4	98.6	0	0	0
HFPO-DA	69	0	0	98.6	1.4	0	0
ADONA	69	0	2.9	91.3	5.8	0	0
PFEESA	69	0	1.4	98.6	0	0	0
9Cl-PF3ONS	68	4.4	7.4	85.3	2.9	0	0
11Cl-PF3OUdS	67	10.4	16.4	71.6	1.5	0	0
3:3FTCA	69	1.4	5.8	91.3	1.4	0	0
5:3FTCA	69	0	0	100	0	0	0
7:3FTCA	69	1.4	0	98.6	0	0	0

file: Exa GW_TRG_PCT_REC_summary_20230512.xls

Table 8-10. Proportion of groundwater matrix %recovery results for EIS compounds within ranges

EIS Compound	All Labs proportion % recovery					
	n	<10%	≥10% to <20%	≥20% to <150%	≥150% to <200%	≥200%
$^{13}C_4$-PFBA	168	3.0	8.3	88.7	0	0
$^{13}C_5$-PFPeA	168	0	0	100	0	0
$^{13}C_5$-PFHxA	168	0.6	0	99.4	0	0
$^{13}C_4$-PFHpA	168	0.6	0	99.4	0	0
$^{13}C_8$-PFOA	168	1.2	1.2	97.6	0	0
$^{13}C_9$-PFNA	168	2.4	0	97.6	0	0
$^{13}C_6$-PFDA	167	1.8	0	98.2	0	0
$^{13}C_7$-PFUnA	167	1.8	0.6	97.6	0	0
$^{13}C_2$-PFDoA	167	2.4	1.2	96.4	0	0
$^{13}C_2$-PFTeDA	167	4.2	0	95.8	0	0
$^{13}C_3$-PFBS	168	0.6	0	99.4	0	0
$^{13}C_3$-PFHxS	171	2.3	0	97.7	0	0
$^{13}C_8$-PFOS	168	2.4	0.6	97.0	0	0
$^{13}C_2$-4:2FTS	168	0.6	0	98.2	1.2	0
$^{13}C_2$-6:2FTS	168	1.2	1.2	97.6	0	0
$^{13}C_2$-8:2FTS	168	2.4	0	92.9	4.2	0.6
$^{13}C_8$-PFOSA	168	2.4	0	97.6	0	0
D_3-NMeFOSA	168	2.4	0.6	97.0	0	0
D_5-NEtFOSA	168	3.0	1.2	95.8	0	0
D_3-NMeFOSAA	168	3.0	0	97.0	0	0
D_5-NEtFOSAA	168	3.0	0.6	96.4	0	0
D_7-NMeFOSE	168	5.4	0.6	94.0	0	0
D_9-NEtFOSE	168	6.0	1.2	92.9	0	0
$^{13}C_3$-HFPO-DA	168	0.6	0	99.4	0	0

file: Exa Groundwater EIS 20230512.xls

9 SUMMARY FOR WASTEWATER, GROUNDWATER, AND SURFACE WATER

9.1 PREPARATORY BATCH QC

Per EPA Method 1633, a sample preparation batch consists of up to 20 study samples, a method blank, an OPR sample, and an LLOPR sample. Due to EIS compound spiking errors all wastewater data from Laboratories 8 and 10 were omitted from the statistical analysis. In addition, the same EIS compound spiking error affected all groundwater and surface water from Laboratory 8; therefore, these data were also omitted from the statistical analysis. Laboratory 9 stated in their data packages that a sample preparation error occurred that affected one of their preparatory batches that contained both groundwater and surface water samples. During the extraction process, these samples were loaded onto the SPE too quickly. As a result, the contact time the sample had with the SPE media was insufficient for analyte retention, as indicated by the extremely low EIS compound recoveries. Because the cause of these failures was human error, all results associated with this preparation batch were omitted from the statistical analysis, including method blank, LLOPR, and OPR. Method blank, OPR, and LLOPR discussions contained in Section 6 relate to the data sets that resulted from these exclusions.

The MLVS Method did not prescribe definitive acceptance criteria for OPR, LLOPR, NIS, and EIS compound recoveries; however, it did provide target acceptance criteria. The target percent recovery for target analytes in OPRs and LLOPRs was 40–150%, 20–150% for EIS compounds, and greater than 30% for NIS compounds. These target criteria were based on the results from the SLVS. Since the statistical evaluation from the MLVS will be the basis for the acceptance criteria included in future versions of EPA Method 1633, the laboratories were instructed to follow their routine corrective action process when the target criteria were not met. This includes reanalysis and dilution. If the reanalysis or dilution met the target criteria, the reanalysis was reported, otherwise, the first analysis was reported. By doing so, results that were extremely biased due to events such as a miss-injection or carryover, were eliminated from the statistical analysis.

9.1.1 Method Blank

Method blanks are included in the method to evaluate the potential for background contamination to be introduced during sample preparation in the laboratory. A 500-mL aliquot of PFAS-free reagent water was used to prepare each method blank associated with wastewater, groundwater, and surface water samples and all were prepared in exactly the same manner as study samples. A total of 57 method blanks were included in the statistical analysis.

Of these 57 method blanks, eight included detections of target analyte concentrations above the laboratories' MDLs. All but two of these reported concentrations were above the laboratories' MDL, but below the laboratories' LOQ. All eight method blanks with detections were associated with three laboratories: Laboratories 3, 5, and 9 (Table 9-1). The low rate of detection in method blanks demonstrated by this study, 18 out of 2,282 target analytes reported (0.79%) indicates the processes described in the method are successful in reducing the potential for bias associated with contamination.

Table 9-1. Method Blank Detection Summary

Matrix of Associated Samples	Laboratory ID	Target Analyte	# of Occurrences	Concentrations (ng/L)
WW	3	NEtFOSA	2	1.2 J, 1.2 J
WW	3	NMeFOSA	2	0.96 J, 0.88 J
WW	5	PFBA	1	0.801 J
WW	5	PFOA	3	0.552 J, 0.596 J, 0.7 JB
WW	5	PFOS	3	0.497 J, 0.762 J, 0.985 JB
WW	5	PFOSA	3	0.993 J, 1.35 JB, 0.926 JB
WW	9	PFOSA	1	1.5
SW & GW	5	6:2FTS	1	1.91 JB
SW	9	PFHxA	1	0.179 J
SW	9	PFOSA	1	12.4 B

Source File: Chapter 9 GW SW WW summary 06082023.xlx

Notes:
J = Analyte concentration >MDL but <LOQ; estimated value.
B = The concentration found in the method blank was ≥ ½ LOQ and ≥ 1/10th the concentration of the target analyte in an associated sample.

The concentration of each target analyte in the method blank was required to be <½ the laboratory's LOQ or <1/10th the concentration of the target method in associated samples. When a method blank failed to meet this criterion, the laboratory applied a "B" data qualifier to the result for the affected target method in the associated sample. Four out of the 57 method blanks reported failed to meet the study criteria. One method blank reported by Laboratory 5 failed to meet the study criteria for PFOA and PFOSA. The concentrations of PFOA and PFOSA that were detected in this method blank fell between the MDL and the LOQ; therefore, a "J" qualifier was applied to these results in the method blank as an indication these concentrations are qualitative. As a result of these detections, a "B" qualifier was applied to PFOA results in two associated samples and PFOSA results were qualified in five associated samples. Another method blank reported by Laboratory 5 failed to meet the study criteria for 6:2FTS. The concentration of 6:2FTS that was detected in this method blank fell between the MDL and the LOQ, therefore a "J" qualifier was applied to this result in the method blank and a "B" qualifier was applied to the 6:2FTS result in one associated sample. The one other method blank that did not meet the study criteria was reported by Laboratory 9 for PFOSA. As a result of this detection, a "B" qualifier was applied to the PFOSA result in one associated sample. In cases where the concentration of the detected target analyte in the method blank was greater than 1/5th the concentration of the target method in these sample, per the data validation guidelines, a "J+" data qualifier was applied to the target analyte in these samples to indicate these results are potentially biased high. A summary of the affected data is presented in Table 9-2.

Table 9-2. Samples Qualified Due to Method Blank Contamination

Sample Number	Number of Instances	Target method	Target method Concentration (ng/L)	Associated Method Blank Concentration(ng/L)
WWI1	1	PFOA	2.45 JB+	0.70 JB
WWI1	1	PFOSA	0.746 JB+	0.926 JB
WWJ1	1	PFOSA	0.397 JB+	0.926 JB
WWM1	1	PFOSA	0.359 JB+	0.926 JB
WWN1	1	PFOA	1.82 JB+	0.70 JB
WWN1	1	PFOSA	0.344 JB+	0.926 JB
WWO1	1	PFOSA	0.228 JB+	0.926 JB
SWF2	1	PFOSA	54.5 JB+	12.4 B
SWG2	1	PFOSA	53.7 JB+	12.4 B
GWC1	1	6:2FTS	3.02 JB+	1.91 JB

Source File: Chapter 9 GW SW WW summary 06082023.xlx

Notes:

J = Analyte concentration >MDL but <LOQ; estimated value.

B+ = Estimated value due analyte concentration being greater than the MDL but less than or equal to five times the concentration detected in the Method Blank.

B = The concentration found in the method blank was ≥ ½ LOQ and ≥ 1/10th the concentration of the target analyte in an associated sample.

Method blank contamination resulted in the "B" qualification of 10 results out of 27,024 wastewater, groundwater, and surface water sample results reported. Thus, these measured concentrations were only sufficient to warrant "B" flags for what ultimately represented <0.037% of the final data set. The method blanks demonstrate that any bias associated with background contamination introduced during sample preparation was negligible.

9.1.2 Ongoing Precision and Recovery Analyses

OPR samples, sometimes referred to in other methods as Laboratory Control Samples (LCS), were included in the method to evaluate the efficiency of the sample preparation process. An OPR was included in each preparation batch, which consisted of a 500-mL aliquot of PFAS-free reagent water that was spiked with all 40 target analytes such that the final concentration of each PFAS in the OPR was greater than or equal to the LOQ and less than or equal to the midpoint of the laboratory's calibration. This spiked aliquot of PFAS-free reagent water was prepared and analyzed in exactly the same manner as study samples.

OPR recoveries across all media for all labs was relatively tight, generally at or above 90% with narrow pooled between laboratory standard deviation (s_b), within laboratory standard deviation (s_w), and Relative Standard Deviation (RSD). (Table 9-3, Figure 9-1A). The concentration the OPR was spiked at by each laboratory did not vary greatly (Figure 9-1B).

A total of 58 OPRs were included in the statistical analysis. All 58 OPRs met the study OPR NIS criteria (>30% recovery). Of the 2,320 target analyte results reported from OPRs, two failed to

meet the target analyte criteria (40–150%), resulting in a failure rate of 0.086%. Laboratory 5 reported two target analyte exceedances in one OPR: 9Cl-PF3ONS (155%) and 11Cl-PF3OUdS (157%). Of the 1,392 EIS compound results reported from OPRs, three failed to meet the EIS compound acceptance criteria (20–150%), resulting in a failure rate of 0.21%. Laboratory 3 reported an exceedance of one EIS compound, D_3-NMeFOSAA, in each of the three OPRs they reported, with recoveries of 187%, 205%, and 205%. Overall, the recoveries of Laboratory 6 OPR recoveries trended lower than all other laboratories, while those of Laboratory 9 exhibited slightly higher OPR recoveries than most (Figure 9-1A).

Following EPA guidance (EPA 821-B-18-001), lower and upper percent recovery limits for target analytes were generated (Table 9-4). The lower percent recovery limit is the mean % recovery minus two times the RSD and the upper percent recovery limit is the mean % recovery plus two times the RSD. All statistically derived lower control limits are greater than MLVS target lower limit of 40% and all upper control limits are lower than the MLVS target upper limit of 150%. In addition, all lower limits are greater than 70% with the exception of PFDoS (63%) and 7:3FTCA (68%), and all upper limits were less than or equal to 130% with the exception of NFDHA (137%).

9.1.3 Low-Level Ongoing Precision and Recovery Analyses

LLOPR samples, sometimes referred to as Low-Level Laboratory Control Samples (LLLCS), were included in the method to evaluate the efficiency of the sample preparation process. An LLOPR was included in each preparation batch, consisting of a 500-mL aliquot of PFAS-free reagent water that was spiked with all 40 target analytes such that the final concentration of each PFAS in the LLOPR was two times the laboratory's LOQ. This spiked aliquot of PFAS-free reagent water was prepared and analyzed in exactly the same manner as study samples.

All of the 57 LLOPRs included in the statistical analysis met the study LLOPR NIS compound recovery criteria (>30%). Of the 2,280 target analyte results reported from LLOPRs, seven failed to meet the target analyte criteria (40 – 150%), resulting in a failure rate of 0.31%. Laboratory 5 reported one target analyte exceedances in one LLOPR, 9Cl-PF3ONS (160%), while Laboratory 9 reported six exceedances, with one exceedance in one LLOPR (PFOSA, 176%) and five in another LLOPR (3:3FTCA (151%), PFOS (152%), NMeFOSA (153%), 5:3FTCA (156%), and NEtFOSA (167%)). Of the 1,368 EIS compound results reported from LLOPRs, two failed to meet the EIS compound criteria, resulting in a failure rate of 0.14%. Laboratory 3 reported an exceedance of one EIS compound, D_3-NMeFOSAA, in two of the three LLOPRs they reported, with recoveries of 183% and 191%. These low failure rates demonstrate the target criteria adopted by this study are routinely achievable. A summary of the LLOPR target analyte and EIS compound recoveries is presented in Table 9-5. Overall, Laboratory 9 exhibited slightly higher LLOPR recoveries than most (Figure 9-2).

