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

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LIST OF APPENDICES

Appendix A

**Multi-Laboratory Study
Plan and Analytical Method
Standard Operating
Procedure**

Study Plan for Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

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AFCEC = Air Force Civil Engineer Center
CalEPA DTSC = California Environmental Protection Agency Department of Toxic Substances Control
ELLET = Eurofins Lancaster Laboratories Environmental Testing, LLC
EPA OLEM = U.S. Environmental Protection Agency, Office of Land and Emergency Management
EPA ORD = U.S. Environmental Protection Agency, Office of Research and Development
EPA OW = U.S. Environmental Protection Agency, Office of Water
ERA = ERA, A Waters Company
ETA = Eurofins-TestAmerica
Exa = Exa Data & Mapping Services Inc.
GDIT = General Dynamics Information Technology
HGL = HydroGeoLogic, Inc.
IDA = Institute for Defense Analyses

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LIST OF ACRONYMS AND ABBREVIATIONS

°C	degrees Celsius
%	percent
AFCEC	Air Force Civil Engineer Center
CalEPA	California Environmental Protection Agency
CEHNC	USACE Huntsville Engineering and Support Center
DoD	U.S. Department of Defense
DTSC	Department of Toxic Substances Control
DVG	data validation guideline
EDD	electronic data deliverable
EIS	extracted internal standard
ELAP	Environmental Laboratory Accreditation Program
ELLET	Eurofins Lancaster Laboratories Environmental Testing, LLC
EM-CX	Environmental and Munitions Center of Expertise
EPA	U.S. Environmental Protection Agency
ERA	ERA, A Waters Company
ESTCP	Environmental Security Technology Certification Program
ETA	Eurofins-TestAmerica
Exa	Exa Data & Mapping Services, Inc.
GCC	©Microsoft Government Community Cloud
GDIT	General Dynamics Information Technology, Inc.
HGL	HydroGeoLogic, Inc.
IDA	Institute for Defense Analysis
IDC	initial demonstration of capability
IPR	initial precision and recovery
ISO	International Organization for Standardization
LC-MS/MS	liquid chromatography mass spectrometry/mass spectrometry
LLOQ	lower limit of quantitation
LOQ	limit of quantitation
LOQVER	LOQ Verification
LQAO	Laboratory Quality and Accreditation Office
MDH	Maryland Department of Health
MDL	method detection limit
MDL_b	MDL based on method blanks
MDL_s	MDL based on spiked samples
mg/L	milligram per liter
MLV	multi-laboratory validation
MVS	Method Validation Study

LIST OF ACRONYMS AND ABBREVIATIONS (Continued)

NAPT	North American Proficiency Testing Program
NAVSEA	Naval Sea Systems Command
NIS	non-extracted internal standard
OLEM	Office of Land and Emergency Management
ORD	Office of Research and Development
OW	Office of Water
PFAS	per- and polyfluoroalkyl substances
QA	quality assurance
QC	quality control
QSM	Quality Systems Manual
RF	response factor
RR	response ratio
RSD	relative standard deviation
SERDP	Strategic Environmental Research and Development Program
SLV	single-laboratory validation
SGS AXYS	SGS AXYS Analytical Services Ltd.
SOP	standard operating procedure
SOW	statement of work
SW	solid waste
TDS	total dissolved solids
TOC	total organic carbon
TSS	total suspended solids
USACE	U.S. Army Corps of Engineers
Wellington	Wellington Laboratories, LLC

Study Plan for Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

1.0   BACKGROUND

Per- and polyfluoroalkyl substances (PFAS) contamination and the composition of other PFAS sources within the environment change over time due to properties of the individual PFAS analytes. Heightened interest in PFAS and ambiguities in PFAS identification led several researchers to investigate and identify over 4,000 PFAS analytes using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS). Detecting the individual PFAS analytes provides a quantitative measure of the PFAS contamination in an environmental sample.

Through the *Strategic Environmental Research and Development Program/Environmental Security Technology Certification Program (SERDP/ESTCP)*, the U.S. Department of Defense (DoD), the U.S. Environmental Protection Agency (EPA), and their contractor, General Dynamics Information Technology, Inc. (GDIT), formed the *Method Validation Study (MVS) Team.* The *MVS Team* conducted a single-laboratory validation (SLV) study of isotope dilution methods for quantifying PFAS in environmental matrices. The results of the SLV study were used by the EPA to support development of *Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS*, which EPA published on 2 September 2021. In December 2022, EPA published the 3rd Draft Method 1633 (Reference 7.1, https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas). It presents multiple-laboratory validation (MLV) data for the wastewater matrix, which includes required quality control (QC) criteria for the wastewater matrix. This revision provided additional clarifications and added flexibilities in response to formal comments received from multiple parties.

