SERDP

TECHNICAL REPORT

Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633 (Volume IV): Tissue

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Abstract

This report is the second 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, Tissue, Biosolids, and Tissue Samples by LC-MS/MS* (the Study). The Study was conducted as a joint effort by the U.S. Department of Defense (DoD) and the Environmental Protection Agency (EPA).

This report is the second in the series of MLVS reports to be published. The first report, titled *Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633: Wastewater, Surface Water, and Groundwater Matrices* (herein identified as *Volume I*) provides the detailed project information that applies to this and subsequent reports in addition to this report. That report provides the project background, the overall project management structure, data validation, and data management procedures. The processes, evaluation, and procedures of the previous report are incorporated by reference.

The objective of the Study was to demonstrate the efficacy of the method using PFAS-spiked environmental samples. Tissue matrices were prepared by shaking an aliquot of the sample with methanolic ammonium hydroxide, followed by carbon clean-up, and then concentrated via tissue-phase extraction (SPE). 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. Analytes were quantified and reported as their acid form.

Ten laboratories participated in the Study: eight commercial laboratories and two state laboratories. All laboratories had previously demonstrated their initial calibrations (ICAL) and were required to complete an initial demonstration of capabilities study for tissue media. Upon successful completion, unspiked, and PFAS-spiked tissue samples were sent to each of the laboratories. Three tissue sample series were analyzed, each series consisting of an unspiked sample, three replicate low-spiked samples, and three replicate high-spiked samples for each participating laboratory.

All data packages were reviewed for completeness and compliance with the requirements of the MLVS Method and the Study Data Validation Guidelines (DVGs); the validation team and process is described in detail in *Volume I*.

Evaluation of the calibration demonstrations submitted by each laboratory as part of Phase 3 of the Study is included in *Volume I.* For the tissue study, the laboratories conducted an initial demonstration of capabilities (IDC) that included of a method detection limit (MDL) determination, an Initial Precision and Recovery (IPR) study, and the limit of quantitation verification (LOQVER). The pooled average MDL for all laboratories was less than 1 µg/kg, and generally less than 0.4 µg/kg for most PFAS. For PFOA and PFOS the pooled MDL was less than 0.1 µg/kg. MDLs were highest for the three FTCA compounds. All laboratories met the Study IPR NIS compound target criterion of >30% recovery, and the EIS compound target acceptance criteria

of 20–150%. All of the valid target analyte results reported from IPRs were within the study target analyte criterion of between 40–150%. For the LOQVER, of the nine laboratories included in the statistical analysis, all met the Study NIS compound target acceptance criterion of >30% recovery. Of the valid target analyte results reported from the LOQVERs, less than 1% of the results exceeded the target criterion of 40–150%. Of the valid EIS compound results reported from LOQVERs, the failure rate relative to the EIS compound acceptance criterion of 20–150% was less than 0.5%.

Three individual tissue matrices were analyzed for an unspiked sample, three low-spiked samples and three high-spiked samples, for a total of 21 samples per laboratory. All sample results from the 10 laboratories were evaluated.

Matrix spike recoveries were statistically evaluated by Analysis of Variance (ANOVA) as described in *Volume I*. All main effects were significant with greater than 99% confidence. On average all PFAS were observed with mean recoveries 70-130% of the target spike concentration. Matrix, Spike Concentration, and Laboratory main effects were also relatively consistent and close to the target spike concentration (i.e., 100% recovery).

The results for the tissue samples support a finding that EPA Method 1633 measures PFAS concentrations as well as or better than most EPA methods for similar sized organic contaminants in real-world samples of these matrices.

EXECUTIVE SUMMARY

E.S.1 INTRODUCTION

This report is the fourth 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, Tissue, 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); EPA's Offices of Water, of Land and Emergency Management, of Research and Development; the U.S. Navy; the U.S. Air Force; and the U.S. Army Corps of Engineers (USACE). SERDP/ESTCP, EPA OW, the U.S. Navy, the U.S. Air Force and the USACE approved and are co-signers to the Study Plan developed for the project.

E.S.2 OBJECTIVES

The 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 report is focused on demonstrating EPA 1633 for tissues. The first report, *Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633 Volume I: Wastewater, Surface Water, and Groundwater Matrices*, (*Volume I*), provides the detailed project information that applies to this and subsequent reports.

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 in this report were to:

- Identify and quantify up to 40 per- and polyfluoroalkyl substances (PFAS) in tissues (tissue) using the isotope dilution liquid chromatography–tandem mass spectrometry (LC-MS/MS) method.
- Achieve a low parts per billion (ppb) method detection limits and levels of quantitation in tissue.
- Demonstrate that the method can be implemented at a typical mid-sized full-service environmental laboratory.
- Validate the method using spiked real-world tissue.

Volume I provided validation of EPA Method 1633 for wastewater, surface water, and groundwater. *Volume II* provided validation for soil and sediment matrices. *Volume III* for landfill leachate and biosolids. This *Volume IV* provides validation of the method for fish and shellfish.

E.S.3 METHOD DESCRIPTION

Methods followed are detailed in the *Volume I* report. Briefly, tissue 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. 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. 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. Updates reflecting those changes was have been iteratively released by EPA, the most recent is the 4th Draft Method 1633 (EPA 2023). The complete method used for this study is provided in Appendix A to the *Volume I*.

Ten laboratories (eight commercial contract laboratories and two state laboratories) participated in the Study. For the purposes of this study, the laboratories were randomly assigned numbers, which were used to maintain the anonymity of the results. All laboratories had previously demonstrated their initial calibrations (ICAL) (*Volume I*) and were required to complete an initial demonstration of capabilities study for tissue media. Upon successful completion, unspiked, and PFAS-spiked tissue samples were sent to each of the laboratories.

All data packages were reviewed for completeness and compliance with the requirements of the MLVS Method and the Study Data Validation Guidelines (DVGs); the validation team and process is described in detail in *Volume I*. While ten laboratories contributed data packages for the tissue IDC, only 8 laboratories contributed data for the PFAS-spiked tissue evaluation. Two laboratories declined to participate, and one laboratory's data did not pass the quality assurance requirements, resulting in data from seven laboratories being included in the evaluation.

E.S.5 TISSUE IDOC FINDINGS

Initial Demonstration of Capabilities

The laboratories next submitted documentation of an IDOC that consisted of a method detection limit (MDL) determination, an Initial Precision and Recovery (IPR) study, and the limit of quantitation verification (LOQVER). The process for setting the MDL is discussed in more detail in *Volume I.*

[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.

Tissue 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.

Tissue Initial Precision and Recovery

For the IPR studies, four aliquots of 2 g of chicken breast or similar animal tissue 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.

Tissue Limits of Quantitation Verification Analyses

A single aliquot of 2.0 g of chicken breast or similar animal tissue was spiked with all 40 target analytes such that the final concentration of each PFAS for the LOQVER was one and two times the laboratory's LOQ.

E.S. 6 TISSUE PERFORMANCE EVALUATION

The results demonstrated the efficacy of EPA Method 1633 to accurately report PFAS concentrations in real-world tissue samples. Three individual tissue matrices were analyzed for an unspiked sample, three low-spiked samples and three high-spiked samples, for a total of 21 samples per laboratory. All sample results from the seven laboratories were evaluated.

E.S. 9 COMBINED TISSUE PERFORMANCE EVALUATION

Overall, the recoveries (especially the mean recoveries) were excellent considering the complexity of the tissue matrix. There were roughly 4,800 matrix spike results. Roughly 90% of the MS data achieved a recovery between 40 to 140%, and roughly 99% of the MS data recovered between 20 to 200%. Only one matrix spike result was below 10% and only 4 were above 300%.

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 Precision and 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 tissue was successfully demonstrated through the analysis of spiked real-world samples of those matrix types. Overall, the recoveries (especially the mean recoveries) were excellent considering the complexity of the tissue matrix. There were roughly 4,800 matrix spike results. Roughly 90% of the MS data achieved a recovery between 40 to 140%, and roughly 99% of the MS data were recovered between 20 to 200%. Only one matrix spike result was below 10% and only 4 were above 300%. However, the percent probability of observing results with less than 30% error for PFDoS (39.8%), 3:3FTCA (47%), 7:3FTCA (46.4%), and NEtFOSE (49.4%) spiked tissue samples across all seven laboratories indicated recovery of this analyte in tissue samples may be biased low. OPR and LLOPR data associated with tissue sample results for these analytes should be considered when determining the usability of data for these analytes in tissue samples.

Method blank results demonstrated that there was negligible bias associated with background contamination introduced during sample preparation. 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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Appendix A PFAS MLVS Institute for Defense Analyses Report

Appendix B Tissue Supporting Tables

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
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
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

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
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
SOW	statement of work
SPE	solid-phase extraction
TS	tissue
µg/kg	microgram per kilogram
USACE	U.S. Army Corps of Engineers
Waters ERA	ERA – A Waters Company
Wellington	Wellington Laboratories, LLC

1 INTRODUCTION

This report is the fourth and final in a series presenting the results of a multi-laboratory validation study (MLVS or "the Study") undertaken to validate the Environmental Protection Agency's (EPA) draft Office of Water (OW) *Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Tissue, Biosolids, and Tissue Samples by LC-MS/MS* (EPA Method 1633). The Study 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), tissues (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.

This report addresses the multi-laboratory study results for tissues. The methods for conducting the Study are presented in the following documents and are incorporated herein by reference.

- *Single Laboratory Validation Study of PFAS by Isotope Dilution LC-MS/MS*
- *Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633 Volume I: Wastewater, Surface Water, and Groundwater Matrices*
- *Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633 Volume II: Soil and Sediment Matrices*
- *Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633 Volume III: Biosolids and Landfill Leachate Matrices*
- 4th Draft Method 1633 (EPA 2023)

The first report, *Multi-Laboratory Validation Study for Analysis of PFAS by EPA Draft Method 1633 Volume I: Wastewater, Surface Water, and Groundwater Matrices*, provides the detailed project information that applies to all subsequent reports. *Volume I* provides the project background, the overall project management structure, data validation, and data management procedures. It describes the processes for laboratory selection, selection of study sample sources, and study sample creation and instructions to each laboratory with sample delivery. Volume I includes results from evaluation of the overall EPA Method 1633 capabilities of each laboratory for aqueous media. This included the evaluation of each laboratory's Standard Operating Procedure (SOP) and documentation of Initial Calibrations (ICAL), the Initial Demonstration of Capabilities (IDOC), method detection limit (MDL) determination, and verification of their sample limit of quantitation (LOQ) for aqueous matrices. The processes, evaluation, and procedures of the previous reports are incorporated herein by reference and are not repeated herein.

1.1 BACKGROUND

The background supporting the undertaking of the Study is presented in Volumes I and II. Briefly, the Study was undertaken as a joint effort that included SERDP&ESTCP, EPA, the US Navy, US Air Force, and the US Army Corps of Engineers. The necessity and importance of validating EPA Method 1633 (and by extension the 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).

1.2 METHOD SUMMARY

The Study Plan used for the MLVS is provided in Appendix A to the *Volume I*. The Study Plan 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 for the Study. The laboratory sample preparation and analysis procedure was EPA Method 1633 with interim quality assurance and quality control criteria included (*Volume I*, MLVS Method, Appendix A).

The analytical method includes both sample preparation and sample analysis procedures that are applicable to a variety of environmental matrices. The matrices evaluated by the Study include wastewater, surface water, groundwater, landfill leachate, soil, sediment, biosolids, and tissue. The tissue matrices were prepared via solvent extraction and SPE, followed by carbon clean-up processes. The method utilizes 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. 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) 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.

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

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
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

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

Analyte Name	Abbreviation	CASRN
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	
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	NA
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	
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:
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, Tissue, 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, Solids, Biosolids, and Tissue Samples by LC-MS/MS* (Study Plan), which is included as Appendix A to *Volume I.*

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: 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) initially agreed to participate in the Study. The initial ten laboratories participating are listed in Table 2-1. For the MLVS 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 (Table 2-2).

The overall MLVS objectives and design are detailed in Section 2 of the *Volume I* and *Volume II* report. For this report the study design involved:

- Eight laboratories, with a goal of complete tissue data sets from at least six of those laboratories
- Three tissue samples included a freshwater low-lipid fish, a marine high-lipid fish, and a shellfish
- Multi-point calibration of the target analytes by each laboratory
- Initial Demonstration of Capabilities (IDOC) in tissue media by each laboratory
- Determination of MDLs for tissue by each laboratory
- Analyses of matrix spike samples prepared from each of the tissue samples.

The calibration, IDOC, and MDL studies of water and solids were previously conducted by each laboratory; those results are presented for aqueous samples in *Volume I*, Section 4, and for solid samples in *Volume II*, Section 4. Tissue specific studies are described in this report.

2.2 MATRICES AND SAMPLE SELECTION

The MLVS was designed to provide a test of the method by analyses of real-world environmental matrices. To obtain a wide diversity and sufficient quantity of matrices and samples, SERDP and the USACE coordinated collection of representative species and sufficient volumes/mass used in the Study.

The list of all tissue samples acquired for this Study is found in the Study Plan ***(****Volume I*, Appendix A, Attachment 2***)***. The specific tissue samples included in the MLVS included:

- Freshwater fish low-lipid fish - walleye (*Sander vitreum****).*** Market purchase of frozen fish caught in Lake Michigan. For the MLVS, skin-on filet was processed at Waters ERA.
- Marine high-lipid fish - King Salmon (*Oncorhynchus tshawytscha*) Market purchase of fish caught in the Gulf of Alaska. For the MLVS, skin-on filet was processed at Waters ERA.
- Shellfish - butter clams (*Saxidomus gigantea)***.** Market purchase of shell-on clams collected in Washington state**.** Whole clams were sent to Waters ERA, where the tissues were shucked from the clams and processed.

Lipid levels were confirmed for each species as part of the baseline characterization. The lipid reported measures are shown in Table 2-4. For walleye the percent measured lipid was 0.38% (with a duplicate measure of 0.41%), and 8.1% for the King Salmon. Lipid concentration in the clams was measured at 0.63%.

The MLVS design specified that each of the tissue field-collected samples were sub-sampled to create a pre-spiked characterization sample, an unspiked (or "native") sample, three replicates at a low-spiked concentration, and three replicates at a high-spiked concentration (Table 2-3). Each sample was assigned a matrix code: TS. To distinguish individual samples, a single letter sample identifier was assigned. The native sample was assigned the number 0, the unspiked study sample assigned the number 1, low-spiked replicates 2–4, and the high-spiked replicates 5–7.

2.3 SELECTION OF SPIKING LEVELS

The three tissue matrices were screened for baseline PFAS levels. ERA-Waters homogenized all sample matrices and shipped aliquots of composite samples collected from each to SGS AXYS for native PFAS analyses. Levels of PFAS measured in those three samples are provided in Table 2-5. PFAS native concentrations were below detection limits for most of the 40 target PFAS with the following exceptions: walleye – PFDA, PFUnA, and PFOS; salmon – PFOS. PFAS were not detected in the baseline clam tissues.

From these results, the EPA and the Study Quality Assurance (QA) Manager determined appropriate low-spiked, and high-spiked concentrations for each target PFAS. 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. Table 2-4 also shows the appropriate target calibration level set of each PFAS by EPA and the DoD.

2.4 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. Specific spiking procedures for tissues followed by Waters ERA are provided in *Volume II,* Appendix A.

High and low spiking levels were set by the Study QA Manager and EPA based upon review of the baseline (background) PFAS concentrations for the tissue samples (Table 2-4).

Study samples of 2.0 grams wet-weight basis were spiked by Waters ERA at two concentrations per analyte using spiking concentrates prepared from concentrated stock solutions procured from Wellington. Bulk matrices were homogenized prior to packaging. Spiking concentrates were vortexed prior to use. 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 spiked samples (high and low). An example certificate is shown in Figure 2-1.

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 15-mL amber glass screw-top vials of each of the tissue samples: one bottle for analyses of the unspiked sample, three bottles spiked at a low-spiked level, and three bottles spiked at a high-spiked 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.

The sample preparation procedure found in the MLV Study Method was followed, with the following exceptions:

- Instead of homogenizing the sample and weighing out an aliquot of the sample, the laboratories were instructed to transfer the entire contents (2.0 g) of the container received to a 15-mL polypropylene centrifuge tube.
- The laboratories were instructed to record 2.0 g as the mass of sample prepared.

2.4.1 Tissue samples

The tissues samples prepared and shipped by Waters ERA are listed in Table 2-3. The three parent tissue matrices were each prepared as one unspiked, three replicates at the low-spiked level, and three replicate at the high-spiked level (Table 2-5). This resulted in 21 individual Tissue samples at each laboratory for analysis.

Tissue samples were spiked on 19 July 2022, frozen at -20° C over the weekend, shipped on 27 October under chain of custody, and generally arrived within one day 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 tissue sample preparation guidelines accompanied each shipment to the laboratory (Figure 2-2).

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		
Waters ERA	Golden, CO	PFAS-spiked matrices and sample shipment for all aqueous, tissue and tissues
SGS AXYS Analytical Services, Ltd.	Sydney, BC, Canada	Native PFAS measures for all aqueous, tissue, and tissue samples
Eurofins-TestAmerica (ETA) Denver	Arvada, CO	Ancillary analytical measures for wastewater, surface water, groundwater, soils, tissues, 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

Laboratory Number	PFAS Matrix Analyses												
		Initial Dem. Capabilities			Aqueous Matrices				Solid Matrices			Tissue Matrices	
	Initial Calibration	Aqueous	Tissue	Tissue	Wastewater	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. Tissues Used for the Low/High PFAS Matrix Spikes

Sample 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	
Tissue												
Walleye (low lipid fish)	*S. vitreum*	TS	AB	TSAB0	TSAB1	TSAB2	TSAB3	TSAB4	TSAB5	TSAB6	TSAB7	10/27/2022
Salmon (high lipid fish)	*O. tshawytscha*	TS	AC	TSAC0	TSAC1	TSAC2	TSAC3	TSAC4	TSAC5	TSAC6	TSAC7	
Clams	*S. gigantea*	TS	AD	TSAD0	TSAD1	TSAD2	TSAD3	TSAD4	TSAD5	TSAD6	TSAD7	

Table 2-4. Results of Lipid Analyses on Tissue Samples

Analyte	Tissue and Sample ID			
	Walleye	Walleye Duplicate	Salmon	Clam
	TSAB0	TSAB0DU	TSAC0	TSAD0
Percent Lipids	0.38	0.408	8.1	0.63

Table 2-5. Target Low/High PFAS Spike Concentrations and Calibration Range based on Native PFAS Analyses in Tissue Samples

Target PFAS	Target Calibration (in 2.0 g samples)		Target PFAS Spike Concentrations		Final PFAS Spike Concentrations		PFAS Target Compound Analytical Results (µg/kg)		
	Low Cal [1]	High Cal [1]	Low Spike[1]	High Spike[1]	Low Spike[1]	High Spike[1]	TSAB0	TSAC0	TSAD0
PFBA	1.6	500	10	100	10	100	< 0.3941	< 0.3922	< 0.3941
PFPeA	0.8	250	5	100	5	100	< 0.1970	< 0.1961	< 0.1970
PFHxA	0.4	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
PFHpA	0.4	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
PFOA	0.4	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
PFNA	0.4	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
PFDA	0.4	125	2	50	2	50	0.154	< 0.09804	< 0.09852
PFUnA	0.4	125	2	50	2	50	0.1926	< 0.09804	< 0.09852
PFDoA	0.4	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
PFTrDA	0.4	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
PFTeDA	0.4	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
PFBS	0.4	125	2	50	2.04	49.7	< 0.09852	< 0.09804	< 0.09852
PFPeS	0.4	125	2	50	1.97	49.8	< 0.09902	< 0.09853	< 0.09902
PFHxS	0.4	125	2	50	2.01	50.2	< 0.09852	< 0.09804	< 0.09852
PFHpS	0.4	125	2	50	2	49.6	< 0.09852	< 0.09804	< 0.09852
PFOS	0.4	125	2	50	2.05	50.2	0.3888	0.2545	< 0.09852
PFNS	0.4	125	2	50	2.02	50	< 0.09852	< 0.09804	< 0.09852
PFDS	0.4	125	2	50	2.02	50.1	< 0.09852	< 0.09804	< 0.09852
PFDoS	0.4	125	2	50	2.04	50.4	< 0.09852	< 0.09804	< 0.09852
4:2FTS	1.6	100	5	50	4.97	49.7	< 0.3941	< 0.3922	< 0.3941
6:2FTS	1.6	100	5	50	5.05	49.5	< 0.3552	< 0.3534	< 0.3552
8:2FTS	1.6	100	5	50	4.99	49.9	< 0.3941	< 0.3922	< 0.3941
PFOSA	1	125	2	50	2	50	< 0.09852	< 0.09804	< 0.09852
NMeFOSA	1	125	2	50	2	50	< 0.1133	< 0.1128	< 0.1133
NEtFOSA	1	125	2	50	2	50	< 0.2463	< 0.2451	< 0.2463
NMeFOSAA	1	25	2	20	2	20	< 0.09852	< 0.09804	< 0.09852
NEtFOSAA	1	125	2	25	2	25	< 0.09852	< 0.09804	< 0.09852
NMeFOSE	4	250	10	100	10	100	< 0.9852	< 0.9804	< 0.9852
NEtFOSE	4	250	10	100	10	100	< 0.7370	NQ [2]	< 0.7370
HFPO-DA	1.6	100	5	50	5	50	< 0.3744	< 0.3726	< 0.3744

Table 2-5. Target Low/High PFAS Spike Concentrations and Calibration Range based on Native PFAS Analyses in Tissue Samples (Continued)

Target PFAS	Target Calibration (in 2.0 g samples)		Target PFAS Spike Concentrations		Final PFAS Spike Concentrations		PFAS Target Compound Analytical Results (µg/kg)		
	Low Cal [1]	High Cal [1]	Low Spike[1]	High Spike[1]	Low Spike[1]	High Spike[1]	TSAB0	TSAC0	TSAD0
ADONA	1.6	100	5	50	5	50	< 0.3941	< 0.3922	< 0.3941
9CL-PF3ONS	1.6	100	5	50	5.04	50.4	< 0.3951	< 0.3931	< 0.3951
11CL-PF3OUdS	1.6	100	5	50	5	50	< 0.3946	< 0.3927	< 0.3946
3:3FTCA	2	125	5	50	5	50	< 0.3941	< 0.3922	< 0.3941
5:3FTCA	10	624	20	200	20	200	< 2.463	< 2.451	< 2.463
7:3FTCA	10	624	20	200	20	200	< 2.463	< 2.451	< 2.463
PFEESA	0.8	50	5	25	5	25	< 0.09852	< 0.09804	< 0.09852
PFMPA	0.8	250	5	100	5	100	< 0.1970	< 0.1961	< 0.1970
PFMBA	0.8	250	5	100	5	100	< 0.09852	< 0.09804	< 0.09852
NFDHA	2	50	5	25	5	25	< 0.1970	< 0.1961	< 0.1970

Source: Chapter 2 Tissue 01122024.xlsx

Notes:
[1] All spiked concentrations are presented as acid concentrations; as final concentration in sample in µg/kg.
[2] NQ = Compound not quantitated because labeled compound was not detected

ERA
A Waters Company

▪ Certificate of Spiking ▪

Hydrogeologic MLV Study Samples

ERA Project Number: 11252101

Matrix Type: Tissues
Spike Level: High Level
Certificate Issue Date: 31-Oct-2022
Revision Number: 1.0

CERTIFICATION

Compound	Spiked Concentration[1] ng/g
PFBA	100.0
PFPEA	100.0
PFHXA	50.0
PFHPA	50.0
PFOA	50.0
PFNA	50.0
PFDA	50.0
PFUNA	50.0
PFDOA	50.0
PFTRDA	50.0
PFTEDA	50.0
PFBS	49.7
PFPES	49.8
PFHXS	50.2
PFHPS	49.6
PFOS	50.2
PFNS	50.0
PFDS	50.1
PFDOS	50.4
4:2FTS	49.7
6:2FTS	49.5
8:2FTS	49.9
PFOSA	50.0
NMeFOSA	50.0
NEtFOSA	50.0
NMeFOSAA	20.0
NEtFOSAA	25.0
NMeFOSE	100.0
NEtFOSE	100.0
HFPO-DA	50.0
ADONA	50.0
9CL-PF3ONS	50.4
11CL-PF3OUDS	50.0
3:3FTCA	50.0
5:3FTCA	200.0
7:3FTCA	200.0
PFEESA	25.0
PFMPA	100.0
PFMBA	100.0
NFDHA	25.0

SAMPLE-MATRIX TABLE

Lot Number:	Matrix Name:	Sampling Date[2]:	Sampling Time[2]:
TSAB5 TSAB6 TSAB7	Walleye (low lipid fish)	27-Oct-2022	10:00 AM
TSAC5 TSAC6 TSAC7	Salmon (high lipid fish)	27-Oct-2022	10:00 AM
TSAD5 TSAD6 TSAD7	Clams	27-Oct-2022	10:00 AM

Figure 2-1. Tissue Certificate of Spiking

PFAS Method Validation Study:

Tissue Sample Preparation Guidelines

Shipment Contents

- 18"x14"x15" Styrofoam box cooler
- (3) Tissue Lots - packaged in (21) x 15-mL amber glass wide mouth screw-top bottles
- Temperature blank
- Ice packs
- Sample Preparation Guidelines
- Sample Chain of Custody (COC)

Sample Description

- Samples are packaged in 15-mL amber glass wide mouth screw-top bottles containing approximately 2.00 g of spiked sample.
- Samples should be received at < 6°C.
- Samples are not preserved.
- Samples must be stored immediately at ≤-20°C until sample preparation.
- Each sample except the sample designated as the unspiked matrix blank 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 prepared and analyzed as soon as possible.

Sample Instructions

1. The sample preparation procedure found in the MLV Study Method is to be followed, with some exceptions.

- Instead of homogenizing the sample (Section 11.4) and weighing out an aliquot of the sample (Section 11.4.1), the entire contents of the container received is to be transferred into a 15-mL polypropylene centrifuge tube. Record 2.00 g as the aliquot of sample prepared. This is the mass to be used when calculating PFAS concentrations in each sample.

- Reserve the sample container for rinsing. Follow the steps in Sections 11.4.2 and 11.4.3. For Section 11.4.4, instead of adding 10 mL of 0.05M KOH in methanol to the centrifuge tube containing the sample, add it to the sample container that the sample was shipped to the laboratory in that was held in reserve. Vortex, then transfer the solution to the centrifuge tube and proceed with the method as written for the rest of Section 11.4.4 from the point after the addition of the solution to the centrifuge tube.

2. Report your results as ng/g 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: 03

Figure 2-2. Example Tissue 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 *Volume I* Section 3 and in the Study Plan (*Volume I,* Appendix A).

This chapter briefly recaps 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 tissues data used for the statistical analyses. 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; application of those data are presented in Appendix B (IDA Report) and the subsequent chapters of this report.

3.1 DATA MANAGEMENT

Procedures for Data Management are detailed in the Data Management Report (*Volume I,* 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. The data management processes for tissues were the same as those previously described in *Volumes I, II,* and *III.*

3.2 DATA VALIDATION

All data packages were reviewed for completeness and compliance with the requirements of the MLVS Method (*Volume I,* Appendix A), and the Study Data Validation Guidelines (DVGs) (*Volume I,* 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 data validation procedures employed for tissues were the same as those previously described in *Volumes I, II,* and *III.*

As with the data packages and EDDs submitted for *Volumes I, II,* and *III*, some errors and omissions were prevalent and required an iterative process with the laboratories. Problems with the data included, but were not limited to:

- Laboratory did not use the highest MDL_B value as the MDL when it was greater than the concentration calculated from the MDL.
- Ion ratio exceedances for one or multiple analytes that were unreported by the laboratory.
- Miscalculation or non-reported percent recoveries.
- Incorrect EIS compound associations (e.g., PFTrDA quantified using $^{13}C_2$-PFTeDA, not an average of $^{13}C_2$-PFDoA and $^{13}C_2$-PFTeDA or being quantified using $^{13}C_3$-PFDoA).
- Retention time outside of acceptance criteria for target and EIS compounds.

- Incorrect or missing ion transition summaries.
- Incorrect manual integration of peaks from chromatographs with an inability to confirm the laboratories' calculations.

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. Validator-added qualifiers were either confirmed, nulled, or a different data qualifier after additional review of the laboratory report. The qualifiers 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 and are maintained in the Project record.

Table 3-1 present a summary of the total type and number of analyses reviewed for the tissue study. A total of 29,951 individual results were submitted by the laboratories: 12,011 valid data points for the IDC tissue samples and 16,804 for the PFAS-spiked tissue matrices.

3.3 DATA USED IN THE STATISTICAL ANALYSES

The IDA Statistical Data Analysis Report for Tissue is Appendix B to this volume. 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 (AFCEC) and EPA's contractor General Dynamics Information Technology (GDIT). The AFCEC and GDIT findings are reported separately in Section 7.3 (AFCEC) and 7.4 (GDIT) of this report.

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. Of those submitted data, 26,194 data points (87%) passed all quality assurance reviews and were advanced for statistical analyses.

Table 3-1. Summary of Type and Number of Tissue 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 IDC: Reagent Tissue							
MDL Study (7 method blanks [MDLB])	7	3,868	53	2,040	1,272	371	3,683
MDL Study (7 MDL spiked samples [MDLS])	7	3,788	53	2,000	1,278	375	3,653
Initial Precision and Recovery (IPR) Study	7	2,075	29	1,114	700	205	2,019
Method Blanks	6	1,569	22	856	530	155	1,541
Limit of Quantification Verification	7	711	10	389	240	70	699
Tissue							
Unspiked Samples	7	1,940	21	786	514	158	1,458
Low-Level Spike	7	5,682	63	2353	1,544	479	4,376
High-Level Spike	7	5,927	63	2386	1,588	524	4,498
Low-Level Ongoing Precision and Recovery	7	1,085	15	567	371	113	1,051
Method Blanks	7	1,085	15	567	371	113	1,051
Ongoing Precision and Recovery	7	1,085	15	564	371	113	1,048
Total Number of Results		**28,815**	**359**	**13,622**	**8,779**	**2,676**	**25,077**

Source: Chapter Tissue 01122024.xlsx

Notes:
[1]Number of results submitted by the laboratories (i.e., pre-validation).
[2]Post-validation results included in the dataset used in statistical analysis.

4 CALIBRATION AND QUANTIFICATION

Tissue media sample extracts were analyzed by LC-MS/MS in MRM mode. *Volume I*, Section 4 provides a description of the calibration and quantification scheme used. Since the publication of *Volume I*, two more qualitative standards have become commercially available. These are for PFOA and PFNA. Therefore, since the completion of this Study, seven additional quantitative isomeric standards have become commercially available for the target analytes (PFOA, PFNA, PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE). In accordance with EPA Method 1633, these standards must be used when creating calibration standards, calibration verification standards, and spiking solutions and these seven 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 instrument manufacturer's instructions. Please see *Volume I*, Section 4.1, for additional details on the mass calibration and mass calibration verification.