Table 9-3. Summary of Aqueous OPR Percent Recoveries

Analytes	Number of Labs	Number of Results	Mean % Recovery	s_b	s_w	s_c	RSD
PFBA	9	58	103	9.56	7.54	12.2	7.29
PFPeA	9	58	104	9.75	9.12	13.3	8.8
PFHxA	9	58	103	8.44	9.97	12.8	9.65
PFHpA	9	58	103	8.78	9.43	12.7	9.15
PFOA	9	58	108	9.37	11.4	14.4	10.5
PFNA	9	58	105	10.7	11.0	15.2	10.5
PFDA	9	58	103	9.92	10.1	14.0	9.85
PFUnA	9	58	104	8.47	10.5	13.1	10.1
PFDoA	9	58	104	11.7	11.5	16.2	11.1
PFTrDA	9	58	103	13.7	10.2	17.2	9.92
PFTeDA	9	58	107	10.3	11.4	15.1	10.7
PFBS	9	58	102	11.0	10.9	15.4	10.7
PFPeS	9	58	102	11.5	8.37	14.4	8.17
PFHxS	9	58	101	9.94	11.6	15.0	11.5
PFHpS	9	58	103	10.2	9.17	13.7	8.88
PFOS	9	58	103	9.35	10.7	13.9	10.4
PFNS	9	58	102	11.1	11.6	15.8	11.4
PFDS	9	58	98.9	10.3	11.0	14.8	11.2
PFDoS	9	58	89.0	11.6	11.5	16.1	12.9
4:2FTS	9	58	106	10.3	11.9	15.4	11.2
6:2FTS	9	58	109	8.09	17.0	17.8	15.6
8:2FTS	9	58	108	10.3	11.3	15.0	10.5
PFOSA	9	58	105	9.81	8.33	12.9	7.95
NMeFOSA	9	58	108	11.9	12.1	16.8	11.2
NEtFOSA	9	58	106	10.8	9.32	14.3	8.84
NMeFOSAA	9	58	102	12.8	12.1	17.5	11.9
NEtFOSAA	9	58	102	7.40	9.46	11.7	9.3
NMeFOSE	9	58	107	9.09	9.82	13.2	9.18
NEtFOSE	9	58	106	8.71	8.85	12.3	8.32
PFMPA	9	58	102	12.9	9.39	16.1	9.23
PFMBA	9	58	106	10.3	10.3	14.4	9.7
NFDHA	9	58	105	8.52	16.6	17.7	15.9
HFPO-DA	9	58	107	10.1	8.44	13.1	7.89
ADONA	9	58	108	10.1	9.9	14.0	9.15
PFEESA	9	58	104	9.24	10.0	13.4	9.65
9Cl-PF3ONS	9	58	109	12.1	11.6	16.6	10.7
11Cl-PF3OUdS	9	58	104	15.3	12.5	19.8	12.0
3:3FTCA	9	58	95.8	6.97	9.17	11.2	9.58
5:3FTCA	9	58	99.2	10.1	8.47	13.2	8.53
7:3FTCA	9	58	92.4	10.7	11.2	15.3	12.1
$^{13}C_4$-PFBA	9	58	84.9	9.18	13.5	15.8	16.0
$^{13}C_5$-PFPeA	9	58	88.4	8.51	9.18	12.3	10.4
$^{13}C_5$-PFHxA	9	58	88.1	8.35	6.33	10.5	7.18
$^{13}C_4$-PFHpA	9	58	86.4	7.28	7.99	10.6	9.25

Table 9-3. Summary of Aqueous OPR Percent Recoveries

Analytes	Number of Labs	Number of Results	Mean % Recovery	s_b	s_w	s_c	RSD
$^{13}C_8$-PFOA	9	58	87.9	5.83	8.68	10.1	9.88
$^{13}C_9$-PFNA	9	58	87.7	6.73	6.75	9.43	7.7
$^{13}C_6$-PFDA	9	58	87.9	7.09	8.86	11.1	10.1
$^{13}C_7$-PFUnA	9	58	85.7	5.96	10.6	11.6	12.3
$^{13}C_2$-PFDoA	9	58	82.1	8.23	10.6	13.0	12.9
$^{13}C_2$-PFTeDA	9	58	74.3	8.11	9.35	12.1	12.6
$^{13}C_3$-PFBS	9	58	87.7	8.43	9.63	12.5	11.0
$^{13}C_3$-PFHxS	9	58	87.9	7.62	9.82	12.1	11.2
$^{13}C_8$-PFOS	9	58	85.5	6.77	6.88	9.53	8.04
$^{13}C_2$-4:2FTS	9	58	101	11.8	14.3	18.1	14.1
$^{13}C_2$-6:2FTS	9	58	93.2	9.88	15.4	17.5	16.5
$^{13}C_2$-8:2FTS	9	58	93.6	10.4	14.0	16.9	15.0
$^{13}C_8$-PFOSA	9	58	75.4	11.3	8.79	14.4	11.7
D_3-NMeFOSA	9	58	55.7	13.8	9.04	16.8	16.2
D_5-NEtFOSA	9	58	53.2	13.8	8.45	16.5	15.9
D_3-NMeFOSAA	9	58	87.2	17.3	23.2	28.0	26.6
D_5-NEtFOSAA	9	58	80.5	6.65	8.62	10.6	10.7
D_7-NMeFOSE	9	58	64.6	15.1	7.53	17.4	11.6
D_9-NEtFOSE	9	58	64.0	15.7	7.64	18.0	11.9
$^{13}C_3$-HFPO-DA	9	58	86.0	8.09	7.58	11.0	8.82

Source file: OPR_results_V1_230607_124749.csv

Notes:
s_b - The pooled between lab standard deviation of the percent recovery. Equation from EPA 821-B-18-001 page G-25.
s_w - The pooled within-laboratory standard deviation. Equation from EPA 821-B-18-001 page G-25.
sc - The combined within and between lab standard deviations. Equation EPA 821-B-18-001 page G-26
RSD - The pooled within-laboratory relative standard deviation (RSD, (s_w/(mean % recovery) *100).

Table 9-4. Statistically Derived OPR Acceptance Criteria

Analytes	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
PFBA	103	14.6	88	118
PFPeA	104	17.6	86	122
PFHxA	103	19.3	84	122
PFHpA	103	18.3	85	121
PFOA	108	21.0	87	129
PFNA	105	21.0	84	126
PFDA	103	19.7	83	123
PFUnA	104	20.2	84	124
PFDoA	104	22.2	82	126
PFTrDA	103	19.84	83	123
PFTeDA	107	21.4	86	128
PFBS	102	21.4	81	123
PFPeS	102	16.34	86	118
PFHxS	101	23.0	78	124
PFHpS	103	17.76	85	121
PFOS	103	20.8	82	124
PFNS	102	22.8	79	125
PFDS	98.9	22.4	77	121
PFDoS	89	25.8	63	115
4:2FTS	106	22.4	84	128
6:2FTS	109	31.2	78	140
8:2FTS	108	21.0	87	129
PFOSA	105	15.9	89	121
NMeFOSA	108	22.4	86	130
NEtFOSA	106	17.68	88	124
NMeFOSAA	102	23.8	78	126
NEtFOSAA	102	18.6	83	121
NMeFOSE	107	18.36	89	125
NEtFOSE	106	16.64	89	123
PFMPA	102	18.46	84	120
PFMBA	106	19.4	87	125
NFDHA	105	31.8	73	137
HFPO-DA	107	15.78	91	123
ADONA	108	18.3	90	126

Table 9-4. Statistically Derived OPR Acceptance Criteria

Analytes	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
PFEESA	104	19.3	85	123
9Cl-PF3ONS	109	21.4	88	130
11Cl-PF3OUdS	104	24.0	80	128
3:3FTCA	95.8	19.16	77	115
5:3FTCA	99.2	17.06	82	116
7:3FTCA	92.4	24.2	68	116

Source: RSDs from OPR_results_V1_230607_124749.csv

Notes:

[1] Two times the pooled within-laboratory relative standard deviation (RSD, (sw/(mean % recovery) *100)

[2] Lower % Recovery acceptance limit calculated as the Mean % Recovery – (2 x RSD) expressed as whole number.

[3] Upper % Recovery acceptance limit calculated as the Mean % Recovery – (2 x RSD) expressed as whole number.

Table 9-5. Aqueous LLOPR Results Summary

Analyte	Number of Labs	Number of Results	Minimum Concentration (ng/L)	Maximum Concentration (ng/L)	Mean % Recovery	s_b	s_w	s_c	RSD
PFBA	9	57	8.56	29.5	103	9.99	9.17	13.5	8.92
PFPeA	9	57	4.17	14.9	105	9.75	9.79	13.6	9.34
PFHxA	9	57	2.17	7.83	109	9.96	11.3	14.8	10.4
PFHpA	9	57	2.08	8.14	107	10.8	14.9	17.8	14
PFOA	9	57	2.24	8.87	113	12.3	13.2	17.7	11.7
PFNA	9	57	2.09	7.67	108	10.8	13.1	16.6	12.2
PFDA	9	57	2.35	7.31	103	12.3	10.8	16.3	10.5
PFUnA	9	57	2.12	7.57	106	8.88	14.2	16.1	13.4
PFDoA	9	57	2.13	8.06	104	8.80	11.6	14.1	11.1
PFTrDA	9	57	2.09	7.73	102	11.6	12.1	16.5	11.9
PFTeDA	9	57	2.15	7.53	109	11.9	11.8	16.6	10.8
PFBS	9	57	1.69	6.82	106	10.4	11.3	15.1	10.6
PFPeS	9	57	2.01	6.82	102	9.57	9.11	13.1	8.89
PFHxS	9	57	2.03	7.17	106	11.0	11.0	15.4	10.3
PFHpS	9	57	1.98	8.11	106	8.33	13.8	15.4	13
PFOS	9	57	2.04	7.73	108	10.0	13.9	16.6	12.9
PFNS	9	57	2.12	7.05	104	9.39	13.5	15.9	13
PFDS	9	57	1.73	6.83	101	9.58	14.0	16.4	13.9
PFDoS	9	57	1.71	6.45	88.3	14.5	12.7	19.2	14.3
4:2FTS	9	57	8.35	28.2	108	10.4	12.3	15.7	11.4
6:2FTS	9	57	7.97	32.4	110	10.4	14.3	17.1	13.1
8:2FTS	9	57	8.86	33.2	110	11.6	14.7	18.1	13.3
PFOSA	9	57	2.26	7.65	107	14.3	13.3	19.4	12.4
NMeFOSA	9	57	2.24	7.90	110	12.4	12.7	17.5	11.6
NEtFOSA	9	57	2.5	7.97	107	13.7	14.2	19.5	13.2
NMeFOSAA	9	57	1.44	7.88	101	16.5	14.0	21.6	13.9
NEtFOSAA	9	57	2.32	8.57	105	10.5	13.3	16.5	12.6
NMeFOSE	9	57	22.9	73.5	108	12.5	10.8	16.5	10.1
NEtFOSE	9	57	22.8	71.9	108	12.1	12.5	17.2	11.6
PFMPA	9	57	3.34	14.4	104	11.2	12.3	16.3	11.9
PFMBA	9	57	3.96	14.7	107	13.9	11.2	17.8	10.4
NFDHA	9	57	4.03	16.5	108	8.99	13.3	15.5	12.4
HFPO-DA	9	57	9.57	31.3	108	9.65	8.89	13.0	8.22
ADONA	9	57	8.85	29.0	109	9.59	9.26	13.2	8.52
PFEESA	9	57	3.44	13.2	105	9.21	9.15	12.8	8.69
9Cl-PF3ONS	9	57	8.51	29.3	109	15.2	11.6	19.3	10.7
11Cl-PF3OUdS	9	57	7.52	25.3	102	18.0	13.1	22.4	12.9
3:3FTCA	9	57	12.0	34.1	98.7	19.1	9.92	22.1	10.1
5:3FTCA	9	57	63.6	186	101	17.8	8.47	20.3	8.4
7:3FTCA	9	57	41.4	185	93.7	17.4	12.8	21.8	13.7
$^{13}C_4$-PFBA	9	57	40.9	101	85.1	9.97	11.2	14.7	13.2
$^{13}C_5$-PFPeA	9	57	26.5	55.3	89.1	7.36	9.04	11.4	10.1
$^{13}C_5$-PFHxA	9	57	13.4	25	88.1	6.39	7.07	9.35	8.03

Table 9-5. Aqueous LLOPR Results Summary

Analyte	Number of Labs	Number of Results	Minimum Concentration (ng/L)	Maximum Concentration (ng/L)	Mean % Recovery	s_b	s_w	s_c	RSD
$^{13}C_4$-PFHpA	9	57	13.0	28.4	86.4	7.5	9.33	11.7	10.8
$^{13}C_8$-PFOA	9	57	10.4	28.5	87.3	6.54	9.83	11.4	11.3
$^{13}C_9$-PFNA	9	57	6.43	13.0	87.2	7.48	9.61	11.8	11.0
$^{13}C_6$-PFDA	9	57	6.31	13.8	87.8	6.84	10.0	11.7	11.4
$^{13}C_7$-PFUnA	9	57	5.91	13.2	85.8	6.51	10.1	11.5	11.8
$^{13}C_2$-PFDoA	9	57	5.61	13.4	80.6	8.14	11.3	13.5	14.1
$^{13}C_2$-PFTeDA	9	57	4.55	11.8	72.7	8.88	9.96	13.1	13.7
$^{13}C_3$-PFBS	9	57	12.3	24.0	86.3	8.48	8.34	11.8	9.67
$^{13}C_3$-PFHxS	9	57	11.8	25.6	87.7	7.53	8.52	11.1	9.72
$^{13}C_8$-PFOS	9	57	11.2	24.4	84.5	8.08	8.50	11.6	10.1
$^{13}C_2$-4:2FTS	9	57	25.1	85.0	103	12.9	17.2	20.8	16.6
$^{13}C_2$-6:2FTS	9	57	21.7	81.1	95.8	12	15.5	19	16.2
$^{13}C_2$-8:2FTS	9	57	22.3	88.0	93.7	15.3	18.0	23.1	19.2
$^{13}C_8$-PFOSA	9	57	9.69	22.2	74.2	10.2	6.97	12.5	9.39
D_3-NMeFOSA	9	57	4.69	19.0	52.8	13.8	7.22	15.9	13.7
D_5-NEtFOSA	9	57	4.19	18.8	50.8	13.9	7.32	16.2	14.4
D_3-NMeFOSAA	9	57	20.8	76.2	85.0	16.0	18.5	24	21.8
D_5-NEtFOSAA	9	57	21.6	50	78.0	6.73	8.75	10.7	11.2
D_7-NMeFOSE	9	57	64.6	216	64.3	15.2	9.43	18.2	14.7
D_9-NEtFOSE	9	57	59.5	206	63.2	15.0	8.74	17.8	13.8
$^{13}C_3$-HFPO-DA	9	57	54.0	103	87.3	6.97	8.41	10.7	9.63

Source File: LLOPR_results_V1_230607_124655.csv

Notes:

Min Concentration (ng/L) - The minimum concentration measured across all laboratories.