EPA Method 1633 is an interim draft method and now requires a MLV study. The end goal of the MLV study is to use the findings to revise, as necessary, draft Method 1633, and to submit the supporting data packages to the EPA Office of Water (OW) for consideration as a final method under the Clean Water Act. If recommended for approval, EPA will prepare a proposed rule for approval, as is required for all new wastewater methods, using the information provided by the *MVS Team* in a future report from this study. Then, EPA will compile the rule docket, pass the proposed rule through internal and/or external review at EPA, and then submit it to the Office of the Federal Register for publication.

The information and data from this MLV Study will also be submitted to the EPA Office of Land and Emergency Management (OLEM) for the future development and validation of an EPA solid waste (SW)-846 method. The OW will distribute the method/data package to OLEM.

This study is being undertaken pursuant to the procedures described in EPA's *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (Reference 7.2). During the course of testing, certain elements of this Study Plan may change. The *MVS Team* will evaluate the changes and determine whether they will be documented in an addendum to this Study or in the MLV Study Report.

2.0   STUDY OBJECTIVES

The goals of the MLV study are to achieve the following:

- Obtain data from matrices that are representative of the method's intended use;

- Obtain data from laboratories that are representative of those likely to use the approved method;

- Obtain feedback from laboratory users on the specifics of the Study Method (Reference 7.3, Attachment 1);

- Use study data to characterize performance of the method;

- Develop statistically derived QC acceptance criteria;

- Generate data according to specified analytical and quality assurance (QA)/QC procedures; and

- Obtain data from each participating laboratory subject to verification and validation by an independent review.

As noted in multiple locations in the Study Method (Reference 7.3, Attachment 1), "…Participating laboratories must follow this method without modification…" to meet these goals and the underlying quality objectives. In addition, the *MVS Team* will employ the following QA/QC strategies:

- Perform all activities in accordance with this Study Plan.

- Require that the vendor selected to prepare the study samples must (1) have demonstrated experience in performing work of a similar nature, (2) have a comprehensive and current QA program in place, and (3) submit applicable standard operating procedures (SOPs) for review by the *MVS Team*.

- Require that each participating laboratory must have demonstrated experience with analyses of a similar nature and must have a comprehensive and current QA program in place.

- Ensure that the study report and the final Study Method have been reviewed by the *MVS Team* to ensure the QC requirements meet data quality objectives.

Cumulatively, these requirements are intended to ensure that the data produced in this study are of appropriate and documented quality.

3.0   STUDY MANAGEMENT

3.1   MVS TEAM

This MLV Study is being managed by the *MVS Team*, which includes SERDP/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. Funding for this project was provided by SERDP/ESTCP and EPA's OW to 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, for program management; Exa Data & Mapping Services, Inc., (Exa) for data management; and the following firms for independent, third-party data validation: Chem Val Consulting, Inc.; Jacobs Engineering Group, Inc.; and, Pyron Environmental, Inc. The *MVS Team* structure is given in Figure 3-1. *MVS Team* members and responsibilities are provided in Table 3-1.

3.2   PROCUREMENT OF PFAS REFERENCE STANDARD MIXTURES AND TEST MATRICES

Under the direction of the *MVS Team*, HGL has responsibility for the following:

- Soliciting, reviewing qualifications, and contracting laboratories to participate in the study;

- Procuring certified PFAS reference standard mixtures;

- Acquiring samples and characterizing sample media through third-parties; and

- Creating the Study Samples for this study through the use of a third-party vendor.

Specifics of these tasks are described in Section 4. Participating suppliers and laboratories are described here.

A commercial vendor, Wellington Laboratories, LLC (Wellington), has been selected to provide reference standard mixtures and individual, high-concentration PFAS reference standard mixtures as defined by the *MVS Team* to the laboratories participating in the study. Another commercial vendor, ERA (A Waters Company) (ERA), that specializes in proficiency testing samples, will prepare Study Samples using "real-world" environmental sample matrices provided for use in this study (see Table 3-2). The Oversight Contractor, HGL, is responsible for procuring and providing oversight of both vendors and ensuring sufficient volumes of sample matrices from various sources are delivered to ERA for homogenization, aliquoting, spiking, and then shipping to the participant laboratories listed in Table 3-2. HGL is also responsible for procuring standards from Wellington, which will provide them to the participant laboratories listed in Table 3-2. Attachment 6 includes the Certificate of Analysis Documentation for PFAS Reference Standard Mixtures provided by Wellington Laboratories, Inc. to each participating laboratory.