4.2 MULTI-POINT INITIAL CALIBRATION

Discussion on the multi-point initial calibrations evaluated in Phase 3 of the MLVS can be found in *Volume I*, Section 4.2. It should be noted that while data from Laboratory 8 was eliminated from the evaluation due to a spiking error, ICALs used for quantitation of the tissue IDC and tissue samples were spiked correctly. Therefore, data from Laboratory 8 was included in the statistical analysis of data for the tissue IDC and tissue samples.

4.3 QUALITATIVE STANDARDS

Volume I, Section 4.3 contains information on the Qualitative Standard used in the Study.

4.4 CALIBRATION VERIFICATION

The calibration verification (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 criterion with the exception of one laboratory. Laboratory 10 reported one instance of CV standards failing to meet this criterion that affected the data that was reported (Table 4-1). Per the Study Plan, samples that were bracketed by CV standards whose % recoveries exceeded the acceptance criteria were retained and qualified with a "J+" qualifier in instances when the affected analyte was detected in the sample and a "J" in instances when it was not. The low CV failure rate documented by this study indicates the CV % recovery acceptance criteria required by this study is routinely achievable.

Table 4-1. Summary of Instances of CV Recoveries Outside of MLVS Acceptance Criteria Range

Laboratory ID	Affected Sample ID	Analyte	%Recovery	Data Qualifier Applied
10	TSAC4 DL	4:2FTS	137	J
10	TSAC5 DL	4:2FTS	137	J+
10	TSAC6 DL	4:2FTS	137	J+

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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 a 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 soils and sediment sample analysis were at a concentration that was 0.25 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 four exceptions. 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 in instances when the affected analyte was detected in the sample and a "J" in instances when it was not. 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.

Table 4-2. Summary of Instances of ISC Recoveries Outside of MLVS Acceptance Criteria Range

Laboratory ID	Affected Sample ID	Analyte	% Recovery	Data Qualifier Applied
1	TSDA7	NEtFOSAA	132.9	J+
3	TSAB1	NEtFOSAA	158.8	J
3	TSAB1	NFDHA	159.5	J
3	TSAB2	NEtFOSAA	158.8	J+
3	TSAB2	NFDHA	159.5	J+
3	TSAB3	NEtFOSAA	158.8	J+
3	TSAB3	NFDHA	159.5	J+
3	TSAB4	NEtFOSAA	158.8	J+
3	TSAB4	NFDHA	159.5	J+
3	TSAB5	NEtFOSAA	158.8	J+
3	TSAB5	NFDHA	159.5	J+
3	TSAB6	NEtFOSAA	158.8	J+
3	TSAB6	NFDHA	159.5	J+
3	TSAB7	NEtFOSAA	158.8	J+
3	TSAB7	NFDHA	159.5	J+
3	TSAC1	NFDHA	132.4	J

Table 4-2. Summary of Instances of ISC Recoveries Outside of MLVS Acceptance Criteria Range (Continued)

Laboratory ID	Affected Sample ID	Analyte	% Recovery	Data Qualifier Applied
3	TSAC2	NFDHA	132.4	J+
3	TSAC3	NFDHA	132.4	J+
3	TSAC4	NFDHA	132.4	J+
3	TSAC5	NFDHA	132.4	J+
3	TSAC6	NFDHA	132.4	J+
3	TSAC7	NFDHA	132.4	J+
3	TSAD1	NFDHA	132.4	J
3	TSAD2	NFDHA	132.4	J+
3	TSAD3	NFDHA	132.4	J+
3	TSAD4	NFDHA	132.4	J+
3	TSAD5	NFDHA	132.4	J+
3	TSAD6	NFDHA	132.4	J+
3	TSAD7	NFDHA	132.4	J+
4	TSAB1	PFTeDA	130.5	J
4	TSAB2	PFTeDA	130.5	J+
4	TSAB2	PFTeDA	130.5	J+
4	TSAB3	PFTeDA	130.5	J+
4	TSAB4	PFTeDA	130.5	J+
4	TSAB5	PFTeDA	130.5	J+
4	TSAB5	PFTeDA	130.5	J+
4	TSAB6	PFTeDA	130.5	J+
4	TSAB7	PFTeDA	130.5	J+
4	TSAC1	PFTeDA	130.5	J
4	TSAC2	PFTeDA	130.5	J+
4	TSAC3	PFTeDA	130.5	J+
4	TSAC4	PFTeDA	130.5	J+
4	TSAC5	PFTeDA	130.5	J+
4	TSAC6	PFTeDA	130.5	J+
4	TSAC7	PFTeDA	130.5	J+
4	TSAD1	PFTeDA	130.5	J
4	TSAD2	PFTeDA	130.5	J+
4	TSAD3	PFTeDA	130.5	J+
4	TSAD4	PFTeDA	130.5	J+
4	TSAD5	PFTeDA	130.5	J+
4	TSAD6	PFTeDA	130.5	J+
4	TSAD7	PFTeDA	130.5	J+

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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 (*Volume I,* Appendix A). The IDOC consisted of the MDL determination, the IPR study, 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.

5.1 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 PFAS-free tissue (chicken breast or other similar tissue) 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 tissue (chicken breast or other similar tissue) was the reference media used to prepare the seven MDL method blank replicates. Each replicate was spiked with the 24 EIS and seven NIS compounds to create at least seven MDL method blanks. At least 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. (Laboratories 6, 8, and 9 opted to prepare eight blanks and eight spiked replicates, which is allowed by the revised MDL procedure. Laboratory 1 prepared eight blanks and seven spiked replicates.) All MDL method blanks and MDL spiked samples were prepared per EPA Method 1633, in at least three batches on three separate calendar dates and analyzed on three separate calendar dates.

During the validation process, it was discovered that an error had occurred in the MDL Study submitted by Laboratory 3 that affected the quantitation of 6:2FTS, ADONA, PFHpS, PFHxA, PFNA, PFTrDA, NEtFOSA, and PFUnA. The laboratory did not use the highest MDL_b value in instances when its value was greater than the value calculated from the analysis of MDL_s samples. Due to this error, all data for these eight analytes have been eliminated from the rest of the IDC as well as the tissue statistical analyses.

The EIS and NIS compounds were spiked at the same concentrations as in the ICAL standards. The MDL values 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.

The only MDL_b value in Table 5-1 that was used as the final MDL came from Laboratory 4, for PFMPA. In this instance, the MDL_b value was used in the calculation of the final pooled MDL value in the table.

The distribution of detected analytes in the MDL_b aliquots for tissues is shown in Table 5-2 and was heavily influenced by Laboratory 1, with 34 of the 37 MDL_b detections across the 7 laboratories. The 34 detections by Laboratory 1 were spread across 12 target analytes, with 1 to 8 detections in the 8 blanks for those 13 analytes. However, those blank results were sufficiently low that none of the final MDL values from Laboratory 1 were based on an MDL_b, even in the case of PFUnA, where all 8 of the blanks contained the analyte. (The MDL_b for PFUnA calculated by Laboratory 1 was 0.18 µg/kg and the MDL_s was 0.28 µg/kg.)

Table 5-1. Tissue Method Detection Limit Study Results

Target Analyte	Number of Labs[1]	Max MDL_b[2]	Minimum Concentration of MDL (µg/kg)[3]	Maximum Concentration of MDL (µg/kg)[4]	# Labs Using MDL_b as Final MDL[5]	Pooled MDL (µg/kg)[6]
PFBA	7	U	0.151	0.391	0	0.208
PFPeA	7	U	0.0971	0.284	0	0.155
PFHxA	6	U	0.0613	0.196	0	0.111
PFHpA	7	0.0162	0.0385	0.187	0	0.0988
PFOA	7	0.0519	0.0681	0.215	0	0.105
PFNA	6	0.0416	0.0699	0.254	0	0.119
PFDA	7	0.0816	0.0664	0.357	0	0.149
PFUnA	6	0.176	0.0572	0.28	0	0.125
PFDoA	7	U	0.0665	0.245	0	0.101
PFTrDA	6	U	0.0248	0.37	0	0.142
PFTeDA	7	U	0.0492	0.433	0	0.159
PFBS	7	U	0.0644	0.18	0	0.0974
PFPeS	7	U	0.0436	0.139	0	0.0762
PFHxS	7	U	0.0499	0.159	0	0.0808
PFHpS	6	U	0.0324	0.214	0	0.119
PFOS	6	0.207	0.0906	0.303	0	0.145
PFNS	7	U	0.0284	0.264	0	0.108
PFDS	7	U	0.0365	0.212	0	0.114
PFDoS	7	U	0.0406	0.395	0	0.153
4:2FTS	7	U	0.117	0.704	0	0.369
6:2FTS	6	0.0396	0.252	1.39	0	0.537
8:2FTS	7	U	0.201	0.769	0	0.378
PFOSA	7	0.0135	0.0539	0.116	0	0.0688
NMeFOSA	6	0.225	0.0372	0.383	0	0.162
NEtFOSA	6	U	0.0702	0.397	0	0.163
NMeFOSAA	7	U	0.078	0.265	0	0.145
NEtFOSAA	7	U	0.0683	0.278	0	0.148
NMeFOSE	7	0.289	0.365	1.96	0	0.832
NEtFOSE	6	U	0.323	4.88	0	1.77
PFMPA	7	0.204	0.0792	0.655	1	0.273
PFMBA	7	U	0.0828	0.288	0	0.168
NFDHA	7	U	0.11	0.407	0	0.216
HFPO-DA	7	U	0.196	0.625	0	0.339
ADONA	6	U	0.096	0.479	0	0.274
PFEESA	7	U	0.0972	0.248	0	0.123
9Cl-PF3ONS	7	0.0214	0.208	0.697	0	0.362
11Cl-PF3OUdS	7	U	0.24	0.751	0	0.352
3:3FTCA	7	U	0.202	2.22	0	0.716
5:3FTCA	7	U	1.56	4.62	0	2.38
7:3FTCA	7	0.21	1.5	3.41	0	2.02

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Notes:

1 The number of laboratories for which an MDL value was calculated.

2 The maximum MDLb value across individual spiked samples. "U" indicates analyte was not detected.

3 The minimum MDL calculated across laboratories. 4 The maximum MDL calculated across laboratories

5 The number of laboratories for which the MDLb value was the final MDL value.

6 Pooled MDL using the individual laboratory MDL values calculated. Equation from EPA 821-B-18-001 page G-22.

Table 5-2. Tissue Method Detection Limit Study Results

# MDL_b Detections	Lab 1	Lab 3	Lab 4	Lab 6	Lab 8	Lab 9	Lab 10
	34	2	1	0	0	0	0

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Figure 5-1 shows the distribution of individual laboratory MDLs relative to the pooled value calculated in Table 5-1. The figures shows that the individual MDLs reported by the laboratories are relatively similar and clustered around the pooled MDL for PFBA through PFMBA, with the exception of Laboratory 1, which typically exhibited higher MDLs. Beginning at NFDHA and continuing through the FTCAs, a much wider distribution of MDLs is seen, with the FTSs exhibiting the highest variability. This is also reflected in the minimum and maximum MDL for those same PFAS in Table 5-1.

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 the tissue matrices. Four aliquots of 2.0 grams wet-weight of PFAS-free tissue (e.g., chicken breast or similar tissue) 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 were prepared and analyzed in exactly the same manner as study samples, per EPA Method 1633.

A total of 24 to 29 IPRs were included in the statistical analysis, depending on the target analyte. The mean percent recovery, standard deviations, and RSD of recoveries are presented in Table 5-3. All IPRs met the Study IPR NIS criteria (>30% recovery). All of the 1,114 valid target analyte results reported from IPRs were within the target analyte criteria (40–150%), except for nine results. Six of these results were below the 40% criteria for PFDoS; Laboratory 9 reported four instances (32%, 32%, 35%, and 35%), Laboratory 4 reported one instance (33.8), and Laboratory 1 reported one instance (39%). The three results that exceeded the 150% criteria were for three different target analytes; Laboratory 3 reported one instance for 8:2FTS (152%) and one instance for 7:3FTCA (155%) and Laboratory 4 reported a single instance for NEtFOSE (180%). None of these results can be explained by their EIS compound recoveries since they were not statistically different than those from the other laboratories.

Most recoveries reported by laboratories were consistent, with the exception of Laboratory 6, which was biased low and Laboratory 8, which was biased high, comparatively (Figure 5-2).

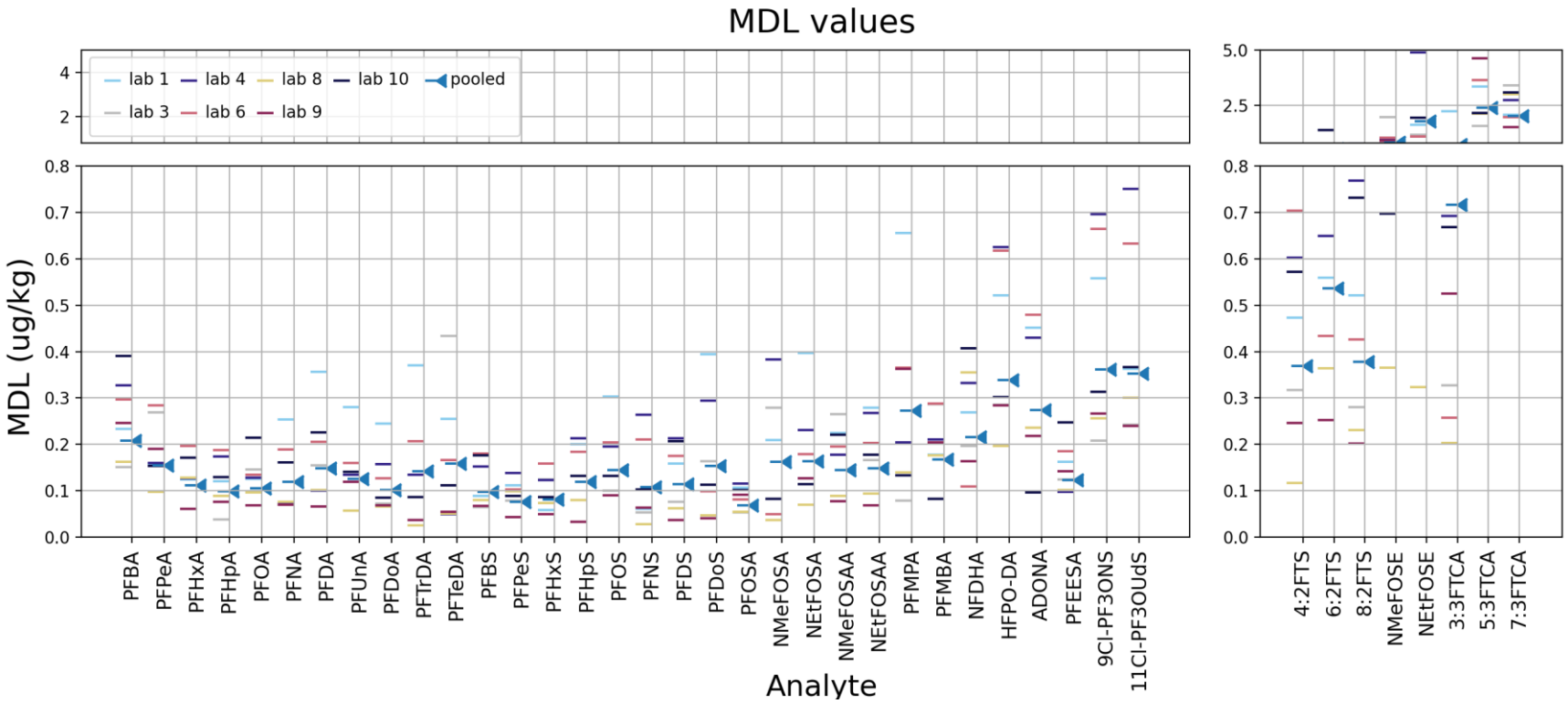

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Figure 5-1. Tissues Method Detection Limit Study Results.
Figure includes individual and pooled Results (Table 5-1)

The lowest mean recovery for a target analyte was associated with PFDoS (75.4%), while the highest mean recovery was associated with 8:2FTS (118%). All but five target analyte mean recoveries were at or greater than 100%.

Of the 700 valid EIS compound results reported from IPRs, 20 results failed to meet the target EIS compound acceptance criteria (20–150%), resulting in a 2.9% exceedance rate. These exceedances were reported by Laboratory 4 (4 instances), Laboratory 6 (8 instances), and Laboratory 9 (8 instances). Ten of these exceedances were for D_9-NEtFOSE; Laboratory 9 reported four instances (0.3%, 0.4%, 0.6%, 0.8%), Laboratory 4 reported four instances (3.56%, 10.5%, 17.7%, 18.6%), and Laboratory 6 reported two instances (13%, 17%). There were five exceedances for D_3-NMeFOSA reported; Laboratory 9 reported four instances (4%, 5%, 5%, 5%) while Laboratory 6 reported one instance (17.9%). The remaining exceedances were reported by Laboratory 6 for D_7-NMeFOSE (10.9%, 13.2%, 13.6%, 14.8%) and $^{13}C_4$-PFBA (9.15%).

5.3 LIMIT OF QUANTITATION VERIFICATION ANALYSES

Since a low level ongoing precision and recovery (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 2.0 grams of PFAS-free tissue (chicken breast or other similar tissue) 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 was prepared and analyzed in exactly the same manner as study samples, 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 four. All valid data submitted for LOQVER samples was included in the statistical analysis.

A total of 10 LOQVERs were included in the statistical analysis. Table 5-4 shows the pooled results across all laboratories by PFAS; the results are graphically shown in Figure 5-3. All 10 LOQVERs met the Study NIS target acceptance criteria (>30% recovery). Of the 389 valid target analyte results reported from LOQVERs, six target analytes recoveries failed to meet the target criteria (40–150%), resulting in an exceedance rate of 1.80%. Three of these recoveries were below the 40% criteria, ranging from 26% to 39% while the remaining instances ranged from 159% to 192%. The recoveries reported below the 40% criteria were associated with PFDoS (2) and 3:3FTCA (1). The recoveries above the 150% criteria were associated with PFOA, 7:3FTCA, and NEtFOSE.

Of the 240 valid EIS compound results reported from LOQVERs, twenty-three failed to meet the EIS compound acceptance criteria (20–150%), resulting in a failure rate of 9.6%. Fourteen of these recoveries were below the 20% criteria, ranging from 7.7% to 19.6% while the remaining nine instances ranged from 151% to 253%. The recoveries reported below the 20% criteria were associated with $^{13}C_2$-PFTeDA, $^{13}C_4$-PFBA, D_3-NMeFOSA, D_7-NMeFOSE, and D_9-NEtFOSE. The recoveries reported above the 150% criteria were associated with $^{13}C_2$-PFTeDA, $^{13}C_3$-PFBS, $^{13}C_2$-4:2FTS, $^{13}C_2$-6:2FTS, $^{13}C_2$-8:2FTS, D_5-NEtFOSAA, and D_7-NMeFOSE. The 3 most frequent failures were for D_3-NMeFOSA (5), D_9-NEtFOSE (4), and D_7- NMeFOSE (4).

Table 5-5 provides the range of LOQs the laboratories used to report tissue samples in this Study. Concentrations are based on a sample mass of 2.0 grams; LOQs that were elevated due to extract dilutions prior to analysis were omitted from the summary.

Table 5-3. Tissue 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	7	28	110	13.3	2.83	14.2	2.58
PFPeA	7	29	108	9.69	4.3	10.4	3.96
PFHxA	6	25	112	13.0	6.00	14.1	5.35
PFHpA	7	29	112	14.2	5.63	15.2	5.04
PFOA	7	29	110	10.8	9.08	11.6	8.22
PFNA	6	25	112	12.7	6.11	13.8	5.44
PFDA	7	29	110	10.6	6.08	11.3	5.53
PFUnA	6	25	110	11.3	6.04	12.3	5.50
PFDoA	7	29	107	11.4	4.70	12.2	4.39
PFTrDA	6	25	103	30.7	5.71	33.2	5.54
PFTeDA	7	29	112	15.1	8.55	16.1	7.66
PFBS	7	29	110	11.4	5.98	12.2	5.44
PFPeS	7	29	106	16.7	5.38	17.8	5.08
PFHxS	7	29	110	11.0	8.69	11.8	7.88
PFHpS	6	25	105	16.6	5.69	17.9	5.41
PFOS	6	24	108	8.11	11.6	8.76	10.7
PFNS	7	29	96.9	14.8	4.18	15.8	4.31
PFDS	7	29	94.2	20.1	4.23	21.5	4.49
PFDoS	7	29	75.4	37.6	10.9	40.2	14.4
4:2FTS	7	29	107	15.6	10.3	16.7	9.63
6:2FTS	6	25	111	11.3	9.64	12.2	8.65
8:2FTS	7	29	118	13.3	9.92	14.3	8.42
PFOSA	7	29	111	12.8	3.11	13.7	2.80
NMeFOSA	6	25	116	9.33	11.5	10.1	9.88
NEtFOSA	6	25	111	11.2	12.7	12.2	11.4
NMeFOSAA	7	29	111	14.6	7.29	15.6	6.59
NEtFOSAA	7	29	105	16.0	7.73	17.1	7.36
NMeFOSE	7	29	95.7	22.8	3.59	24.3	3.75
NEtFOSE	6	25	116	17.6	17.6	19.1	15.2

Table 5-3. Tissue IPR Results (Continued)

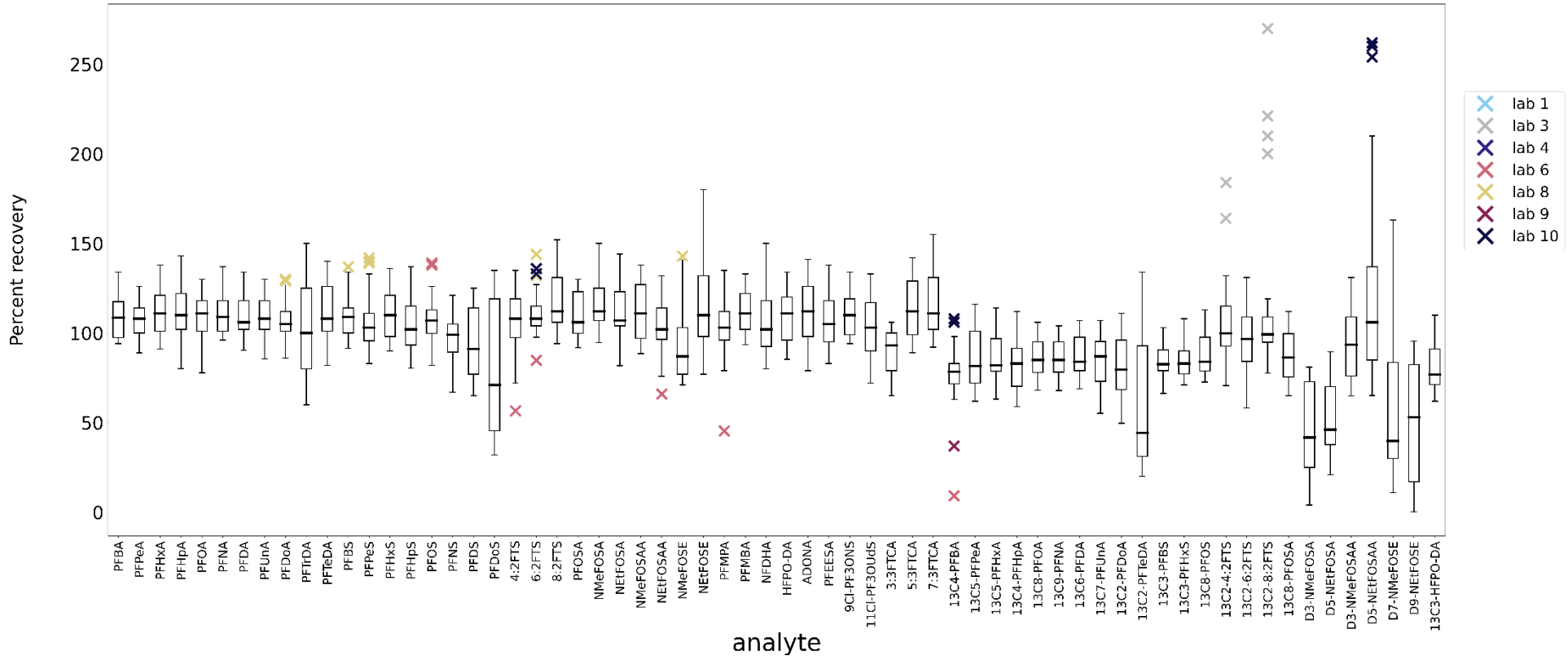
Analyte	Number of Labs[1]	Number of Results[2]	Mean % Recovery[3]	Pooled Between-Lab std. dev. (s_b)[4]	Pooled Within-Lab std. dev. (s_w)[5]	Pooled Between- and Within-Lab std. dev. (s_c)[6]	RSD (s_w)[7]
PFMPA	7	29	105	15.4	11.1	16.5	10.7
PFMBA	7	29	110	11.2	4.17	12.0	3.77
NFDHA	7	29	107	19.2	10.4	20.6	9.71
HFPO-DA	7	29	109	12.4	7.61	13.3	6.98
ADONA	6	25	111	17.5	6.88	18.9	6.18
PFEESA	7	29	108	15.1	7.68	16.2	7.12
9Cl-PF3ONS	7	29	111	11.5	6.76	12.3	6.12
11Cl-PF3OUdS	7	29	102	18.3	6.75	19.5	6.59
3:3FTCA	7	29	88.7	12.9	5.64	13.8	6.36
5:3FTCA	7	29	114	16.3	6.93	17.4	6.09
7:3FTCA	7	29	116	16.8	6.79	17.9	5.84
EIS Compounds							
$^{13}C_4$-PFBA	7	29	77.4	14.8	16.2	15.9	21.0
$^{13}C_5$-PFPeA	7	29	84.3	16	5.73	17.1	6.79
$^{13}C_5$-PFHxA	7	29	86.3	11.5	5.36	12.4	6.21
$^{13}C_4$-PFHpA	7	29	82.7	14.1	4.06	15.0	4.91
$^{13}C_8$-PFOA	7	29	86.5	10.5	5.76	11.2	6.66
$^{13}C_9$-PFNA	7	29	86.1	10.4	4.42	11.2	5.14
$^{13}C_6$-PFDA	7	29	87.2	10.8	4.62	11.6	5.31
$^{13}C_7$-PFUnA	7	29	83.0	15.2	3.51	16.3	4.22
$^{13}C_2$-PFDoA	7	29	80.6	17.6	6.05	18.8	7.51
$^{13}C_2$-PFTeDA	7	29	61.9	38.2	8.75	40.9	14.1
$^{13}C_3$-PFBS	7	29	84.0	9.69	5.48	10.4	6.53
$^{13}C_3$-PFHxS	7	29	85.4	9.49	5.62	10.2	6.58
$^{13}C_8$-PFOS	7	33	87.6	11.6	5.15	12.5	5.88
$^{13}C_2$-4:2FTS	7	29	47.0	27.5	5.30	29.4	11.3
$^{13}C_2$-6:2FTS	7	29	51.5	19.7	7.17	21.0	13.9
$^{13}C_2$-8:2FTS	7	29	93.2	18.1	7.76	19.3	8.33
$^{13}C_8$-PFOSA	7	29	122	59.8	11.6	63.9	9.46
D_3-NMeFOSA	7	29	61.5	46.6	6.26	49.8	10.2

Table 5-3. Tissue IPR Results (Continued)

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]
D_5-NEtFOSA	7	29	45.6	35.3	7.54	37.7	16.5
D_3-NMeFOSAA	7	29	82.0	14.0	6.10	15.0	7.44
D_5-NEtFOSAA	7	29	47.0	27.5	5.30	29.4	11.3
D_7-NMeFOSE	7	29	51.5	19.7	7.17	21.0	13.9
D_9-NEtFOSE	7	29	93.2	18.1	7.76	19.3	8.33
$^{13}C_3$-HFPO-DA	7	29	122	59.8	11.6	63.9	9.46

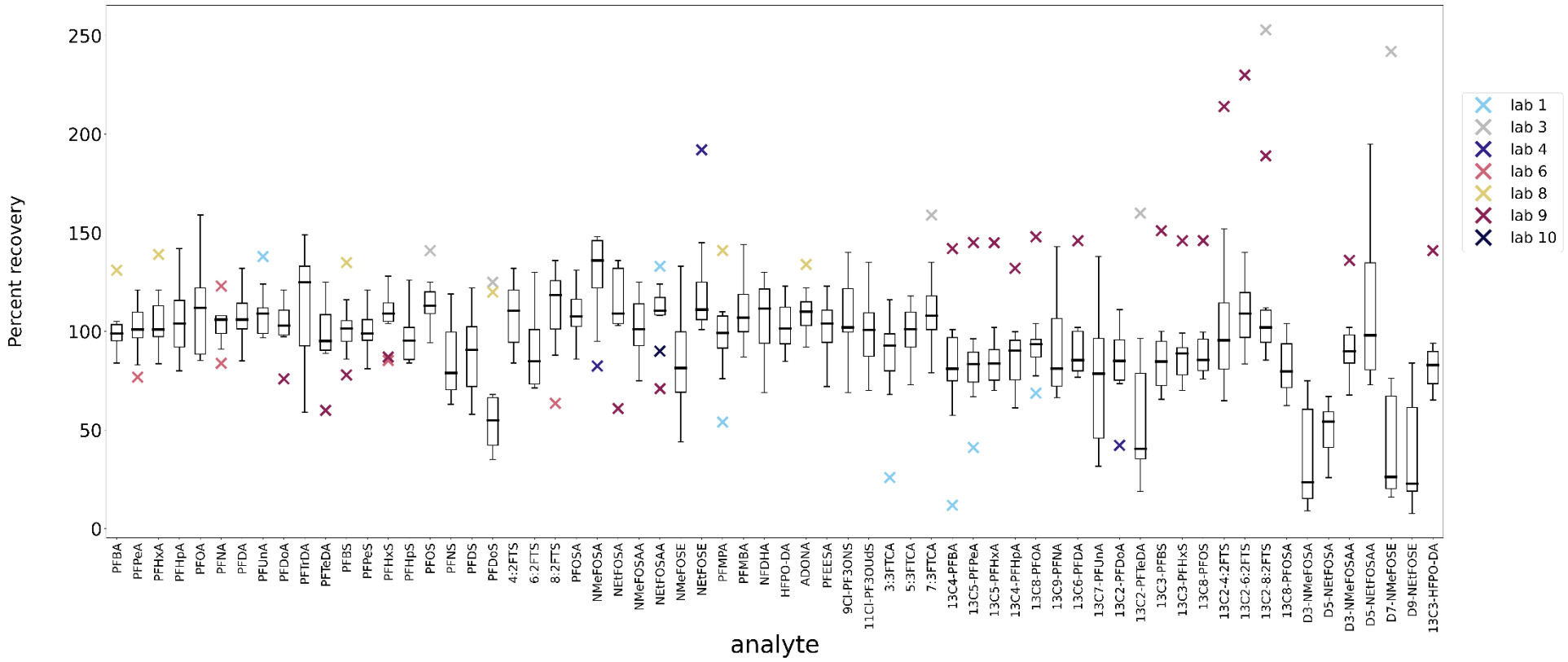
Source: RT_IPR_results_V0_231215_004710.csv

Notes:
1 The number of laboratories reporting initial precision recovery (IPR) results.
2 The number of individual IPR results that do not have a U flag included in the calculations.
3 Mean % Recovery - The mean percent recovery for IPR samples across all labs for the given analyte.
4 The combined within and between-laboratory standard deviations. Equation from EPA 821-B-18-001 page G-25.
5 The pooled between-laboratory standard deviation of the percent recoveries. 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).