Max Concentration (ng/L) - The maximum concentration measured across all laboratories.

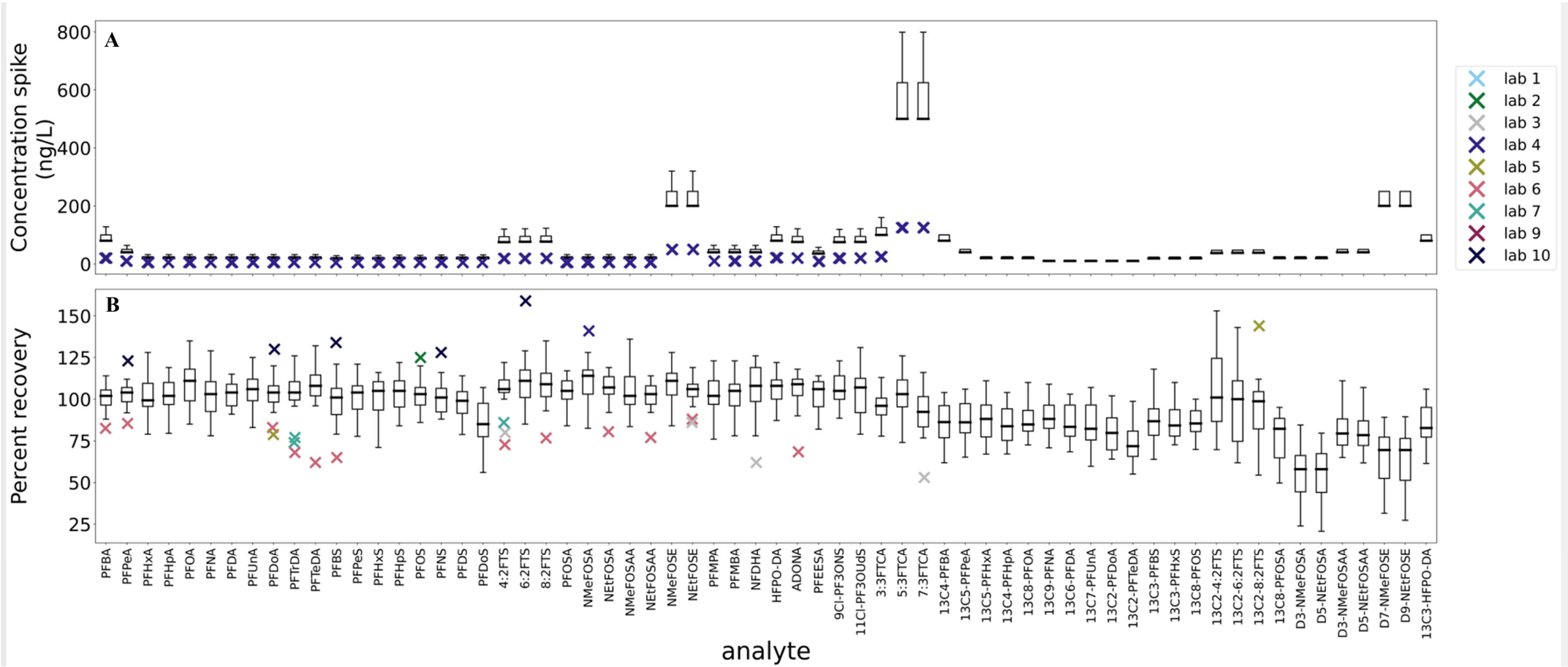
Mean % Recovery - The mean percent recovery across all samples across all laboratories.

s_b - The pooled between laboratory standard deviation of the percent recovery. Equation EPA 821-B-18-001 page G-25.

s_w - The pooled within-laboratory standard deviation of the percent recovery. Equation EPA 821-B-18-001 page G-25.

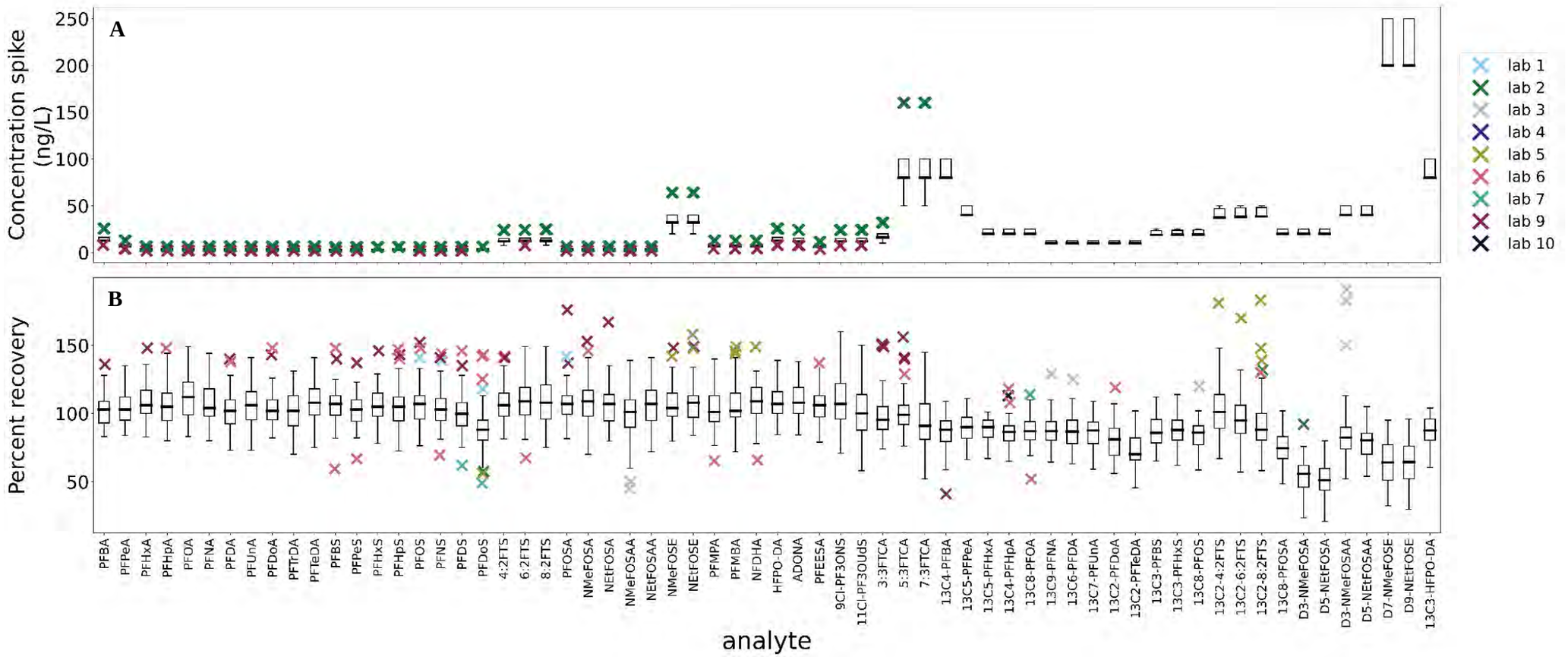
s_c - The combined within and between-laboratory standard deviations. Equation from EPA 821-B-18-001 page G-26.

RSD - The pooled within-laboratory relative standard deviation (RSD, (s_w/(mean % recovery) *100).



Source File: IDA file: All_OPR_Boxplot_V1_230607.

Figure 9-1. Wastewater, Surface Water, and Groundwater OPR results by compound by laboratory.
(A) Spiked Concentration. (B) Calculated percent recovery.
Figure includes all OPR data batched with unspiked and spiked samples.



Source File: IDA file: All_LLOPR_Boxplot_V1_230607.

Figure 9-2. Wastewater, Surface Water, and Groundwater LLOPR results by compound by laboratory.
(A) Spiked Concentration. (B) Calculated percent recovery.
Figure includes all LLOPR data batched with unspiked and spiked samples.

Following EPA guidance (EPA 821-B-18-001), the LLOPR percent recovery and RSD values in Table 9-5 were used to calculate lower and upper percent recovery limits for target analytes (Table 9-6). The lower percent recovery limit is the mean % recovery minus two times the RSD and the upper percent recovery limit is the mean % recovery plus two times the RSD. All statistically derived lower control limits are greater than MLVS target lower limit of 40% and all statistically derived upper control limits are lower than the MLVS target upper limit of 150%. In addition, all of the statistically derived lower control limits are greater than 70% with the exception of 7:3FTCA (66%) and PFDoS (60%). Multiple (15/40) statistically derived upper control limits exceeded 130% but all were less than 140%.

Utilizing the lower of the two statistically derived lower control limits (OPR and LLOPR) and upper control limits (OPR and LLOPR), a single % recovery acceptance criteria range is presented in Table 9-7.

Table 9-6. Statistically Derived LLOPR Acceptance Criteria

Analyte	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
PFBA	103	17.84	85	120
PFPeA	105	18.68	86	124
PFHxA	109	20.8	88	130
PFHpA	107	28	79	135
PFOA	113	23.4	90	136
PFNA	108	24.4	84	132
PFDA	103	21	82	124
PFUnA	106	26.8	79	133
PFDoA	104	22.2	82	126
PFTrDA	102	23.8	78	126
PFTeDA	109	21.6	87	131
PFBS	106	21.2	85	127
PFPeS	102	17.78	84	120
PFHxS	106	20.6	85	127
PFHpS	106	26	80	132
PFOS	108	25.8	82	134
PFNS	104	26	78	130
PFDS	101	27.8	73	129
PFDoS	88.3	28.6	60	117
4:2FTS	108	22.8	84	131
6:2FTS	110	26.2	78	136
8:2FTS	110	26.6	83	137
PFOSA	107	24.8	82	132
NMeFOSA	110	23.2	86	133
NEtFOSA	107	26.4	81	133

Table 9-6. Statistically Derived LLOPR Acceptance Criteria

Analyte	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
NMeFOSAA	101	27.8	73	129
NEtFOSAA	105	25.2	80	130
NMeFOSE	108	20.2	88	128
NEtFOSE	108	23.2	85	131
PFMPA	104	23.8	80	128
PFMBA	107	20.8	86	128
NFDHA	108	24.8	73	133
HFPO-DA	108	16.44	91	124
ADONA	109	17.04	90	126
PFEESA	105	17.38	85	122
9Cl-PF3ONS	109	21.4	88	130
11Cl-PF3OUdS	102	25.8	76	128
3:3FTCA	98.7	20.2	77	119
5:3FTCA	101	16.8	82	118
7:3FTCA	93.7	27.4	66	121

Source File: Chapter 9 GW SW WW summary 06082023.xlx

Notes:

[1] Two times the pooled within-laboratory relative standard deviation (RSD, (sw/(mean % recovery) *
[2] Lower % Recovery acceptance limit calculated as the Mean % Recovery – (2 x RSD) expressed as whole numb
[3] Upper % Recovery acceptance limit calculated as the Mean % Recovery – (2 x RSD) expressed as whole number

Table 9-7. Combined Control Limits Applicable to OPRs and LLOPRs

Analytes	LCL[1]	UCL[2]
Perfluoroalkyl carboxylic acids		
PFBA	85	120
PFPeA	86	124
PFHxA	88	130
PFHpA	79	135
PFOA	87	136
PFNA	84	132
PFDA	82	124
PFUnA	79	133
PFDoA	82	126
PFTrDA	78	126
PFTeDA	86	131
Perfluoroalkyl sulfonic acids		
PFBS	81	127
PFPeS	84	120
PFHxS	78	127
PFHpS	80	132
PFOS	82	134
PFNS	78	130
PFDS	73	129
PFDoS	60	117
Fluorotelomer sulfonic acids		
4:2FTS	85	131
6:2FTS	84	140
8:2FTS	83	137

Analytes	LCL[1]	UCL[2]
Perfluorooctane sulfonamides		
PFOSA	82	132
NMeFOSA	87	133
NEtFOSA	81	133
Perfluorooctane sulfonamidoacetic acids		
NMeFOSAA	73	129
NEtFOSAA	80	130
Perfluorooctane sulfonamide ethanols		
NMeFOSE	88	128
NEtFOSE	85	131
Per- and Polyfluoroether carboxylic acids		
HFPO-DA	92	124
ADONA	92	126
PFMPA	80	128
PFMBA	86	128
NFDHA	83	137
Ether sulfonic acids		
9Cl-PF3ONS	88	130
11Cl-PF3OUdS	76	128
PFEESA	88	123
Fluorotelomer carboxylic acids		
3:3FTCA	79	119
5:3FTCA	84	118
7:3FTCA	66	121

Source File: IDA Chapter 9 GW SW WW summary 06082023.xlx

[1] Lower % Recovery acceptance limit calculated as the Mean % Recovery – (2 x RSD) expressed as whole number.
[2] Upper % Recovery acceptance limit calculated as the Mean % Recovery – (2 x RSD) expressed as whole number.