Storage requirements for source samples and samples prepared by the Study Sample vendor were determined by the *MVS Team*, based on the holding time study results from the SLV study and consistent with requirements in draft Method 1633. In addition, a separate bench-scale cooler study has been conducted by ERA to determine the potential effects on sample temperatures during shipment. Findings from this cooler study completed by ERA are included in Attachment 7.

3.3   PARTICIPATING LABORATORIES

HGL is responsible for procuring and providing oversight of eight commercial contract laboratories and two state laboratories that will participate in the MLV study. The number and identity of participating laboratories was determined by the *MVS Team* based on factors such as cost and ability to support the study. In keeping with the approach described in *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (Reference 7.2), HGL solicited participation from the 10 laboratories listed in Table 3-2, recognizing the possibility that some participants may drop out or otherwise fail to provide usable data. Candidate laboratories were added to the study after review and confirmation with the *MVS Team.*

To comply with policies regarding laboratory competency, HGL required accreditations from the DoD Environmental Laboratory Accreditation Program (ELAP) for all commercial laboratories relevant to the analysis of PFAS in environmental matrices and International Organization for Standardization (ISO) accreditation for government laboratories. Table 3-2 includes the relevant accreditations for participating laboratories and vendors. HGL maintains copies of QA program documentation obtained during the solicitation process. Laboratories that are unable to demonstrate competency in PFAS analyses and that do not have an adequate QA program in place were not included as participants in the study. HGL is responsible for ensuring all accredited laboratories maintain all method and data reporting requirements contained in this Plan throughout the period of MLV study testing. Additionally, HGL will compile all documentation from the studies, analytical data packages, and results with the associated communication records.

HGL will ensure each submittal includes a data package and an electronic data deliverable (EDD), as defined in this Study Plan. Both will be reviewed for completeness relative to ensuring that all required analytes, extracted internal standard (EIS) analytes, and non-extracted internal standard (NIS) analytes are reported for each sample received; only one result will be reported for each analyte for each sample in both the data packages and EDDs. Before providing the EDDs to the Government, HGL will review them to ensure all data for samples and QC samples reported in the data packages have been included and that all fields are completed, as required by this Study Plan. HGL will not send data packages and EDDs to the *MVS Team* for data validation until this review is complete and any issues are resolved.

3.4   VALIDATORS

The contracted, independent, third-party validators will receive the EDDs and data packages after HGL and Exa have completed their reviews and resolved any issues with submittals from the laboratories (Attachment 4). The validator will validate the data packages and EDDs in accordance with the study data validation guidelines (DVGs) (Attachment 5) that the *MVS Team* has reviewed and approved for use for this study. The validator will provide a data validation report and the associated amended EDD (amended per DVGs) and data package submittals for each EDD/data package that has been validated to the Study QC Manager. The *MVS Team* will review the validator's submittals. If changes are required, the validator will be responsible for making the requested changes.

3.5   STATISTICAL ANALYSIS

The *MVS Team* and Institute for Defense Analyses (IDA) will review and evaluate all data collected during this study to characterize the performance of the Study Method. This includes data on calibration, initial precision and recovery (IPR), method detection limits (MDL), performance in real-world matrices, and labeled compound recoveries. The *MLV Study Report* will contain statistically derived QC limits that will be calculated from the data collected during this study. The laboratories that participate are representative of the real-world laboratories that will potentially conduct this method, and the matrices are typical of matrices that a laboratory using this method would analyze.

4.0   TECHNICAL APPROACH

The study will be performed in six phases. Work on some phases may occur simultaneously.

- Phase 1 (Section 4.1) involves soliciting laboratories to participate in the study.

- Phase 2 (Section 4.2) involves procuring the reference standard mixtures, acquiring and characterizing sample media, and creating the Study Samples for this study.

- Phase 3 (Section 4.3) involves using the Study Method (Attachment 1, dated October 2021), which includes MLV Study-specific requirements and guidance to (1) perform the initial steps (calibration, initial demonstrations of capability [IDCs], IPR, MDLs, and verify limits of quantitation [LOQs]), (2) demonstrate laboratory capability with standards and clean matrices, and (3) generate an applicable SOP. Phase 3 submittals must be received, validated (by the validator), and deemed acceptable by an independent, third-party data validation entity, and reviewed and approved by the Project QA Manager and Technical Manager prior to the laboratory starting Phase 4.

- Phase 4 (Section 4.3) involves all participant laboratories using the Study Method to analyze the aqueous, solid, and tissue Study Samples after receiving approval from the *MVS Team* to proceed.

- Phase 5.1 (Section 4.5) involves data verification of all study results by the HGL Project Chemist and automated checks of the EDDs. HGL's Project Chemist will perform an initial evaluation of the data from each phase of the study with the *MVS Team* before authorization is given to proceed with the next phase of the study.