Source File: RT_IPR_Boxplot_V0_231215_004710

Figure 5-2. Initial Precision and Recovery (IPR) Results by Analyte by Laboratory
Figure includes both target compound recoveries, and EIS compound recoveries.



Source file: RT_LOQVER_Boxplot_V0_231215_004710

Figure 5-3. Limit of Quantitation Verification (LOQVER) Results by Analyte by Laboratory
Figure includes both target compound recoveries, and EIS compound recoveries.

Table 5-4. Tissue LOQVER Summary

Target Analyte	Number of Laboratories[1]	Minimum Concentration (µg/kg)[2]	Maximum Concentration (µg/kg)[3]	Minimum Percent Recovery[4]	Maximum Percent Recovery[5]
Target Analyte					
PFBA	7	1.39	26.1	83.5	131
PFPeA	7	0.719	13.7	76.9	121
PFHxA	6	0.438	6.44	83.6	139
PFHpA	7	0.346	7.38	80.4	141
PFOA	7	0.418	6.8	85.2	159
PFNA	6	0.433	7.66	83.9	123
PFDA	7	0.368	7.17	85.4	132
PFUnA	6	0.494	6.99	96.8	138
PFDoA	7	0.357	6.87	75.6	121
PFTrDA	6	0.295	9.32	59	149
PFTeDA	7	0.301	5.93	60.2	126
PFBS	7	0.314	5.77	77.9	135
PFPeS	7	0.332	6.5	81.3	121
PFHxS	7	0.37	6.23	85.3	128
PFHpS	6	0.4	6.37	83.9	127
PFOS	6	0.468	6.38	94.3	141
PFNS	7	0.304	4.68	63.2	119
PFDS	7	0.278	5.87	57.6	122
PFDoS	7	0.172	3.73	35.5	125
4:2FTS	7	1.21	30.8	84	132
6:2FTS	6	1.39	20	71.4	130
8:2FTS	7	1.46	32.4	63.6	136
PFOSA	7	0.423	6.81	85.8	131
NMeFOSA	6	0.412	9.2	82.4	148
NEtFOSA	6	0.306	7.23	61.2	136
NMeFOSAA	7	0.332	7.22	75.2	125
NEtFOSAA	7	0.356	7.13	71.2	133
NMeFOSE	7	2.19	48.3	43.8	134
NEtFOSE	6	4.96	77.9	101	192
PFMPA	7	0.543	13.7	53.5	141
PFMBA	7	0.87	13.6	87	144
NFDHA	7	0.71	15.6	68.5	130
HFPO-DA	7	1.46	26.7	84.8	123
ADONA	6	1.75	25.8	92.6	134
PFEESA	7	0.459	12.2	71.9	123
9Cl-PF3ONS	7	0.924	24.3	69	140
11Cl-PF3OUdS	7	0.949	26.1	69.8	135
3:3FTCA	7	1.09	30.7	26.5	116
5:3FTCA	7	6.52	184	72.7	118
7:3FTCA	7	8.91	184	78.6	159

Data file: Source: RT_LOQVER_results_V0_231215_004710.csv

Notes:
1 The number of laboratories reporting limit of quantitation verification (LOQVER) results.
2 The minimum concentration measured across all laboratories.
3 The maximum concentration measured across all laboratories.
4 The minimum percent recovery across all laboratories.
5 The maximum percent recovery across all laboratories.

Table 5-5. Summary of Verified LOQs for Tissues

Target Analyte	Number of Laboratories	LOQ Minimum Concentration (µg/kg)	LOQ Maximum Concentration (µg/kg)	LOQ Average Concentration (µg/kg)
PFBA	7	1.6	4	2.27
PFPeA	7	0.8	1	0.97
PFHxA	6	0.4	0.5	0.482
PFHpA	7	0.4	0.5	0.485
PFOA	7	0.4	0.5	0.485
PFNA	6	0.4	0.5	0.483
PFDA	7	0.4	0.5	0.485
PFUnA	6	0.4	1	0.567
PFDoA	7	0.4	0.5	0.486
PFTrDA	6	0.4	0.5	0.482
PFTeDA	7	0.4	1	0.56
PFBS	7	0.4	0.5	0.47
PFPeS	7	0.4	0.5	0.477
PFHxS	7	0.4	0.5	0.474
PFHpS	6	0.4	0.5	0.475
PFOS	6	0.4	2	0.728
PFNS	7	0.4	0.5	0.48
PFDS	7	0.4	0.5	0.481
PFDoS	7	0.4	0.5	0.481
4:2FTS	7	1.6	2	1.89
6:2FTS	6	1.6	2	1.88
8:2FTS	7	1.6	2	1.91
PFOSA	7	0.4	0.5	0.485
NMeFOSA	6	0.4	0.5	0.482
NEtFOSA	6	0.4	1	0.523
NMeFOSAA	7	0.4	0.5	0.486
NEtFOSAA	7	0.4	0.5	0.484
NMeFOSE	7	4	5	4.87
NEtFOSE	6	4	5	4.95
PFMPA	7	0.8	2	1.12
PFMBA	7	0.8	1	0.969
NFDHA	7	0.8	1	0.971
HFPO-DA	7	1.6	2.09	1.96
ADONA	6	1.6	2	1.91
PFEESA	7	0.8	1	0.94
9Cl-PF3ONS	7	1.6	2	1.92
11Cl-PF3OUdS	7	1.6	2	1.92
3:3FTCA	7	2	4	2.58
5:3FTCA	7	10	20	12.9
7:3FTCA	7	10	12.5	11.8

Version: Summary_tables_Exa_Tissue_CH5_12222023.xlsx

Notes:
[1] Concentrations based on an extract dilution factor of 1.

6 TISSUE RESULTS

A total of 21 study samples were spiked and shipped to each participating laboratory as described in Section 2 of this report. These included one native (unspiked), three low-spiked, and three high-spiked samples. All tissue study samples were prepared and analyzed by each laboratory as required by EPA Method 1633. Data were reported and validated in accordance with the requirements of the Study Plan. The rules used for including individual analyte results are presented in Section 3 of this report.

The methods used to calculate the percent recoveries, within-laboratory standard deviation, within- and between-laboratory standard deviation, and within-laboratory RSDs followed the ATP-protocol prescribed methods (EPA 2018). The specific detailed methods followed are presented in *Volume I,* Appendix D. Methods adapted for evaluating the tissues by IDA are given in Appendix A of this report.

6.1 PFAS CONCENTRATIONS IN UNSPIKED TISSUE

Each laboratory received and analyzed a single unspiked sample of each tissue (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. Table 6-1 also includes the results of the reconnaissance analysis (by SGS AXYS) used to set the low/high spike concentrations (Table 2-2). The total number of PFAS target analytes detected by at least one laboratory is given in Table 6-2.

Table 6-1 also shows that the detections of PFAS reported from the three tissue samples across the 8 laboratories ranged from no PFAS detections across all three tissue samples, to 34 detections for Laboratory 8. With few exceptions the detected values were just above the MDL and less than 1 µg/kg. The one apparent difference is in the number of reported values for Laboratory 8: 34 reported detections and all of those for one walleye sample (Sample TSAB1). All of those reported values were between the MDL and the LOQ, and hence were J-qualified. There were no detected PFAS for Laboratory 8 in the salmon or clam tissue samples. There were no PFAS detected consistently across the eight laboratories. PFOS was detected in the walleye tissue for Laboratories 1, 4 and by SGS AXYS, but was non-detected by the other laboratories. A summary of the reported values for the unspiked sample across all laboratories is found in Appendix B, Table B-1.

6.2 MATRIX SPIKE RESULTS

The compiled PFAS-spiked tissue sample results from the eight laboratories are given in Table 6-3. Overall, the pooled laboratory mean percent recoveries across the 40 target analytes was 95%, with individual PFAS pooled percent recoveries ranging from 66% (PFDoS) to 138% (7:3FTCA).

For the low-spiked tissue samples (Table 6-3), the pooled mean percent recovery across all 40 target analytes was 96%, with a range of 69.3 (PFDoS) to 145% (7:3FTCA). As evident in Figure 6-1, there are differences in reported recoveries by individual laboratories and specific compounds (data in Appendix B, Table B-2). For the low-spiked tissue matrices, recoveries for Laboratory 1 are consistently well-below those of the other seven laboratories. Recoveries for Laboratory 8 were consistently high, and especially for 9Cl-PF3ONS, 11Cl-PF3OUdS, and 7:3FTCA in two of the salmon replicates (TSAC2 and TSAC3). Recoveries of 9Cl-PF3ONS were 3075% and 1062%,

for 11Cl-PF3OUdS 3460% and 1166%, and for 7:3FTCA 700% and 480%. The calculated pooled between-laboratory standard deviation (s_b) and the pooled within-laboratory standard deviation (s_c) was at 24.7%. The higher standard deviation is likely in part skewed by these high recoveries by Laboratory 8.

For the high-spiked samples (Table 6-3), the pooled recoveries were similar to that observed in the low-spiked samples. Mean percent recovery was 93.5%, with a range of 63 (PFDoS) to 132% (7:3FTCA). Figure 6-2 shows the notable differences for individual laboratories and specific target analytes (data in Appendix B, Table B-3). Percent recoveries for Laboratory 1 were again below those reported by the other seven laboratories. For the high-spiked samples, Laboratory 8 recoveries were generally consistent with the other seven laboratories: the elevated recoveries observed for the low-spiked salmon tissues were not observed in the high-spiked samples.

Results comparing the three different tissue samples using the pooled laboratory results are given in Table 6-4. Generally, the mean percent recoveries were similar for all target PFAS across the fish and clam samples.

Table 6-5 provides a summary of the relative proportions of the pooled low-/high-spiked results for all laboratories that that fell between the target percent recovery acceptance criteria that were used to evaluate the OPR and LLOPR (40 - 150%). For 39 of the 40 PFAS analytes, > 75% of all values reported were between 40 – 150% recovery. For the single remaining PFAS, 7:3FTCA, > 65% of all values were within that same recovery range.

Tissue matrix spike-recoveries are discussed further in Section 7.3.

6.3 EXTRACTED INTERNAL STANDARD RESULTS

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

The target percent recovery range for EIS compounds in this Study is 20–150%. The combined results for the minimum, maximum, and average percent recovery are given in Table 6-7. Supporting individual laboratory results are provided in Appendix B, Table B-4. For the eight laboratories, the pooled average EIS percent recovery ranged between 22.9 (D_9-NEtFOSE) and 229.1% ($^{13}C_2$-8:2FTS). Table 6-8 presents the pooled tissue EIS percent recovery; all mean percent recoveries were within the MLVS method-specified target recovery.

Figure 6-4 show that the highest variability in EIS compound recoveries for all laboratories were for $^{13}C_4$-PFBA, $^{13}C_5$-PFPeA, D_3-NMeFOSA, D_5-NEtFOSA, D_7-NMeFOSE and D_9-NEtFOSE. Of those six EIS compounds, percent recoveries were less than 5%. Laboratories 1 and 8 had lower recoveries in particular for $^{13}C_4$-PFBA, D_7-NMeFOSE and D_9-NEtFOSE. Five of the eight laboratories all had low recoveries for D_7-NMeFOSE and D_9-NEtFOSE.

Performance variability is further reflected in Table 6-9. Of the 24 EIS compounds, 15 compounds had > 80% of all values reported between 20 - 150% recovery. For all three of the FTS EIS

compounds, greater than 50% of all reported percent recoveries above 150%. For $^{13}C_2$-8:2FTS approximately 80% of reported percent recoveries across all laboratories exceeded 150%. EIS recovery of >150% was also high for D_3-NMeFOSA and D_5-NEtFOSA (36 and 39% of all values, respectively). Conversely, for D_7-NMeFOSE and D_9-NEtFOSE the proportion of reported values less than 20% was approximately 39% and 56%, respectively. Individual laboratory performance is given in Appendix B, Table B-4.

Finally, Table 6-10 provides a comparison of the mean individual laboratory EIS compound percent recoveries relative to the acceptance limits for EIS compounds that EPA determined for all aquatic matrices and QC samples in the most recent draft of EPA Method 1633 (Version 4, Table 6). For that comparison, average EIS percent recoveries for all compounds and all laboratories were tissue within the acceptance criteria range.

EIS compound results are further discussed in Section 7.4.2.

Table 6-1. Summary of Target Analytes Detected in Unspiked Tissue Samples in µg/kg

Analyte	Number of Labs	Lab 1		Lab 3		Lab 4		Lab 6		Lab 8		Lab 9		Lab 10		SGS-AXYS Baseline	
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual [1]
TSAB1 - Walleye (low lipid fish)																	
PFBA	7	0.229	U	0.2	U	0.323	U	0.278	U	1.66	J	0.666	U	0.39	U	0.3941	U
PFPeA	7	0.157	U	0.1	U	0.161	U	0.266	U	0.645	J	0.559	J	0.154	U	0.197	U
PFHxA	6	0.124	U	--	X	0.126	U	0.184	U	0.268	J	0.119	U	0.171	U	0.09852	U
PFHpA	7	0.121	U	0.05	U	0.174	U	0.175	U	0.219	J	0.076	U	0.129	U	0.09852	U
PFOA	7	0.125	U	0.05	U	0.128	U	0.125	U	0.286	J	0.07	U	0.215	U	0.09852	U
PFNA	6	0.254	U	--	X	0.0725	U	0.177	U	0.261	J	0.108	U	0.161	U	0.09852	U
PFDA	7	0.357	U	0.05	U	0.12	J	0.193	U	0.452	J	0.133	J	0.226	U	0.154	
PFUnA	6	0.28	U	--	X	0.169	JI	0.15	U	0.35	J	0.19	U	0.14	U	0.1926	
PFDoA	7	0.245	U	0.05	U	0.156	U	0.119	U	0.226	J	0.068	U	0.085	U	0.09852	U
PFTrDA	6	0.37	U	--	X	0.134	U	0.194	U	0.38	J	0.052	U	0.087	U	0.09852	U
PFTeDA	7	0.255	U	0.05	U	0.0491	UJ	0.155	U	0.278	J	0.158	U	0.112	U	0.09852	U
PFBS	7	0.089	U	0.05	U	0.152	U	0.168	U	0.208	J	0.067	U	0.177	U	0.09852	U
PFPeS	7	0.111	U	0.05	U	0.138	U	0.0965	U	0.159	J	0.044	U	0.089	U	0.09902	U
PFHxS	7	0.0981	JI	0.05	U	0.123	U	0.148	U	0.152	U	0.063	U	0.086	U	0.09852	U
PFHpS	6	0.216	U	--	X	0.214	U	0.172	U	0.305	J	0.032	U	0.132	U	0.09852	U
PFOS	6	0.233	JI	0.05	U	0.287	JI	0.191	U	--	X	0.418	U	0.211	J	0.3888	
PFNS	7	0.0984	U	0.05	U	0.264	U	0.197	U	0.231	J	0.063	U	0.103	U	0.09852	U
PFDS	7	0.173	U	0.05	U	0.212	U	0.164	U	0.263	J	0.037	U	0.207	U	0.09852	U
PFDoS	7	0.425	U	0.05	U	0.294	U	0.0923	U	0.176	J	0.04	U	0.113	U	0.09852	U
6:2FTS	6	0.555	U	--	X	0.647	U	0.405	U	1.05	J	0.251	U	13.9	UD	0.3552	U
8:2FTS	7	0.516	U	0.2	UJ	0.767	U	0.398	U	0.66	J	0.199	U	7.32	UD	0.3941	U
PFOSA	7	0.107	U	0.05	U	0.116	U	0.0755	U	0.259	J	0.091	U	0.102	U	0.09852	U
NMeFOSA	6	0.209	U	0.05	U	0.383	U	0.0459	U	0.253	J	--	X	0.083	U	0.1133	U
NEtFOSA	5	0.397	U	--	X	0.231	U	0.167	U	0.312	J	--	X	0.113	U	0.2463	U
NMeFOSAA	7	0.225	U	0.05	U	0.177	U	0.183	U	0.237	J	0.078	U	0.22	U	0.09852	U
NEtFOSAA	7	0.279	U	0.05	UJ	0.268	U	0.189	U	0.253	J	0.069	U	1.78	UD	0.09852	U
PFMPA	7	0.656	U	0.1	U	0.204	U	0.342	UJ	0.363	J	0.205	U	0.132	U	0.197	U
PFMBA	7	0.17	U	0.1	U	0.212	U	0.269	U	0.65	J	0.205	U	0.083	U	0.09852	U
NFDHA	7	0.269	U	0.1	UJ	0.332	U	0.103	U	0.872	JI	0.163	U	0.407	U	0.197	U
HFPO-DA	7	0.514	U	0.2	U	0.626	U	0.577	U	0.783	J	0.285	U	0.297	U	0.3744	U
ADONA	6	0.447	U	--	X	0.432	U	0.448	U	0.652	J	0.215	U	0.1	U	0.3941	U
PFEESA	7	0.162	U	0.1	U	0.0971	U	0.174	U	0.704	J	0.141	U	0.248	U	0.09852	U
9Cl-PF3ONS	7	0.564	U	0.2	U	0.703	U	0.622	U	0.697	J	0.268	U	0.313	U	0.3951	U
11Cl-PF3OUdS	7	0.363	U	0.2	U	0.746	U	0.592	U	0.651	J	0.238	U	0.368	U	0.3946	U

Table 6-1. Summary of Target Analytes Detected in Unspiked Tissue Samples in µg/kg (Continued)

Analyte	Number of Labs	Lab 1		Lab 3		Lab 4		Lab 6		Lab 8		Lab 9		Lab 10		SGS-AXYS Baseline	
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual [1]
3:3FTCA	7	2.22	U	0.25	U	0.698	U	0.241	U	0.667	U	1.48	J	0.665	U	0.3941	U
5:3FTCA	7	3.34	U	1.25	U	2.07	U	3.42	U	5.53	J	4.6	U	2.16	U	2.463	U
7:3FTCA	7	2.05	U	1.25	UJ	2.66	U	1.85	U	6.79	J	1.51	U	3.1	U	2.463	U
TSAC1 - Salmon (high lipid fish)																	
PFPeA	6	0.157	U	0.1	U	0.161	U	0.266	U	--	X	0.326	J	0.154	U	0.1961	U
PFHxA	6	0.124	U	--	X	0.126	U	0.184	U	0.167	U	0.119	U	1.33	I	0.09804	U
PFHpA	7	0.121	U	0.05	U	0.174	U	0.175	U	0.167	U	0.107	J	0.129	U	0.09804	U
PFHxS	7	0.058	U	0.05	U	0.123	U	0.148	U	0.152	U	0.117	J	0.086	U	0.09804	U
PFHpS	6	0.216	U	--	X	0.214	U	0.172	U	0.159	U	0.048	J	0.132	U	0.09804	U
NMeFOSA	5	0.272	J	0.05	U	0.383	U	0.0459	U	--	X	--	X	0.083	U	0.1128	U
NEtFOSA	5	0.664		--	X	0.231	U	0.167	U	0.167	U	--	X	0.113	U	0.2451	U
TSAD1 - Clams																	
PFPeA	7	0.157	U	0.1	U	0.161	U	0.266	U	0.333	U	1.03		0.154	U	0.197	U
PFHxA	6	0.124	U	--	X	0.126	U	0.184	U	0.167	U	0.164	J	5.21	I	0.09852	U
PFOA	7	0.125	U	0.05	U	0.128	U	0.125	U	0.167	U	0.072	J	0.215	U	0.09852	U
PFHxS	7	0.109	J	0.05	U	0.123	U	0.148	U	0.152	U	0.1	J	0.086	U	0.09852	U
PFHpS	6	0.216	U	--	X	0.214	U	0.172	U	0.159	U	0.115	J	0.132	U	0.09852	U
PFDS	7	0.173	U	0.05	U	0.212	U	0.164	U	0.161	U	1.58		0.207	U	0.09852	U
NFDHA	7	0.269	U	0.1	UJ	0.332	U	0.103	U	0.333	U	0.191	J	0.407	U	0.197	U
Total # Analytes Reported Across All Samples		5		0		3		0		34		14		3		3	

Version: Summary_tables_Exa_Tissue_CH6_12222023.xlsx

Notes:
-- : X-flagged results

Table 6-2. Numbers of Detected Analytes in Unspiked Tissue Samples

Unspiked Tissue Sample	Total Number of Analytes Detected by at least One Laboratory
TSAB1 - Walleye (low-lipid fish)	37
TSAC1 - Salmon (high-lipid fish)	7
TSAD1 - Clams	7

Version: Summary_tables_Exa_Tissue_CH6_12222023.xlsx

Table 6-3. Pooled Laboratory PFAS-Spiked Tissue 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	7	56	97.4	14.1	9.5	9.7	60	97.7	14.5	8.3	8.5	116	97.6	14.2	8.64	8.86
PFPeA	7	60	92.2	19.8	14.8	16.0	63	89.9	17.9	9.7	10.8	123	91	18.3	12.9	14.2
PFHxA	6	50	103	19.8	16.9	16.3	54	98.1	20.8	11.1	11.3	104	101	19.4	15.2	15.1
PFHpA	7	63	87.7	11.3	10.1	11.5	63	90.2	12.8	10.5	11.6	126	88.9	11.8	10.3	11.6
PFOA	7	63	102	17.3	31.8	31.2	63	92.2	17.8	11.9	12.9	126	97.1	15.2	25.3	26
PFNA	6	54	90	18.5	11.2	12.4	54	88.7	20.3	9.8	11.1	108	89.4	19.3	10.4	11.6
PFDA	7	63	101	9.75	22.9	22.7	63	96.2	11.8	19.3	20.1	126	98.7	10.1	21	21.3
PFUnA	6	54	91.8	16.1	13.7	15.0	54	89.6	17.3	10.4	11.6	108	90.7	16.5	12.1	13.4
PFDoA	7	63	91.1	17	12.7	14.0	63	86.3	17.6	9.0	10.4	126	88.7	17.1	11.2	12.7
PFTrDA	6	54	87.9	33.4	21.1	24.0	54	82.5	28	19.8	24.0	108	85.2	30.6	20.4	23.9
PFTeDA	7	63	89	23.8	12.0	13.4	63	84.7	26.3	9.2	10.9	126	86.9	25	10.8	12.4
PFBS	7	62	89.8	12.5	11.7	13.0	63	92.1	15.6	10.3	11.1	125	91	14	11.1	12.2
PFPeS	7	63	85.6	18.7	18.4	21.4	63	86.4	17.8	10.8	12.5	126	86	17.9	15	17.4
PFHxS	7	63	81	16.3	16.3	20.1	63	81.6	15.1	10.6	13.0	126	81.3	15.6	13.5	16.5
PFHpS	6	54	94.7	14.3	17.4	18.4	54	93.4	17.6	13.4	14.3	108	94	15.6	15.4	16.4
PFOS	6	54	94.1	14.4	14.9	15.8	54	85.9	12.4	12.3	14.3	108	90	13.1	14.2	15.8
PFNS	7	63	86.4	17.9	19.3	22.3	63	83.4	15.4	12.5	15.0	126	84.9	16.4	16.1	19
PFDS	7	63	89.3	24.4	27.0	30.2	63	81.5	19	12.7	15.6	126	85.4	21.4	21.3	24.9
PFDoS	7	60	69.3	24.7	21.0	30.3	63	63.2	20.9	17.7	27.9	123	66.2	22.2	19.8	30
4:2FTS	6	53	92	14.4	10.0	10.9	63	91.8	16.1	12.2	13.3	116	91.9	14.8	12.3	13.4
6:2FTS	5	45	95.2	27.8	16.2	17.0	54	97.3	28.5	20.5	21.1	99	96.3	27.7	20.4	21.2
8:2FTS	7	56	102	28.3	18.0	17.6	63	103	22.8	14.9	14.5	119	102	22	16.2	15.8
PFOSA	7	63	96.9	21.1	13.3	13.7	63	96.4	20.6	10.1	10.5	126	96.7	20.7	11.7	12.1
NMeFOSA	6	49	107	35.5	24.1	22.5	54	107	34.1	18.8	17.6	103	107	34.5	21.2	19.8
NEtFOSA	6	45	95	22.2	13.8	14.5	49	90.6	23	10.7	11.8	94	92.7	22	13	14.1
NMeFOSAA	7	63	95	13.1	14.8	15.6	63	97.2	16.5	12.4	12.8	126	96.1	14.7	13.5	14
NEtFOSAA	7	62	93.4	17.5	13.8	14.7	63	92.8	21.2	11.2	12.1	125	93.1	19.3	12.3	13.2
NMeFOSE	7	45	101	33.4	14.4	14.2	48	96.6	33.2	13.4	13.8	93	98.8	33.2	13.5	13.6

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

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)
NEtFOSE	6	41	126	55.2	66.6	53.0	40	112	38.6	8.3	7.4	81	119	43.1	54.2	45.5
PFMPA	7	60	73.9	19.2	22.8	30.8	63	75.7	17.7	20.9	27.6	123	74.8	17.6	21.8	29.1
PFMBA	7	61	98.7	21.6	19.9	20.2	63	98	24.3	17.1	17.5	124	98.3	22.8	18.3	18.6
NFDHA	7	62	95.8	24.1	13.2	13.7	63	97.3	24.7	14.1	14.5	125	96.6	24.3	13.4	13.8
HFPO-DA	7	62	104	26.3	12.9	12.3	63	104	29.3	11.1	10.6	125	104	27.7	11.9	11.4
ADONA	6	53	107	19.1	23.4	21.8	54	103	20.7	12.0	11.6	107	105	19.4	18.4	17.6
PFEESA	7	62	102	18.1	14.9	14.6	63	104	20.7	9.5	9.1	125	103	19.3	12.3	11.9
9Cl-PF3ONS	7	62	123	58.6	125.0	102.0	63	107	23.8	18.3	17.1	125	115	38.8	86.5	75.2
11Cl-PF3OUdS	7	62	111	67.5	139.0	125.0	63	94.6	26.9	17.0	18.0	125	103	44.9	95.8	93.2
3:3FTCA	7	54	83	35	18.5	22.3	63	77.8	46.8	16.2	20.8	117	80.2	41.8	18.1	22.6
5:3FTCA	7	62	98.1	25.5	26.5	27.0	63	98.7	28.2	25.8	26.2	125	98.4	26.9	25.4	25.9
7:3FTCA	7	62	145	60.6	46.7	32.2	63	132	42.9	25.7	19.5	125	138	50.3	38.8	28.1

Source file: TS_Matrix_compiled_results_V0_231214_135747.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 Samples Results by Individual Tissue Sample

Analyte	TSAB					TSAC					TSAD				
	Number of Labs	Number of Results	Mean % Recovery	Min % Recovery	Max % Recovery	Number of Labs	Number of Results	Mean % Recovery	Min % Recovery	Max % Recovery	Number of Labs	Number of Results	Mean % Recovery	Min % Recovery	Max % Recovery
PFBA	7	40	91.1	51.7	117	7	35	103	91.5	133	7	41	98.9	58.8	130
PFPeA	7	42	86	39.2	114	7	40	89.6	49.6	136	7	41	97.6	56	157
PFHxA	6	36	91.9	46.4	137	6	35	107	80	148	6	33	104	51.5	172
PFHpA	7	42	85.7	34.9	103	7	42	94.5	70	117	7	42	86.6	47	108
PFOA	7	42	89.8	40.4	124	7	42	110	71.4	226	7	42	91.5	43.8	148
PFNA	6	36	83.6	26.6	114	6	36	95.5	63.5	120	6	36	89	37	120
PFDA	7	42	89.9	22.8	116	7	42	112	70.5	185	7	42	94.3	40.8	138
PFUnA	6	36	85.5	23.5	114	6	36	99.5	85.8	123	6	36	87.2	34	110
PFDoA	7	42	84.2	24.2	113	7	42	95.1	68.8	113	7	42	86.8	29	131
PFTrDA	6	36	79.6	24.6	185	6	36	95.1	56	195	6	36	81	27.4	146
PFTeDA	7	42	82.5	28.1	116	7	42	92.8	40.4	140	7	42	85.3	35.2	125
PFBS	7	42	85.8	46	106	7	41	98.2	75	136	7	42	89	49	124
PFPeS	7	42	76	30.6	119	7	42	89.2	29	127	7	42	92.9	45.7	133
PFHxS	7	42	72.2	21.4	99.5	7	42	84.7	55.2	111	7	42	86.9	34.3	129
PFHpS	6	36	84.3	31	110	6	36	101	56.5	118	6	36	96.9	40.3	130
PFOS	6	36	81.9	22.1	109	6	36	99	79.7	116	6	36	89.2	37.6	127
PFNS	7	42	76.8	22.6	98.3	7	42	98.5	75.6	203	7	42	79.3	37.9	113
PFDS	7	42	76.2	18.2	107	7	42	98.9	75.8	206	7	42	81.1	30.5	223
PFDoS	7	41	57	21.9	90.2	7	42	81.8	38.5	176	7	40	59.1	10.8	131
4:2FTS	7	39	88	44.1	122	7	38	98.9	68.8	144	7	39	89	43.5	121
6:2FTS	6	33	82.4	33.7	137	6	33	110	70.9	193	6	33	96.9	41	152
8:2FTS	7	39	93.3	23.6	121	7	41	115	85.6	174	7	39	98.1	37.5	154
PFOSA	7	42	90.5	33.8	137	7	42	104	84.4	131	7	42	95.8	34.5	182
NMeFOSA	6	36	92.3	28	144	6	31	116	77.9	154	6	36	114	40.1	280
NEtFOSA	6	32	83.9	29	110	5	26	102	50.8	146	6	36	93.9	43.2	124
NMeFOSAA	7	42	91.1	39.3	112	7	42	105	79	133	7	42	92.3	40.6	118
NEtFOSAA	7	41	85.6	27.6	116	7	42	103	70	142	7	42	91	35.6	122
NMeFOSE	7	38	97.6	23.4	151	3	13	122	101	151	7	42	92.8	29.2	146
NEtFOSE	5	29	110	36.4	155	4	19	161	103	387	6	33	103	32.1	145