9.2 EXTRACTED INTERNAL STANDARDS

One of the most important aspects of draft EPA Method 1633 is its use of isotope dilution quantitation to determine the concentrations of the target analytes. As described in Section 4 of this report, each sample to be analyzed is spiked with a suite of 24 labeled analogs of the target PFAS that are used as quantitation reference standards for both true isotope dilution quantitation and a modified form of isotope dilution for other target analytes. Those 24 labeled compounds are referred to as EIS compounds in EPA Method 1633. They are exact analogs of 24 of the 40 target

analytes. The relationship of the EIS compounds to the target analytes and their use in quantification is spelled out in Table 4-1 of Section 4.

This use of the EIS compounds for quantification results is an inherent correction of the target analyte concentration for the loss (or apparent gain) of the EIS compound throughout the entire analytical process, including the extraction steps as well as any extract cleanup steps. Relative to the more commonly employed internal standards that are injected into the final sample extract shortly before the instrumental analysis, isotope dilution quantitation yields data that are both more accurate (less biased) and more precise.

Methods that rely on the analysis of MS/MSD samples to estimate accuracy and precision as a QC measure typically limit those MS/MSD analyses to a small subset of all the samples prepared together, with the typical frequency of 5%, or one in every 20 field samples. Whatever accuracy and precision information is generated is often assumed to apply to the entire sample batch, even when samples from different sources or locations are prepared and analyzed together. In contrast, the labeled EIS compounds are added to every sample in the batch, so the analysis generates sample-specific accuracy data for those target analytes with isotopically-labelled analogs commercially available (24 of the 40 target analytes), in the form of the measured recovery of each of the labeled compounds in each sample. MS/MSDs are still a useful tool for target analytes that isotopically-labelled analogs are not commercially available for (16 of the 40 target analytes).

EIS compound recovery data was compiled from all analyses of spiked and unspiked aqueous samples. The EIS compound recoveries from the nine laboratories that completed the aqueous sample portion of the study are summarized in Table 9-8 below. These data represent the analyses of the unspiked samples and matrix spike aliquots of all 12 aqueous samples at two spiking levels ("low" and "high"), for a total of 686 to 693 observations for each EIS compound (16,625 observations in all). The only data which were not included were in situations that were identified in previous sections of this report, which were caused by spiking errors or extraction errors. Outliers identified in previous sections were included in the table below. The table contains the observed mean, minimum, and maximum recoveries from those observations for each labeled compound, across all of the nine laboratories (8 for wastewater and groundwater). Table 9-9 provides a summary of the relative proportions for all laboratories that fell between the study EIS compound target percent recovery acceptance criteria.

Table 9-8. All Aqueous Media Samples EIS Compound Recovery Analysis

EIS Compound	Number of Labs	Number of Results	Mean % Recovery	s_b	s_w	RSD
$^{13}C_4$-PFBA	9	686	55.9	20.7	27.1	48.6
$^{13}C_5$-PFPeA	9	693	80.9	8.21	15.4	19.1
$^{13}C_5$-PFHxA	9	693	84.7	7.77	13.6	16.1
$^{13}C_4$-PFHpA	9	693	84.1	9.44	15.7	18.7
$^{13}C_8$-PFOA	9	694	84.1	6.75	14.3	17
$^{13}C_9$-PFNA	9	693	82.3	6.63	14.5	17.6
$^{13}C_6$-PFDA	9	692	78.9	6.39	16.2	20.6
$^{13}C_7$-PFUnA	9	692	72.1	7.73	17.1	23.8
$^{13}C_2$-PFDoA	9	692	65.8	9.95	18.9	28.7
$^{13}C_2$-PFTeDA	9	692	51.8	9.81	17.9	34.5
$^{13}C_3$-PFBS	9	693	85.6	10.1	15	17.5
$^{13}C_3$-PFHxS	9	696	84.3	8.32	16	19
$^{13}C_8$-PFOS	9	693	77.3	7.51	16.9	21.8
$^{13}C_2$-4:2FTS	9	693	134	40.3	54.1	40.5
$^{13}C_2$-6:2FTS	9	693	107	18	43.7	40.8
$^{13}C_2$-8:2FTS	9	693	115	24.1	67.4	58.8
$^{13}C_8$-PFOSA	9	693	71.8	9.94	16.7	23.3
D_3-NMeFOSA	9	693	53.5	10.7	15.6	29.2
D_5-NEtFOSA	9	693	49.3	11.1	15.6	31.6
D_3-NMeFOSAA	9	693	81	12.9	30.8	38.1
D_5-NEtFOSAA	9	693	76.4	7.89	24.7	32.3
D_7-NMeFOSE	9	693	53.6	13.4	20.1	37.5
D_9-NEtFOSE	9	693	49.7	14	20.1	40.4
$^{13}C_3$-HFPO-DA	9	693	81.5	10.9	16.7	20.5

Source File: IDA file Matrix_EIS_results_V1_23.0607_124828

Notes:

Number of Labs - The number of laboratories reporting matrix (native & spiked) results.
Number of Results - The total number of matrix results that do not have a U flag.

Mean % Recovery - The mean percent recovery across all of the EIS compounds individual samples across all laboratories for the given analyte.
s_b - The pooled between-laboratory standard deviation. Equation from EPA 821-B-18-001 page G-25.
s_w - The pooled within-laboratory standard deviation. Equation from EPA 821-B-18-001 page G-25.
RSD - The pooled within-laboratory relative standard deviation (RSD, (s_w/(mean % recovery) *100).

Table 9-9. Proportion of All Aqueous Media % recovery results for EIS compounds within ranges.

EIS Compound	n	<10%	≥10% to <20%	≥20% to <150%	≥150% to <200%	≥200%
$^{13}C_4$-PFBA	686	7.7	11.5	80.2	0.4	0.1
$^{13}C_5$-PFPeA	693	0.1	0.1	99.3	0.3	0.1
$^{13}C_5$-PFHxA	693	0.1	0	99.3	0.4	0.1
$^{13}C_4$-PFHpA	693	0.1	0.3	98.8	0.6	0.1
$^{13}C_8$-PFOA	694	0.6	0.3	98.6	0.4	0.1
$^{13}C_9$-PFNA	693	0.9	0.1	98.6	0.3	0.1
$^{13}C_6$-PFDA	692	0.9	0	98.8	0.1	0.1
$^{13}C_7$-PFUnA	692	1.0	0.4	98.3	0.1	0.1
$^{13}C_2$-PFDoA	692	1.6	1.3	96.8	0.1	0.1
$^{13}C_2$-PFTeDA	692	3.6	2.9	93.5	0	0
$^{13}C_3$-PFBS	693	0.1	0.3	98.8	0.6	0.1
$^{13}C_3$-PFHxS	696	0.9	0.1	98.4	0.4	0.1
$^{13}C_8$-PFOS	693	1.2	0.4	98.0	0.3	0.1
$^{13}C_2$-4:2FTS	693	0.1	0	75.0	14.1	10.7
$^{13}C_2$-6:2FTS	693	0.6	0.4	88.6	6.1	4.3
$^{13}C_2$-8:2FTS	693	1.0	0	85.6	5.1	8.4
$^{13}C_8$-PFOSA	693	1.6	0.3	97.8	0.1	0.1
D_3-NMeFOSA	693	2.3	1.2	96.2	0.1	0.1
D_5-NEtFOSA	693	2.9	1.6	95.4	0	0.1
D_3-NMeFOSAA	693	1.3	0.7	95.8	1.4	0.7
D_5-NEtFOSAA	693	1.9	0.4	96.2	1.3	0.1
D_7-NMeFOSE	693	4.3	1.9	93.5	0	0.3
D_9-NEtFOSE	693	5.6	3.6	90.5	0.1	0.1
$^{13}C_3$-HFPO-DA	693	0.1	0.1	98.8	0.7	0.1

Source File: IDA Chapter 9 GW SW WW summary 06082023.xlx

9.3 NON-EXTRACTED INTERNAL STANDARD RECOVERY ANALYSES

The seven NIS compounds are: $^{13}C_3$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{18}O_2$-PFHxS, and $^{13}C_4$-PFOS. These labeled standards are added to the final sample extract shortly before the instrumental analysis, in a manner similar to the use of the "internal standards" in many EPA non-isotope dilution methods for organic contaminants that rely on mass spectrometric determination (e.g., EPA Methods 624.1 and 625.1).

The responses of the seven NIS compounds are used to calibrate the 24 EIS compounds and to calculate the recoveries of those EIS compounds in samples. It is important to note that the NIS compounds do not play a role in quantifying the target analytes. As with the internal standards in non-isotope dilution methods, the laboratory monitors the areas of the NIS compounds in each analysis and compares those areas to the areas observed during instrument calibration as a check on instrument operating conditions and interferences. The NIS compound responses typically are reported in "area counts" from the chromatograms, because there is no other standard present in the sample extract against which the NIS compound response can be quantified.

The NIS compound responses can serve as a diagnostic function in any method. Simultaneous significant changes in the areas of all NIS compounds in a given analysis may indicate a bad injection of the extract, or an overall loss of sensitivity indicative of ion source issues in the mass spectrometer. Changes in the area of a single NIS compound may indicate an interference that either enhances or suppresses the responses of the NIS, but also may affect the EIS compounds and target analytes in that portion of the chromatogram.

Some non-isotope dilution methods place bounds on the responses of the internal standards as a factor of two around the mean response in most recent ICAL (e.g., the area of internal standard X in Sample Y must be within 50–200% of its mean area in the ICAL standards). For the purposes of the EPA Method 1633 validation study, DoD required the laboratories to normalize their NIS compound responses against the mean responses in the ICAL and report the normalized responses as "recoveries." A target lower limit of recovery of greater than or equal to 30% was utilized in the MLVS; no target upper limit was provided to the laboratories.

All of the NIS compound "recovery" data from the unspiked and spiked aqueous samples were compiled and descriptive statistics for each NIS compound were generated across all aqueous matrices. Table 9-10 summarizes 4,954 NIS compound recoveries data across all aqueous media and nine laboratories (eight for wastewater and groundwater), reported to the nearest percent. All NIS compound recoveries met the target recovery criteria (>30%), with the exception of one result reported by Laboratory 2 for $^{13}C_2$-PFDA (27.5%). All of the minimum % recoveries were reported by Laboratory 2. Figure 9-6 clearly illustrates that recoveries reported by Laboratory 2 are statistically different than those reported by the other laboratories. Eighty-four percent (37 of the 44) of the NIS compound recoveries that were below 40% were reported by Laboratory 2 while the remaining seven recoveries below 40% were reported by Laboratory 3. In addition, all but five of the 105 NIS compound recoveries reported below 50% were reported by laboratories other than Laboratories 2 and 3. All of the maximum % recoveries were reported by Laboratory 5 and were associated with one sample (WWO1), with the exception of $^{13}C_2$-PFHxA (Laboratory 2, WWL5). Eighty-one percent (18 out of 22) of the NIS compound recoveries that exceeded 200% were reported by Laboratory 5, with the remaining four reported by Laboratory 2. All instances of exceedance of the target recovery criteria were associated with samples that required dilution.

Table 9-10. All Aqueous Media Samples NIS Compound Recovery Analysis[1]

NIS Compound	Number of Labs	Number of Results[1]	Min % Recovery	Max % Recovery	Mean % Recovery	s_b	s_w	RSD
^{13}C3-PFBA	9	693	32.3	192	96.8	14.5	18	18.6
$^{13}C_2$-PFHxA	9	693	33.6	222	104	14.0	21.3	20.5
$^{13}C_4$-PFOA	9	693	36.5	255	104	11.2	22.3	21.5
$^{13}C_5$-PFNA	9	693	33.2	286	104	12.9	20.1	19.5
$^{13}C_2$-PFDA	9	697	27.5	302	106	13.8	24.0	22.7
$^{18}O_2$-PFHxS	9	724	32.7	283	100	18.4	23.5	23.5
$^{13}C_4$-PFOS	9	761	33.9	308	103	19.4	23.6	22.8

Source File: IDA file Matrix_NIS_results_V1_230607_124909

[1] Analysis does not include recoveries associated with samples extracts that required dilution prior to analysis.

Following EPA guidance (EPA 821-B-18-001), lower and upper percent recovery limits for NIS compounds were generated (Table 9-11). The lower percent recovery limit is the mean % recovery minus two times the RSD and the upper percent recovery limit is the mean % recovery plus two times the RSD. All statistically derived lower control limits are greater than MLVS target lower limit of 30%.

Table 9-11. Statistically-Derived NIS Compound Recovery Acceptance Criteria

NIS Compound	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
$^{13}C_3$-PFBA	96.8	37.2	59.6	134
$^{13}C_2$-PFHxA	104	41.0	63.0	145
$^{13}C_4$-PFOA	104	43.0	61.0	147
$^{13}C_5$-PFNA	104	39.0	65.0	143
$^{13}C_2$-PFDA	106	45.4	60.6	151.4
$^{18}O_2$-PFHxS	100	47.0	53.0	147
$^{13}C_4$-PFOS	103	45.6	57.4	148.6

Derived from RSD provided by IDA file Matrix_NIS_results_V1_230607_124909

[1] Relative Standard Deviation (RSD) from Table 9-10.

[2] Lower % Recovery acceptance limit calculated as the Mean % Recovery – (2 x RSD).

[3] Upper % Recovery acceptance limit calculated as the Mean % Recovery + (2 x RSD).

9.4 MATRIX SPIKE ANALYSES

Matrix spike recoveries were statistically evaluated by Analysis of Variance (ANOVA) to test for differences among the various independent experimental factors (i.e., main effects). Main effects included the target analytes ("PFAS"), the different matrices ("Matrix"), laboratories ("Laboratory"), and spike concentrations ("Spike Concentration"). Because the final working dataset consisted of missing permutations of main effects, 1) no interaction effects were evaluated, and 2) the Least Squares Means from the ANOVA predictions are reported to more accurately reflect mean differences (i.e., marginal means that control for other main effects). All main effects were significant with greater than 99% confidence (Table 9-12). Specific to the PFAS main effect, PFDoS (the largest perfluoroalkyl sulfonic acid evaluated) and the two perfluorooctane sulfonoamidoacedic acids (NMeFOSAA and NEtFOSAA) were the only three target analytes with mean recoveries outside 70–130% of the target analyte spike concentration; PFDoS was observed with a low bias, whereas, both NMeFOSAA and NEtFOSAA were observed with a high bias (Figure 9-3). Mean recoveries for the Matrix, Spike Concentration, and Laboratory main effects were all much more consistent and closer to the target spike concentration (i.e., 100% recovery) (Figure 9-4).