- Phase 5.2 (Section 4.5) involves data validation of all study results by an independent third-party validation entity. Validation will begin as data becomes available from Phase 4.

- Phase 6 (Section 4.6) involves a statistical analysis of the data, development of QC acceptance criteria, and revision to the draft Method to be recommended in the MLV Study Report that will be submitted to the EPA.

4.1   PHASE 1 – SOLICITING LABORATORIES

Phase 1 of the study involves executing subcontracts or other agreements with 10 laboratories to participate in the MLV study. As shown in Table 3-2, HGL has established subcontracts with eight commercial environmental laboratories and has written agreements with two state government laboratories.

Prior to the award of this contract, the Government Technical Manager and QC Manager developed a broad list of likely participants and contacted them in advance of a formal solicitation to determine their potential interest. From the list of potential participants, HGL solicited bids using competitive, government-approved procurement procedures. Since the Study Method has changed from the original bids, HGL has sent to all participating laboratories the Study Method (Attachment 1), an interim final version of this MLV Study Plan, and a brief statement of work (SOW) to update price estimates. This Study Plan and the Study Method detail the requirements for sample preparation, storage, shipment, analysis, and QA/QC needed for laboratories to conduct the testing as well as reporting requirements.

All laboratories must have a comprehensive laboratory QA program in place and operating at all times during the MLV study, and this program must be consistent with the *Guidance for Developing Quality Systems for Environmental Programs* (Reference 7.4) and the general laboratory procedures specified in the *Handbook for Analytical Quality Control in Water and Wastewater Laboratories* (Reference 7.5).

Regardless of the nature of a laboratory's participation (contract or agreement), the same study requirements will apply. If laboratories are unable to successfully complete Phase 3 or provide the required documentation, they will not proceed to Phase 4.

4.2 PHASE 2 – PROCURING REFERENCE STANDARD MIXTURES AND STUDY SAMPLES

For Phase 2, HGL has procured and provided sufficient quantities of the reference standard mixtures (Table 4-1) from Wellington needed for laboratories to perform sample analysis per the method. HGL also procured laboratory support services to analyze pre-spiked sample matrices that were provided to the *MVS Team* and transferred under chain of custody to ERA.

After the *MVS Team* reviewed the pre-spike characterization results, they finalized the agreed-upon spike levels for all samples. ERA procured the reference standard mixtures, homogenized the matrices used for the study, spiked the Study Samples required for the MLV Study, and shipped the Study Samples to each participating laboratory.

4.2.1 Reference Standard Mixtures

A list of the method analytes, NIS compounds, and EIS compounds is provided in Table 4-1 and was developed by each laboratory using the reference standard mixtures provided by Wellington in Attachment 6. Each laboratory participating in the study utilized these standard mixtures provided by Wellington to generate all study data. This requirement avoided having each laboratory prepare their own standards from neat materials or available stock solutions, which would add significant variability to the study results as well as not likely reflect routine laboratory practice when performing the method.

HGL has centrally purchased an initial lot of the reference standard mixtures specified by the Government Technical Manager and the QC Manager from Wellington, who distributed the lots to the participating laboratories. Additional volumes of these standard mixtures may be purchased by the laboratories.

ERA purchased separate, high-concentration stock reference standard mixtures of the method analytes listed in Table 4-1 to create the spiking solutions needed to create the Study Samples that were provided to the participating laboratories.

Laboratories were responsible for purchasing qualitative method analyte standards (stock standards) (including branched isomers, as noted in the table) listed in Table 4-1 as well as the bile salt standards required by the Study Method.

4.2.2 Pre-Spike Sample Matrix Characterization

The focus of this study is on the analysis of real-world environmental matrices, including wastewater, groundwater, surface water (fresh and marine), soil, sediment (fresh and marine),

fish/clam tissue, landfill leachate, and biosolids. To obtain a wide diversity and sufficient quantity of matrices and samples, the *MVS Team* worked with EPA and municipal, state, and regional contacts to obtain sufficient volumes/mass of several media to be used in the study. A list of the sample types and quantities that were provided to the *MVS Team* are listed in Table 4-2. All media were shipped to and held at ERA at ≤ 6 degrees Celsius (°C).