Table 6-4. PFAS-Spiked Samples Results by Individual Tissue Sample (Continued)

Analyte	TSAB					TSAC					TSAD				
	Number of Labs	Number of Results	Mean % Recovery	Min % Recovery	Max % Recovery	Number of Labs	Number of Results	Mean % Recovery	Min % Recovery	Max % Recovery	Number of Labs	Number of Results	Mean % Recovery	Min % Recovery	Max % Recovery
PFMPA	7	42	67.4	13.5	105	7	39	65.4	17.4	104	7	42	91	16.2	129
PFMBA	7	42	97	47.2	154	7	40	99.4	54.4	208	7	42	98.6	53.2	138
NFDHA	7	42	99.3	52	160	7	41	99.6	67.6	159	7	42	90.9	57.4	135
HFPO-DA	7	42	97.5	51.6	152	7	41	112	81.4	194	7	42	104	57.2	178
ADONA	6	36	94	40.6	116	6	35	118	81.6	218	6	36	103	45.2	144
PFEESA	7	42	97	54.4	135	7	41	111	89.2	183	7	42	102	57.6	149
9Cl-PF3ONS	7	42	96.9	30.6	123	7	41	141	78.6	1060	7	42	108	43.3	174
11Cl-PF3OUdS	7	42	89	25.8	129	7	41	132	78.6	1170	7	42	88.1	39.8	157
3:3FTCA	7	40	76.3	13.7	159	7	37	68.5	3.7	160	7	40	94.9	38.6	200
5:3FTCA	7	42	104	42.6	175	7	41	121	51.5	172	7	42	70.4	25	100
7:3FTCA	7	42	119	38.6	212	7	41	158	87.5	480	7	42	138	47	298

Source file: TS_Matrix_sample_results_V0_231214_135747.csv

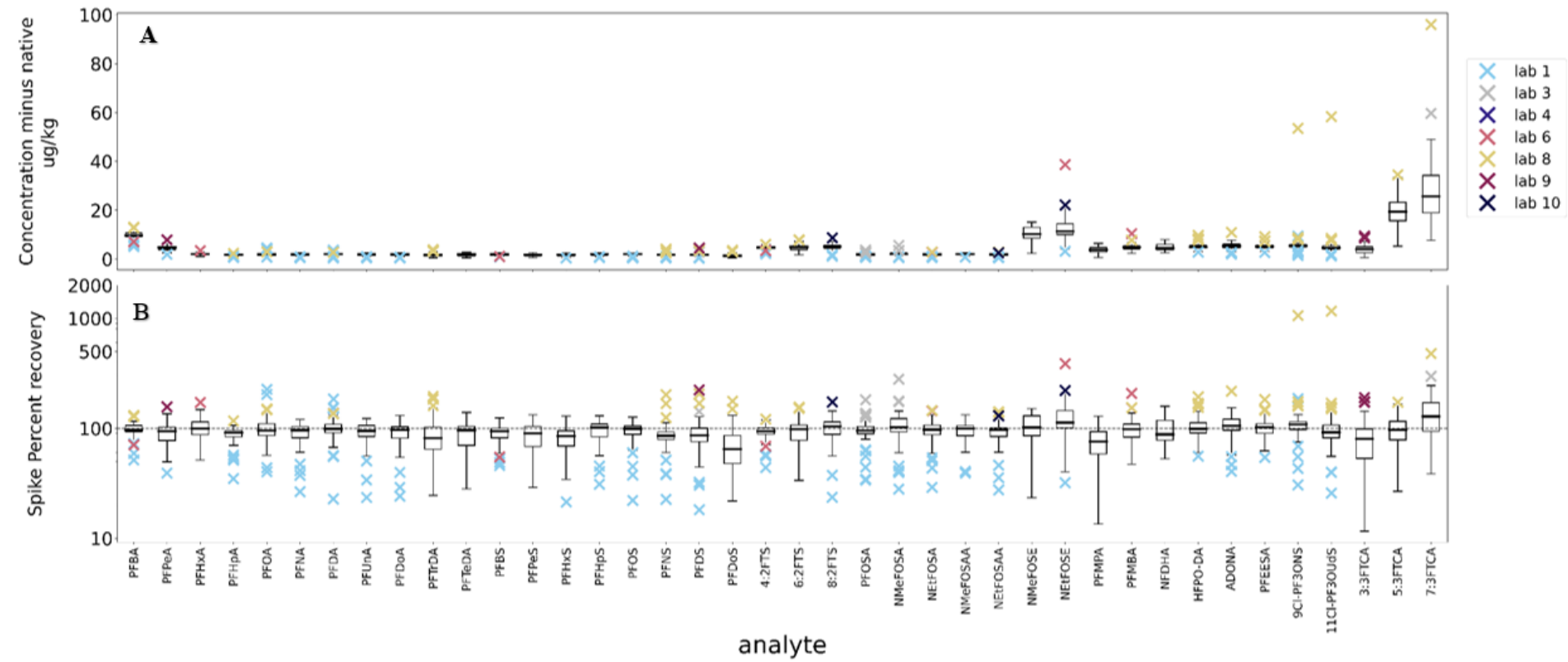
Notes:
Number of Labs - The number of laboratories reporting matrix spiked sample results.
Number of Results - The total number of matrix sample results categorized as low spike concentration (indicated in Row 1) that do not have a U flag.
Mean % Recovery - The mean percent recovery for spiked samples across all laboratories.
Min % Recovery - The minimum percent recovery for the matrix spike samples across all labs.
Max % Recovery - The maximum percent recovery for the matrix spike samples across all labs.

Table 6-5. Proportion of Tissue Matrix Spike Percent Recovery Results for Target Analytes within Ranges (Pooled High/Low-Spiked Samples)

Analyte	Low-Spiked Samples						
	n	<40%	≥40% to <70%	≥70% to <130%	≥130% to <150%	≥150% to <200%	≥200%
PFBA	116	0	6	91.4	2.6	0	0
PFPeA	123	0.8	17.1	78.9	2.4	0.8	0
PFHxA	104	0	8.7	79.8	10.6	1	0
PFHpA	126	0.8	8.7	90.5	0	0	0
PFOA	126	0	11.9	78.6	6.3	0.8	2.4
PFNA	108	6.5	5.6	88	0	0	0
PFDA	126	0.8	6.3	83.3	6.3	3.2	0
PFUnA	108	4.6	6.5	88.9	0	0	0
PFDoA	126	4.8	6.3	88.1	0.8	0	0
PFTrDA	108	7.4	27.8	54.6	2.8	7.4	0
PFTeDA	126	7.1	18.3	72.2	2.4	0	0
PFBS	125	0	12.8	86.4	0.8	0	0
PFPeS	126	4.8	19	75.4	0.8	0	0
PFHxS	126	4	21.4	74.6	0	0	0
PFHpS	108	1.9	10.2	87	0.9	0	0
PFOS	108	3.7	7.4	88.9	0	0	0
PFNS	126	4	11.9	82.5	0	0.8	0.8
PFDS	126	6.3	15.9	74.6	0.8	0.8	1.6
PFDoS	123	20.3	37.4	39.8	0.8	1.6	0
4:2FTS	116	0	11.2	87.9	0.9	0	0
6:2FTS	99	2	17.2	65.7	9.1	6.1	0
8:2FTS	119	2.5	6.7	80.7	6.7	3.4	0
PFOSA	126	2.4	7.1	85.7	4	0.8	0
NMeFOSA	103	3.9	7.8	70.9	9.7	4.9	2.9
NEtFOSA	94	3.2	13.8	78.7	4.3	0	0
NMeFOSAA	126	0.8	11.1	87.3	0.8	0	0
NEtFOSAA	125	4	5.6	84.8	5.6	0	0
NMeFOSE	93	7.5	12.9	58.1	17.2	4.3	0
NEtFOSE	81	4.9	8.6	44.4	25.9	12.3	3.7
PFMPA	123	12.2	26	61.8	0	0	0
PFMBA	124	0	18.5	71.8	5.6	3.2	0.8
NFDHA	125	0	10.4	76	9.6	4	0
HFPO-DA	125	0	8	77.6	4.8	9.6	0
ADONA	107	0	10.3	78.5	9.3	0.9	0.9
PFEESA	125	0	8	82.4	7.2	2.4	0
9Cl-PF3ONS	125	1.6	6.4	77.6	4.8	8.8	0.8
11Cl-PF3OUdS	125	2.4	12.8	75.2	2.4	6.4	0.8
3:3FTCA	117	13.7	27.4	47	1.7	9.4	0.9
5:3FTCA	125	4.8	12	64	8.8	10.4	0
7:3FTCA	125	1.6	5.6	46.4	13.6	20.8	12

Version: Summary_tables_Exa_Tissue_CH6_12222023.xlsx

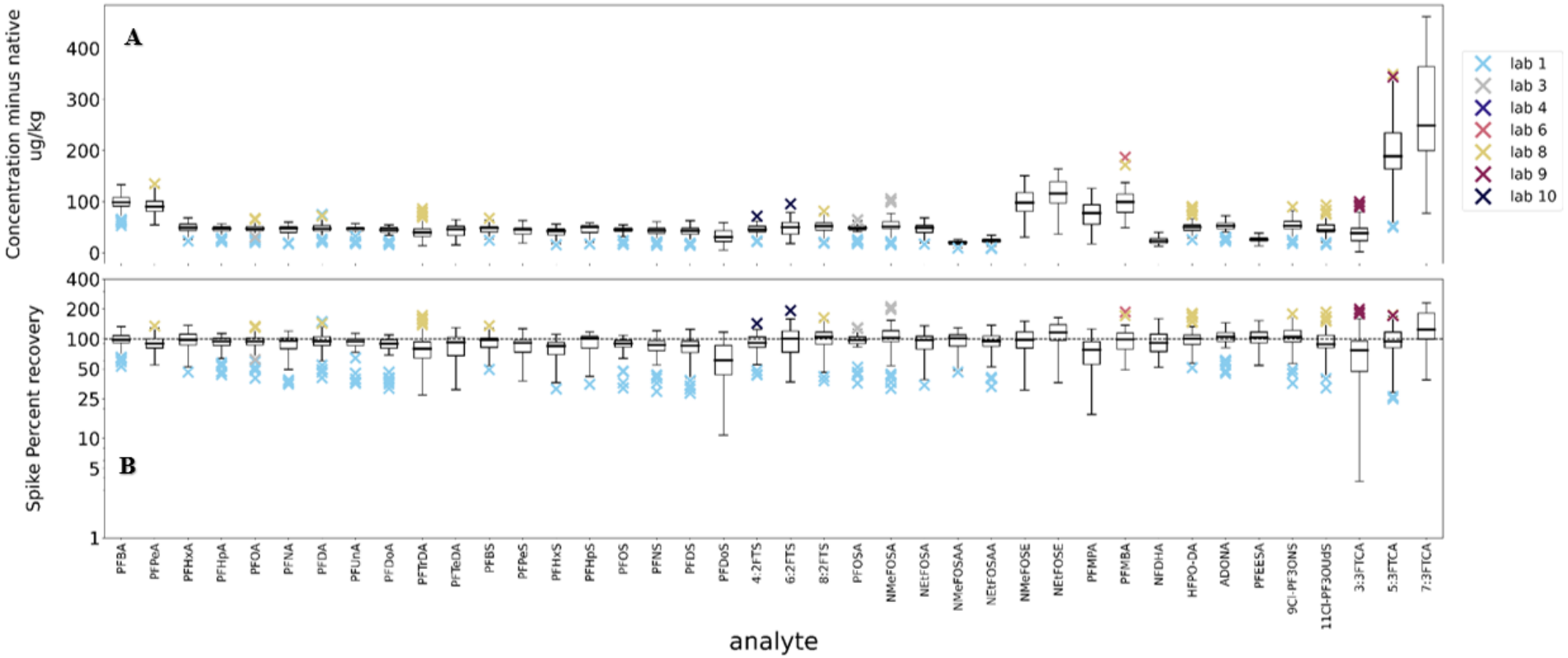
[1] Based on validated data. Does not include MB, OPR, LLOPR QC samples.



Source File: TS_LowSpike_Boxplot_V0_231214_135747

Figure 6-1. Tissue Low Matrix Spiked Results by Analyte by Laboratory

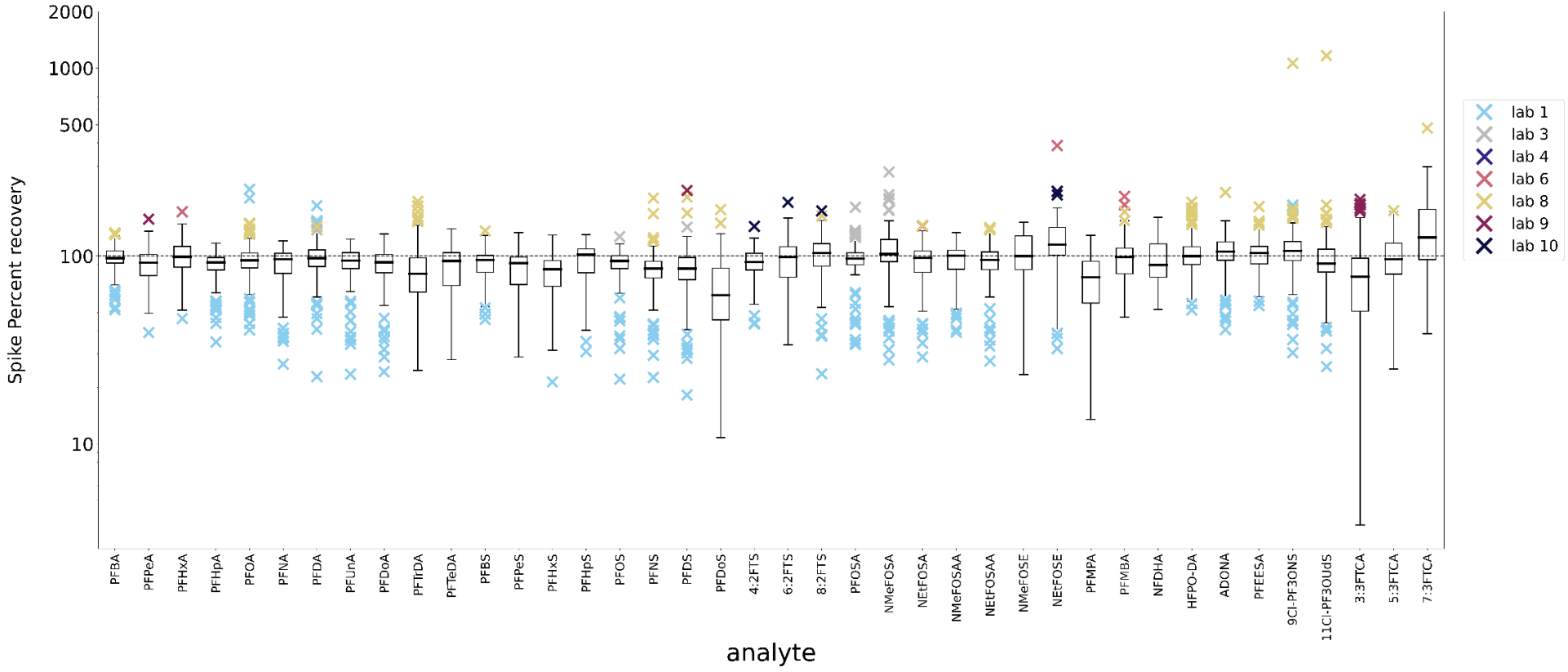
(A) Spiked concentration minus the laboratory-reported native concentration. (B) Low-spiked percent recovery.



Source File: TS_HighSpike_Boxplot_V0_231214_135747

Figure 6-2. Tissue High Matrix Spiked Results by Analyte by Laboratory

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



Source File: TS_LowHighCombinedSpike_Boxplot_V0_231214_135747

Figure 6-3. Pooled Low- and High-spiked Tissue Percent Recovery Results by Analyte by Laboratory

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

EIS Compound	Minimum Concentration (µg/kg)	Maximum Concentration (µg/kg)
$^{13}C_4$-PFBA	20	25
$^{13}C_5$-PFPeA	10	12.5
$^{13}C_5$-PFHxA	5	6.25
$^{13}C_4$-PFHpA	5	6.25
$^{13}C_8$-PFOA	5	6.25
$^{13}C_9$-PFNA	2.5	3.13
$^{13}C_6$-PFDA	2.5	3.13
$^{13}C_7$-PFUnA	2.5	3.13
$^{13}C_2$-PFDoA	2.5	3.13
$^{13}C_2$-PFTeDA	2.5	3.13
$^{13}C_3$-PFBS	4.65	6.25
$^{13}C_3$-PFHxS	4.74	6.25
$^{13}C_8$-PFOS	4.79	6.25
$^{13}C_2$-4:2FTS	9.38	12.5
$^{13}C_2$-6:2FTS	9.5	12.5
$^{13}C_2$-8:2FTS	9.6	12.5
$^{13}C_8$-PFOSA	5	6.25
D_3-NMeFOSA	5	6.25
D_5-NEtFOSA	5	6.25
D_3-NMeFOSAA	10	12.5
D_5-NEtFOSAA	10	12.5
D_7-NMeFOSE	50	62.5
D_9-NEtFOSE	50	62.5
$^{13}C_3$-HFPO-DA	20	25

Version: Summary_tables_Exa_Tissue_CH6_12222023.xlsx

Notes:
Does not include MB, OPR, LLOPR QC samples.

Table 6-7. Summary of EIS Compound Percent Recovery in Tissue Samples for All Laboratories

EIS Compound	All Labs % recovery			
	n	Min	Max	Mean
$^{13}C_4$-PFBA	149	1.82	118	60.5
$^{13}C_5$-PFPeA	169	2.3	185	87.5
$^{13}C_5$-PFHxA	176	5.8	171	84.4
$^{13}C_4$-PFHpA	147	15.6	231	93.8
$^{13}C_8$-PFOA	152	19.7	157	88.1
$^{13}C_9$-PFNA	147	23.5	194	92.8
$^{13}C_6$-PFDA	147	22.3	158	88.1
$^{13}C_7$-PFUnA	153	25.6	203	87.7
$^{13}C_2$-PFDoA	166	27.3	213	83.1
$^{13}C_2$-PFTeDA	151	11.5	176	71.4
$^{13}C_3$-PFBS	151	9.7	194	90.8
$^{13}C_3$-PFHxS	161	29.5	185	98.3
$^{13}C_8$-PFOS	156	24.3	172	94.0
$^{13}C_2$-4:2FTS	147	6.74	373	159.2
$^{13}C_2$-6:2FTS	147	29.7	342	164.0
$^{13}C_2$-8:2FTS	147	36.9	485	229.1
$^{13}C_8$-PFOSA	148	22.3	191	96.2
D_3-NMeFOSA	147	0.3	76.6	36.6
D_5-NEtFOSA	147	0.9	73.2	32.8
D_3-NMeFOSAA	147	25.5	253	132.5
D_5-NEtFOSAA	147	25.7	250	133.5
D_7-NMeFOSE	147	0.51	166	39.6
D_9-NEtFOSE	147	0.08	82.1	22.9
$^{13}C_3$-HFPO-DA	150	7.29	197	83.7

Version: Summary_tables_Exa_CH6_10312023.xlsx

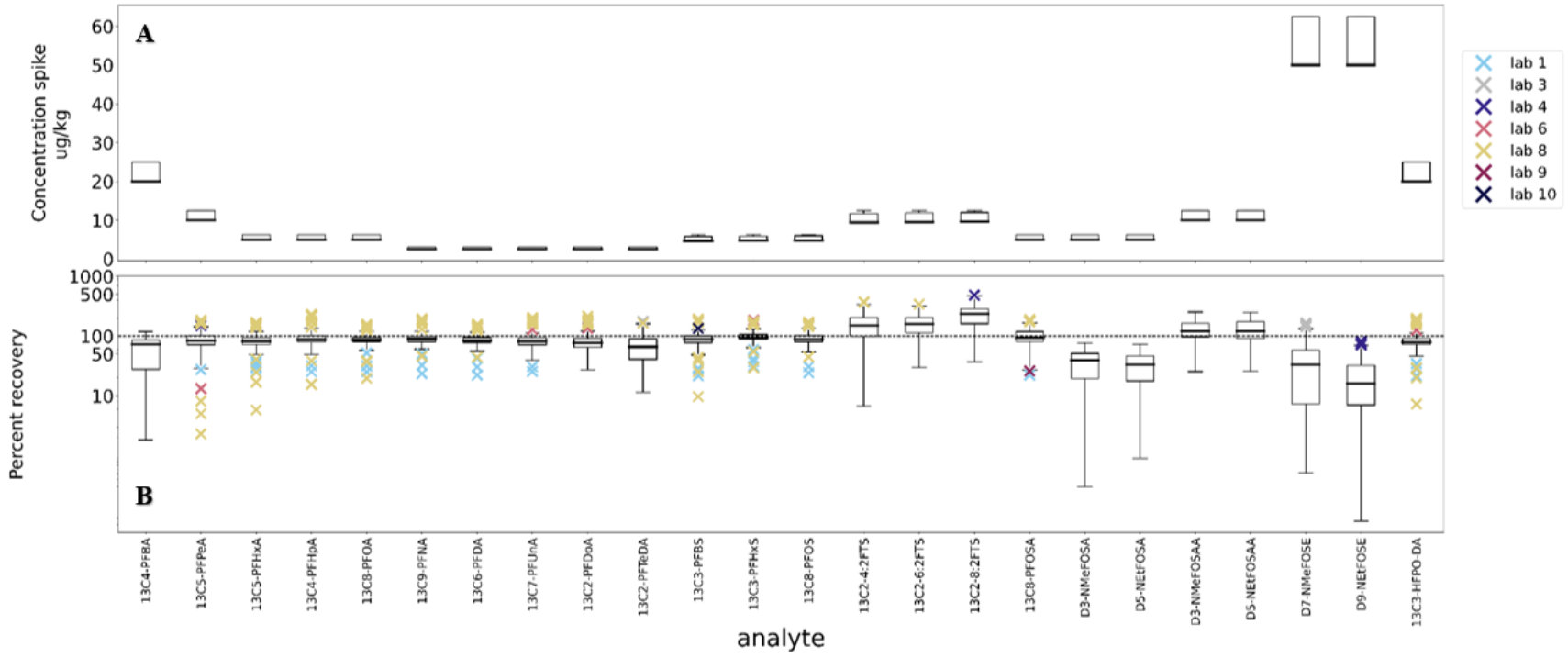
Based on validated data. Does not include MB, OPR, LLOPR QC samples.

Table 6-8. Statistical Evaluation of EIS Compound Results Associated with Tissue 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 (s_w)
$^{13}C_4$-PFBA	7	149	60.5	25.5	22.6	37.4
$^{13}C_5$-PFPeA	7	169	87.5	21.3	26.9	30.7
$^{13}C_5$-PFHxA	7	176	84.4	12.6	21.2	25.1
$^{13}C_4$-PFHpA	7	147	93.8	19.7	26.9	28.6
$^{13}C_8$-PFOA	7	152	88.1	9.13	17.1	19.4
$^{13}C_9$-PFNA	7	147	92.7	16.9	18.9	20.3
$^{13}C_6$-PFDA	7	147	88.1	14	15.7	17.8
$^{13}C_7$-PFUnA	7	153	87.6	22.2	21	23.9
$^{13}C_2$-PFDoA	7	166	83	21.2	23.5	28.3
$^{13}C_2$-PFTeDA	7	151	71.4	29.2	26.2	36.7
$^{13}C_3$-PFBS	7	151	90.8	15.7	23.2	25.5
$^{13}C_3$-PFHxS	7	161	98.3	13.4	20.5	20.8
$^{13}C_8$-PFOS	7	156	94	15.7	16.4	17.4
$^{13}C_2$-4:2FTS	7	147	159	53.7	57.5	36.1
$^{13}C_2$-6:2FTS	7	147	164	45	49.4	30.1
$^{13}C_2$-8:2FTS	7	147	229	68.3	68.8	30
$^{13}C_8$-PFOSA	7	148	96.2	27.3	20.9	21.7
D_3-NMeFOSA	7	147	36.6	15.3	14.5	39.7
D_5-NEtFOSA	7	147	32.8	15	11.4	34.6
D_3-NMeFOSAA	7	147	132	39.1	30.6	23.1
D_5-NEtFOSAA	7	147	133	43	29.9	22.4
D_7-NMeFOSE	7	147	39.6	35	21.7	54.8
D_9-NEtFOSE	7	147	22.9	15.5	14.4	62.9
$^{13}C_3$-HFPO-DA	7	150	83.7	12.9	24.9	29.8

Source file: TS_EIS_results_V0_231214_135747.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).



Source File: TS_EIS_Boxplot_V0_231214_135747

Figure 6-4. Tissue EIS Compound Results by Compound by Laboratory

(A) Spiked Concentration. (B) Calculated percent recovery.

Table 6-9. Proportion of Tissue Percent 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	149	8.1	10.7	81.2	0	0
$^{13}C_5$-PFPeA	169	1.8	0.6	89.9	7.7	0
$^{13}C_5$-PFHxA	176	0.6	0.6	94.9	4	0
$^{13}C_4$-PFHpA	147	0	0.7	94.6	1.4	3.4
$^{13}C_8$-PFOA	152	0	0.7	98.7	0.7	0
$^{13}C_9$-PFNA	147	0	0	95.2	4.8	0
$^{13}C_6$-PFDA	147	0	0	97.3	2.7	0
$^{13}C_7$-PFUnA	153	0	0	93.5	5.2	1.3
$^{13}C_2$-PFDoA	166	0	0	94	5.4	0.6
$^{13}C_2$-PFTeDA	151	0	1.3	94	4.6	0
$^{13}C_3$-PFBS	151	0.7	0	94.7	4.6	0
$^{13}C_3$-PFHxS	161	0	0	93.2	6.8	0
$^{13}C_8$-PFOS	156	0	0	96.2	3.8	0
$^{13}C_2$-4:2FTS	147	0.7	0	48.3	25.2	25.9
$^{13}C_2$-6:2FTS	147	0	0	46.3	26.5	27.2
$^{13}C_2$-8:2FTS	147	0	0	19.7	20.4	59.9
$^{13}C_8$-PFOSA	148	0	0	95.3	4.7	0
D_3-NMeFOSA	147	15.6	10.2	74.1	0	0
D_5-NEtFOSA	147	10.9	18.4	70.7	0	0
D_3-NMeFOSAA	147	0	0	63.9	25.9	10.2
D_5-NEtFOSAA	147	0	0	60.5	29.3	10.2
D_7-NMeFOSE	147	26.5	12.2	59.9	1.4	0
D_9-NEtFOSE	147	30.6	25.2	44.2	0	0
$^{13}C_3$-HFPO-DA	150	0.7	0	94.7	4.7	0

Version: Summary_tables_Exa_CH6_10312023.xlsx

Based on validated data. Does not include MB, OPR, LLOPR QC samples.

Table 6-10. Tissue Percent Recovery Results for EIS Compounds Compared to Acceptance Limits for Aqueous Matrices in EPA Method 1633

EIS Compound	Acceptance Limits for EIS Compounds in Tissue Matrices and QC Samples [1]		Average EIS % Recovery by Laboratory for Tissues (Appendix B-4)							All Labs % Recovery	
			Lab 1	Lab 3	Lab 4	Lab 6	Lab 8	Lab 9	Lab 10	n	Avg
$^{13}C_4$-PFBA	5	130	50.6	68.5	98.4	21.5	46.2	55.7	84.1	149	60.5
$^{13}C_5$-PFPeA	10	185	77.3	70.0	128.1	79.1	103.0	69.2	85.6	169	87.5
$^{13}C_5$-PFHxA	25	170	72.0	73.3	102.5	91.4	99.6	79.8	77.2	176	84.4
$^{13}C_4$-PFHpA	25	150	72.0	93.9	103.9	97.3	130.3	79.0	80.4	147	93.8
$^{13}C_8$-PFOA	25	150	74.5	83.7	101.7	88.2	97.9	84.9	89.4	152	88.1
$^{13}C_9$-PFNA	35	185	76.4	87.5	103.1	90.5	126.0	80.5	85.6	147	92.8
$^{13}C_6$-PFDA	30	150	69.6	82.8	98.1	91.9	112.2	79.1	83.1	147	88.1
$^{13}C_7$-PFUnA	30	180	69.8	81.0	91.5	82.8	136.6	78.0	79.2	153	87.7
$^{13}C_2$-PFDoA	35	180	63.0	91.3	94.8	81.6	125.6	72.2	71.2	166	83.1
$^{13}C_2$-PFTeDA	20	160	52.5	111.9	101.2	40.1	91.8	52.8	49.6	151	71.4
$^{13}C_3$-PFBS	25	190	67.2	81.8	93.7	91.8	113.2	78.8	104.7	151	90.8
$^{13}C_3$-PFHxS	35	175	88.4	92.3	101.0	121.6	112.5	88.0	89.4	161	98.3
$^{13}C_8$-PFOS	40	160	74.8	82.4	106.2	101.5	116.5	78.6	88.2	156	94.0
$^{13}C_2$-4:2FTS	30	300	82.1	189.8	158.3	169.4	206.9	217.3	90.6	147	159.2
$^{13}C_2$-6:2FTS	35	300	134.0	156.2	120.1	198.6	211.9	217.4	110.0	147	164.0
$^{13}C_2$-8:2FTS	40	365	227.7	241.3	325.1	204.7	280.0	219.5	105.2	147	229.1
$^{13}C_8$-PFOSA	25	180	54.4	124.8	117.6	91.2	125.9	75.5	86.2	148	96.2
D_3-NMeFOSA	5	130	30.8	45.4	52.6	40.7	32.8	6.6	46.9	147	36.6
D_5-NEtFOSA	5	130	24.6	32.4	57.5	36.7	23.0	11.6	43.8	147	32.8
D_3-NMeFOSAA	30	250	85.0	170.1	181.4	149.0	138.5	80.5	122.7	147	132.5
D_5-NEtFOSAA	30	235	89.4	152.5	191.0	172.9	145.6	80.1	102.7	147	133.5
D_7-NMeFOSE	5	160	25.3	110.2	30.8	25.0	12.2	13.3	60.4	147	39.6
D_9-NEtFOSE	5	130	24.7	37.3	46.3	8.2	11.9	4.8	27.1	147	22.9
$^{13}C_3$-HFPO-DA	20	185	74.1	77.9	86.1	102.2	99.8	78.8	68.5	150	83.7

Version: Summary_tables_Exa_Tissue_CH6_12222023.xlsx

Notes:
1 EIS Limits from Table 7-12
Does not include MB, OPR, LLOPR QC samples.