Despite statistically significant differences among the various levels of each main effect evaluated, the overall method accuracy and precision was quantified. Method accuracy was calculated as the mean percent bias (% recovery–100%) for each spike concentration and laboratory and matrix averaging over the method analytes to avoid an impracticable number of permutations. Similarly, precision was calculated as the intra-laboratory percent RSD among replicate measures of the various spiked samples. Figure 9-5 illustrates the calculated accuracy and precision on a unit scale such that the results can be interpreted quantitatively (i.e., a literal bullseye target). Overall, the method as validated by this multi-laboratory study can be summarized to result in less than 40% error for aqueous samples (Figure 9-5).

Table 9-12. Accuracy analysis: ANOVA results for the observed matrix spike recoveries

Effect	F Value	P Value
Matrix	11.48	<0.0001
Laboratory	76.82	<0.0001
PFAS	85.85	<0.0001
Spike Concentration	39.58	<0.0001

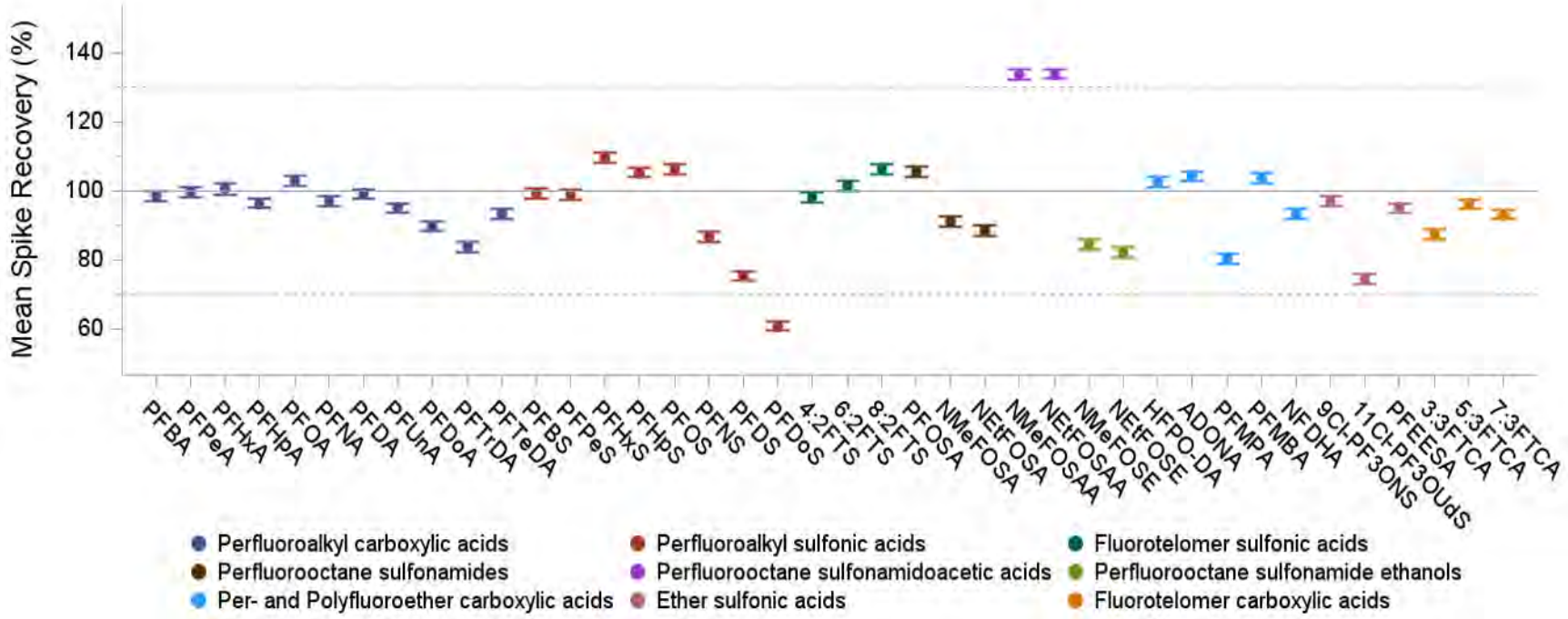


Figure 9-3. Mean spike recoveries summarized for each target analyte (i.e., the "PFAS" effect).

Error bars reflect one standard error. Reference lines are provided ± 30% of the target spike concentration for illustration only.

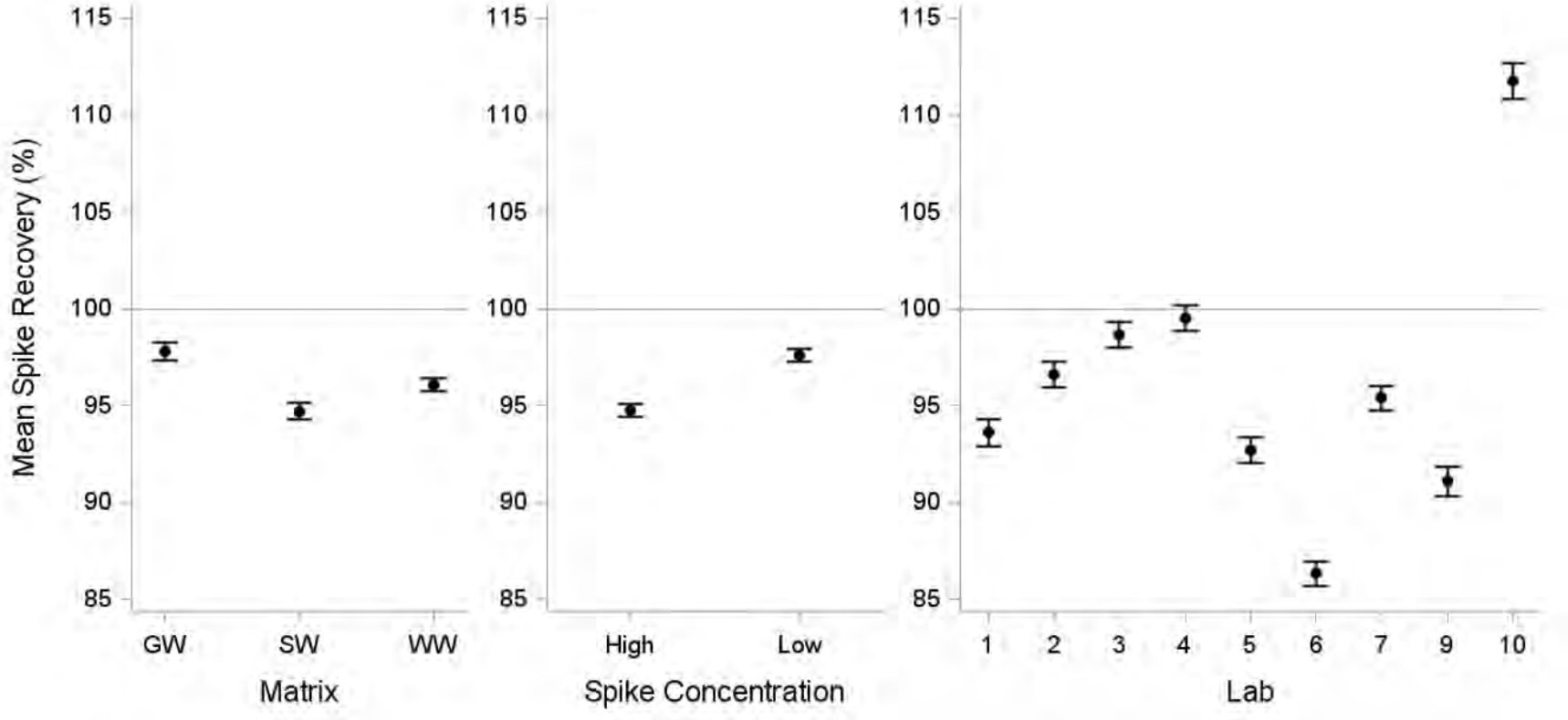


Figure 9-4. **Mean spike recoveries summarized for each matrix, spike concentration, and laboratory (i.e., the "Matrix", "Spike Conc." and "Lab" effects).**
Error bars reflect one standard error.

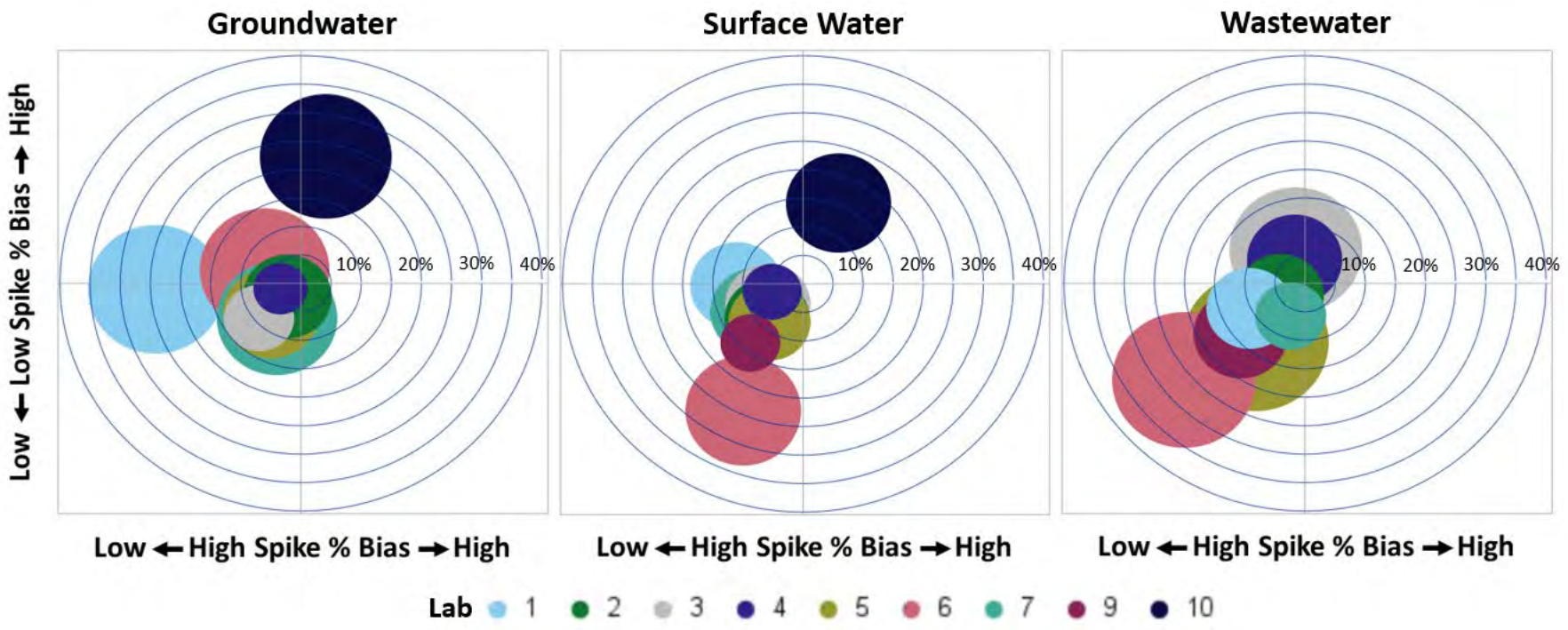


Figure 9-5. Summary illustration of the overall method accuracy and precision.

Bubble sizes reflect precision calculated as the intra-laboratory percent relative standard deviation (RSD) among replicate measures of the various spiked samples. Bubble centroids reflect mean bias (% recovery - 100%). The RSDs are scaled to the axes such that the illustration can be interpreted quantitatively.

9.5 DETERMINATION OF FINAL QC SPECIFICATIONS FOR METHOD 1633

Following completion of the statistical calculations described in Sections 9.1, 9.2, and 9.3, EPA and DoD examined the initial acceptance limits and agreed to take several additional steps that will allow EPA to establish the final QC specifications for Method 1633 for IPRs, OPRs, LLOPRs, EIS compound, and NIS compound recoveries. This is in part, due to the fact there appeared to be true outliers included in the final data set and the resulting acceptance criteria were more stringent that the acceptance criteria included in EPA Method 1633 for analytical standards that did not undergo sample preparation. Among those steps were:

- Additional analyses using statistical procedures previously applied to evaluate IPR and OPR QC acceptance criteria to inter-laboratory validation studies of EPA Methods 1600 and 1603. These calculation routines developed by GDIT in the Statistical Analysis Software (SAS) package, were conducted on the final MLVS data set and includes an allowance for simultaneous testing of multiple analytes.
- Combining the IPR, OPR, and LLOPR data from all three aqueous matrix types (wastewater, groundwater, and surface water) because the IPR, OPR, and LLOPR aliquots are all prepared in reagent water, so there is no risk of a "matrix effect" related to the aqueous matrix type of the associated study samples. This change will allow EPA to develop a single set of QC specifications that can be applied to all three aqueous matrix types, thus simplifying the implementation of the method in laboratories.
- Similarly combining the EIS compound data from all three aqueous matrix types and all QC and study samples and developing a single set of EIS compound QC specifications that will be applied to all study samples and QC samples, further simplifying the implementation of the method in laboratories. (Note: Often, a laboratory may not know if a sample is a wastewater, surface water, or groundwater, so implementing QC criteria for sub-groups of aqueous samples would be burdensome for the laboratory community.)
- Applying the Grubbs outlier test to the data sets to look for anomalous data and then rerunning the calculations in SAS.
- Comparing the newly calculated limits to the study data set and where appropriate, applying professional judgement to manually establish QC limits that cutoff at the 1st and 99th percentile of the observed data, and then rounding those values to the nearest multiple of 5%.