ERA will homogenize all sample matrices and ship aliquots of composite samples collected from each to SGS AXYS Analytical Services Ltd. (SGS AXYS) for native PFAS analyses and to Eurofins-TestAmerica (ETA)-Denver for conventional physical and chemical analyses. Shipments to SGS AXYS will be coordinated with HGL to ensure proper paperwork is included with shipments. Shipments to ETA-Denver will be coordinated with a courier from the laboratory. HGL is responsible for transmission of these results to the *MVS Team.*

All sample matrices will be characterized before spiking to ensure the appropriate amounts of high-concentration PFAS reference standard mixtures are used to create the Study Samples. SGS AXYS will analyze all samples for the 40 PFAS method analytes listed in Table 4-3. HGL will conduct a preliminary data review, and the *MVS Team* will identify contracted, independent, third-party entities to conduct data validation, as described in Section 4.5 and Attachment 5 of this Plan. The *MVS Team* will review the results and determine the spiking levels for PFAS method analytes for each Study Sample/matrix. The *MVS Team* will also determine if any adjustments to the samples are necessary prior to spiking (e.g., dilutions due to potentially elevated native PFAS concentrations).

The seven wastewater matrices will include effluents from a publicly owned treatment works, a substitute wastewater as specified in ASTM International Reference D5905-98, *Standard Practice for the Preparation of Substitute Wastewater* (Reference 7.6), and wastewaters from specific industrial discharges. As recommended in the EPA *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (Reference 7.2), at least one of the wastewater matrix types will have one of the following characteristics, such that each criterion below is represented by at least one wastewater sample:

- Total suspended solids (TSS) greater than 40 milligrams per liter (mg/L),

- Total dissolved solids (TDS) greater than 100 mg/L,

- Oil and grease greater than 20 mg/L,

- Sodium chloride greater than 120 mg/L, and

- Calcium carbonate greater than 140 mg/L.

In anticipation of future data user needs, ETA-Denver will also analyze the samples for other physical and chemical characteristics (Table 4-4) as follows:

- ***Aqueous Samples (9 Wastewaters, 3 Landfill Leachates, 3 Groundwaters, and 4 Surface Waters):*** alkalinity (total, carbonate, and bicarbonate), ammonia, calcium, chloride, conductivity, oil and grease, pH, sodium, sulfate, TDS, and TSS;

- ***Sediment Samples (3) and Soil Samples (8):*** grain size, moisture, pH, salinity (sediment only), and total organic carbon;

- ***Tissue Samples (3):*** Lipids; and

- ***Biosolid Samples (3):*** pH.

After HGL conducts data verification and an independent, third-party entity conducts data validation, the *MVS Team* will review the results to determine if any adjustments (e.g., adding water to increase moisture content in soil samples, other additions to adjust solids, oil and grease, sodium chloride or calcium carbonate content) are needed for any of the sample matrices prior to their spiking with PFAS compounds. If adjustments are needed, ERA may need to conduct additional follow-on chemical analyses to ensure the characteristics meet specifications stipulated by the *MVS Team* prior to spiking with PFAS.

Requirements for sample nomenclature are included in Attachment 2. Additional matrix-specific sample preparation guidelines and chain of custody forms will be included in the coolers used for shipping packages from ERA. Participating laboratories will use the nomenclature provided on sample labels and chain of custody forms and follow the matrix-specific sample preparation guidelines received from ERA.

4.2.3   Study Sample Development and Handling

The *MVS Team* and HGL ensured that the required matrices were delivered to ERA to be homogenized, characterized, spiked, aliquoted into study-specific sizes, and distributed to each laboratory in accordance with the Study Method (Attachment 1).

While SGS AXYS and ETA-Denver analyze the samples, ERA will procure the necessary stock solutions for the list of required PFAS analytes from the qualified vendor (Wellington). ERA will also procure all necessary sample containers and shipping supplies (e.g., coolers, ice, packaging material).

After the *MVS Team* determines the spiking levels and if any adjustments are needed (e.g., dilutions due to native PFAS concentrations), ERA will create spiking solutions containing the PFAS compounds including two solutions for the aqueous matrices and two for the solid matrices, as noted in Table 4-5. ERA will certify the spiking solutions based on weights and measures.

After ERA confirms the certified values for each spiking solutions using LC-MS/MS analysis in their laboratory, they will create, label, package, and ship the Study Samples specified for each matrix to all 10 participating laboratories. All samples are to be frozen to -20°C and then shipped frozen with sufficient blue ice to maintain the samples in a frozen state during transit.

ERA will create at least two replacement samples for each matrix/level, should breakage occur during shipment or handling by laboratories. Depending on the remaining volume of the matrices selected for the Study Samples, additional sets may be created. When complete, ERA will provide a Certificate of Analysis for each Study Sample. Each Certificate will be stored with other documentation for this study.

HGL staff will work with ERA to schedule and direct the shipments of materials to each participating laboratory. HGL staff will notify each laboratory of impending shipments, track each shipment from ERA to the laboratory, and confirm the condition of the materials on receipt by each laboratory. HGL will work with ERA and participating laboratories to resolve any issues or discrepancies and will communicate with the *MVS Team* regularly.