7 SUMMARY

7.1 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.

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 was 40–150% in OPRs and LLOPRs, 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 included 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.

7.1.1 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 2.0 g aliquot of PFAS-free tissue (e.g., chicken breast or similar tissue) was used to prepare each method blank associated with tissue samples and all were prepared in exactly the same manner as study samples. A total of 15 method blanks were included in the statistical analysis.

Of these 15 method blanks, four included detections of target analytes concentrations above the laboratories' MDLs. A total of four target analytes were detected (Table 7-1). 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, whichever is greater. 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. Since all four of the detections met these criteria, no sample results were "B" data qualified. The method blanks demonstrate that any bias associated with background contamination introduced during sample preparation was negligible.

Table 7-1. Method Blank Detection Summary

Laboratory ID	Target Analyte	# of Occurrences	Concentrations (µg/kg)
3	NMeFOSA	1	0.16 JI
9	PFOA	1	0.077 J
9	PFOSA	1	0.099 J
9	PFTrDA	1	0.057 J

Source File: IDA FILE TS_MB_results_231214_135747.csv

Notes:
J = Analyte concentration >MDL but <LOQ; estimated value.
I = Ion abundance ratio did not meet acceptance criteria

7.1.2 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 2.0 g aliquot of PFAS-free tissue (e.g., chicken breast or similar tissue) 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 was prepared and analyzed in exactly the same manner as study samples.

OPR recoveries across all media for all laboratories were relatively tight, generally at or above 100% with narrow pooled between-laboratory standard deviation (s_b), within-laboratory standard deviation (s_w), and RSD. (Table 7-2). The concentration at which the OPR was spiked by each laboratory did not vary greatly (Figure 7-1A), however, the concentrations spiked by Laboratory 4 were slightly higher than all other laboratories.

A total of 15 OPRs were included in the statistical analysis. All 15 OPRs met the Study NIS criteria (>30% recovery). Of the 564 valid target analyte results reported from OPRs, 22 failed to meet the target analyte criteria (40–150%), resulting in a failure rate of 3.90%. Five of these recoveries were below the 40% criteria, ranging from 19.5% to 33% while the remaining 17 instances ranged from 155% to 349%. The recoveries reported below the 40% criteria were associated with PFDoS, PFPeS, 3:3FTCA, and PFMPA. The recoveries above the 150% criteria were associated 5:3FTCA, 7:3FTCA, NFDHA, NEtFOSE, 8:2FTS, 11Cl-PFOUdS, and 9Cl-PFONS. Of the 371 valid EIS compound results reported from OPRs, 36 failed to meet the EIS compound acceptance criteria (20–150%), resulting in a failure rate of 9.7%. Sixteen of these recoveries were below the 20% criteria, ranging from 0.3% to 19.3% while the remaining 20 instances ranged from 155% to 321%. The recoveries reported below the 20% criteria were associated with $^{13}C_4$-PFBA, D_3-NMeFOSA, D_5-NEtFOSA, D_7-NMeFOSE, and D_9-NEtFOSE. The recoveries reported above the 150% criteria were associated with $^{13}C_2$-4:2FTS, $^{13}C_2$-6:2FTS, $^{13}C_2$-8:2FTS, D_3-NMeFOSAA, and D_5-NEtFOSAA. The 3 most frequent exceedance were for D_9-NEtFOSE (7), D_5-NEtFOSAA (5), and D_3-NMeFOSAA (5).

A graphical representation of the performance of the variance in the tissue OPR results across all laboratories, all analytes, and concentrations is given in Figure 7-1. From the data presented in Table 7-2, the plot shows the percent relative standard deviation (%RSD) of the four replicates analyzed by each laboratory, pooled vs. concentration. The shaded area in the plot represents the minimum (3.21) and maximum (28.9) %RSDs. The preponderance of the points in Figure 7-2 are below 20% RSD for each analyte by laboratory; the exceptions to that are observed for Laboratories 1, 4, and 9.

Following EPA guidance (EPA 821-B-18-001), lower and upper percent recovery limits for target analytes were generated (Table 8-4). 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 the MLVS target lower limit of 40% and all upper control limits are lower than the MLVS target upper limit of 150% with the exception of NEtFOSE, 9Cl-PFONS, 11Cl-PFOUdS, 5:3FTCA, and 7:3FTCA, which all exceeded the upper control limit (Table 7-3).

7.1.3 Low-Level Ongoing Precision and Recovery Analyses

LLOPR samples were included in the method to evaluate the efficiency of the sample preparation process near the quantitation limit. An LLOPR was included in each preparation batch, consisting of a 2.0 g aliquot of PFAS-free tissue (e.g., chicken breast or similar tissue) 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 was prepared and analyzed in exactly the same manner as study samples.

LLOPR recoveries across all media for all laboratories were relatively tight, generally at or above 100% with narrow pooled between-laboratory standard deviation (s_b), within-laboratory standard deviation (s_w), and RSD (Table 7-4). The concentration at which the LLOPR was spiked by each laboratory did not vary greatly (Figure 7-3A).

All of the 15 LLOPRs included in the statistical analysis met the Study LLOPR NIS compound recovery criteria (>30%). Of the 567 valid target analyte results reported from LLOPRs, 37 failed to meet the target analyte criteria (40–150%), resulting in a failure rate of 6.53%. Three of these recoveries were below the 40% criteria, ranging from 0% to 35% while the remaining 34 instances ranged from 151% to 316%. The recoveries reported below the 40% criteria were associated with PFPeS, NMeFOSE, and NEtFOSE. The recoveries above the 150% criteria were associated with PFHxA, PFHxS, PFOS, PFUnA, PFEESA, ADONA, 6:2FTS, 8:2FTS, 5:3FTCA, 7:3FTCA, 11Cl-PFOUdS, 9Cl-PFONS, NEtFOSE, and NMeFOSE. Of the 371 valid EIS compound results reported from OPRs, 44 failed to meet the EIS compound acceptance criteria (20–150%), resulting in a failure rate of 11.9%. Eighteen of these recoveries were below the 20% criteria, ranging from 0.7% to 18.5% while the remaining 26 instances ranged from 151% to 280%. The recoveries reported below the 20% criteria were associated with $^{13}C_4$-PFBA, $^{13}C_5$-PFPeA, D_3-NMeFOSA, D_5-NEtFOSA, D_7-NMeFOSE, and D_9-NEtFOSE. The recoveries reported above the 150% criteria were associated with $^{13}C_2$-4:2FTS, $^{13}C_2$-6:2FTS, $^{13}C_2$-8:2FTS, D_3-NMeFOSAA, and D_5-NEtFOSAA. The 3 most frequent exceedance were for D_9-NEtFOSE (9), D_5-NEtFOSAA (7), and D_3-NMeFOSAA (5).

A graphical representation of the performance of the variance in the tissue LLOPR results across all laboratories, all analytes, and concentrations is given in Figure 7-4. From the data presented in Table 7-4, the plot shows the percent relative standard deviation (%RSD) of the individual replicates analyzed by each laboratory, and the pooled results, vs. concentration. The shaded area in the plot represents the minimum (4.74) and maximum (28.5) %RSDs. The preponderance of the points in Figure 7-4 are below 30% RSD for each analyte by laboratory; the exceptions to that are observed for Laboratories 1, 4, and 9.

Following EPA guidance (EPA 821-B-18-001), the LLOPR percent recovery and RSD values in Table 7-4 were used to calculate lower and upper percent recovery limits for target analytes.

The lower percent recovery limit is the mean percent recovery minus two times the RSD and the upper percent recovery limit is the mean percent recovery plus two times the RSD. All statistically derived lower control limits are greater than the MLVS target lower limit of 40% and all upper control limits are lower than the MLVS target upper limit of 150% with the exception of PFDoS, NEtFOSE, PFEESA, 9Cl-PFONS, 11Cl-PFOUdS, 5:3FTCA, and 7:3FTCA, which all exceeded the upper control limit with the exception of PFDoS, which fell below the lower limit (Table 7-5).

7.2 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. Further discussion of the relationship of the NIS compounds to the EIS compounds, their use as a diagnostic tool to assess instrument sensitivity, and the benefits of their use is spelled out in Section 4 of *Volume I.*

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 tissue samples were compiled and descriptive statistics for each NIS compound were generated across all tissue samples. Table 7-6 summarizes 1,161 NIS compound recoveries data across all tissue samples and seven laboratories, reported to the nearest percent. All NIS compound recoveries met the target recovery criteria (>30%) and the 50-200% recovery criteria as well, with the exception of 4 recoveries. All four of the NIS recoveries reported below 50% were marginally below that limit and were reported by Laboratory 8; 3 instances for $^{13}C_2$-PFHxA (46.4%, 46.6%, 46.8%) and one instance for $^{13}C_5$-PFNA (49.2%). Figure 7-5 clearly illustrates that overall, recoveries reported by the 7 laboratories indicated no true outliers.

Table 7-2. Summary of Tissue OPR Percent Recoveries

Analyte	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	Combined std. dev. (s_c)	RSD (s_w)
PFBA	6	13	113	13.2	3.62	14.5	3.21
PFPeA	7	15	110	13.5	7.18	15.3	6.53
PFHxA	6	12	111	12.5	3.83	13.8	3.46
PFHpA	7	15	108	13.8	7.07	15.6	6.53
PFOA	7	15	106	12.4	8.17	14.5	7.7
PFNA	6	12	110	13.2	10.3	16	9.35
PFDA	7	15	113	10.5	6.93	12.3	6.15
PFUnA	6	12	110	9.92	6.76	11.7	6.12
PFDoA	7	15	111	12.1	4.59	13.4	4.16
PFTrDA	6	12	96.7	14.9	5.61	16.6	5.8
PFTeDA	7	15	104	11.8	8.37	14	8.07
PFBS	7	15	111	19.9	10.5	22.6	9.46
PFPeS	7	15	109	28.8	13.1	32.2	12
PFHxS	7	15	106	18.8	9.98	21.3	9.41
PFHpS	6	12	105	13	7.85	15.1	7.45
PFOS	6	13	108	19	4.35	20.8	4.02
PFNS	7	15	94.1	23.9	6.79	26	7.21
PFDS	7	15	103	21.7	9.69	24.3	9.45
PFDoS	7	15	71.8	26.4	11.4	29.4	15.8
4:2FTS	7	15	108	20.4	6.11	22.3	5.63
6:2FTS	6	12	111	13.5	7.26	15.4	6.53
8:2FTS	7	15	119	23.2	9.22	25.7	7.78
PFOSA	7	15	114	10.3	5.11	11.6	4.49
NMeFOSA	6	13	110	10.6	6.67	12.5	6.08
NEtFOSA	5	10	108	13.2	6.76	15.2	6.26
NMeFOSAA	7	15	112	6.43	9.5	9.77	8.45
NEtFOSAA	7	15	105	11.4	10.6	14.5	10.1
NMeFOSE	6	13	111	18.4	5.28	20.2	4.78
NEtFOSE	5	11	139	41.9	30.6	51.2	22.1
PFMPA	7	15	92.1	33.9	12.8	37.4	13.9

Table 7-2. Summary of Tissue OPR Percent Recoveries (Continued)

Analyte	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	Combined std. dev. (s_c)	RSD (s_w)
PFMBA	7	15	116	16.9	5.09	18.4	4.4
NFDHA	7	15	126	19.6	12.2	22.8	9.65
HFPO-DA	7	15	110	14.7	7.51	16.6	6.8
ADONA	6	12	108	25.3	9.66	28.1	8.95
PFEESA	7	15	111	18.7	21.1	25.2	18.9
9Cl-PF3ONS	7	15	149	82.2	10.4	88.2	6.97
11Cl-PF3OUdS	7	15	131	76	13	81.8	9.96
3:3FTCA	7	15	84	24	12.6	27.2	15
5:3FTCA	7	15	118	27.9	21	33.6	17.8
7:3FTCA	7	15	130	27.1	19.4	32.2	14.9
$^{13}C_4$-PFBA	7	15	68.8	35.9	19.9	41.1	28.9
$^{13}C_5$-PFPeA	7	16	87.1	15.6	10.2	18.3	11.7
$^{13}C_5$-PFHxA	7	17	89.5	14.5	6.46	16.2	7.21
$^{13}C_4$-PFHpA	7	15	85.6	31.9	9.32	34.7	10.9
$^{13}C_8$-PFOA	7	16	90.8	11.8	6.9	13.7	7.6
$^{13}C_9$-PFNA	7	15	90.6	13.7	7.38	15.6	8.15
$^{13}C_6$-PFDA	7	16	87.3	11.7	7.72	13.8	8.85
$^{13}C_7$-PFUnA	7	16	82.9	11.6	7.76	13.7	9.36
$^{13}C_2$-PFDoA	7	17	87.5	16.4	9.22	18.9	10.5
$^{13}C_2$-PFTeDA	7	15	59.4	23	11	25.8	18.5
$^{13}C_3$-PFBS	7	15	93.4	8.07	10.3	11.4	11
$^{13}C_3$-PFHxS	7	16	89.9	13.1	9.29	15.6	10.3
$^{13}C_8$-PFOS	7	15	92.8	10.6	7.22	12.5	7.78
$^{13}C_2$-4:2FTS	7	15	123	16.4	16.4	21.3	13.4
$^{13}C_2$-6:2FTS	7	15	116	25.4	14.2	29	12.3
$^{13}C_2$-8:2FTS	7	15	149	58.5	38.7	68.6	26
$^{13}C_8$-PFOSA	7	15	95.2	14.9	7.42	16.8	7.79
D_3-NMeFOSA	7	15	59.2	32.4	10.5	35.4	17.7
D_5-NEtFOSA	7	15	45.9	20.1	11.3	23.1	24.7
D_3-NMeFOSAA	7	15	118	38.4	17.1	42.9	14.6

Table 7-2. Summary of Tissue OPR Percent Recoveries (Continued)

Analyte	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	Combined std. dev. (s_c)	RSD (s_w)
D_5-NEtFOSAA	7	15	143	66	21.2	72.3	14.9
D_7-NMeFOSE	7	15	66.5	35.1	15.9	39.3	24
D_9-NEtFOSE	7	15	31.9	30.2	7.01	32.7	22
$^{13}C_3$-HFPO-DA	7	17	82.5	20.9	12.6	24.3	15.3

Source file: TS_OPR_Phase4_py_log_V0_231214_135747,csv

Notes:
Number of Results - The number of individual OPR results that do not have a U flag included in the calculations.
Mean % Recovery - The mean percent recovery for OPR samples across all labs for the given analyte.
sb - The pooled between-laboratory standard deviation of the percent recoveries. Equation from EPA 821-B-18-001 page G-25.
sw - The pooled within-laboratory standard deviation of the percent recoveries. Equation from EPA 821-B-18-001 page G-25.
sc - The combined within- and between-laboratory standard deviations. Equation from EPA 821-B-18-001 page G-26.

Table 7-3. Statistically Derived Tissue OPR Acceptance Criteria

Analyte	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
PFBA	113	6.42	107	119
PFPeA	110	13.06	97	123
PFHxA	111	6.92	104	118
PFHpA	108	13.06	95	121
PFOA	106	15.40	91	121
PFNA	110	18.70	91	129
PFDA	113	12.30	101	125
PFUnA	110	12.24	98	122
PFDoA	111	8.32	103	119
PFTrDA	96.7	11.60	85	108
PFTeDA	104	16.14	88	120
PFBS	111	18.92	92	130
PFPeS	109	24.00	85	133
PFHxS	106	18.82	87	125
PFHpS	105	14.90	90	120
PFOS	108	8.04	100	116
PFNS	94.1	14.42	79	109
PFDS	103	18.90	84	122
PFDoS	71.8	31.60	40	103
4:2FTS	108	11.26	97	119
6:2FTS	111	13.06	98	124
8:2FTS	119	15.56	103	135
PFOSA	114	8.98	105	123
NMeFOSA	110	12.16	98	122
NEtFOSA	108	12.52	95	121
NMeFOSAA	112	16.90	95	129
NEtFOSAA	105	20.20	85	125
NMeFOSE	111	9.56	101	121
NEtFOSE	139	44.20	95	183
PFMPA	92.1	27.80	64	120
PFMBA	116	8.80	107	125
NFDHA	126	19.30	107	145
HFPO-DA	110	13.60	96.	124
ADONA	108	17.90	90.	126
PFEESA	111	37.80	73.	149
9Cl-PF3ONS	149	13.94	135	163
11Cl-PF3OUdS	131	19.92	111	151
3:3FTCA	84	30.00	54	114
5:3FTCA	118	35.60	82	154
7:3FTCA	130	29.80	100	160

Source File: derived from Table 7-2 and IDA file: TS_OPR_result_V0_231214_135747.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 7-4. Summary of Tissue LLOPR Results

Analyte	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	Combined std. dev. (s_c)	RSD (s_w)
PFBA	7	15	107	21.8	9.88	24.4	9.23
PFPeA	7	15	109	18.5	7.98	20.6	7.31
PFHxA	6	12	111	23.6	8.69	26.2	7.82
PFHpA	7	15	102	13.8	8.06	15.9	7.93
PFOA	7	15	102	18.6	7.98	20.7	7.81
PFNA	6	12	111	23.5	6.65	25.8	6.01
PFDA	7	15	113	14.5	15.9	19.4	14.1
PFUnA	6	12	118	26.8	11	30	9.26
PFDoA	7	15	109	13.4	5.24	14.8	4.79
PFTrDA	6	12	95.9	26.9	5.19	29.3	5.41
PFTeDA	7	15	105	18.2	11.3	21.1	10.7
PFBS	7	15	106	12.9	12.1	16.4	11.4
PFPeS	7	15	106	27.2	12.8	30.5	12
PFHxS	7	15	108	20.2	16.9	24.8	15.6
PFHpS	6	12	101	14.3	10.7	17.2	10.6
PFOS	6	13	121	36.9	12.1	40.9	9.99
PFNS	7	15	92.9	26.6	8.27	29.1	8.9
PFDS	7	15	98.2	23.9	9.74	26.6	9.92
PFDoS	6	13	69.8	17.9	14	21.9	20.1
4:2FTS	7	15	105	18.5	6.31	20.3	6.01
6:2FTS	6	12	109	23.8	7.84	26.3	7.19
8:2FTS	7	15	120	26.5	13.2	29.9	11
PFOSA	7	15	111	18.4	4.74	19.9	4.29
NMeFOSA	6	13	109	19.6	9.25	22.2	8.49
NEtFOSA	5	10	109	13.2	14.1	17.6	13
NMeFOSAA	7	15	109	14.7	13.8	18.7	12.7
NEtFOSAA	7	15	105	14.7	8.06	16.8	7.68
NMeFOSE	5	11	107	21.6	5.26	24	4.91
NEtFOSE	4	9	128	38.9	15.2	45	11.9
PFMPA	6	13	98.1	13.2	18.1	19.5	18.5

Table 7-4. Summary of Tissue LLOPR Results (Continued)

Analyte	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	Combined std. dev. (s_c)	RSD (s_w)
PFMBA	7	15	112	16.7	15.4	21.1	13.7
NFDHA	7	15	115	16.6	15.4	21	13.4
HFPO-DA	7	15	111	18.1	13	21.5	11.7
ADONA	6	12	116	29.1	14	32.9	12
PFEESA	7	15	108	24.3	23.4	31.1	21.7
9Cl-PF3ONS	7	15	149	71	18.9	77.2	12.7
11Cl-PF3OUdS	7	15	132	69.2	20.5	75.5	15.6
3:3FTCA	6	13	91.7	16.2	10	19	11
5:3FTCA	7	15	124	48.6	20.4	54	16.5
7:3FTCA	7	15	135	45.8	14.2	50	10.5
$^{13}C_4$-PFBA	7	15	70.2	24.5	28.5	33.5	40.6
$^{13}C_5$-PFPeA	7	16	88.7	16.2	22.6	24.2	25.5
$^{13}C_5$-PFHxA	7	17	86.5	15.1	11	18.2	12.7
$^{13}C_4$-PFHpA	7	15	88.7	35.7	9.57	38.8	10.8
$^{13}C_8$-PFOA	7	16	90.7	12.3	7.01	14.2	7.73
$^{13}C_9$-PFNA	7	15	92.1	15	11.9	18.2	12.9
$^{13}C_6$-PFDA	7	16	89.8	13.2	10.2	16.1	11.3
$^{13}C_7$-PFUnA	7	16	85.6	16.3	13.1	20	15.3
$^{13}C_2$-PFDoA	7	17	89.8	20.9	17.9	26.3	20
$^{13}C_2$-PFTeDA	7	15	61.4	24.7	24.9	32	40.5
$^{13}C_3$-PFBS	7	15	89.4	10.8	11.9	14.5	13.3
$^{13}C_3$-PFHxS	7	16	88.7	14.4	8.82	16.8	9.95
$^{13}C_8$-PFOS	7	15	92.3	14.3	9.67	16.9	10.5
$^{13}C_2$-4:2FTS	7	15	129	35.6	22.9	41.6	17.7
$^{13}C_2$-6:2FTS	7	15	131	48.1	21.1	53.7	16.1
$^{13}C_2$-8:2FTS	7	15	141	50	22.3	55.8	15.8
$^{13}C_8$-PFOSA	7	15	95.4	15.1	12.6	18.6	13.2
D_3-NMeFOSA	7	15	52.8	27.5	14.6	31.3	27.8
D_5-NEtFOSA	7	15	39.6	16.7	10.5	19.4	26.5
D_3-NMeFOSAA	7	15	118	34.4	12.7	37.9	10.8

Table 7-4. Summary of Tissue LLOPR Results (Continued)

Analyte	Number of Labs	Number of Results	Mean % Recovery	Pooled Between-Lab std. dev. (s_b)	Pooled Within-Lab std. dev. (s_w)	Combined std. dev. (s_c)	RSD (s_w)
D_5-NEtFOSAA	7	15	148	67.8	19.1	73.8	12.9
D_7-NMeFOSE	7	15	57	35.7	15.3	39.8	26.9
D_9-NEtFOSE	7	15	25.8	27.4	7.83	29.8	30.3
$^{13}C_3$-HFPO-DA	7	17	76.6	24.8	10.5	27.7	13.7

Source File: TS_LLOPR_results_V0_231214_1357

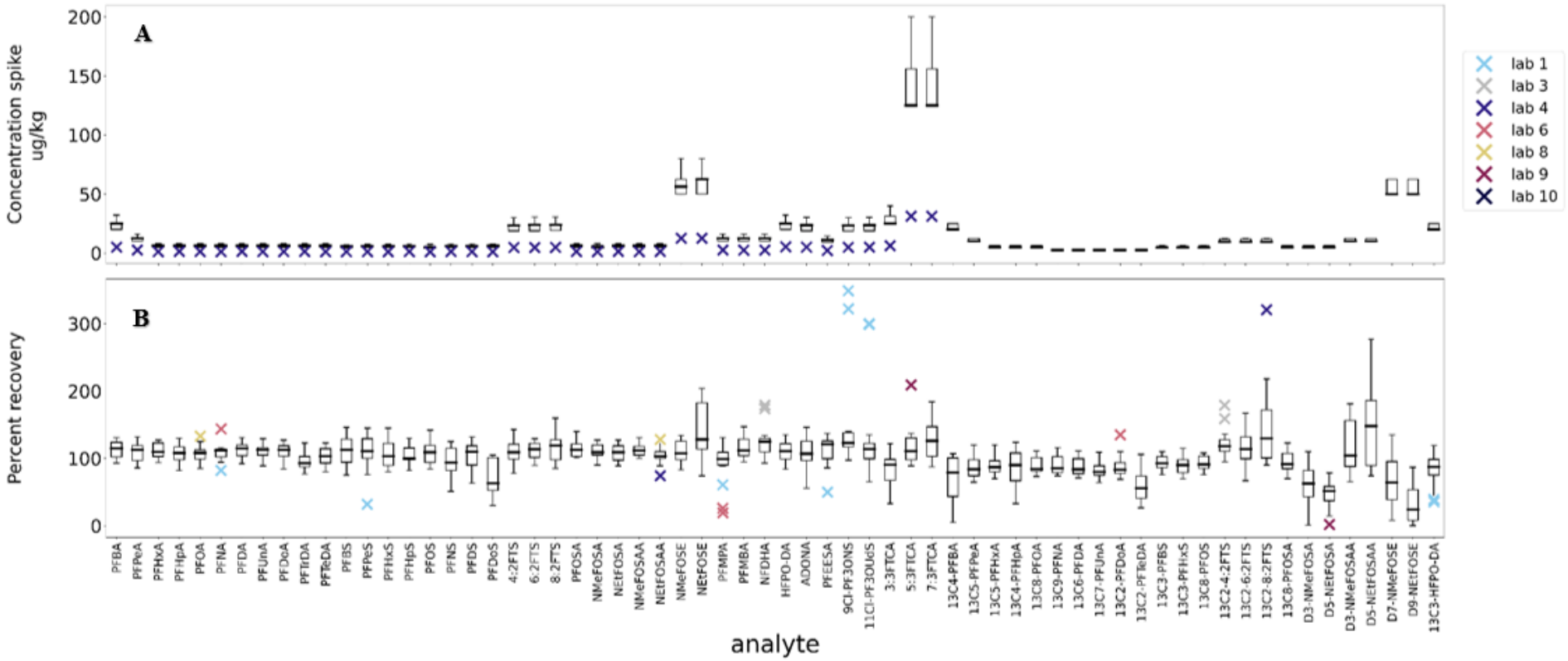
Notes:
Number of Results - The number of individual OPR results that do not have a U flag included in the calculations.
Mean % Recovery - The mean percent recovery for OPR samples across all labs for the given analyte.
s_b - The pooled between-laboratory standard deviation of the percent recoveries. Equation from EPA 821-B-18-001 page G-25.
s_w - The pooled within-laboratory standard deviation of the percent recoveries. Equation from 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.

Table 7-5. Statistically Derived Tissue LLOPR Acceptance Criteria

Analyte	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
PFBA	107	18.46	89	125
PFPeA	109	14.62	94	124
PFHxA	111	15.64	95	127
PFHpA	102	15.86	86	118
PFOA	102	15.62	86	118
PFNA	111	12.02	99	123.
PFDA	113	28.20	85	141
PFUnA	118	18.52	99	137
PFDoA	109	9.58	99	119
PFTrDA	95.9	10.82	85	107
PFTeDA	105	21.40	84	126
PFBS	106	22.80	83	129
PFPeS	106	24.00	82	130
PFHxS	108	31.20	77	139
PFHpS	101	21.20	80	122
PFOS	121	19.98	101	141
PFNS	92.9	17.80	75	110
PFDS	98.2	19.84	78	118
PFDoS	69.8	40.20	30	110
4:2FTS	105	12.02	93	117
6:2FTS	109	14.38	95	123
8:2FTS	120	22.00	98	142
PFOSA	111	8.58	102	120
NMeFOSA	109	16.98	92	126
NEtFOSA	109	26.00	83	135
NMeFOSAA	109	25.40	84	134
NEtFOSAA	105	15.36	90	120
NMeFOSE	107	9.82	97	117
NEtFOSE	128	23.8	104	152
PFMPA	98.1	37.00	61	135
PFMBA	112	27.40	85	139
NFDHA	115	26.80	88	142
HFPO-DA	111	23.40	88	134
ADONA	116	24.00	92	140
PFEESA	108	43.40	65	151
9Cl-PF3ONS	149	25.40	124	174
11Cl-PF3OUdS	132	31.20	101	163
3:3FTCA	91.7	22.00	70	114
5:3FTCA	124	33.00	91	157
7:3FTCA	135	21.00	114	156

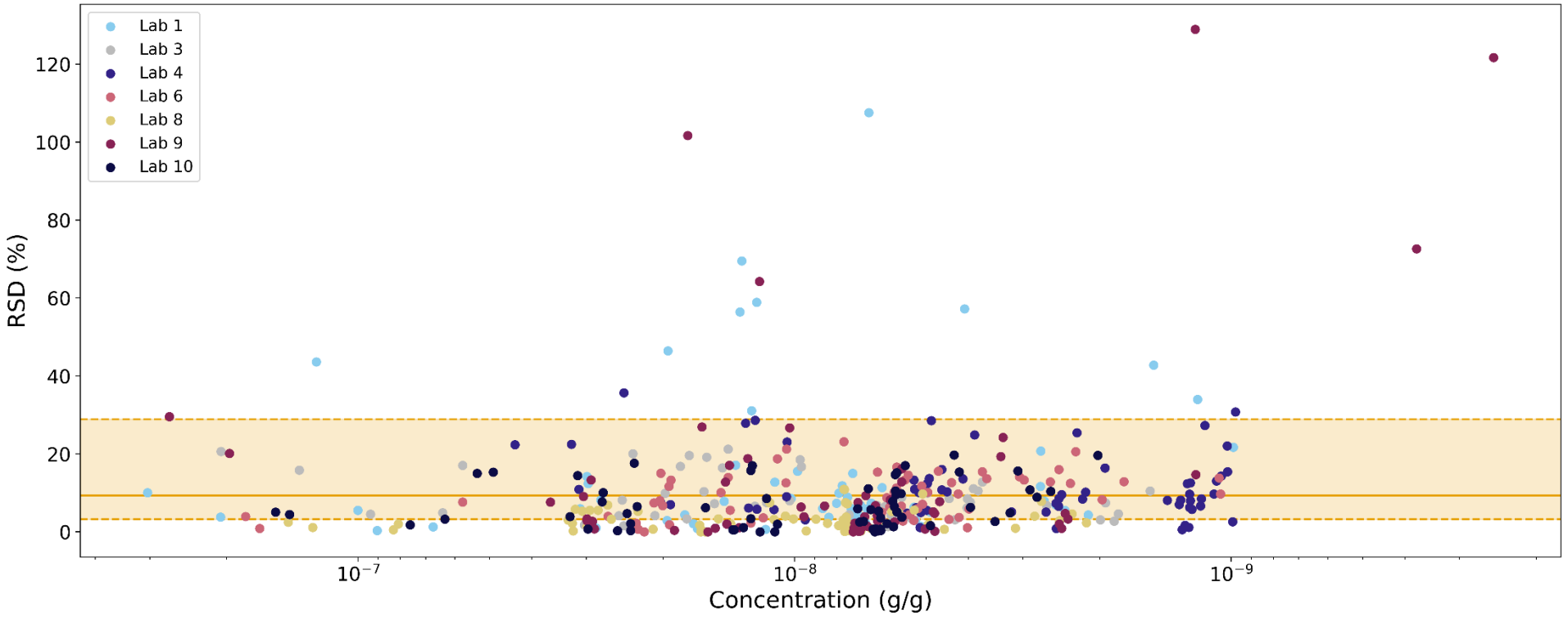
Source File: derived from Table 7-4 and IDA file: TS_LLOPR_result_V0_231214_135747.csv

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.