9.5.1 Initial SAS Calculations

Table 9-13 contains the initial SAS calculations of the IPR and OPR limits for the 40 target analytes using the entire data set (all 9 laboratories and all aqueous QC matrices), with the calculated recoveries rounded to the nearest whole percent and the IPR RSD limits rounded to the nearest 0.1%. The minimum and maximum observed recoveries ("Obs. Rec.") below 100% are reported to the nearest 0.1%.

Table 9-14 contains the corresponding EIS compound results from the IPR and OPR analyses using the entire data set (all 9 laboratories and all aqueous QC matrices).

Table 9-13. Initial SAS Calculations of the IPR and OPR Limits for the 40 Target Analytes Using the Entire Data Set

Analyte	n	# labs	Mean	Max. RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR Lower Limit (%)	OPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
PFBA	94	9	103.7	19.1	70	137	66	141	82.5	133
PFPeA	94	9	103.4	20.4	66	141	62	145	66.5	135
PFHxA	94	9	103.8	24.5	70	138	61	146	79.0	137
PFHpA	94	9	104.4	25.7	73	136	62	147	76.5	131
PFOA	94	9	108.0	24.2	65	151	59	157	76.5	138
PFNA	94	9	104.6	25.4	72	137	62	147	77.0	134
PFDA	94	9	101.5	23.9	65	138	59	144	79.0	140
PFUnA	94	9	104.6	27.7	69	140	58	151	69.0	136
PFDoA	94	9	104.1	24.3	75	133	65	144	58.5	143
PFTrDA	94	9	101.0	25.7	56	146	51	151	68.0	137
PFTeDA	94	9	105.1	25.2	61	149	55	155	49.5	136
PFBS	94	9	102.6	23.9	68	138	60	145	65.0	134
PFPeS	94	9	104.0	24.2	67	141	60	148	76.1	138
PFHxS	94	9	101.6	23.5	69	134	61	142	69.4	136
PFHpS	94	9	104.1	23.6	67	141	60	148	75.4	140
PFOS	94	9	103.4	23.1	69	138	62	145	82.3	133
PFNS	94	9	101.2	25.0	68	134	60	143	66.2	142
PFDS	94	9	99.6	25.5	66	133	58	142	73.0	141
PFDoS	94	9	91.5	31.5	52	131	43	140	56.0	122
4:2FTS	94	9	105.7	24.7	76	135	65	146	72.7	143
6:2FTS	94	9	107.1	30.5	79	135	61	153	83.0	159

Table 9-13. Initial SAS Calculations of the IPR and OPR Limits for the 40 Target Analytes Using the Entire Data Set

Analyte	n	# labs	Mean	Max. RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR Lower Limit (%)	OPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
8:2FTS	94	9	108.1	28.4	81	135	65	152	62.5	137
PFOSA	94	9	104.4	20.8	73	136	67	142	74.0	140
NMeFOSA	94	9	105.3	27.5	79	132	64	147	75.0	148
NEtFOSA	94	9	103.1	22.9	80	126	68	138	80.5	143
NMeFOSAA	94	9	102.9	27.7	72	134	60	146	67.5	140
NEtFOSAA	94	9	103.1	22.4	74	132	66	140	72.0	135
NMeFOSE	94	9	104.2	21.1	79	129	70	138	81.0	141
NEtFOSE	94	9	103.3	21.4	84	123	72	135	84.0	141
HFPO-DA	94	9	104.6	22.7	76	133	67	142	83.6	133
ADONA	94	9	106.7	22.1	80	134	70	143	68.4	141
9Cl-PF3ONS	94	9	106.8	28.3	77	137	62	151	72.1	155
11Cl-PF3OUdS	94	9	102.6	33.3	64	142	50	156	70.6	157
3:3FTCA	94	9	96.4	21.0	74	119	65	128	65.5	114
5:3FTCA	94	9	99.7	21.0	71	129	64	135	74.0	126
7:3FTCA	94	9	93.5	28.1	57	130	49	138	53.0	118
PFEESA	94	9	104.1	23.8	70	138	62	146	74.2	136
PFMPA	94	9	100.3	23.3	61	140	56	145	56.8	125
PFMBA	94	9	102.6	24.5	68	137	60	145	63.2	138
NFDHA	94	9	104.6	36.5	66	143	48	161	61.0	142

Source file: IPR-OPR specs for all aqueous matrices 5-1-2023.xlsx

Table 9-14. Initial SAS Calculations of the IPR and OPR Limits for the 24 EIS Compounds Using the Entire Data Set

EIS Compound	n	# labs	Mean	Max. RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR Lower Limit (%)	OPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
$^{13}C_4$-PFBA	94	9	85.3	31.6	56	114	44	126	28.0	108
$^{13}C_5$-PFPeA	94	9	90.0	28.6	61	119	50	130	58.0	114
$^{13}C_5$-PFHxA	94	9	88.0	23.4	58	118	52	124	67.0	111
$^{13}C_4$-PFHpA	94	9	85.5	24.7	48	123	44	127	67.1	106
$^{13}C_8$-PFOA	94	9	87.6	25.6	67	108	55	120	65.0	110
$^{13}C_9$-PFNA	94	9	87.5	24.3	66	109	56	119	70.8	110
$^{13}C_6$-PFDA	94	9	89.1	30.2	62	116	49	129	68.4	111
$^{13}C_7$-PFUnA	94	9	86.8	30.4	63	110	49	124	59.7	107
$^{13}C_2$-PFDoA	94	9	82.3	33.1	54	111	41	123	57.5	114
$^{13}C_2$-PFTeDA	94	9	77.7	36.7	34	122	26	129	53.2	109
$^{13}C_3$-PFBS	94	9	90.9	46.1	57	125	33	149	63.9	118
$^{13}C_3$-PFHxS	94	9	88.0	27.5	58	118	49	127	72.6	130
$^{13}C_8$-PFOS	94	9	86.3	24.1	61	111	53	119	66.7	113
$^{13}C_2$-4:2FTS	94	9	99.3	35.2	48	150	38	160	69.7	153
$^{13}C_2$-6:2FTS	94	9	93.0	36.4	57	129	42	144	61.8	143
$^{13}C_2$-8:2FTS	94	9	95.3	38.4	57	133	41	150	54.4	144
$^{13}C_8$-PFOSA	94	9	78.8	35.1	29	128	24	133	45.7	102
D_3-NMeFOSA	94	9	61.1	64.4	7	115	-5	128	23.9	84.5
D_5-NEtFOSA	94	9	59.3	64.2	10	109	-3	122	20.7	79.6
D_3-NMeFOSAA	94	9	87.8	62.5	43	133	12	164	55.2	205

Table 9-14. Initial SAS Calculations of the IPR and OPR Limits for the 24 EIS Compounds Using the Entire Data Set

EIS Compound	n	# labs	Mean	Max. RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR Lower Limit (%)	OPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
D_5-NEtFOSAA	94	9	84.6	37.4	53	116	38	131	59.7	107
D_7-NMeFOSE	94	9	68.0	42.4	15	121	10	126	31.5	95.0
D_9-NEtFOSE	94	9	67.2	42.7	14	120	9	125	27.3	94.0
$^{13}C_3$-HFPO-DA	94	9	85.6	23.9	55	116	50	122	61.4	111

Source file: IPR-OPR specs for all aqueous matrices 5-1-2023.xlsx

Table 9-15 contains the initial SAS calculations of the LLOPR limits for the 40 target analytes using the entire data set (all laboratories and all aqueous QC matrices), with the calculated recoveries rounded to the nearest whole percent. The minimum and maximum observed recoveries are reported to the nearest 0.1%.

Table 9-15. Initial SAS Calculations of the LLOPR Limits for the 40 Target Analytes Using the Entire Data Set

Analyte	n	# labs	Mean	LLOPR Lower Limit (%)	LLOPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
PFBA	75	9	102.5	53	152	83.0	186
PFPeA	75	9	104.4	64	145	84.0	135
PFHxA	75	9	107.2	67	147	82.8	148
PFHpA	75	9	106.5	57	156	80.0	154
PFOA	75	9	111.9	57	167	83.0	149
PFNA	75	9	106.7	54	159	80.0	163
PFDA	75	9	103.4	56	151	73.0	140
PFUnA	75	9	105.1	57	154	73.0	141
PFDoA	75	9	103.9	64	144	81.2	148
PFTrDA	75	9	100.5	52	149	70.0	131

Table 9-15. Initial SAS Calculations of the LLOPR Limits for the 40 Target Analytes Using the Entire Data Set

Analyte	n	# labs	Mean	LLOPR Lower Limit (%)	LLOPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
PFTeDA	75	9	107.8	58	158	75.0	141
PFBS	75	9	104.9	62	148	59.5	148
PFPeS	75	9	101.9	62	141	66.8	137
PFHxS	75	9	103.2	55	151	65.1	146
PFHpS	75	9	104.3	54	155	72.4	149
PFOS	75	9	106.0	53	159	58.8	152
PFNS	75	9	101.7	55	149	66.5	144
PFDS	75	9	100.0	50	150	62.0	146
PFDoS	75	9	87.9	28	148	49.0	143
4:2FTS	75	9	107.3	60	154	81.4	142
6:2FTS	75	9	106.6	52	161	67.5	149
8:2FTS	75	9	108.2	49	168	64.4	149
PFOSA	75	9	105.9	58	154	81.6	176
NMeFOSA	75	9	107.5	58	157	60.0	153
NEtFOSA	75	9	105.8	54	158	68.0	167
NMeFOSAA	75	9	100.3	39	161	45.0	139
NEtFOSAA	75	9	104.8	55	154	72.0	141
NMeFOSE	75	9	105.9	59	152	79.7	148
NEtFOSE	75	9	106.1	58	155	84.0	158
HFPO-DA	75	9	107.4	66	149	84.4	139
ADONA	75	9	107.1	64	151	75.0	138
9Cl-PF3ONS	75	9	107.0	48	166	70.9	160

Table 9-15. Initial SAS Calculations of the LLOPR Limits for the 40 Target Analytes Using the Entire Data Set

Analyte	n	# labs	Mean	LLOPR Lower Limit (%)	LLOPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
11Cl-PF3OUdS	75	9	99.3	30	169	58.0	150
3:3FTCA	75	9	98.8	39	158	74.0	151
5:3FTCA	75	9	100.0	46	154	76.0	156
7:3FTCA	75	9	93.8	36	151	52.0	145
PFEESA	75	9	102.9	62	144	74.0	137
PFMPA	75	9	101.8	53	150	65.3	140
PFMBA	75	9	104.5	48	161	71.9	149
NFDHA	75	9	105.1	46	165	53.3	150

Source file: LLOPR specs for all aqueous matrices 5-1-2023.xlsx

Table 9-16 contains the corresponding EIS compound recoveries for the LLOPR aliquots.

Table 9-16. Initial SAS Calculations of the LLOPR Limits for the 24 EIS Compounds Using the Entire Data Set

EIS Compound	n	# labs	Mean	LLOPR Lower Limit (%)	LLOPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
$^{13}C_4$-PFBA	75	9	86.5	46	127	41.0	109
$^{13}C_5$-PFPeA	75	9	91.0	56	126	66.2	111
$^{13}C_5$-PFHxA	75	9	90.0	60	120	67.0	106
$^{13}C_4$-PFHpA	75	9	87.8	49	127	59.2	118
$^{13}C_8$-PFOA	75	9	88.3	55	122	52.0	123
$^{13}C_9$-PFNA	75	9	89.1	52	126	64.3	129
$^{13}C_6$-PFDA	75	9	89.9	51	128	63.1	125
$^{13}C_7$-PFUnA	75	9	88.3	48	129	59.1	117
$^{13}C_2$-PFDoA	75	9	82.9	39	127	56.1	119
$^{13}C_2$-PFTeDA	75	9	74.3	31	117	45.5	102
$^{13}C_3$-PFBS	75	9	89.3	45	134	65.2	156
$^{13}C_3$-PFHxS	75	9	88.9	55	123	62.1	114
$^{13}C_8$-PFOS	75	9	86.7	47	127	58.6	123
$^{13}C_2$-4:2FTS	75	9	102.7	39	167	66.1	181
$^{13}C_2$-6:2FTS	75	9	96.0	38	154	57.1	170
$^{13}C_2$-8:2FTS	75	9	96.1	23	169	58.1	183
$^{13}C_8$-PFOSA	75	9	78.0	29	127	48.4	108
D_3-NMeFOSA	75	9	59.2	-6	124	23.4	111
D_5-NEtFOSA	75	9	56.5	-4	117	20.9	98
D_3-NMeFOSAA	75	9	87.0	13	161	52.0	191

Table 9-16. Initial SAS Calculations of the LLOPR Limits for the 24 EIS Compounds Using the Entire Data Set

EIS Compound	n	# labs	Mean	LLOPR Lower Limit (%)	LLOPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
D_5-NEtFOSAA	75	9	81.8	38	126	54.0	113
D_7-NMeFOSE	75	9	66.3	2	131	32.3	103
D_9-NEtFOSE	75	9	65.0	-2	132	29.7	114
$^{13}C_3$-HFPO-DA	75	9	88.4	54	123	60.4	112

Source file: LLOPR specs for all aqueous matrices 5-1-2023.xlsx

The calculated recovery limits for all 40 of the target analytes for the IPR, OPR, and LLOPR analyses were positive numbers. However, for some of the deuterated EIS compounds, the lower recovery limits were negative numbers, or positive values below 10% recovery.