4.2.4   Bench-Scale Cooler Temperature Study

ERA will conduct a bench-scale cooler temperature study at their laboratory to assess the ability of water samples to remain frozen during shipping. This study will consist of freezing bottles of water identical to the bottles that will be used for the MLV study and then placing them in coolers filled with ice. The coolers will be packed as they normally would for typical sample shipping purposes, and they will be the same type as will be used to ship the Study Samples to the laboratories. The coolers will be chilled before the beginning of this study. The ice that is used for this study will be the same type as will be used to ship the Study Samples. The use of "blue ice" is acceptable for the bench-scale cooler temperature and to ship Study Samples for this MLV study.

The temperature of a bottle of water from each of the coolers will be checked using an infrared temperature gun after 12 hours have transpired. Similar temperature checks will be made using bottles from additional coolers checked after 24, 36, 48, and 72 hours have elapsed. A separate frozen bottle of water and cooler with blue ice will be used for each time interval such that the cooler is not opened before the temperature of the bottle is taken. ERA will photograph the cooler, ice, packing materials, and bottle when recording temperatures at each time interval. These photographs will be maintained with other project records.

4.3   PHASE 3 – CALIBRATION AND DEMONSTRATION OF CAPABILITY

Prior to analyzing any of the environmental sample matrices, each laboratory will (1) develop and submit an SOP that is compliant with the requirements of the Study Method (Attachment 1), (2) perform a minimum of three initial multi-point calibrations, and (3) conduct an IDC for 'clean' sample matrices, as described in the sections below.

4.3.1   Initial Calibration

Each laboratory will use the reference standard mixtures provided by Wellington specifically for the project to create all laboratory standards (initial calibration and calibration verification) and spiking solutions needed. A list of the method analytes, EIS compounds, and NIS compounds required is provided in Table 4-3. The laboratory must purchase all other supplies and standards (e.g., the qualitative branched standards and bile salt interference check standards) required by the draft Study Method (Attachment 1). The laboratory will perform (or compile) at least three initial calibrations, including verifications, to demonstrate the applicable range of the procedure in accordance with requirements in the draft Study Method for initial calibration and initial calibration verification requirements. Each laboratory will report the calibration linearity metric that they use (e.g., the relative standard deviation [RSD] of response factors [RFs]) for each analyte. A data package that includes all elements required for Stage 4 validation per the DVGs used for this study (Attachment 5) must be submitted for each initial calibration. In addition to the Stage 4 required documentation, the data packages provided for the initial calibrations and their verifications must include the following:

- Documentation of the mass calibration and mass calibration verification;

- A list of the concentrations of the method analytes, NIS compounds, and EIS compounds in each calibration standard and calibration verification standard;

- Tabulation of the response ratios (RRs) or RFs for each method analyte and EIS compound in each of the initial calibration standards in each of the initial calibrations, and the mean RR or RF and mean RSD across the three initial calibrations for each method analyte and EIS compound;

- Tabulation of the ion ratios of applicable method analytes;

- Data for the calibration linearity metric that is used (e.g., the RSD of RRs and RFs) for each analyte; and

- Tabulation of the concentrations (reported and spiked) and recoveries of the method analytes, NIS compounds, and EIS compounds in each calibration verification and instrument sensitivity check.

One data package will be submitted for the three initial calibrations. No EDD is required for the initial calibrations submittal.

4.3.2   Initial Demonstration of Capability (IDC)

Each laboratory will perform an IDC for clean media similar to each of the matrix types in the study using a suitable spiked reference matrix. Each laboratory is required to prepare spiking solutions from the reference standard mixtures provided for this project by Wellington. Each laboratory will spike a set of clean reference matrices for subsequent analysis in accordance with the Study Method (Attachment 1). The spiked reference matrix is created from a clean matrix (void of target compounds at or above the MDL/limit of detection). If background contamination cannot be resolved for any of the method analytes, the *MVS Team* will be contacted for additional guidance. For this project, the reference matrices are blank media consisting of PFAS-free reagent water (aqueous samples), wetted Ottawa sand and/or PFAS-free reagent grade sand (solid samples), and PFAS-free fish tissue (tissue samples). Each laboratory will provide and spike their own clean reference matrices.

Each IDC will include an IPR determination, an MDL study, and a LOQ Verification (LOQVER). A method blank must be prepared with each IDC batch of samples. For data package requirements for the IDC, see Section 5.1 of this document. The IPR consists of a blank and four replicate samples of each reference matrix spiked with native PFAS analytes (replicate samples only) and labeled compounds and carried through the entire analytical process (sample preparation and analysis). Each laboratory will perform an IPR study for each matrix.

Each laboratory will establish MDLs for all method analytes using the MDL procedure in 40 Code of Federal Regulations Part 136, Appendix B for each matrix.