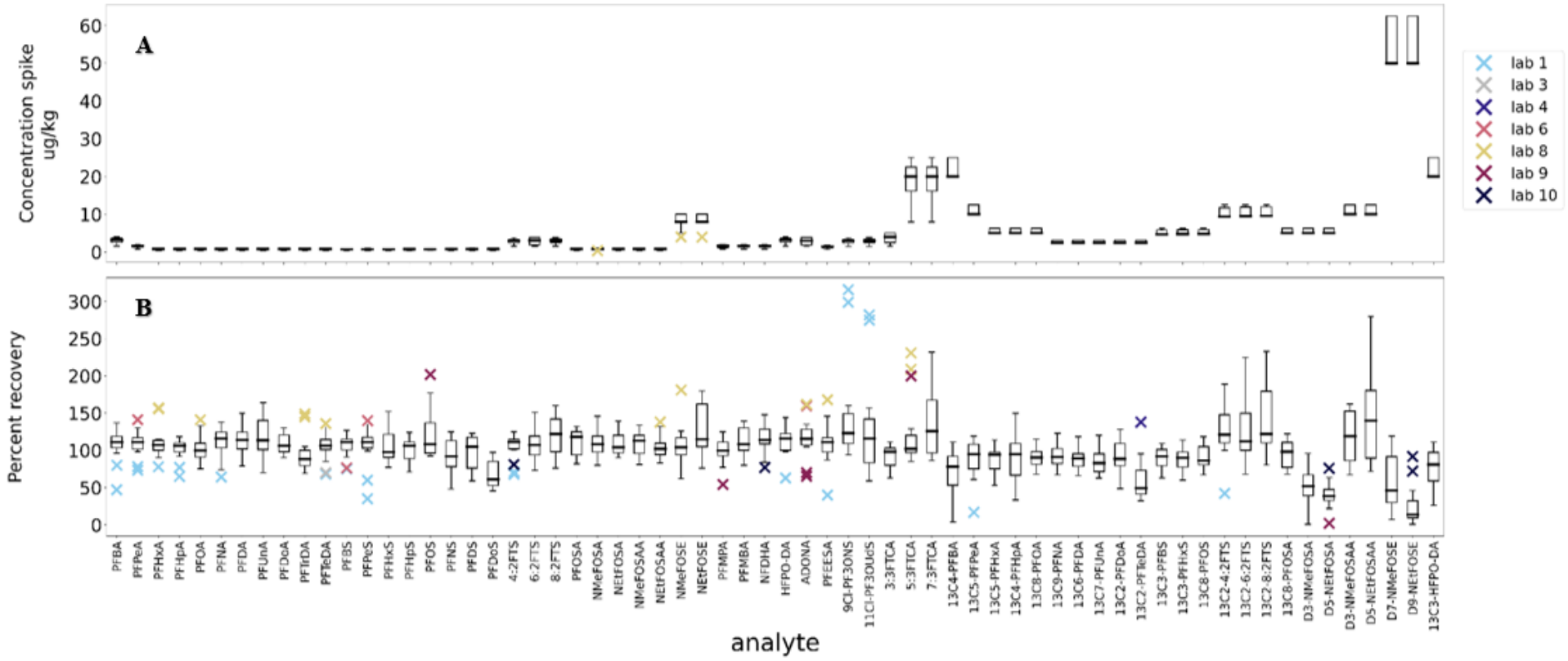
Source file: TS_OPR_Boxplot_V0_231214__135747

Figure 7-1. Tissue OPR Results by Compound by Laboratory
(A) Spiked Concentration. (B) Calculated percent recovery.
Figure includes all OPR data batched with unspiked and spiked samples.



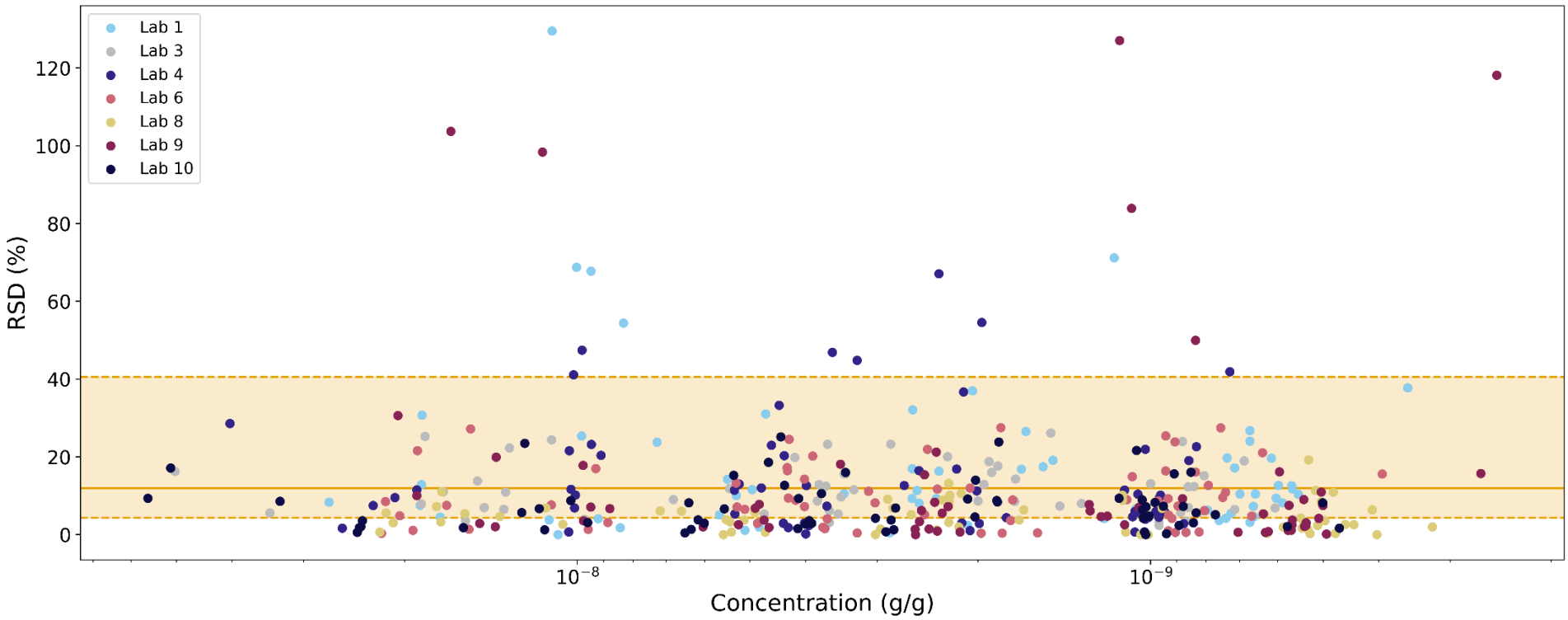
Source file: TS_OPR_Horwitz_V0_231214__135747

Figure 7-2. Individual Laboratory and Pooled OPR Relative Standard Deviation (from Table 7-2)
Shaded Area is the range (minimum and maximum) OPR RSD from Table 5-3. Solid line is the median %RSD
Figure includes both target compound recoveries, and EIS compound recoveries.
The concentrations on the Y-axis is arrayed from highest to lowest. Limits of detection would be at the right tail of the graphic.



Source file: TS_LLOPR_Boxplot_V0_231214__135747

Figure 7-3. Tissue LLOPR Results by Compound by Laboratory
(A) Spiked Concentration. (B) Calculated percent recovery.
Figure includes all LLOPR data batched with unspiked and spiked samples.



Source file: TS_LLOPR_Horwitz_V0_231214__135747

Figure 7-4. Individual Laboratory and Pooled LLOPR Relative Standard Deviation (from Table 7-4)

Shaded Area is the range (minimum and maximum) LLOPR RSD from Table 5-3. Solid line is the median %RSD
Figure includes both target compound recoveries, and EIS compound recoveries.
The concentrations on the Y-axis is arrayed from highest to lowest. Limits of detection would be at the right tail of the graphic.

Table 7-6. Pooled Tissue Media Samples NIS Compound Recovery Analysis

Analyte	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)
$^{13}C_3$-PFBA	7	149	98.7	17.9	15	15.2
$^{13}C_2$-PFHxA	7	183	98.3	22.6	13.4	13.6
$^{13}C_4$-PFOA	7	152	104	21.9	16.7	16.0
$^{13}C_5$-PFNA	7	147	103	13.3	14.9	14.4
$^{13}C_2$-PFDA	7	192	115	21.8	17.2	15.0
$^{18}O_2$-PFHxS	7	166	99.9	17.3	12.3	12.3
$^{13}C_4$-PFOS	7	172	100	22.9	13.0	13.0

Source File: IDA file: TS_NIS_results_V0_231214_135747.csv

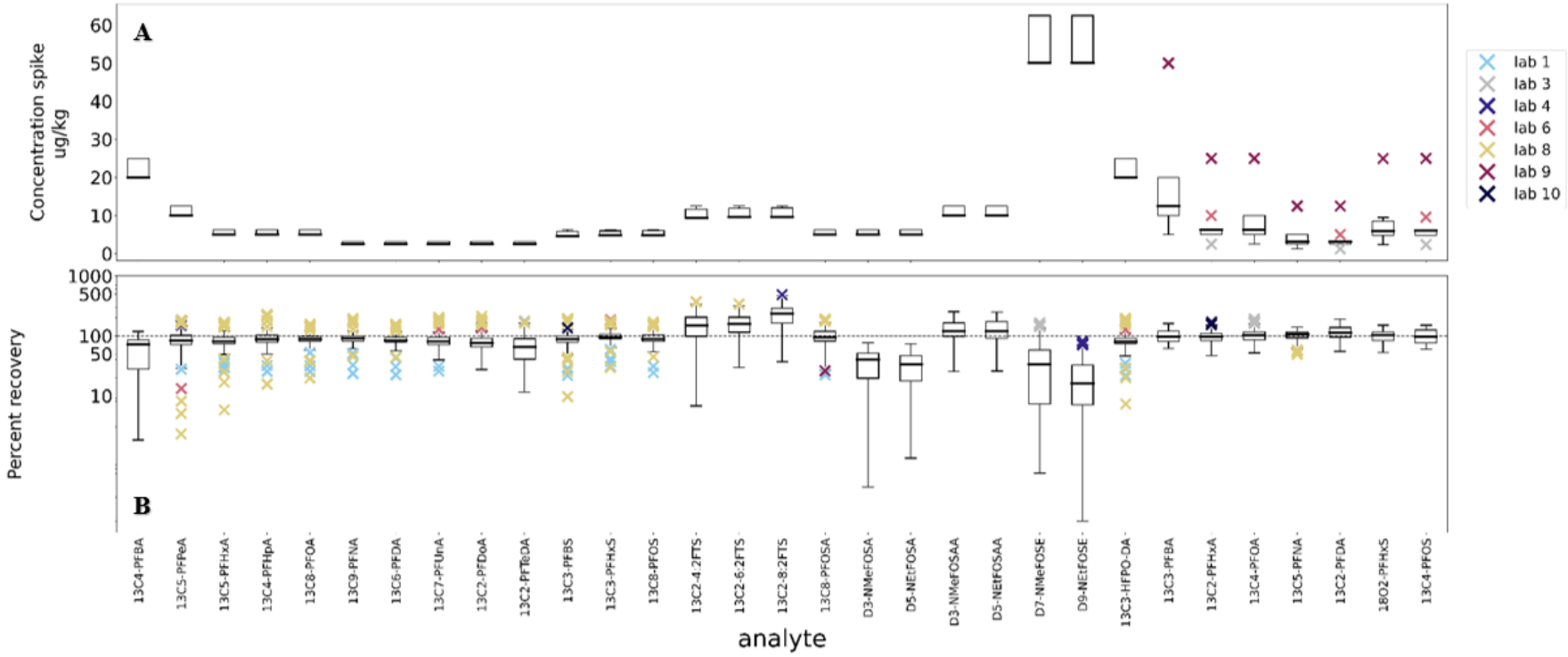
Notes:
Number of Results - The total number of matrix results that do not have a U flag.
Mean % Recovery - The mean percent recovery across all individual matrix samples and labs for the given analyte.
s_b - The pooled between-laboratory standard deviation of the percent recoveries. Equation from EPA 821-B-18-001 page G-25.
s_w - The pooled within-laboratory standard deviation of the percent recoveries. Equation from EPA 821-B-18-001 page G-25.
RSD - The pooled within-laboratory relative standard deviation (RSD, (s_w/(mean % recovery) *100). Equation from EPA 821-B-18-001 page G-26.

Table 7-7. Statistically-Derived NIS Compound Recovery Acceptance Criteria

NIS Compound	Mean % Recovery	2 x RSD[1]	LCL[2]	UCL[3]
$^{13}C_3$-PFBA	98.7	30.4	68	129
$^{13}C_2$-PFHxA	98.3	27.2	71	126
$^{13}C_4$-PFOA	104	32.0	72	136
$^{13}C_5$-PFNA	103	28.8	74	132
$^{13}C_2$-PFDA	115	30.0	85	145
$^{18}O_2$-PFHxS	99.9	24.6	75	125
$^{13}C_4$-PFOS	100	26.0	74	126

Source File: \ Source File: Derived from data in Table 7-6.

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.



Source file: TS_NIS_Boxplot_V0_231214_135747

Figure 7-5. Tissue NIS and EIS Compound Results by Compound by Laboratory
(A) Spiked Concentration. (B) Calculated percent recovery.
Figure includes all EIS and NIS compound data from unspiked and spiked samples.

7.3 MATRIX SPIKE ANALYSES

Spike recoveries for the tissue samples 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"), laboratories ("Lab"), and spike concentrations ("Spike Conc."). Because the final working dataset consisted of missing permutations of main effects (see Section 6), 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 (Table 7-8). All PFAS on average were observed with mean recoveries 70-130% of the target spike concentration, with exception for PFDoS and the 7:3FTCA (Figure7-6). Spike Conc. and Lab main effects were also relatively consistent and close to the target spike concentration (i.e., 100% recovery) (Figure 7-7).

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 averaging over the method analytes to avoid an impracticable number of permutations. Similarly, precision was calculated as the inter-laboratory percent relative standard deviation (RSD) among replicate measures of the various spiked samples. Figure 7-6 illustrates the calculated accuracy and precision on a unit scale such that the results can be interpreted quantitively (i.e., a literal bullseye target). Overall, the method as validated by this multi-laboratory study can be summarized to result in less than 70% error for the tissue matrix. Table 7-9 provides the percent probability of observing a result with <30% error for tissue matrix.

Table 7-8. Accuracy Analysis: ANOVA Results for the Observed Matrix Spike Recoveries

Effect	F Value	P Value
Laboratory	216	<0.0001
PFAS	19.1	<0.0001
Spiked Concentration	13.2	0.0003

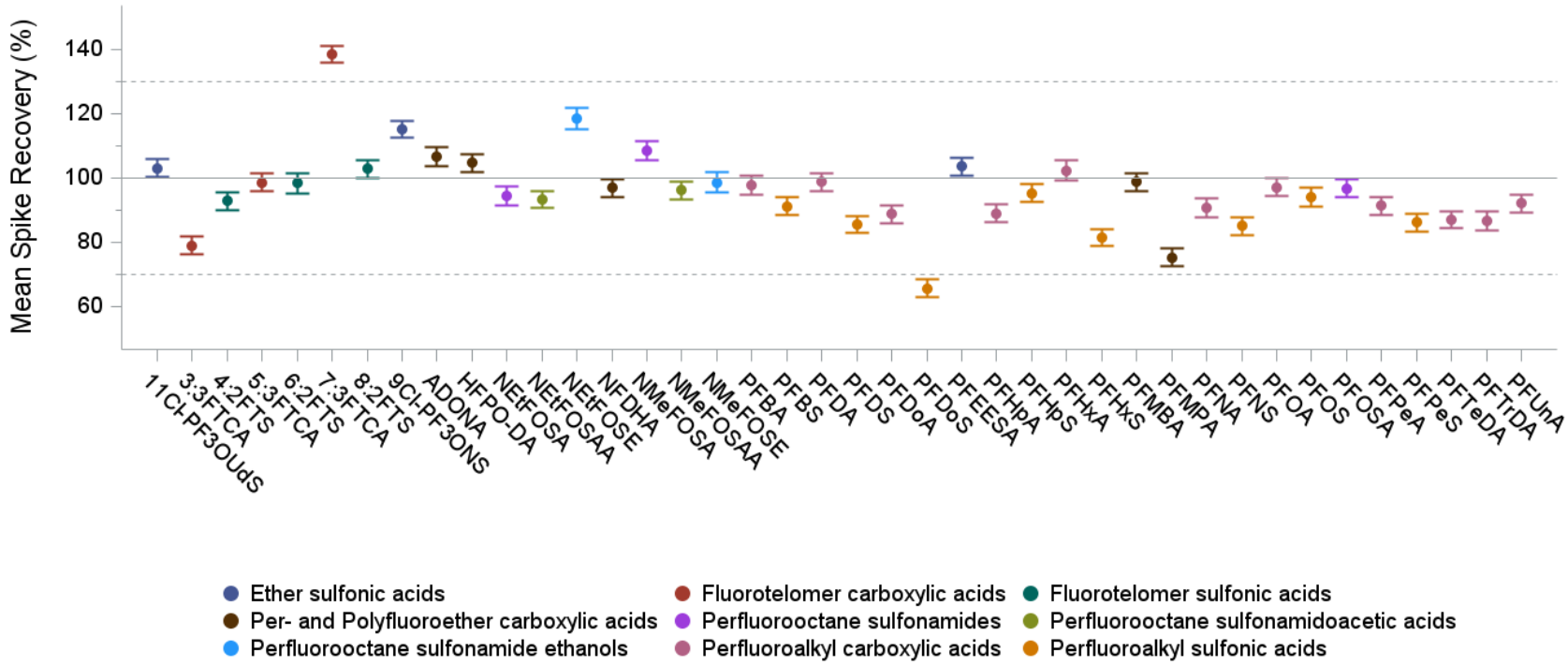


Figure 7-6. Mean Spike Recoveries Summarized for Each Target Analyte (i.e., the "PFAS" Effect in Table 7-8)

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

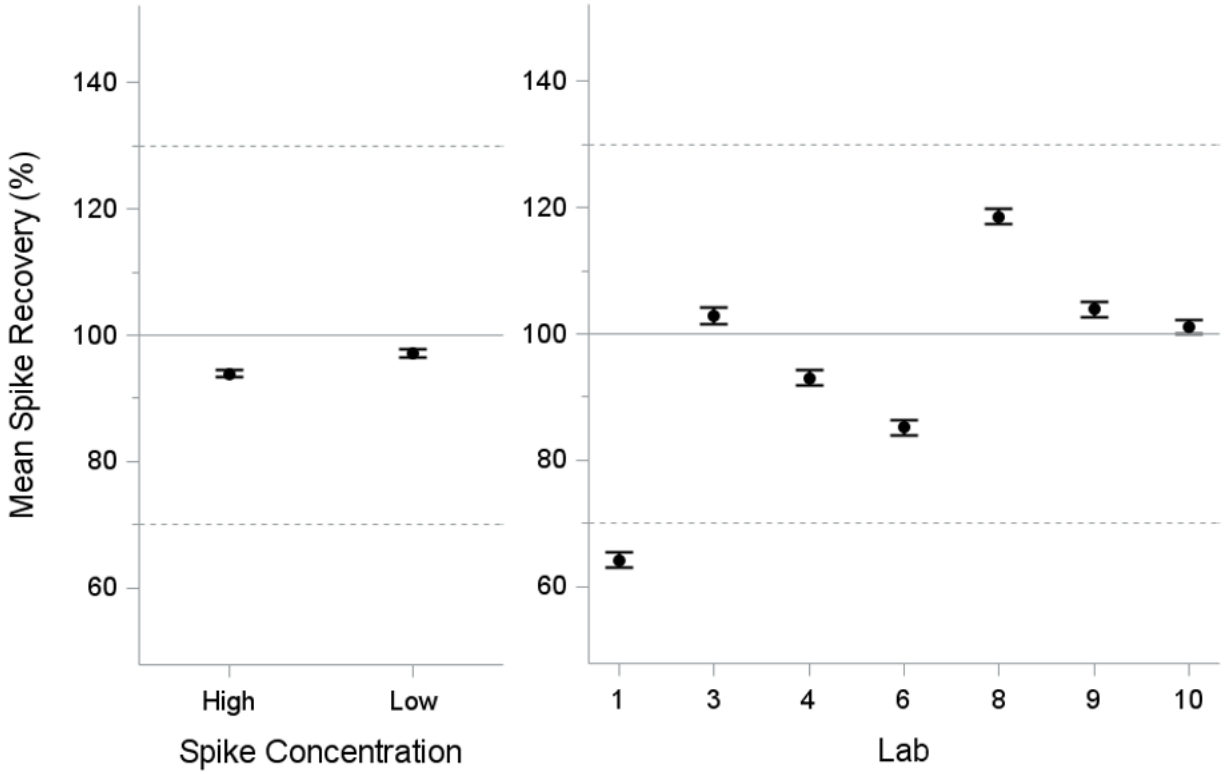


Figure 7-7. Mean spike recoveries summarized for each spike concentration and laboratory (i.e., the "Spike Conc." and "Lab" effects in Table 7-8, respectively)

Error bars reflect one standard error.

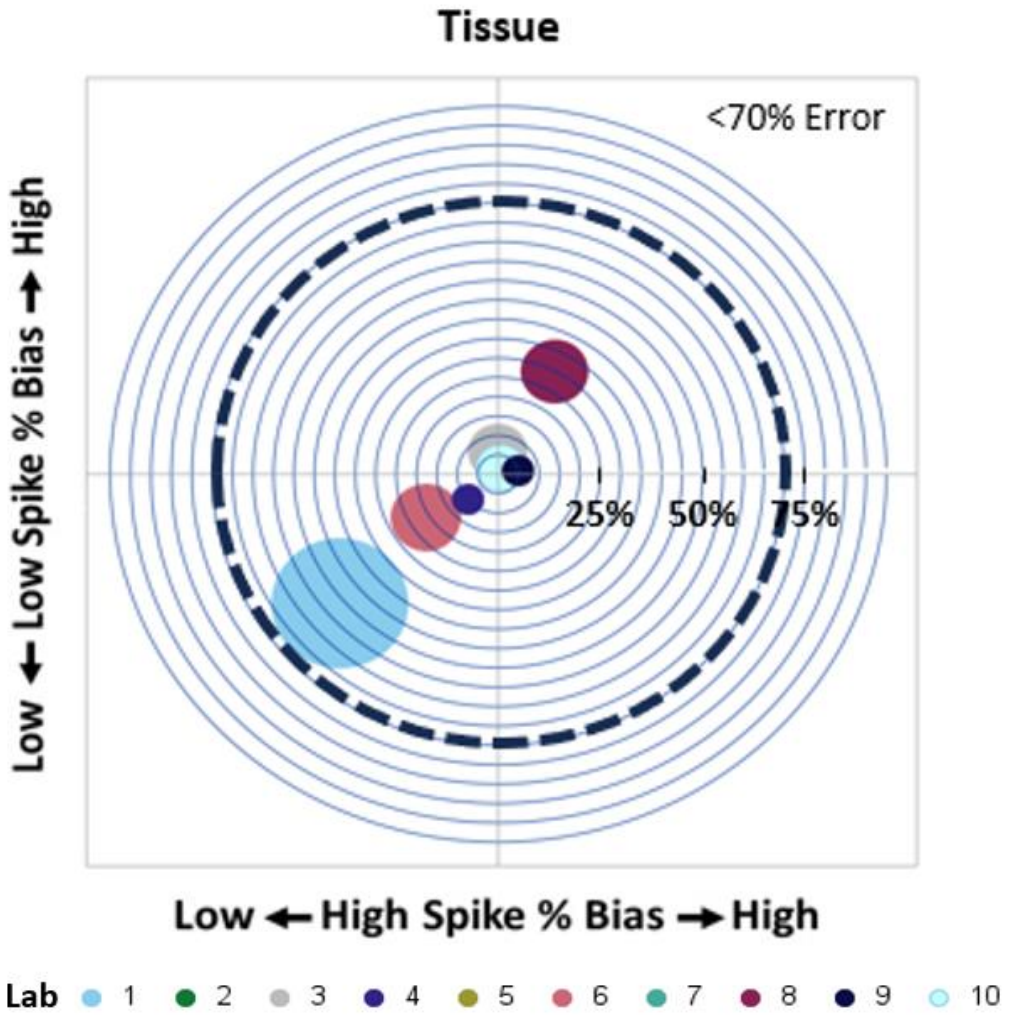


Figure 7-8. Summary Illustration of the Overall Method Accuracy and Precision

Bubble sizes reflect precision calculated as the intra-laboratory %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.

Table 7-9. Probability (%) of observing a result with <30% error

Analyte	Tissue Probability (%)
Perfluoroalkyl carboxylic acids	
PFBA	92.2
PFPeA	78.9
PFHxA	79.8
PFHpA	90.5
PFOA	78.6
PFNA	88
PFDA	83.3
PFUnA	88.9
PFDoA	88.1
PFTrDA	54.6
PFTeDA	73
Perfluoroalkyl sulfonic acids	
PFBS	86.4
PFPeS	75.4
PFHxS	74.6
PFHpS	88
PFOS	88.9
PFNS	82.5
PFDS	74.6
PFDoS	39.8
Fluorotelomer sulfonic acids	
4:2FTS	87.9
6:2FTS	65.7
8:2FTS	80.7

Analyte	Tissue Probability (%)
Perfluorooctane sulfonamides	
PFOSA	85.7
NMeFOSA	71.8
NEtFOSA	78.7
Perfluorooctane sulfonamidoacetic acids	
NMeFOSAA	87.3
NEtFOSAA	85.6
Perfluorooctane sulfonamide ethanols	
NMeFOSE	61.3
NEtFOSE	49.4
Per- and Polyfluoroether carboxylic acids	
PFMPA	61.8
PFMBA	72.6
NFDHA	76
HFPO-DA	77.6
ADONA	78.5
Ether sulfonic acids	
PFEESA	82.4
9Cl-PF3ONS	77.6
11Cl-PF3OUdS	75.2
Fluorotelomer carboxylic acids	
3:3FTCA	47
5:3FTCA	64
7:3FTCA	46.4

Source File: Prop_30%_error.csv

7.4 DETERMINATION OF FINAL QC SPECIFICATIONS FOR METHOD 1633

EPA and DoD used the same approach to determine the QC acceptance criteria for the fish and shellfish tissue samples that they used for the results from the aqueous and solids portion of the method validation study (see Sections 8.5 and 9.5). Following completion of the statistical calculations, 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 due to the fact there appeared to be some true outliers included in the final data set, and that the standard deviation based approach produced QC criteria that were much wider than what was actually observed. 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.
- Comparing the individual laboratory minimum and maximum means and relative standard deviation for the Initial Precision and Recovery (IPR) Study.
- 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 percentiles of the observed data, and then rounding those values to the nearest multiple of 5%.

7.4.1 Initial SAS Calculations

Table 7-10 contains the initial SAS calculations of the IPR and OPR limits for the 40 target analytes using the entire data set (all 7 laboratories and both tissue reference QC matrix analyses), with the calculated recoveries, RSDs, minimum, and maximum observed recoveries rounded to the nearest 1%.

Table 7-10. Initial SAS Calculations of the IPR and OPR/LLOPR Limits for the 40 Target Analytes Using the Entire Data Set of Fish and Shellfish Tissue QC Sample Results

Analyte	n	# labs	Mean	Max. RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
PFBA	66	7	108	25	56	161	53	164	47	137
PFPeA	69	7	108	26	71	145	62	154	73	141
PFHxA	58	6	111	25	45	176	48	174	78	157
PFHpA	69	7	108	30	68	147	57	159	65	143
PFOA	69	7	108	32	64	152	53	163	75	141
PFNA	58	6	110	30	65	155	56	164	64	144
PFDA	69	7	111	27	83	139	68	153	79	150
PFUnA	58	6	112	30	65	158	57	167	70	164
PFDoA	69	7	108	20	65	151	63	153	84	130
PFTrDA *	58	6	102	44	-4	208	0	204	60	150
PFTeDA	69	7	106	30	51	162	46	167	68	140
PFBS	69	7	108	27	58	158	53	163	75	146
PFPeS	69	7	106	36	28	184	26	186	32	145
PFHxS	69	7	109	30	56	161	50	168	77	152

Table 7-10. Initial SAS Calculations of the IPR and OPR/LLOPR Limits for the 40 Target Analytes Using the Entire Data Set of Fish and Shellfish Tissue QC Sample Results (Continued)

Analyte	n	# labs	Mean	Max. RSD	IPR Lower Limit (%)	IPR Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)	Min. Obs. Rec.	Max. Obs. Rec.
PFHpS	58	6	103	27	45	162	45	161	71	137
PFOS	59	6	112	45	71	153	43	181	82	202
PFNS	69	7	94	33	12	176	14	174	48	125
PFDS	69	7	96	34	9	183	12	180	59	132
PFDoS *	69	7	71	55	-65	208	-58	201	30	135
4:2FTS	69	7	107	38	73	141	50	164	56	143
6:2FTS	58	6	107	34	50	165	43	172	73	151
8:2FTS	69	7	117	32	44	191	41	194	76	160
PFOSA	69	7	111	20	62	161	61	161	82	140
NMeFOSA	60	6	115	27	55	175	53	176	80	150
NEtFOSA	56	6	110	33	74	147	57	164	81.8	144
NMeFOSAA	69	7	109	29	71	148	60	159	81	138
NEtFOSAA	69	7	105	28	56	155	51	160	66	138
NMeFOSE	67	7	98	57	20	176	5	191	0	181
NEtFOSE	57	6	122	68	42	202	3	240	0	204
HFPO-DA	69	7	109	28	65	153	57	161	63	144
ADONA	58	6	112	27	10	213	19	204	56	162
9Cl-PF3ONS *	69	7	127	81	-33	286	-54	308	94	349
11Cl-PF3OUdS*	69	7	114	80	-49	277	-61	290	58.8	300
3:3FTCA	69	7	87	41	23	151	18	156	33	122
5:3FTCA	69	7	115	50	29	201	15	215	85	231
7:3FTCA	69	7	123	45	27	219	19	227	86.1	232
PFEESA	69	7	108	39	50	166	38	178	40	168
PFMPA	69	7	99	49	33	164	19	178	19.5	135
PFMBA	69	7	112	27	65	159	59	165	80	147
NFDHA	69	7	112	39	46	179	36	189	77	179

Source files: Tissue IPR OPR LLOPR specs 2024-01-07.xlsx

* The negative values for the lower IPR and OPR/LLOPR limits for PFTrDA, PFDoS, 9Cl-PF3ONS, and 11Cl-PF3OudS have no physical basis, but are a function of the effect of the wide within-laboratory and between-laboratories variabilities for these analytes on the statistical calculations for multi-laboratory validation study . The occurrence of such negative values is one of the reasons that EPA and DoD employed the non-parametric approach to establishing acceptance criteria described elsewhere in this report.