9.5.2 Grubbs Outlier Test Results

Those low or negative recovery limits lead GDIT, EPA, and DoD to examine the data set in more detail and GDIT, EPA, and DoD agreed to combine the OPR and LLOPR results and to apply the Grubbs Outlier Test to the data set with a maximum of 3 outliers removed per EIS compound and matrix type and recalculate the QC acceptance limits. Table 9-17 contains the list of the 33 results that were removed from the IPR and OPR data set. Of those 33 results, 15 were for target analytes and 18 were for EIS compounds. For the target analytes, 10 of the 15 results removed were above 100% recovery, and 5 were below 100% recovery. In contrast, only 2 of the 18 EIS compound results that were removed were below 100% recovery.

Table 9-17. Results Removed by the Grubbs Outlier Test

Analyte	Grubbs Removal Round	Recovery (%)	Lab #
PFHpA	1	152	5
PFUnA	1	165	5
PFDoA	1	58.5	6
PFDoA	2	143	9
PFTeDA	1	49.5	6
6:2FTS	1	159	10

Table 9-17. Results Removed by the Grubbs Outlier Test

Analyte	Grubbs Removal Round	Recovery (%)	Lab #
NEtFOSA	1	143	9
NEtFOSA	2	138	9
NMeFOSE	1	141	9
NEtFOSE	1	141	9
ADONA	1	68.4	6
9Cl-PF3ONS	1	155	5
PFMPA	1	56.8	6
PFMPA	2	60.0	3
NFDHA	1	164	5
$^{13}C_4$-PFBA	1	28.0	3
$^{13}C_4$-PFBA	2	42	9
$^{13}C_9$-PFNA	1	130	2
$^{13}C_2$-PFTeDA	1	133	6
$^{13}C_3$-PFBS	1	174	5
$^{13}C_3$-PFBS	2	170	5
$^{13}C_3$-PFBS	3	155	5
$^{13}C_3$-PFHxS	1	130	6
$^{13}C_2$-4:2FTS	1	164	5
D_3-NMeFOSA	1	138	5
D_3-NMeFOSA	2	137	5
D_3-NMeFOSA	3	125	5
D_5-NEtFOSA	1	130	5
D_5-NEtFOSA	2	128	5

Table 9-17. Results Removed by the Grubbs Outlier Test

Analyte	Grubbs Removal Round	Recovery (%)	Lab #
D_5-NEtFOSA	3	116	5
D_3-NMeFOSAA	1	205	3
D_3-NMeFOSAA	2	205	3
D_3-NMeFOSAA	3	187	3

Source file: grubbsoutliers_watermatrices CM.xlsx

Following the application of the Grubbs Outlier Test, the IPR and OPR/LLOPR acceptance limits were recalculated and the results of those recalculations for the 40 target analytes are shown in Table 9-18.

Table 9-18. Recalculation of the IPR and Combined OPR/LLOPR Limits for the 40 Target Analytes After Application of the Grubbs Outlier Test

Analyte	n	# labs	Mean	Max RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
PFBA	168	9	102.7	20.6	70	135	65	141
PFPeA	169	9	103.9	23.1	73	135	64	143
PFHxA	168	9	105.1	24.4	78	132	66	144
PFHpA	168	9	105.0	27.7	75	135	62	149
PFOA	169	9	109.7	26.5	67	152	59	161
PFNA	168	9	105.2	28.1	75	136	61	149
PFDA	169	9	102.3	25.7	67	137	58	146
PFUnA	168	9	104.5	29.0	76	133	60	149
PFDoA	164	9	103.3	21.1	81	126	71	136
PFTrDA	169	9	100.8	28.5	60	141	51	150
PFTeDA	168	9	106.6	27.2	72	141	61	153

Table 9-18. Recalculation of the IPR and Combined OPR/LLOPR Limits for the 40 Target Analytes After Application of the Grubbs Outlier Test

Analyte	n	# labs	Mean	Max RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
PFBS	167	9	103.6	23.2	72	136	63	144
PFPeS	169	9	103.1	25.2	71	135	61	145
PFHxS	169	9	102.3	27.4	72	133	59	145
PFHpS	169	9	104.2	29.5	73	136	58	150
PFOS	169	9	104.5	29.2	72	137	58	151
PFNS	169	9	101.4	28.7	72	131	58	145
PFDS	169	9	99.8	30.1	69	131	55	145
PFDoS	169	9	89.9	34.8	46	134	36	144
4:2FTS	169	9	106.4	26.9	77	135	64	149
6:2FTS	168	9	106.6	31.6	78	135	59	154
8:2FTS	169	9	108.2	33.3	79	138	58	158
PFOSA	168	9	104.6	22.3	77	133	67	142
NMeFOSA	169	9	106.2	30.4	81	132	62	151
NEtFOSA	166	9	103.5	26.2	81	126	66	141
NMeFOSAA	169	9	101.7	32.2	65	138	51	152
NEtFOSAA	169	9	103.9	27.6	76	132	62	146
NMeFOSE	168	9	104.7	22.8	77	132	67	142
NEtFOSE	165	9	103.4	21.2	84	123	72	135
HFPO-DA	169	9	105.8	22.7	76	135	67	144
ADONA	168	9	107.1	22.9	80	135	69	145
9Cl-PF3ONS	168	9	106.6	29.5	72	142	58	155
11Cl-PF3OUdS	169	9	101.1	35.4	53	149	41	161

Table 9-18. Recalculation of the IPR and Combined OPR/LLOPR Limits for the 40 Target Analytes After Application of the Grubbs Outlier Test

Analyte	n	# labs	Mean	Max RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
3:3FTCA	166	9	96.5	23.3	79	114	66	127
5:3FTCA	166	9	99.0	24.0	78	120	65	133
7:3FTCA	169	9	93.6	34.1	58	129	44	143
PFEESA	169	9	103.6	25.2	74	133	63	144
PFMPA	167	9	101.5	23.3	64	139	58	145
PFMBA	169	9	103.4	26.6	65	142	56	151
NFDHA	168	9	104.5	36.7	69	140	49	160

Source file: iproprlloprspecs_3watertypescombined_05252023_withgrubbsremoval CM2.xlsx

Table 9-19 contains the corresponding new calculations for the EIS compound recoveries after the Grubbs Outlier Test was applied.

Table 9-19. Recalculation of the IPR and Combined OPR/LLOPR Limits for the 24 EIS Compounds After Application of the Grubbs Outlier Test

EIS Compound	n	# labs	Mean	Max RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
$^{13}C_4$-PFBA	166	9	86.7	22.0	57	117	52	122
$^{13}C_5$-PFPeA	169	9	90.4	25.6	62	119	53	128
$^{13}C_5$-PFHxA	169	9	88.9	21.6	61	117	55	122
$^{13}C_4$-PFHpA	169	9	86.6	25.1	51	122	46	127
$^{13}C_8$-PFOS	167	9	87.9	23.5	68	108	58	118
$^{13}C_9$-PFNA	167	9	87.7	23.1	66	109	57	119
$^{13}C_6$-PFDA	169	9	89.5	29.1	63	116	50	129
$^{13}C_7$-PFUnA	169	9	87.5	30.8	63	112	49	126
$^{13}C_2$-PFDoA	169	9	82.6	34.1	54	111	40	125
$^{13}C_2$-PFTeDA	168	9	75.8	34.0	38	114	30	121
$^{13}C_3$-PFBS	165	9	88.4	26.1	57	120	49	128
$^{13}C_3$-PFHxS	168	9	88.2	24.9	62	114	53	123
$^{13}C_8$-PFOS	169	9	86.5	26.8	60	113	51	122
$^{13}C_2$-4:2FTS	167	9	99.9	34.4	56	144	44	156
$^{13}C_2$-6:2FTS	168	9	93.9	34.7	57	131	43	145
$^{13}C_2$-8:2FTS	168	9	95.2	40.5	54	137	37	154
$^{13}C_8$-PFOSA	169	9	78.4	34.0	32	125	26	131
D_3-NMeFOSA	166	9	58.9	48.7	5	112	1	117
D_5-NEtFOSA	166	9	56.9	51.0	8	106	1	112

Table 9-19. Recalculation of the IPR and Combined OPR/LLOPR Limits for the 24 EIS Compounds After Application of the Grubbs Outlier Test

EIS Compound	n	# labs	Mean	Max RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
D_3-NMeFOSAA	163	9	83.8	34.8	61	107	43	124
D_5-NEtFOSAA	169	9	83.4	38.0	56	111	38	128
D_7-NMeFOSE	169	9	67.3	39.4	11	123	8	126
D_9-NEtFOSE	169	9	66.2	40.0	9	124	6	126
$^{13}C_3$-HFPO-DA	169	9	86.8	23.2	57	116	51	122

Source file: iproprlloprspecs_3watertypescombined_05252023_withgrubbsremoval CM2.xlsx

The EIS compound recoveries in Table 9-19 do not include any negative values, but the lower recovery limits for several of the deuterated EIS compounds are still below 10%, with several at 1% recovery.

Those findings lead DoD and EPA to further examine the entire data set, including the study sample results. Based on issues discussed in Sections 6, 7, and 8 for the three aqueous matrix types, DoD and EPA concluded that the EIS compound results from Laboratory 2 may have been the cause of the very low EIS compound lower acceptance limits. This was partially confirmed by removing the Laboratory 2 EIS compound data from the data set and recalculating the IPR, OPR, and LLOPR QC acceptance limits using a non-parametric approach using 1st and 99th percentiles (p1 and p99) as the lower and upper bounds (to approximate a 98% confidence level criteria). A comparison of the calculations before and after the removal of the Laboratory 2 EIS compound data is shown in Table 9-20.

Table 9-20. Comparison of Calculated EIS Compound Acceptance Limits with and without Laboratory 2 Results

EIS Compound	All Labs		Laboratory 2 Removed	
	p1	p99	p1	p99
$^{13}C_4$-PFBA	2.4	111	2.4	112
$^{13}C_5$-PFPeA	43.7	112	42.2	112
$^{13}C_5$-PFHxA	57.0	111	58.0	112
$^{13}C_4$-PFHpA	59.6	119	60.8	119
$^{13}C_8$-PFOA	58.0	110	61.0	110
$^{13}C_9$-PFNA	53.0	111	53.0	111
$^{13}C_6$-PFDA	40.2	109	40.1	112
$^{13}C_7$-PFUnA	31.0	104	30.2	105
$^{13}C_2$-PFDoA	13.3	122	13.3	124
$^{13}C_2$-PFTeDA	2.0	92	2.0	92
$^{13}C_3$-PFBS	58.0	129	58.4	131
$^{13}C_3$-PFHxS	54.4	117	54.4	114
$^{13}C_8$-PFOS	47.5	103	43.3	104
$^{13}C_2$-4:2FTS	62.0	371	63.0	290
$^{13}C_2$-6:2FTS	53.7	332	53.7	208
$^{13}C_2$-8:2FTS	28.3	378	40.0	375
$^{13}C_8$-PFOSA	33.3	104	36.4	101
D_3-NMeFOSA	3.5	83	6.0	83
D_5-NEtFOSA	1.9	81	2.2	81
D_3-NMeFOSAA	33.0	168	33.8	168
D_5-NEtFOSAA	26.0	159	29.6	134
D_7-NMeFOSE	0.1	98	0.8	98

Table 9-20. Comparison of Calculated EIS Compound Acceptance Limits with and without Laboratory 2 Results

EIS Compound	All Labs		Laboratory 2 Removed	
	p1	p99	p1	p99
D_9-NEtFOSE	0.1	96	0.6	96
$^{13}C_3$-HFPO-DA	31.9	124	49.1	125

Source file: iproprlloprspecs_3watertypescombined_05252023_withgrubbsremoval CM2.xlsx

As can be seen in Table 9-20, the removal of the EIS compound results from Laboratory 2 made slight improvements in the some of the calculated EIS compound acceptance limits, largely raising many of the lower acceptance limits, but still resulting in limits below 10% for $^{13}C_2$-PFTeDA and 4 of the deuterated EIS compounds. The most notable changes were for the upper recovery limits for $^{13}C_2$-4:2FTS and $^{13}C_2$-6:2FTS, which dropped from 371% and 332% to 290% and 208%, respectively. (Among the 84 observations from Laboratory 2 for each of these EIS compounds, eighteen $^{13}C_2$-4:2FTS recoveries and nine $^{13}C_2$-6:2FTS recoveries were greater than 300%.)

9.5.3 Final IPR, OPR, LLOPR, EIS Compound, and NIS Compound QC Acceptance Criteria for Method 1633

Following the review of the statistically-derived acceptance limits and the various recalculated acceptance limits, EPA and DoD decided to apply both a non-parametric approach and professional judgement (e.g., elimination of a specific laboratory for an analyte or EIS compound) to establish the QC acceptance limits for the:

- IPR
- Combined OPR/LLOPR limits (e.g., one set of limits for both types of OPR)
- EIS compound recoveries in study samples

The goal of the non-parametric approach was to set the limits such that no more than 1% of the observed results would fail either the lower or upper limits. The IPR recoveries were generated using the original statistical calculation in Table 9-19. All of the IPR and OPR/LLOPR recovery limits were then expressed to a multiple of 5% (the mean IPR recovery and the corresponding RSD are expressed to the nearest 1%). Furthermore, none of the criteria were made more stringent than 70% for the lower recovery or 130% for the upper recovery, which are the bounds for the calibration verification criteria, as it does not make sense to make the IPR or OPR recovery more stringent than that criteria. The final IPR and OPR/LLOPR limits for the target analytes are shown in Table 9-21.