Each laboratory will perform LOQVERs in each reference matrix in accordance with the requirements of the *DoD Quality Systems Manual (QSM) for Environmental Laboratories, Version 5.4* (Reference 7.7). The requirements for LOQVER can be found in the DoD QSM, Module 4, Section 1.5.2. Each LOQVER will consist of a method blank and a reference matrix sample spiked with method analytes, EIS compounds, and NIS compounds and will be carried through the entire analytical process (sample preparation and analysis) in accordance with the Study Method (Attachment 1). Samples used in the MDL study can overlap with samples used for the LOQVER. Sample volumes are defined in the Study Method (Attachment 1). Exact spike concentrations will be determined by each participating laboratory based on the results of the MDL study and

acceptable initial calibration range. EIS compounds will be spiked around the midpoint of the calibration curve.

The LOQ is the smallest concentration that produces a quantitative result with known and recorded precision and bias. A LOQVER meets all requirements of a lower limit of quantitation (LLOQ), as defined by EPA SW-846. The LOQVER is spiked between 1-2 times the LOQ and will go through the same sequence of preparation and analytical steps as used when analyzing a normal sample. For this study, the following limits will apply to the IPR and LOQVER: 40 to 150 percent (%) for target analytes, 20 to 150% for EIS compounds, and >30% for NIS compounds. The Project Chemist should be contacted if these limits are not achieved. The stated criteria for EIS and NIS compound recoveries also apply to the MDL Study.

For data package requirements for the IDC, see Section 5.1 of this document. One data package and EDD per sample matrix will be submitted. In addition to the elements required by Section 5.1, IDC data packages must include the following:

- *IPR:* Tabulation of individual sample results for each matrix, and the mean % recovery, standard deviation, and RSD of the results; and

- *IPR:* Tabulation of IPR spiking levels for each matrix.

- *MDL:* Tabulation of individual MDL sample results; and

- *MDL:* Tabulation of the computed MDL based on method blanks (MDL_b), the MDL based on spiked samples (MDL_s), and the final MDL; and

- *LOQVER:* Tabulation of individual sample results and spike concentrations for each matrix, and the % recovery.

One data package and one EDD per matrix (aqueous, solid, and tissue; three EDDs and three data packages per laboratory) will be submitted for the IDCs after the completion of each matrix.

4.4   PHASE 4 – ANALYSES OF STUDY SAMPLES

The focus of Phase 4 is to evaluate the Study Method (Attachment 1) in various real-world matrices. Phase 4 will be staged such that wastewater/groundwater/surface water are analyzed first, followed by soils/sediments, tissues, and lastly, landfill leachate and biosolids. These are described further, below. One data package and EDD per matrix (groundwater, surface water, soil, sediment, tissue, landfill leachate, and biosolids) will be submitted. The number of samples for each data package will require multiple sample delivery groups (or QC batches) for each of these data packages that require more than 20 samples to be analyzed (i.e., 8 EDDs per laboratory).

4.4.1   Groundwater, Surface Water, and Wastewater

Aqueous sample matrices are to be extracted following the procedures described in Attachment 1. A total of three groundwater, three surface water, and seven wastewater matrices of different origin (see Table 4-2 and Attachment 2) will be used to create the first sets of Study Samples to be sent to the laboratories. For each real-world aqueous matrix, ERA will prepare and send to each laboratory sets of spiked (six) and unspiked (one) Study Samples required for the MLV Study as listed in Table 4-5 (13 sets of aqueous Study Samples). Laboratories will follow the instructions

provided to them by the ERA. A total of 91 aqueous Study Samples will be prepared and analyzed by each laboratory in accordance with the draft Study Method (Attachment 1).

Assuming that 10 laboratories successfully complete the study, the study design will yield 490 results for wastewater Study Samples and 210 results each for groundwater and surface water Study Samples for each method analyte and labeled compound. Even if fewer than 10 laboratories participate, or if some are unable to produce usable results, the *MVS Team* will still have a significant body of performance data with which to judge the method's capabilities.

4.4.2 Soil and Sediment

Soil and sediment sample matrices are to be extracted following the procedures described in Attachment 1. A total of three soil and three sediment matrices of different origin (see Table 4-2 and Attachment 2) will be used to create the next set of Study Samples sent to the laboratories. For each real-world solid matrix, ERA will prepare and send to each laboratory sets of spiked (six) and unspiked (one) Study Samples required for the MLV Study as listed in Table 4-5 (six sets of solid Study Samples). Laboratories will follow the instructions provided to them by ERA. A total of 42 solid Study Samples will be prepared and analyzed by each laboratory, in accordance with the draft Study Method (Attachment 1).