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

As was done for the aqueous and solid sample portions of the study, following the review of the statistically derived acceptance limits, EPA and DoD decided to apply both a non-parametric approach and professional judgement (e.g., elimination of results from a specific laboratory for an analyte or EIS compound or elimination of a few data points far outside of what was observed from the rest of the data) to establish the QC acceptance limits. Each use of professional judgement is documented below. The following QC criteria for fish and shellfish tissue are discussed in this section:

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

The initial calculations of the IPR recoveries in Table 7-10 were generated using a 99.875% confidence interval. The 99.875% confidence level was used because it targets an overall 5% false positive probability (i.e., a compound failing the criterion despite not having any analytical problems) of at least one failure across the 40 target analytes; 99.875 = 100 - ([5/40]/100). 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.

All of the non-parametric IPR and OPR/LLOPR recovery limits were then expressed to a multiple of 5% and the RSD limits were expressed to the nearest 1%. Some of the calculated OPR/LLOPR criteria were tightened when none of the 50 to 69 observed OPR/LLOPR results were within 10% of the calculated values. 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. Ultimately, the OPR criteria were made no more stringent than 60-140% because tissue is known as a more challenging matrix. The individual laboratory IPR means and %RSD were also evaluated, and the IPR criteria were made such that all seven laboratories would pass the IPR specifications below. The final IPR and OPR/LLOPR limits for the target analytes are shown in Table 7-11.

Table 7-11. Final IPR and OPR/LLOPR Acceptance Limits

Analyte	IPR Max RSD	IPR Mean Lower and Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
PFBA	20	70 - 135	60	140
PFPeA	20	70 - 130	60	145
PFHxA	20	70 - 140	60	160
PFHpA	20	70 - 140	60	145
PFOA	25	70 - 130	60	150
PFNA	20	70 - 140	60	145
PFDA	20	70 - 135	60	150
PFUnA	20	70 - 135	60	155
PFDoA	20	70 - 135	60	140
PFTrDA	20	55 - 160	60	150
PFTeDA	25	70 - 140	60	140
PFBS	20	70 - 145	60	150
PFPeS	20	70 - 150	60	145
PFHxS	25	70 - 140	60	155
PFHpS	20	70 - 145	60	140
PFOS	30	70 - 135	60	160
PFNS	20	60 - 130	45	140
PFDS	20	55 - 135	50	140
PFDoS	45	25 - 145	25	140
4:2FTS	30	65 - 140	55	150
6:2FTS	25	70 - 140	60	150
8:2FTS	25	70 - 150	60	170
PFOSA	20	70 - 140	60	150

Table 7-11. Final IPR and OPR/LLOPR Acceptance Limits (Continued)

Analyte	IPR Max RSD	IPR Mean Lower and Upper Limit (%)	OPR/LLOPR Lower Limit (%)	OPR/LLOPR Upper Limit (%)
NMeFOSA	30	70 - 140	60	160
NEtFOSA	40	70 - 140	60	150
NMeFOSAA	20	70 - 140	60	145
NEtFOSAA	25	70 - 140	60	145
NMeFOSE	20	60 - 150	40	180
NEtFOSE	40	70 - 145	60	205
HFPO-DA	25	70 - 140	60	145
ADONA	20	70 - 145	55	165
9Cl-PF3ONS	20	70 - 140	60	170
11Cl-PF3OUdS	20	65 - 140	50	170
3:3FTCA	25	55 - 130	30	140
5:3FTCA	20	70 - 145	60	160
7:3FTCA	20	70 - 155	60	200
PFEESA	25	70 - 145	50	150
PFMPA	40	70 - 145	25	145
PFMBA	20	70 - 140	60	150
NFDHA	30	70 - 155	60	180

Source files: Tissues IPR and OPR-LLOPR specs 11-7-23_ah CM.xlsx and Comparison of IPR-OPR specs for aqueous and tissues_ah GDIT.xlsx

Most of the OPR/LLOPR acceptance criteria in Table 7-11 are inclusive of the highest or lowest observed results from Table 7-10, which included 56 to 69 data points from 6 or 7 laboratories, depending on the analyte. Below are the exceptions:

- The lowest observed recovery for PFBA was 47%. The second lowest recovery was 80% and the mean recovery was 108%, so a lower recovery criteria of 60% was selected because that one data point was an anomaly (33% lower than any other data point).
- The two highest recoveries for PFOS are from Laboratory 9 (202 and 177%). The third highest recovery is 151%. Because the two highest recoveries were well above the rest of the data set, the upper recovery criterion was set at 160%.
- The three highest recoveries for 6:2FTS are from Laboratory 8 (151, 145, and 144%). The next highest recovery is 136%. 150% was selected as the upper criterion rather than 151%, and because the Laboratory 8 data were significantly different than the rest of the data set.
- The lowest recovery of NMeFOSE is 0%, from Laboratory 8. The second lowest recovery is 44%, and the third lowest 61%, both from different laboratories. The low recovery criterion was set at 40% because the 0% recovery is so different from the rest of the data set (by 44%). The six highest recoveries are from Laboratory 8 (181, 143, 141, 138, 137, and 137). The next highest recovery is 134%. The QC criterion was set at 150%, which is inclusive of most of the Laboratory 8 data.
- The lowest recovery for NEtFOSE is 0% from Laboratory 8. The second and third lowest recoveries are 74 and 76%, from two other laboratories. The low recovery criterion was set at 60% because the lowest point was so different from the rest of the data set.

- The four highest recoveries for 9Cl-PF3ONS are from Laboratory 1 (349, 322, 316, and 299%). The highest recovery from any other laboratory is 160%. The 4 highest recoveries were all run with the spiked tissue samples (2 LLOPRs and 2 OPRs). The IPR and LOQVER samples from Laboratory 1 that were run on a different day all had very reasonable recoveries. Given the extremely high recoveries in these four QC samples, there is some concern that they were double spiked. Therefore, the high recovery criterion was set at 170%.
- The four highest recoveries for 11Cl-PF3OUdS are from Laboratory 1 (300, 299, 282, and 275%). The highest recovery from any other laboratory is 157%. As with the recoveries for 9Cl-PF3ONS, there is some concern that these four QC samples were double spiked. Therefore, the high recovery criterion was set at 170%.
- The three lowest recoveries for 3:3FTCA are from Laboratory 1 (26, 33, and 55%). The lowest recovery from any other laboratory is 55%. Therefore, the low recovery criterion was set at 30%.
- The highest four recoveries for 5:3FTCA were much higher than the rest of the data, at 231, 209, 209, and 200%, from two different laboratories. These four data points are about double what is typical of the data, which suggests that these QC samples may have been double spiked. The next highest recovery is 142%. Therefore, the high recovery criterion was set at 160%.
- The two highest recoveries for 7:3FTCA are from Laboratory 8 (232 and 198%) and the next highest recovery is 184%. Given the large difference between the two highest points (34%), and smaller difference between the next two (14%), the second highest point was used to set the high recovery criterion at 200%
- The five highest recoveries for PFEESA are from Laboratory 8 (168, 146, 138, 137, and 137%). The high recovery criterion was set at 150% to include all but the highest data point.

As was done for the aqueous and solids portion of the study, 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 goal was to simplify the application of the EIS compound acceptance limits in the laboratory. The ranges of 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 acceptance limits in Table 7-12 were developed from the entire study sample data set of 147 to 192 recoveries per EIS compound, using both a non-parametric approach and professional judgement (including the decision to eliminate the EIS compound recoveries from one laboratory for a specific parameter). Also, none of the acceptance criteria were made more stringent than 40% to 130%. Professional judgement was used to prevent the worst performing laboratories or data points from overly influencing the method criteria.

The spiked sample data from the aqueous portion of the study 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. Given those observations from the aqueous portion of the study, the tissue

EIS criteria below include lower recovery limits of 5% for several EIS and upper recovery limits as high as 365% for several others. Overall, these EIS limits were attainable by the overwhelming majority of the laboratories participating in the tissue sample portion of the study. It should be noted that a minority of the laboratories were unable to achieve a 5% recovery with the D_7-NMeFOSE and D_9-NEtFOSE. Data associated with these low recoveries may be inaccurate and should be considered estimated (as indicated on the table and the published final method criteria).

The comments section of the table explains all of the cases where the criteria were stricter than the non-parametric 1 or 99th percentile of the data rounded to the nearest 5%.

Table 7-12. EIS Compound Acceptance Limits Applicable to Tissue Sample Types

EIS Compound	Lower Limit (%)	Upper Limit (%)	Notes
$^{13}C_4$-PFBA	5	130	
$^{13}C_5$-PFPeA	10	185	
$^{13}C_5$-PFHxA	25	170	
$^{13}C_4$-PFHpA	25	150	The highest 9 recoveries are all from Laboratory 8: 231, 228, 221, 216, 212, 182, 174, 142, and 135%. The highest recovery from any other laboratory is 127%. If the data from Laboratory 8 were not used, the p1 value would be 32% and the 99th percentile (p99) value would be 125%.
$^{13}C_8$-PFOA	25	150	
$^{13}C_9$-PFNA	35	185	
$^{13}C_6$-PFDA	30	150	
$^{13}C_7$-PFUnA	30	180	The ten highest recoveries are from Laboratory 8 (203, 201, 197, 186, 179, 175, 175, 166, 165, and 155). The highest recovery from any other laboratory is 128%. An upper criterion of 180% is inclusive of most of the Laboratory 8 data but does not use the highest recoveries.
$^{13}C_2$-PFDoA	35	180	The ten highest recoveries are from Laboratory 8 (213, 187, 184, 182, 173, 166, 160, 160, 159, and 151). The highest recovery from any other laboratory is 136%. An upper criterion of 180% is inclusive of most of the Laboratory 8 data but does not use the highest recoveries.
$^{13}C_2$-PFTeDA	20	160	
$^{13}C_3$-PFBS	25	190	
$^{13}C_3$-PFHxS	35	175	
$^{13}C_8$-PFOS	40	160	
$^{13}C_2$-4:2FTS	30	300	
$^{13}C_2$-6:2FTS	35	300	
$^{13}C_2$-8:2FTS	40	365	
$^{13}C_8$-PFOSA	25	180	
D_3-NMeFOSA	5	130	The five lowest recoveries were from Laboratory 9 (0.3, 0.4, 0.5, 0.5, and 5%). Without Laboratory 9, the first percentile (p1) would be 7%. A lower criterion of 5% is inclusive of most of the Laboratory 9 data but does not use the lowest recoveries.

Table 7-12. EIS Compound Acceptance Limits Applicable to Tissue Sample Types (Continued)

EIS Compound	Lower Limit (%)	Upper Limit (%)	Notes
D_5-NEtFOSA	5	130	The eleven lowest recoveries were from Laboratory 9 (0.9, 1, 1, 1, 2, 2, 3, 3, 3, 3, and 4%). Without Laboratory 9, the p1 would be 5%. A lower criterion of 5% is inclusive of half of the Laboratory 9 data but does drastically change the performance metric of the method to accommodate one laboratory that has significantly worse performance than the others for this EIS standard.
D_3-NMeFOSAA	30	250	
D_5-NEtFOSAA	30	235	
D_7-NMeFOSE *	5	160	24 of the 147 results are below 5% (about 16% of the data). The fourteen highest recoveries are all from Laboratory 3 (91, 114, 122, 131, 132, 132, 133, 142, 143, 145, 149, 149, 158, and 166%). The highest recovery from any other laboratory is 90%. *The method will state a 5% recovery as a goal but acknowledge that it may not be possible for all labs in all tissue matrices. Any result associated with an EIS recovery below 5% should be considered estimated.
D_9-NEtFOSE *	5	130	20 of the 147 results are below 5% (about 14% of the data). *The method will state a 5% recovery as a goal but acknowledge that it may not be possible for all laboratories in all tissue matrices. Any result associated with an EIS recovery below 5% should be considered estimated.
$^{13}C_3$-HFPO-DA	20	185	

Source file: 1633 Tissue EIS & NIS Specs 2024-01-08.xlsx

Notes:

* D_7-NMeFOSE and D_9-NEtFOSE can achieve 5% EIS recovery most of the time at most labs, but some laboratories struggled with this criteria. Analyte recovery associated with an EIS below 5% has been shown to be less accurate, should be considered estimated for tissue samples. The method will state a 5% recovery as a goal but acknowledge that it may not be possible for all labs in all tissue matrices. Any result associated with an EIS recovery below 5% should be considered estimated.

The NIS compound data were compiled only using the study samples, which generated 147 to 192 data points for each of the NIS compounds. The criteria were generated by applying professional judgement to establish QC acceptance limits that cutoff at the 1st and 99th percentiles of the observed data, and then rounding those values to the more inclusive multiple of 5%. Based on the percentiles shown in Table 7-13, all of the acceptance criteria were set at 50-200%, which is consistent with the approach used for the aqueous and solids portion of the study.

Table 7-13. NIS Compound Acceptance Limits Applicable to All Tissue Sample Types.

NIS Compound	n	p1	p99	Lower Limit (%)	Upper Limit (%)
$^{13}C_2$-PFDA	192	55	173	50	200
$^{13}C_2$-PFHxA	183	50	155	50	200
$^{13}C_3$-PFBA	149	65	160	50	200
$^{13}C_4$-PFOA	152	55	170	50	200
$^{13}C_4$-PFOS	172	60	150	50	200
$^{13}C_5$-PFNA	147	50	140	50	200
$^{18}O_2$-PFHxS	166	55	135	50	200

Source file: 1633 Tissue EIS & NIS Specs 2024-01-08.xlsx

8 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 Precision and Recovery
- Preparatory batch QC samples (MB, OPR, LLOPR)
- Quantitative and qualitative analyte identification criteria

The suitability of EPA Method 1633 to detect and quantify the 40 target analytes in tissue was successfully demonstrated through the analysis of spiked real-world samples of those matrix types. Overall, the recoveries (especially the mean recoveries) were excellent considering the complexity of the tissue matrix. There were roughly 4,800 matrix spike results. Roughly 90% of the MS data achieved a recovery between 40 to 140%, and roughly 99% of the MS data was between 20 to 200%. Only one matrix spike result was below 10% and only 4 were above 300%. However, the percent probability of observing results with less than 30% error for PFDoS (39.8%), 3:3FTCA (47%), 7:3FTCA (46.4%), and NEtFOSE (49.4%) spiked tissue samples across all seven laboratories (Table 6-3) indicated recovery of this analyte in tissue samples may be biased low. OPR and LLOPR data associated with tissue sample results for these analytes should be considered when determining the usability of data for these analytes in tissue samples.

Method blank results demonstrated that there was negligible bias associated with background contamination introduced during sample preparation. The IPR, OPR, and LLOPR recoveries (Tables 5-3, 8-3, and 8-5) and the EIS and NIS compound recoveries (Tables 8-7 and 8-8) associated with study samples were used to derive QC acceptance criteria (Tables 8-13, 8-14, and 8-15) for inclusion in the finalized method.

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Appendix A

PFAS MLVS Institute for Defense Analyses Report

INTEROFFICE MEMORANDUM

IDA

SCIENCE & TECHNOLOGY
DIVISION

19 January 2024

To: Dr. Kimberly Spangler, Dr. Andrea Leeson, SERDP/ESTCP

CC: Mr. Timothy Thompson, Science, Engineering and the Environment, LLC

From: Dr. Allyson Buytendyk, Institute for Defense Analyses (IDA)

Subject: IDA Statistical Analyses in the PFAS Multi-Laboratory Validation (MLV)

In 2022, SERDP/ESTCP sponsored IDA to be the independent organization to conduct the statistical analyses in the joint Department of Defense (DoD) and Environmental Protection Agency (EPA) multi-laboratory validation (MLV) study of a PFAS measurement method—EPA Draft Method 1633. IDA's role in the PFAS MLV study is to statistically summarize the overall performance of the laboratories for each test. Results from the statistical analyses inform 1) the acceptance criteria for quality control (QC) samples that the EPA will establish for the method and 2) the precision and accuracy of measurements of the PFAS analytes in each environmental matrix studied.

The study plan for the PFAS MLV closely follows the process outlined in the EPA Alternate Test Procedure (ATP) guidance[1] which, describes the tests and statistical formulas for developing QC acceptance criteria based on data generated in a study. The ATP specifies three tiers of statistical formulas based on the number of laboratories analyzing each sample. The PFAS MLV study includes ten participating laboratories and three types of datasets: initial calibration (ICAL), initial demonstration of capability (IDC), and environmental matrix samples. Previously, IDA analyzed the ICAL, aqueous[2] and solids IDC and five environmental matrices: wastewater (WW), surface water (SW), ground water (GW), soils (SS), sediments (SD), biosolids (BS) and landfill leachate (LC) datasets provided by the sponsor.

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[1] U.S. Environmental Protection Agency, Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternative Test Procedure Program, EPA 821-B-18-001. (Washington, DC: Environmental Protection Agency, February 2018). https://www.epa.gov/sites/default/files/2018-03/documents/chemical-atp-protocol_feb-2018.pdf.

[2] The results of the previous analysis of the aqueous datasets are documented in A. Buytendyk, K. Fisher, T. Pleasant, J. Shah, J. Silk, *Statistical Methods in the Multi-Laboratory Validation of a PFAS Measurement Method*. Alexandria: Institute for Defense Analyses, July 2023. IDA Product 3000051

IDA then analyzed the sponsor provided tissue IDC and environmental matrix PFAS MLV datasets using the same statistical methods outlined in the MLV study plan/EPA's ATP at Tier 3[3] for the aqueous dataset. This memo outlines the formulas IDA used in the statistical analyses and also documents the version of the solids datasets that correspond to the tables and figures IDA generated for the PFAS MLV study.[4]

STATISTICAL FORMULAS

IDC DATASET

Method Detection Limit (MDL)

MDL for Spiked Samples for a Lab

The equation for the MDL for spiked samples for a laboratory is represented as:

Equation 1: MDL for Spiked Samples for a Lab ($MDL_{s,lab}$)[5]

$$MDL_{s,j} = S_{s,j} \cdot t_{(n-1,1-\propto=0.99)};$$

where $S_{s,j}$=sample standard deviation of spiked sample measured concentrations for lab j, $t_{(n-1,1-\propto=0.99)}$ = student' s t-value for the one tailed test at the 99% confidence level with n-1 degrees of freedom.

MDL for Blank Samples for a Lab[6]

- If none of the blank samples give a numerical result, the MDL for the blank samples for a laboratory does not apply.
- If some (but not all) of the blank samples give a numerical result, the MDL for the blank samples for a laboratory is the maximum value.
- If all of the blank samples give a numerical result, the MDL for the blank samples for a laboratory is represented as:

Equation 2: MDL for Blank Samples for a Lab ($MDL_{b,lab}$)[7]

$$MDL_{b,j} = \bar{X}_j + S_{b,j} \cdot t_{(n-1,1-\propto=0.99)};$$

where $\bar{X}_j$ = mean measured concentration of the blank samples for lab j, $S_{b,j}$= sample standard deviation, of the blank samples measured concentration for lab j, $t_{(n-1,1-\propto=0.99)}$ = student's t-value for the one tailed test at the 99% confidence level with n-1 degrees of freedom.

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[3] QC acceptance criteria at Tier 3 requires a minimum of nine laboratories. EPA, *Protocol for Review and Validation of New Methods,* G-22.

[4] IDA performs calculations on the dataset using coded scripts in Python version 3.7.8, rounds statistical values based on the number of significant figures reported in the dataset and delivers the outputs as CSV files to the sponsor.

[5] 40 CFR Part 136, Appendix B; EPA, *Protocol for Review and Validation of New Methods,* G-9.

[6] 40 CFR Part 136, Appendix B; EPA, *Protocol for Review and Validation of New Methods,* G-9.

[7] 40 CFR Part 136, Appendix B; EPA, *Protocol for Review and Validation of New Methods,* G-9.

Lab MDL

The equation for the MDL for a laboratory is represented as:

Equation 3: MDL for a Lab (MDL_{lab})[8]

$$MDL_j = \max\{MDL_{s,j}, MDL_{b,j}\};$$

where $MDL_{s,j}$ = the MDL for the spiked samples for lab j, $MDL_{b,j}$ = the MDL for the blank samples for lab j.

Pooled MDL

The equation for MDL that is pooled using individual lab MDL values is represented as:

Equation 4: Pooled MDL (MDL_{pooled})[9]

$$MDL_{pooled} = \sqrt{\sum_{j=1}^{m} \frac{n_j}{N} \left(\frac{MDL_j}{t_{(n_j, 1-\alpha=0.99)}} \right)^2} \; t_{(N, 1-\alpha=0.99)};$$

where m = number of labs, MDL_j = method detection limit for the *jth* lab, n_j = number of replicates for the *jth* lab, N = total number of replicates, $\boldsymbol{t_{(n,1-\alpha=0.99)}}$ = student's t-value for the one tailed test at the 99% confidence level with n degrees of freedom.

Limit of Quantitation Verification (LOQVER)

The equation for percent bias of laboratory measurements near the limit of quantitation (LOQ) is represented as:

Equation 5: LOQ Percent Bias[10]

$$LOQ_{bias,j} = \frac{\text{spike concentration} - \bar{X}_j}{\text{spike concentration}} \cdot 100;$$

where $\bar{X}_j$ = mean of the measured sample concentrations for lab j.

Initial Precision and Recovery (IPR)

The equation for the between laboratory standard deviation is represented as:

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[8] Code of Federal Regulations (CFR), Title 40, Part 136, Appendix B.

[9] EPA, *Protocol for Review and Validation of New Methods,* G-22.

[10] Department of Defense, Department of Energy (DoD, DOE), *DoD Quality Systems Manual Version 5.4*, Module 4, Section 1.5.2 (Washington, DC: DoD, DOE, 2021), 77–78, https://www.denix.osd.mil/edqw/denix-files/sites/43/2021/10/QSM-Version-5.4-FINAL.pdf.

Equation 6: Between Lab Standard Deviation (s_b)[11]

$$s_b = \sqrt{\frac{\sum_{j=1}^{m}(\overline{X_j}-\overline{X})^2}{\mathrm{m}-1}};$$

where m = the number of labs, $\overline{X}$ = overall mean percent recovery, $\overline{X_j}$ = the mean percent recovery for the *jth* lab.

The equation for the within-laboratory standard deviation is represented as:

Equation 7: Within Lab Standard Deviation (s_w)[12]

$$s_w = \sqrt{\frac{\sum_{j=1}^{m}(s_j)^2}{\mathrm{m}}};$$

where m = the number of labs, s_j = the variance of the percent recovery values for the *jth* lab.

The equation for the combined standard deviation for IPR results in the study is represented as:

Equation 8: IPR Combined Standard Deviation (s_{IPR})[13]

$$s_{IPR} = \sqrt{\left(1+\frac{1}{m}\right)s_b^2 + \left(\frac{1}{4}-\frac{1}{n}\right)s_w^2};$$

where m = the number of labs, n = the number of data points per lab, s_b = the between lab standard deviation, s_w = the within lab standard deviation.

The equation for the relative standard deviation (RSD) across all laboratories is represented as:

Equation 9: RSD[14]

$$RSD = \frac{s_w}{\bar{X}} \cdot 100;$$

where s_w = the within lab standard deviation, $\bar{X}$= mean percent recovery across all labs.

ENVIRONMENTAL MATRIX DATASET

Ongoing Precision and Recovery (OPR) & Low-Level Ongoing Precision and Recovery (LLOPR)

The equation for the combined standard deviation for the OPR and LLOPR results in the study is represented as:

[11] EPA, *Protocol for Review and Validation of New Methods,* G-25.

[12] EPA, *Protocol for Review and Validation of New Methods,* G-25.

[13] EPA, *Protocol for Review and Validation of New Methods,* G-25.

[14] EPA, *Protocol for Review and Validation of New Methods,* G-26.

Equation 10: OPR Combined Standard Deviation (s_{OPR})[15]

$$s_{OPR} = \sqrt{\left(1 + \frac{1}{m}\right) s_b^2 + \left(1 - \frac{1}{n}\right) s_w^2} \; ;$$

where m = the number of labs, n = the number of data points per lab, s_b = the between-lab standard deviation, s_w = the within-lab standard deviation.

Equation 9 provides the formula for the RSD for the OPR test. The calculations for the LLOPR test follow those for the OPR using Equations 6, 7, 9 and 10.

Matrix Spike Recovery

The calculations for the matrix spike test include those in Equations 6 and 7 to determine s_b and s_w as well as Equation 9 to find the RSD for the matrix test.

[15] EPA, *Protocol for Review and Validation of New Methods,* G-26.

DATASETS & IDA GENERATED PRODUCTS FILE NAMES

IDA Generated Product	
Tables	**Figures**
Tissue Initial Demonstration of Capabilities Dataset RT_DBexport_V0_20231204.xlsx	
RT_IPR_results_V0_231215_004710.csv RT_LOQVER_results_V0_231215_004710.csv RT_MDL_results_V0_231215_004710.csv	RT_IPR_Boxplot_V0_231215_004710.png RT_IPR_Horwitz_V0_231215_004710.png RT_LOQVER_Boxplot_V0_231215_004710.png RT_MDL_Plot_V0_231215_004710.png
Tissue Matrix Dataset TS_DBexport_V0_20231213.xlsx	
TS_LLOPR_results_V0_231214_135747.csv TS_OPR_results_V0_231214_135747.csv TS_EIS_results_V0_231214_135747.csv TS_Matrix_sample_results_V0_231214_135747.csv TS_Matrix_compiled_results_V0_231214_135747.csv TS_MB_results_V0_231214_135747.csv TS_NIS_results_V0_231214_135747.csv	TS_LLOPR_Boxplot_V0_231214_135747.png TS_LLOPR_Horwitz_V0_231214_135747.png TS_OPR_Boxplot_V0_231214_135747.png TS_OPR_Horwitz_V0_231214_135747.png TS_HighSpike_Boxplot_V0_231214_135747.png TS_LowSpike_Boxplot_V0_231214_135747.png TS_LowHighCombinedSpike_Boxplot_V0_231214_135747.png TS_EIS_Boxplot_V0_231214_135747.png TS_NIS_Boxplot_V0_231214_135747.png

Appendix B

Tissue Supporting Tables

Table B-1. Range of Target Analytes in Unspiked Tissue Samples (µg/kg).

Analyte	Number of Labs	TSAB1		TSAC1		TSAD1	
		Min	Max	Min	Max	Min	Max
PFBA	7[a]	0.2 U	1.66 J	0.2 U	0.666 U	0.2 U	0.667 U
PFPeA	7[b]	0.1 U	0.645 J	0.1 U	0.326 J	0.1 U	1.03
PFHxA	6	0.119 U	0.268 J	0.119 U	1.33 I	0.124 U	5.21 I
PFHpA	7	0.05 U	0.219 J	0.05 U	0.107 J	0.05 U	0.175 U
PFOA	7	0.05 U	0.286 J	0.05 U	0.215 U	0.05 U	0.072 J
PFNA	6	0.0725 U	0.261 J	0.0725 U	0.254 U	0.0725 U	0.254 U
PFDA	7	0.05 U	0.452 J	0.05 U	0.357 U	0.05 U	0.357 U
PFUnA	6	0.14 U	0.35 J	0.135 U	0.28 U	0.135 U	0.28 U
PFDoA	7	0.05 U	0.226 J	0.05 U	0.245 U	0.05 U	0.245 U
PFTrDA	6	0.052 U	0.38 J	0.052 U	0.37 U	0.052 U	0.37 U
PFTeDA	7	0.0491 UJ	0.278 J	0.0491 UJ	0.255 U	0.0491 UJ	0.255 U
PFBS	7	0.05 U	0.208 J	0.05 U	0.177 U	0.05 U	0.177 U
PFPeS	7	0.044 U	0.159 J	0.044 U	0.157 U	0.044 U	0.157 U
PFHxS	7	0.05 U	0.0981 JI	0.05 U	0.117 J	0.05 U	0.109 J
PFHpS	6	0.032 U	0.305 J	0.132 U	0.048 J	0.132 U	0.115 J
PFOS	6	0.05 U	0.287 JI	0.05 UJ	0.418 U	0.05 U	0.418 U
PFNS	7	0.05 U	0.231 J	0.05 U	0.264 U	0.05 U	0.264 U
PFDS	7	0.037 U	0.263 J	0.037 U	0.212 U	0.05 U	1.58
PFDoS	7	0.04 U	0.176 J	0.04 U	0.425 U	0.04 U	0.425 UJ
4:2FTS	7	0.2 U	5.76 UD	0.2 U	5.76 UD	0.2 U	5.76 UD
6:2FTS	6	0.251 U	1.05 J	0.251 U	13.9 UD	0.251 U	13.9 UD
8:2FTS	7	0.199 U	0.66 J	0.199 U	0.767 U	0.199 U	7.32 UD
PFOSA	7	0.05 U	0.259 J	0.05 U	0.167 U	0.05 U	0.167 U
NMeFOSA	6[a]	0.0459 U	0.253 J	0.0459 U	0.272 J	0.0459 U	0.383 U
NEtFOSA	6[c]	0.113 U	0.312 J	0.113 U	0.664	0.113 U	0.397 U
NMeFOSAA	7	0.05 U	0.237 J	0.05 U	0.225 U	0.05 U	0.225 U
NEtFOSAA	7	0.05 UJ	0.253 J	0.05 U	1.78 UD	0.05 U	1.78 UD
NMeFOSE	7[d]	0.5 U	1.67 U	0.5 U	0.693 U	0.5 U	1.67 UJ
NEtFOSE	5[e]	0.5 UJ	4.88 U	0.5 UJ	4.88 U	0.5 UJ	4.88 U
PFMPA	7[b]	0.1 U	0.363 J	0.1 U	0.656 U	0.1 U	0.656 U
PFMBA	7[b]	0.083 U	0.65 J	0.083 U	0.269 U	0.083 U	0.333 U
NFDHA	7	0.1 UJ	0.872 JI	0.1 UJ	0.407 U	0.1 UJ	0.191 J
HFPO-DA	7	0.2 U	0.783 J	0.2 U	0.667 U	0.2 U	0.667 U
ADONA	6	0.1 U	0.652 J	0.1 U	0.63 U	0.1 U	0.63 U
PFEESA	7	0.0971 U	0.704 J	0.0971 U	0.297 U	0.0971 U	0.297 U
9Cl-PF3ONS	7	0.2 U	0.697 J	0.2 U	0.703 U	0.2 U	0.703 U
11Cl-PF3OUdS	7	0.2 U	0.651 J	0.2 U	0.746 U	0.2 U	0.746 U
3:3FTCA	7b	0.241 U	1.48 J	0.241 U	2.22 U	0.241 U	2.22 UJ
5:3FTCA	7	1.25 U	5.53 J	0.333 U	4.6 U	0.333 U	4.6 U
7:3FTCA	7	1.25 UJ	6.79 J	1.25 UJ	3.33 U	1.25 UJ	3.33 U

Version: Summary_tables_Exa_Tissue_App_01182024.xlsx

Notes:

[a] 5 labs for TSAC1

[b] 6 labs for TSAC1

[c] 5 labs for TSAB1, TSAC1

[d] 2 labs for TSAC1, 5 in TSAB1

[e] 3 labs for TSAC1

Table B-2. Summary of Tissue Spike Percent Recoveries in Low Spike Samples for each Laboratory.