Table 9-21. Final IPR and OPR/LLOPR Acceptance Limits

Analyte	IPR Mean	IPR Max RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
PFBA	103	21	70	135	70	140
PFPeA	104	23	70	135	65	135
PFHxA	105	24	70	135	70	145
PFHpA	105	28	70	135	70	150
PFOA	110	27	65	155	70	150
PFNA	105	28	70	140	70	150
PFDA	102	26	65	140	70	140
PFUnA	105	29	70	135	70	145
PFDoA	103	21	70	130	70	140
PFTrDA	101	29	60	145	65	140
PFTeDA	107	27	70	145	60	140
PFBS	104	23	70	140	60	145
PFPeS	103	25	70	135	65	140
PFHxS	102	27	70	135	65	145
PFHpS	104	30	70	140	70	150
PFOS	105	29	70	140	55	150
PFNS	101	29	70	135	65	145
PFDS	100	30	70	135	60	145
PFDoS	90	35	45	135	50	145
4:2FTS	106	27	70	135	70	145
6:2FTS	107	32	70	135	65	155
8:2FTS	108	33	70	140	60	150

Table 9-21. Final IPR and OPR/LLOPR Acceptance Limits

Analyte	IPR Mean	IPR Max RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
PFOSA	105	22	70	135	70	145
NMeFOSA	106	30	70	135	60	150
NEtFOSA	104	26	70	130	65	145
NMeFOSAA	102	32	65	140	50	140
NEtFOSAA	104	28	70	135	70	145
NMeFOSE	105	29	70	135	70	145
NEtFOSE	103	21	70	130	70	135
HFPO-DA	106	23	70	135	70	140
ADONA	107	23	70	135	65	145
9Cl-PF3ONS	107	30	70	145	70	155
11Cl-PF3OUdS	101	35	50	150	55	160
3:3FTCA	96	23	70	130	65	130
5:3FTCA	99	24	70	130	70	135
7:3FTCA	93	34	55	130	50	145
PFEESA	104	25	70	135	70	140
PFMPA	102	23	60	140	55	140
PFMBA	103	27	65	145	60	150
NFDHA	105	37	65	140	50	150

Source file: iproprlloprspecs_3watertypescombined_05252023_withgrubbsremoval CM2.xlsx

Most of the acceptance criteria in Table 9-21 are inclusive of the highest or lowest observed result from Table 9-13 and Table 9-15, which usually included 169 data points from 9 laboratories for most of the analytes. Below are the exceptions:

- The second lowest data point was used for PFDoA, because the lowest five points were 58.5, 75.5, 78.8, 82, and 83% recovery. The 58.5% recovery appeared to be an anomaly, as it was about 17% lower than the second lowest data point out of 164 data points.

- The second lowest data point was used for PFTeDA, since the lowest five points were 49.5, 62, 75, 76, and 82% recovery. The 49.5% recovery appeared to be an anomaly, as it was about 13% lower than the second lowest data point and 25% lower than the third lowest data point out of 168 data points. The statistically calculated result from Table 9-18 was also much closer to 60%.
- The lowest recovery for NMeFOSAA was 45%, but the second lowest recovery was 50%. We used the second lowest data point to generate the criteria because it was in agreement with the statistically calculated value of 51% in Table 9-18.
- The statistically calculated high recovery criteria from Table 9-18 was used for several of the LLOPR/OPR criteria in Table 9-21, because they were more aligned with the second or third highest value observed for all of the LLOPR and OPR data points. This was done for: PFHxA, PFHpA, PFNA, PFDoA, PFBS, PFHxS, PFOS, PFDS, 6:2 FTS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, NEtFOSE, 9Cl-PF3ONS, 3:3FTCA, and 5:3FTCA.

EPA and DoD decided to develop a single set of acceptance limits for EIS compound recoveries that would be applicable to both the study sample results and the IPR and OPR/LLOPR and other QC samples analyses (e.g., method blanks). The EIS compound recoveries in study samples were significantly wider than in method blanks, OPRs, and LLOPRs, so the wider of the two sets was used. The goal was to simplify the application of the EIS compound acceptance limits in the laboratory.

The acceptance limits in Table 9-22 were developed from the entire study sample data set of almost 700 EIS compound recoveries using both a non-parametric approach and professional judgement (including the decision to eliminate the EIS compound recoveries from 1 to 2 laboratories for a specific parameter). Also, none of the acceptance criteria were made more stringent than 40% to 130%. The notes column explains any acceptance criteria where professional judgement was used, and the acceptance criteria are tighter than what is shown in Table 9-20. Professional judgement was used to prevent the worst performing laboratories from overly influencing the method criteria.

The spiked sample data demonstrated that the accuracy of the method was good when the EIS compound recovery was as low as 5%, and as high as 500%, but if the criteria were made this wide, it might encourage poor laboratory technique. Also, a very low acceptance limit could mask sample processing or instrumental issues that would reduce the method's sensitivity. The criteria below were obtainable by the majority of the laboratories participating in the study.

Table 9-22. EIS Compound Acceptance Limits Applicable to All Aqueous Sample Types

EIS Compound	Lower Limit (%)	Upper Limit (%)	Notes
$^{13}C_4$-PFBA	5*	130	Of 686 data points, the bottom fifty-three are below 10%, which is 7.7% of the data. Twenty-eight results are below 5% and 26 of those data points come from Laboratories 4 and 6.
$^{13}C_5$-PFPeA	40	130	
$^{13}C_5$-PFHxA	40	130	
$^{13}C_4$-PFHpS	40	130	
$^{13}C_8$-PFOA	40	130	
$^{13}C_9$-PFNA	40	130	
$^{13}C_6$-PFDA	40	130	
$^{13}C_7$-PFUnA	30	130	
$^{13}C_2$-PFDoA	10	130	
$^{13}C_2$-PFTeDA	10	130	
$^{13}C_3$-PFBS	40	135	
$^{13}C_3$-PFHxS	40	130	
$^{13}C_8$-PFOS	40	130	
$^{13}C_2$-4:2FTS	40	200	Of 693 data points, 73 are above 200, which is 10.5% of the data. These 73 data points above 200% came from four laboratories (37 from Laboratory 2, 24 from Laboratory 9, eight from Laboratory 6, and four from Laboratory 3). If the two worst performing laboratories are removed, then only the 2% of the data is above 200%. Six laboratories had no data above 200%.
$^{13}C_2$-6:2FTS	40	200	Of 693 data points, 29 are above 200, which is 4.2% of the data. These 29 data point above 200% came from four laboratories (fourteen from Laboratory 2, nine from Laboratory 6, five from Laboratory 9, and one from Laboratory 3). If the two worst performing laboratories are removed, then only 1% of data is above 200%. Six laboratories had no data above 200%. Seven laboratories combined only had one data point above 200%.

Table 9-22. EIS Compound Acceptance Limits Applicable to All Aqueous Sample Types

EIS Compound	Lower Limit (%)	Upper Limit (%)	Notes
$^{13}C_2$-8:2FTS	40	300	Of 693 data points, 57 are above 200, which is 8.2% of the data. These 57 data points are from seven laboratories (twenty-one from Laboratory 2, eight from Laboratory 9, seven from Laboratory 6, seven from Laboratory 4, seven from Laboratory 1, five from Laboratory 5, and two from Laboratory 3). It is interesting to note that the Laboratory 4 data above 200% all look as if they were double spiked. These values are all more than twice the other spikes. If the data from Laboratories 2, 9, and 4 are ignored; then only ten of about 500 data points are above 300% recovery. For the low criterion, almost all of the worst recoveries are from Laboratory 2, so Laboratory 2 was ignored.
$^{13}C_8$-PFOSA	40	130	Of 693 data points, 31 data points are below 45%. Almost all of the lowest recovery data come from Laboratories 2 and 5 (seventeen from Laboratory 5 and eleven from Laboratory 2). If these laboratories are ignored, only three data points are below 45.
D_3-NMeFOSA	10	130	Of 693 data points, sixteen data points are below 10%. Fifteen of these are from Laboratories 2 and 5 (seven from Laboratory 2, seven from Laboratory 5, and one from Laboratory 1).
D_5-NEtFOSA	10	130	Of 693 data points, twenty data points are below 10%. Seventeen of these are from Laboratories 2 and 5 (eight from Laboratory 2, nine from Laboratory 5, and one from Laboratories 1, 6, and 9). If you ignore Laboratories 2 and 5, only three data points of about 550 are below 10%.
D_3-NMeFOSAA	40	170	Of 693 data points, twenty-two data points were below 40%. If you ignore Laboratories 2 and 5, the top 99% of the data is above 40%. Without Laboratories 2 and 5, there are only five data points below 40% among the remaining 525 data points.
D_5-NEtFOSAA	25	135	Of 693 data points, fourteen data points are below 15%. Thirteen of these are from Laboratories 2 and 5 (seven from Laboratory 2 and six from Laboratory 5). If you ignore Laboratories 2 and 5, only one data point of about 550 is below 15%, and only four data points are below 25%.
D_7-NMeFOSE	10	130	Of 693 data points, thirty data points are below 10%. Twenty-five of these are from Laboratories 2, 5, and 6 (nine from Laboratory 2, nine from Laboratory 5, seven from Laboratory 6, two from Laboratory 9, and each one from Laboratories 1, 4, and 10). Seven laboratories only had five data points out of about 500 below 10%.

Table 9-22. EIS Compound Acceptance Limits Applicable to All Aqueous Sample Types

EIS Compound	Lower Limit (%)	Upper Limit (%)	Notes
D_9-NEtFOSE	10	130	Of 693 data points, thirty-nine data points are below 10%. Thirty-five of these are from Laboratories 2, 5, 6, and 9 (nine from Laboratory 2, eleven from Laboratory 5, nine from Laboratory 6, six from Laboratory 9, two from Laboratory 10, and one each from Laboratories 1 and 4). Seven laboratories only had ten data points out of about 500 below 10%. If the data from only six laboratories are used, there are only four data points out of about 400 below 10%.
$^{13}C_3$-HFPO-DA	40	130	

Source file: 1633 EIS Specs Statistics and Reality 2023-06-21.xlsx

*Recovery of $^{13}C_4$-PFBA can be problematic in some study samples. Although the lower limit for recovery for this EIS compound is set below 10%, laboratories should routinely track recovery of this EIS compound and take reasonable steps to ensure that recovery is at least 10% in the majority of samples.

The NIS compound data was compiled only using the study samples, which generated approximately 700 data points for each of the NIS compound. The criteria were generated by applying professional judgement to manually establish QC acceptance limits that cutoff at the 1st and 99th percentile of the observed data, and then rounding those values to the more inclusive multiple of 5%. No acceptance criteria were made any more stringent than 50-200% (Table 9-23). The notes column explains any criteria where professional judgement was used.

Table 9-23. NIS Compound Acceptance Limits Applicable to All Aqueous Sample Types

NIS Compound	n	p1	p99	Lower Limit (%)	Upper Limit (%)	Notes
$^{13}C_2$-PFDA	697	41	185	50	200	Twenty of the lowest 25 recoveries came from Laboratory 2. If Laboratory 2 is eliminated the n1 value is 61%. Sixteen results were below 50% from the entire data set, thirteen of these were from Laboratory 2.
$^{13}C_2$-PFHxA	693	40	185	50	200	Twenty-one of the lowest 25 recoveries came from Laboratory 2. If Laboratory 2 is eliminated the n1 value is 62%. Eleven results were below 50%, nine of these were from Laboratory 2.
$^{13}C_3$-PFBA	693	40	158	50	200	Twenty-one of the lowest 25 recoveries came from Laboratory 2. If Laboratory 2 is eliminated the n1 value is 60%. Sixteen results were below 50%, Thirteen of these were from Laboratory 2.

Table 9-23. NIS Compound Acceptance Limits Applicable to All Aqueous Sample Types

NIS Compound	n	p1	p99	Lower Limit (%)	Upper Limit (%)	Notes
$^{13}C_4$-PFOA	693	43	174	50	200	Nineteen of the lowest 25 recoveries came from Laboratory 2. If Laboratory 2 is eliminated the n1 value is 60%. Thirteen results were below 50%, Eleven of these were from Laboratory 2.
$^{13}C_4$-PFOS	761	35	193	50	200	The seven lowest results were all due to diluted extracts (diluted to 10:1). These were excluded. Once excluded the p1 value was 40%. Seventeen undiluted values were below 50%, all but four were from Laboratory 2. The p1 value is above 64% if Laboratory 2 and the diluted results are eliminated.
$^{13}C_5$-PFNA	693	41	160	50	200	Thirteen data points were below 50%, Eleven were from Laboratory 2.
$^{18}O_2$-PFHxS	724	17	180	50	200	The twenty-three lowest results were all due to diluted extracts. These were not included. Once excluded the p1 value was 40%. Seventeen undiluted values were below 50%, all but two were from Laboratory 2. The p1 value is above 64% if Laboratory 2 and the diluted results are eliminated.

Source file: WW_SW_GW_Export_20230605_NIS

10 CONCLUSIONS

The objectives of this MLVS were achieved: validation of EPA Method 1633 and the production of a method that can be implemented at a typical mid-sized full-service environmental laboratory. Overall, the data generated during the MLVS demonstrated that EPA Method 1633, as written, is robust enough to be performed by suitable laboratories using similar instruments of different manufacturers and models. The results generated by participating laboratories in this study routinely met the requirements stated in the method for:

- Mass calibration and mass calibration verification,
- Initial calibration and calibration verification,
- Determination of MDLs and LOQs,
- Initial Performance Recovery,
- Preparatory batch QC samples (MB, OPR, LLOPR), and
- Quantitative and qualitative analyte identification criteria.

The suitability of EPA Method 1633 to detect and quantify the 40 target analytes in wastewater, surface water, and groundwater samples was successfully demonstrated through the analysis of spiked real-world samples of those matrix types. Method blank results demonstrated that there was negligible bias associated with background contamination introduced during sample preparation was negligible. The IPR, OPR, and LLOPR recoveries (Tables 5-3, 9-3, and 9-5) and the EIS and NIS compound recoveries (Tables 9-8 and 9-23) associated with study samples were used to derive QC acceptance criteria (Tables 9-21, 9-22, and 9-23) for inclusion in the finalized method.

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Appendix A

Multi-Laboratory Study Plan and Analytical Method Standard Operating Procedure

Appendix B

Preparation of PFAS-Spiked Samples

Appendix C

Data Management Report (Exa Data and Mapping Services Inc.)

Appendix D

PFAS MLVS Institute for Defense Analyses Report

Appendix E

Wastewater Supporting Tables

Appendix F

Surface Water Supporting Tables

Appendix G

Groundwater Supporting Tables