Analyte	Lab 1 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 6 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	8	51.7	96.2	77.8	9	84.8	106	94.3	9	88	101	94.4	6	70.8	96.3	88.3
PFPeA	8	39.2	66.8	57.2	9	86	120.8	97.4	9	55	97.6	78.0	9	76	135.8	103.5
PFHxA	9	51.5	89	73.2	0	--	--	--	9	86.5	101	94.4	9	80.5	172	115.9
PFHpA	9	34.9	90	63.7	9	71	103	85.9	9	86.5	95	90.1	9	76	98.5	88.4
PFOA	9	40.4	226.5	113.9	9	65	114	84.3	9	86.5	117	101.9	9	79.5	92.5	87.1
PFNA	9	26.6	83.5	55.6	0	--	--	--	9	86	99	93.4	9	76	115	95.2
PFDA	9	22.8	185	97.1	9	95	138	105.3	9	87	101	94.4	9	70.5	110.5	86.8
PFUnA	9	23.5	102	65.7	0	--	--	--	9	84	109	96.1	9	78.5	105	89.2
PFDoA	9	24.2	97.5	58.8	9	81	131	96.7	9	95.5	108.5	101.9	9	68	102	82.8
PFTrDA	9	24.6	114	62.3	0	--	--	--	9	72.5	108	91.7	9	53	68	60.6
PFTeDA	9	28.1	89.5	59.5	9	39	70	54.9	9	88	105.5	97.1	9	64	98.5	85.7
PFBS	9	46	108.3	70.8	9	85.3	124.5	101.0	9	77.9	107.4	97.5	9	54.9	82.8	73.4
PFPeS	9	30.6	104.6	63.3	9	67	133	90.5	9	86.3	105.1	96.1	9	33	91.4	61.9
PFHxS	9	21.4	90	55.3	9	65.7	129.4	89.3	9	85.1	104.5	95.7	9	42.8	90.5	61.9
PFHpS	9	31	104.5	69.2	0	--	--	--	9	83.5	115.5	98.1	9	73	113.5	88.4
PFOS	9	22.1	106.8	66.6	9	93.7	126.8	105.5	9	80.1	105.4	93.6	9	85.9	108.3	94.0
PFNS	9	22.6	93.1	59.8	9	65.3	95	79.6	9	84.2	95.5	89.2	9	64.4	92.6	81.8
PFDS	9	18.2	79.7	51.3	9	92.1	142.6	107.4	9	77.7	94.6	87.3	9	44.5	108.4	75.3
PFDoS	6	22.4	68.1	43.7	9	65.7	131.4	93.0	9	78.9	93.6	84.6	9	21.9	78.4	38.0
4:2FTS	9	44.1	95	68.9	9	80.5	108.7	93.5	9	80.3	99.8	90.2	9	68.8	95	87.8
6:2FTS	9	33.7	99.4	65.2	0	--	--	--	9	75.8	105.3	91.4	9	49.1	98.2	77.1
8:2FTS	9	23.6	104.6	69.1	9	109	141.9	119.4	9	80.8	100.4	89.3	9	73.3	96.4	87.9
PFOSA	9	33.8	101.5	64.0	9	117	182	134.2	9	80	100.5	91.9	9	79.5	97	87.7
NMeFOSA	7	28	77.9	47.5	9	122	280	159.1	9	87	122.5	103.6	9	85	142.5	107.8
NEtFOSA	7	29	67.5	52.0	0	--	--	--	9	79.5	105.5	93.7	9	77	144.5	99.9
NMeFOSAA	9	39.3	125	75.0	9	66	106	88.1	9	89.5	107.5	101.1	9	66	96	83.4
NEtFOSAA	9	27.6	101.5	62.7	9	73	110	83.7	9	84.5	101	94.2	9	78.5	99.5	92.1
NMeFOSE	6	23.4	56	41.6	9	126	151	135.6	6	86.6	141	115.1	6	78.9	92	86.9
NEtFOSE	5	32.1	66.7	47.4	9	122	180	148.4	9	101	111	106.4	3	113	387	205.0
PFMPA	9	16.2	80.8	52.9	9	53.6	121.2	88.0	9	50.4	95.6	75.9	8	23	77.8	42.5
PFMBA	9	47.2	83	63.4	9	98	137.6	108.5	9	54.4	99.6	79.6	9	74	208	113.6
NFDHA	9	52.8	118.4	77.3	9	113.6	159.2	137.4	9	68.8	86.2	77.3	9	78.4	132.2	97.4
HFPO-DA	9	55.8	109.4	77.0	9	98.4	132	117.8	9	83.2	103.6	92.4	9	77.4	102.2	92.9
ADONA	9	40.6	142.4	81.2	0	--	--	--	9	94	133.2	115.7	9	95.2	108.6	101.9
PFEESA	9	54.4	124	81.3	9	88.8	106.8	98.4	9	83	94	88.2	9	84.8	118.2	96.9
9Cl-PF3ONS	9	30.6	186.5	86.7	9	91.7	129.8	106.4	9	97.4	121.4	109.6	9	85.3	120	100.1
11Cl-PF3OUdS	9	25.8	140.6	71.6	9	87.6	112.4	97.9	9	90.4	114.4	99.3	9	61.6	105.4	82.4
3:3FTCA	2	49	55	52.0	9	42.8	101.6	71.3	9	60.6	98.4	80.7	9	11.6	91.6	61.3
5:3FTCA	9	26.7	90.5	56.2	9	78	113	98.6	9	46.1	113	82.3	9	68	127.5	90.8
7:3FTCA	9	38.6	138.5	87.3	9	147.5	298	211.3	9	84	101	89.9	9	82	137.5	107.9

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Table B-2. Summary of Tissue Spike Percent Recoveries in Low Spike Samples for each Laboratory (continued).

Analyte	Lab 8 spike % recovery				Lab 9 spike % recovery				Lab 10 spike % recovery				All Labs spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	6	106.4	131	121.6	9	93.3	110	100.8	9	101	116	107.4	56	51.7	131	97.4
PFPeA	7	96.1	128.4	112.0	9	92.2	157	110.6	9	76	103.6	87.2	60	39.2	157	92.2
PFHxA	8	99.1	148	127.1	9	88.8	102.5	98.2	6	97.5	137	118.1	50	51.5	172	103.2
PFHpA	9	76.6	117	93.3	9	91.5	99.5	95.0	9	87.5	106	97.2	63	34.9	117	87.7
PFOA	9	110.2	150	134.3	9	86.9	98.5	93.9	9	86	113	97.9	63	40.4	226.5	101.9
PFNA	9	70	107	86.0	9	93.5	106.5	100.7	9	102.5	120.5	108.9	54	26.6	120.5	90.0
PFDA	9	91.9	136	117.7	9	93.5	110.5	101.8	9	90.5	116.5	104.4	63	22.8	185	101.1
PFUnA	9	68	102.5	83.9	9	97	111.5	106.6	9	101.5	123	109.4	54	23.5	123	91.8
PFDoA	9	72.2	101	86.2	9	96.5	108.5	102.3	9	103	114.5	108.8	63	24.2	131	91.1
PFTrDA	9	118	195	151.4	9	60.5	98.5	78.6	9	68	98.5	83.0	54	24.6	195	87.9
PFTeDA	9	96.1	139.5	117.4	9	96	104.5	100.4	9	102	116.5	107.9	63	28.1	139.5	89.0
PFBS	8	84.4	110.3	96.4	9	92.2	105.9	98.6	9	81.9	103.4	91.7	62	46	124.5	89.8
PFPeS	9	29	114.7	80.4	9	86.8	96.4	92.9	9	104.6	126.9	114.2	63	29	133	85.6
PFHxS	9	59.2	111.9	85.5	9	80.6	85.1	82.1	9	83.6	110	97.3	63	21.4	129.4	81.0
PFHpS	9	56.5	130	100.8	9	87.5	111.1	101.3	9	99.5	118	110.2	54	31	130	94.7
PFOS	0	--	--	--	9	88.3	108.3	101.3	9	92.6	114.6	104.0	54	22.1	126.8	94.1
PFNS	9	80.1	203	119.5	9	82.7	107.9	92.3	9	69.3	93.6	82.3	63	22.6	203	86.4
PFDS	9	82	205.9	124.4	9	74.8	223.3	103.7	9	64.9	89.1	75.9	63	18.2	223.3	89.3
PFDoS	9	48.2	176.5	100.7	9	37.5	88.2	58.6	9	50	63.2	57.8	60	21.9	176.5	69.3
4:2FTS	8	100	120.9	111.2	9	94.6	106.8	102.4	0	--	--	--	53	44.1	120.9	92.0
6:2FTS	9	107.3	156.2	137.0	9	98.8	112.3	105.3	0	--	--	--	45	33.7	156.2	95.2
8:2FTS	9	105.6	144.1	126.6	9	102	113.8	107.9	2	128.7	173.7	151.2	56	23.6	173.7	101.9
PFOSA	9	86.6	120	105.9	9	90.5	104	96.8	9	92	103.5	98.1	63	33.8	182	96.9
NMeFOSA	6	90.4	125	110.1	0	--	--	--	9	93.5	107.5	101.4	49	28	280	106.9
NEtFOSA	8	87.4	145.5	114.2	3	103	111.5	106.3	9	93.5	114	104.1	45	29	145.5	95.0
NMeFOSAA	9	86.1	133	112.5	9	92.5	110	101.6	9	96.5	110	103.1	63	39.3	133	95.0
NEtFOSAA	9	87.8	141.5	116.9	9	90	108	97.6	8	90	130	108.2	62	27.6	141.5	93.4
NMeFOSE	5	125	136	130.8	4	60.1	107	75.2	9	100	105	102.0	45	23.4	151	101.2
NEtFOSE	6	90.6	103	96.6	0	--	--	--	9	130	221	158.8	41	32.1	387	125.7
PFMPA	7	13.5	128.8	92.5	9	40	111.2	76.7	9	73.6	104.8	89.2	60	13.5	128.8	73.9
PFMBA	7	108	154.4	124.6	9	96.8	132.2	112.0	9	84.2	109.4	94.6	61	47.2	208	98.7
NFDHA	8	111.4	129.4	120.8	9	68.8	93.6	78.1	9	69.4	94.6	84.9	62	52.8	159.2	95.8
HFPO-DA	8	135.1	193.8	158.2	9	90	112.8	97.0	9	83	112	101.5	62	55.8	193.8	104.4
ADONA	8	102	218	136.7	9	85.2	106.2	96.2	9	103.6	124.2	114.9	53	40.6	218	107.2
PFEESA	8	108.1	183.2	136.7	9	102.6	112.2	106.5	9	95.4	120.8	111.4	62	54.4	183.2	102.2
9Cl-PF3ONS	8	102.2	1061.5	256.9	9	97.8	133.3	112.4	9	88.9	114.5	105.4	62	30.6	1061.5	123.2
11Cl-PF3OUdS	8	100.4	1166	264.6	9	83.4	119.8	99.6	9	71.6	85.6	79.7	62	25.8	1166	111.2
3:3FTCA	7	16.3	52.8	41.3	9	116.8	190	145.1	9	75.6	113.2	96.2	54	11.6	190	83.0
5:3FTCA	8	95.5	172.5	131.4	9	70.5	167	123.8	9	75	152.5	107.3	62	26.7	172.5	98.1
7:3FTCA	8	153	480	240.5	9	124.5	191.5	163.7	9	116	135.5	124.2	62	38.6	480	144.9

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Table B-3. Summary of Tissue Spike Percent Recoveries in High Spike Samples for each Laboratory.

Analyte	Lab 1 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 6 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	9	53.3	99.6	74.4	9	90	98	94.2	9	89.3	99.5	95.4	7	82.3	91.5	88.8
PFPeA	9	54.6	90.7	65.7	9	87.2	97.8	93.5	9	55.1	98.9	76.2	9	78.4	100	85.6
PFHxA	9	46.4	98.6	67.6	0	--	--	--	9	83.2	94.6	89.4	9	73.2	97.6	87.2
PFHpA	9	43.8	101	67.6	9	69	91.2	78.4	9	87.4	99.8	94.4	9	78.8	102.8	89.7
PFOA	9	40.4	105.2	67.5	9	62.4	93.8	74.6	9	86.6	100.2	93.0	9	84.6	97.6	89.5
PFNA	9	35.2	79.8	51.9	0	--	--	--	9	85.2	99	93.9	9	83.6	101.2	95.4
PFDA	9	40.8	150.4	85.0	9	84	96.4	89.4	9	84.4	99.2	91.8	9	81.4	97.8	90.0
PFUnA	9	35.8	88.8	58.7	0	--	--	--	9	89.5	99.4	93.9	9	82.4	103.8	94.9
PFDoA	9	31.8	89.2	51.5	9	80	93.4	88.3	9	81.6	95.8	90.8	9	77	90.4	85.3
PFTrDA	9	27.4	106.8	54.8	0	--	--	--	9	63	96.2	82.8	9	53	73.4	60.4
PFTeDA	9	31	72.2	49.8	9	35.2	66.2	48.4	9	79.2	95	90.4	9	75	90.6	82.9
PFBS	9	49.3	95.4	67.8	9	93.8	101.4	98.1	9	94.4	106.8	99.8	9	64.2	78.9	73.3
PFPeS	9	37.8	80.7	56.9	9	65.5	96.2	86.2	9	91.6	103.6	96.1	9	62	74.9	68.5
PFHxS	9	31.5	82.7	56.5	9	65.3	91	82.9	9	89.8	100.6	95.2	9	59.8	75.5	66.3
PFHpS	9	35.1	103	67.2	0	--	--	--	9	83.5	109.9	98.4	9	67.9	81.7	75.6
PFOS	9	32.2	102.6	63.2	9	84.9	91	88.0	9	83.1	100.8	92.2	9	74.9	87.1	80.8
PFNS	9	29.6	95.4	58.1	9	69.2	79.8	76.6	9	83.4	103.8	91.5	9	61.8	89	75.6
PFDS	9	28.5	76.4	48.3	9	94.4	108.4	99.9	9	79.8	100.4	89.1	9	51.1	93.8	69.3
PFDoS	9	10.8	59.1	36.5	9	78.2	96	86.0	9	82.3	93.1	87.4	9	24.6	69.6	37.1
4:2FTS	9	43.5	87.1	61.9	9	81.1	92.2	86.8	9	80.9	96.4	89.7	9	64.2	101.6	87.7
6:2FTS	9	37	102.8	65.0	0	--	--	--	9	59.2	102	87.3	9	46.1	80.2	67.9
8:2FTS	9	38.1	117	67.6	9	109	129.7	117.9	9	84.8	94.8	90.0	9	79.2	92.2	86.6
PFOSA	9	36	93.2	59.4	9	119.4	130.2	125.2	9	83.4	98.8	93.5	9	83	92	88.4
NMeFOSA	9	31.8	89.1	56.3	9	126.8	212	162.2	9	94.4	121	103.5	9	83.6	129.4	100.1
NEtFOSA	8	34.4	81.5	51.7	0	--	--	--	9	91	104.6	97.7	9	65.8	84.8	76.0
NMeFOSAA	9	46.6	110.5	71.8	9	79.5	108.5	88.1	9	94.5	104.5	101.1	9	73.5	99	84.4
NEtFOSAA	9	33.2	90	56.0	9	68.8	85.2	77.3	9	86.4	107.2	97.3	9	84.8	107.6	90.0
NMeFOSE	6	30.6	58.8	38.7	9	110	150	126.6	6	94	151	122.7	6	84	88.6	86.0
NEtFOSE	6	36.4	49.1	42.4	9	128	154	139.4	9	101	120	108.6	2	86.3	89.4	87.9
PFMPA	9	35.2	77.7	61.6	9	79.9	98.9	93.4	9	52	95.9	73.7	9	17.4	77	45.4
PFMBA	9	48.9	67.3	58.4	9	99.5	111	105.3	9	54.6	101	77.2	9	75.9	187	100.9
NFDHA	9	52	106	77.3	9	113.2	160.4	141.8	9	67.6	86.8	77.3	9	87.6	120	101.6
HFPO-DA	9	51.6	107	75.9	9	101.6	123	112.4	9	84	97.4	89.6	9	69.4	93.6	83.3
ADONA	9	45.2	91.6	65.5	0	--	--	--	9	94.8	132	112.2	9	86.4	111.6	95.8
PFEESA	9	54.4	104.8	76.0	9	90.8	110	101.7	9	88	96.4	91.1	9	79.2	103.6	95.6
9Cl-PF3ONS	9	35.9	130.2	75.5	9	78.6	104.8	92.4	9	98.4	131.3	108.5	9	78.2	103	92.6
11Cl-PF3OUdS	9	32.2	108.4	60.4	9	77	108.6	90.8	9	87.4	123	101.1	9	55	87.6	76.7
3:3FTCA	9	13	52.6	34.5	9	54.8	92	76.8	9	57.6	99.8	78.2	9	17.6	82	61.6
5:3FTCA	9	25	87.5	52.7	9	81	112	99.1	9	48.5	107	81.3	9	61.5	119.5	86.4
7:3FTCA	9	38.7	135	76.2	9	131.5	205.5	177.7	9	86	103.5	93.1	9	94	127	106.3

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Table B-3. Summary of Tissue Spike Percent Recoveries in High Spike Samples for each Laboratory (continued)

Analyte	Lab 8 spike % recovery				Lab 9 spike % recovery				Lab 10 spike % recovery				All Labs spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	8	111.3	133	119.5	9	99.5	110	106.1	9	98.1	114	106.2	60	53.3	133	97.7
PFPeA	9	108.4	135	119.6	9	96.8	109	104.2	9	78.4	89.2	84.7	63	54.6	135	89.9
PFHxA	9	100.9	135.4	120.8	9	97.4	106.2	103.3	9	109.4	137	120.5	54	46.4	137	98.1
PFHpA	9	89.2	113.6	98.9	9	95	108.4	102.6	9	89.4	107.8	99.8	63	43.8	113.6	90.2
PFOA	9	110.6	134.6	121.7	9	93.4	104.6	98.8	9	92	113	100.3	63	40.4	134.6	92.2
PFNA	9	74.2	87.4	80.3	9	99.6	110	105.7	9	89.4	120.2	105.3	54	35.2	120.2	88.7
PFDA	9	103.7	143.8	119.2	9	99.3	112	104.9	9	83.8	108.6	93.3	63	40.8	150.4	96.2
PFUnA	9	73.3	93.4	82.1	9	103.4	114	108.0	9	93	107.8	100.1	54	35.8	114	89.6
PFDoA	9	72.5	92	81.1	9	97.4	108.4	103.8	9	95.8	109.8	103.3	63	31.8	109.8	86.3
PFTrDA	9	88	172.4	134.1	9	72.6	100	83.8	9	64	87.6	79.3	54	27.4	172.4	82.5
PFTeDA	9	97.6	129.8	113.1	9	96.8	111.6	104.9	9	96.2	108	103.8	63	31	129.8	84.7
PFBS	9	93.2	135.8	108.9	9	98.2	108.9	104.1	9	77.9	106	92.7	63	49.3	135.8	92.1
PFPeS	9	70.2	114.5	93.3	9	89.4	100.4	95.9	9	93.8	126.7	108.1	63	37.8	126.7	86.4
PFHxS	9	68.3	105.6	85.9	9	79.1	91.2	85.7	9	83.7	111.4	98.4	63	31.5	111.4	81.6
PFHpS	9	96.8	117.7	108.3	9	95.8	111.7	104.1	9	91.5	116.3	106.8	54	35.1	117.7	93.4
PFOS	0	--	--	--	9	89	100.4	95.6	9	82.4	109	95.7	54	32.2	109	85.9
PFNS	9	94.1	121.4	104.6	9	87.8	104.2	96.2	9	64.4	95.6	81.3	63	29.6	121.4	83.4
PFDS	9	84.4	125	99.4	9	83.1	105	92.1	9	59.7	83	72.6	63	28.5	125	81.5
PFDoS	9	33.9	117.3	71.4	9	50.8	96.6	66.7	9	44	66.7	57.2	63	10.8	117.3	63.2
4:2FTS	9	101.8	124.5	110.8	9	98	109.5	104.7	9	73.8	143.7	101.3	63	43.5	143.7	91.8
6:2FTS	9	121.9	159	134.5	9	101.2	123.2	108.9	9	78.8	193.1	120.3	54	37	193.1	97.3
8:2FTS	9	115.1	163.5	136.3	9	104.6	124.2	111.6	9	88.2	139.7	108.8	63	38.1	163.5	102.7
PFOSA	9	97.5	125	112.6	9	94.2	103	98.6	9	92	102.2	97.2	63	36	130.2	96.4
NMeFOSA	9	102.3	136.8	117.7	0	--	--	--	9	96.8	104.6	100.7	54	31.8	212	106.8
NEtFOSA	9	97.8	136	114.9	5	87.8	108	102.8	9	92.8	107.6	101.9	49	34.4	136	90.6
NMeFOSAA	9	105.8	129.5	117.0	9	98	109.5	103.2	9	104	127	114.4	63	46.6	129.5	97.2
NEtFOSAA	9	103	137.6	120.6	9	94.8	109.6	100.5	9	95.2	118.8	108.1	63	33.2	137.6	92.8
NMeFOSE	6	111	130	120.8	6	60.6	72.6	65.5	9	93.6	104	99.3	48	30.6	151	96.6
NEtFOSE	5	93.8	116	104.0	0	--	--	--	9	130	164	145.9	40	36.4	164	112.4
PFMPA	9	24.5	126	75.6	9	75	113	92.9	9	80.5	95.5	87.4	63	17.4	126	75.7
PFMBA	9	118	172	131.3	9	103	137	117.0	9	88.2	108	95.6	63	48.9	187	98.0
NFDHA	9	89.6	137.7	117.9	9	70.4	101.6	80.4	9	72.4	98.8	85.1	63	52	160.4	97.3
HFPO-DA	9	133.6	181.2	164.2	9	94.4	106	100.0	9	95.8	115.6	106.1	63	51.6	181.2	104.5
ADONA	9	101.3	145.4	125.4	9	90.8	114	104.9	9	102.6	124.2	113.4	54	45.2	145.4	102.9
PFEESA	9	124.4	153.2	140.9	9	112.4	120	116.8	9	102.8	118.4	108.7	63	54.4	153.2	104.4
9Cl-PF3ONS	9	116.3	178.8	149.2	9	103.2	137.7	121.1	9	93.1	120.2	108.0	63	35.9	178.8	106.7
11Cl-PF3OUdS	9	112.3	186.8	144.1	9	91.2	125.4	107.8	9	68.8	88.8	81.2	63	32.2	186.8	94.6
3:3FTCA	9	3.7	50.8	27.3	9	154.4	200	167.4	9	89.4	107.2	98.9	63	3.7	200	77.8
5:3FTCA	9	93.5	174.7	133.2	9	82.5	172	129.0	9	77.5	140	108.9	63	25	174.7	98.7
7:3FTCA	9	118.5	231	174.5	9	139.5	195	173.4	9	110.5	133.5	121.4	63	38.7	231	131.8

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-- : X-flagged results

Table B-4. Summary of EIS Compound Percent Recovery in Tissue Samples for Each Laboratory

EIS Compound	Lab 1				Lab 3				Lab 4				Lab 6			
	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean
13C4-PFBA	21	6.28	80.6	50.6	21	12	90	68.5	21	91	113	98.4	21	2.24	58.5	21.5
13C5-PFPeA	34	27.5	113	77.3	21	52	86	70.0	21	91	185	128.1	21	13.2	112	79.1
13C5-PFHxA	41	25.3	90.9	72.0	21	63	90	73.3	21	91.9	117	102.5	21	59.4	117	91.4
13C4-PFHpA	21	25.4	90.1	72.0	21	66	118	93.9	21	94.1	117	103.9	21	81	127	97.3
13C8-PFOA	26	25.3	95.9	74.5	21	72	96	83.7	21	92.3	120	101.7	21	62.4	106	88.2
13C9-PFNA	21	23.5	103	76.4	21	76	104	87.5	21	90.3	120	103.1	21	79.2	103	90.5
13C6-PFDA	21	22.3	92.1	69.6	21	71	96	82.8	21	78.1	115	98.1	21	74	114	91.9
13C7-PFUnA	27	25.6	100	69.8	21	66	98	81.0	21	68	114	91.5	21	63.6	128	82.8
13C2-PFDoA	40	27.3	102	63.0	21	77	109	91.3	21	68.1	136	94.8	21	57.2	134	81.6
13C2-PFTeDA	23	11.5	92.3	52.5	21	42	176	111.9	21	74.2	153	101.2	21	23.9	81.6	40.1
13C3-PFBS	21	21.7	85.6	67.2	21	68	95	81.8	21	80.8	103	93.7	21	51.7	125	91.8
13C3-PFHxS	34	31.1	110	88.4	21	74	109	92.3	21	92.6	112	101.0	21	92.2	185	121.6
13C8-PFOS	21	24.3	94.4	74.8	21	72	94	82.4	21	92.1	126	106.2	21	88.3	120	101.5
13C2-4:2FTS	21	26.4	120	82.1	21	131	258	189.8	21	118	226	158.3	21	96.5	268	169.4
13C2-6:2FTS	21	35.4	200	134.0	21	85	239	156.2	21	101	157	120.1	21	156	244	198.6
13C2-8:2FTS	21	36.9	364	227.7	21	151	345	241.3	21	210	485	325.1	21	122	327	204.7
13C8-PFOSA	22	22.3	97.5	54.4	21	107	137	124.8	21	91.7	145	117.6	21	71	105	91.2
D3-NMeFOSA	21	6.97	58.1	30.8	21	25	71	45.4	21	20.7	76.6	52.6	21	15.8	63.8	40.7
D5-NEtFOSA	21	4.53	49.5	24.6	21	14	52	32.4	21	38.9	73.2	57.5	21	12.3	61.4	36.7
D3-NMeFOSAA	21	25.5	105	85.0	21	98	253	170.1	21	150	244	181.4	21	94.7	190	149.0
D5-NEtFOSAA	21	25.7	120	89.4	21	101	206	152.5	21	162	234	191.0	21	107	250	172.9
D7-NMeFOSE	21	1.18	57	25.3	21	52	166	110.2	21	3.9	66.3	30.8	21	2.26	41	25.0
D9-NEtFOSE	21	3.62	54.4	24.7	21	13	74	37.3	21	19	82.1	46.3	21	4.58	13	8.2
13C3-HFPO-DA	24	21.7	97.5	74.1	21	59	97	77.9	21	72.1	109	86.1	21	78	127	102.2

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Notes:

Does not include MB, OPR, LLOPR QC samples.

Table B-4. Summary of EIS Compound Percent Recovery in Tissue Samples for Each Laboratory (continued).

EIS Compound	Lab 8				Lab 9				Lab 10				All Labs % Recovery			
	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean
13C4-PFBA	23	1.82	118	46.2	21	12	79	55.7	21	64.9	91.8	84.1	149	1.82	118	60.5
13C5-PFPeA	30	2.3	184	103.0	21	49	87	69.2	21	63.1	108	85.6	169	2.3	185	87.5
13C5-PFHxA	30	5.8	171	99.6	21	70	85	79.8	21	58.3	96.7	77.2	176	5.8	171	84.4
13C4-PFHpA	21	15.6	231	130.3	21	69	89	79.0	21	55.2	97.5	80.4	147	15.6	231	93.8
13C8-PFOA	21	19.7	157	97.9	21	74	93	84.9	21	67.2	105	89.4	152	19.7	157	88.1
13C9-PFNA	21	45.9	194	126.0	21	72	86	80.5	21	67.8	104	85.6	147	23.5	194	92.8
13C6-PFDA	21	44.4	158	112.2	21	72	86	79.1	21	62.2	95.6	83.1	147	22.3	158	88.1
13C7-PFUnA	21	84.1	203	136.6	21	68	89	78.0	21	62.3	102	79.2	153	25.6	203	87.7
13C2-PFDoA	21	64.9	213	125.6	21	57	93	72.2	21	53.8	93.1	71.2	166	27.3	213	83.1
13C2-PFTeDA	23	36.9	164	91.8	21	29	90	52.8	21	33	65.9	49.6	151	11.5	176	71.4
13C3-PFBS	25	9.7	194	113.2	21	70	91	78.8	21	79.7	134	104.7	151	9.7	194	90.8
13C3-PFHxS	22	29.5	177	112.5	21	79	96	88.0	21	67.4	110	89.4	161	29.5	185	98.3
13C8-PFOS	30	44.9	172	116.5	21	74	85	78.6	21	63.5	99.5	88.2	156	24.3	172	94.0
13C2-4:2FTS	21	6.74	373	206.9	21	129	293	217.3	21	57.6	149	90.6	147	6.74	373	159.2
13C2-6:2FTS	21	29.7	342	211.9	21	131	287	217.4	21	67.4	298	110.0	147	29.7	342	164.0
13C2-8:2FTS	21	193	474	280.0	21	138	276	219.5	21	50.5	148	105.2	147	36.9	485	229.1
13C8-PFOSA	21	40.9	191	125.9	21	26	93	75.5	21	54.7	101	86.2	148	22.3	191	96.2
D3-NMeFOSA	21	5.26	54.6	32.8	21	0.3	22	6.6	21	30	61.3	46.9	147	0.3	76.6	36.6
D5-NEtFOSA	21	8.82	32.7	23.0	21	0.9	36	11.6	21	28.6	54.2	43.8	147	0.9	73.2	32.8
D3-NMeFOSAA	21	97.3	234	138.5	21	59	96	80.5	21	72.9	146	122.7	147	25.5	253	132.5
D5-NEtFOSAA	21	94.7	214	145.6	21	65	94	80.1	21	66.8	149	102.7	147	25.7	250	133.5
D7-NMeFOSE	21	0.51	27.4	12.2	21	1	35	13.3	21	33.1	89.5	60.4	147	0.51	166	39.6
D9-NEtFOSE	21	0.353	27.1	11.9	21	0.08	17	4.8	21	11.6	46.3	27.1	147	0.08	82.1	22.9
13C3-HFPO-DA	21	7.29	197	99.8	21	71	86	78.8	21	46.1	97.1	68.5	150	7.29	197	83.7

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Notes:
Does not include MB, OPR, LLOPR QC samples.