Assuming that 10 laboratories successfully complete the study, the study design will yield 210 results for each soil and sediment Study Sample for each method analyte and labeled compound. Even if fewer than 10 laboratories participate, or if some are unable to produce usable results, the *MVS Team* will still have a significant body of performance data with which to judge the method's capabilities.

4.4.3 Fish Tissue

Tissue sample matrices are to be extracted following the procedures described in Attachment 1. A total of three tissue sample matrices of different origin (see Table 4-2 and Attachment 2) will be used to create the next set of Study Samples to be sent to the laboratories. For each real-world tissue matrix, ERA will prepare and send to each laboratory sets of spiked (six) and unspiked (one) Study Samples required for the MLV Study as listed in Table 4-5 (three sets of tissue Study Samples). Laboratories will follow the instructions provided to them by ERA. A total of 21 Study Samples will be prepared and analyzed by each laboratory, in accordance with the draft Study Method (Attachment 1).

Assuming that 10 laboratories successfully complete the study, the study design will yield 210 results for each Study Sample for each method analyte and labeled compound. Even if fewer than 10 laboratories participate, or if some are unable to produce usable results, the *MVS Team* will still have a significant body of performance data with which to judge the method's capabilities.

4.4.4 Landfill Leachate and Biosolids

A total of three landfill leachate and three biosolid matrices of different origin (see Table 4-2 and Attachment 2) are to be extracted following the procedures in Attachment 1. For each real-world matrix, ERA will prepare and send to each laboratory sets of spiked (six) and unspiked (one) Study Samples required for the MLV Study as listed in Table 4-5 (6 sets of leachate/biosolids Study Samples). Laboratories will follow the instructions provided to them by ERA. A total of 42 Study

Samples will be prepared and analyzed by each laboratory in accordance with the draft Study Method (Attachment 1).

Assuming that 10 laboratories successfully complete the study, the study design will yield 210 results each for the biosolid and landfill leachate Study Samples for each method analyte and labeled compound. Even if fewer than 10 laboratories participate, or if some are unable to produce usable results, the *MVS Team* will still have a significant body of performance data with which to judge the method's capabilities.

4.5   PHASE 5 – DATA VERIFICATION AND VALIDATION

Laboratories will submit their SOPs to HGL, who will provide them to the *MVS Team.* All data and documents will be posted to the MLV Library SharePoint site (Section 5.2). HGL and Exa will review each data package/EDD to ensure that the following criteria are met:

a) Each data package includes all documentation required by the Study Method and this Study Plan (Section 5.0 specifies documentation required to perform Stage 4 Data Validation per the study DVGs);

b) Each EDD includes a single result for each method analyte required by the Study Method for each sample (batch QC and study samples). Multiple results for EIS and NIS compounds may be required for instances where sample dilution(s) or reanalysis are required in order to report a method analyte(s) result for a sample (batch QC and study samples);

c) Each EDD contains all samples (batch QC and Study Samples) and all results reported in the EDD matches all results reported in the data package, and the EDD contains no additional results for each sample;

d) Each EDD contains all information required by Attachment 3; and

e) Each data field in each EDD is completed in accordance with the instructions included in Attachment 3.

If any issues are identified, HGL will work with the laboratory to clarify the situation, obtain any missing information, document the resolution, and request corrected data packages and EDDs. Once all issues are resolved, HGL will transfer the compliant data package and EDD to Exa for storage, tracking, and additional automated QA/QC (Attachment 4). The data packages and EDDs will then be provided to a contracted, independent, third-party data validation entity. The *MVS Team* will be advised of the status of the review efforts on a regular basis via a Tracking System (Attachment 4).

Because this is a method validation effort, there are no *a priori* QC acceptance criteria, and data from the study will not be excluded from consideration simply because they appear to fail some pre-conceived performance expectations. Every effort will be made to retain as many results as is practical. The validation entity will validate the data in accordance with the study DVGs (Attachment 5), update the EDDs to include the necessary data qualifiers, and submit a validation report, in the format stated in Attachment 5, to the *MVS Team.* The study Government QA Manager and/or study Government Technical Manager will review the report and revised EDDs prior to submitting EDDs to the *MVS Team* and IDA for use in the statistical tests in Phase 6 of the study. The MLV Study Report will include an explanation for any data that is excluded from the statistical

analysis, including discussion of potential and likely root causes of non-conformances, and distinguish the excluded data in the EDD from the acceptable study data.

4.6   PHASE 6 – DEVELOPMENT OF QC ACCEPTANCE CRITERIA

The last major phase of the study will be to develop statistically based QC acceptance criteria and summarize method performance in real-world samples. The overall procedures used for that process are based on guidance in *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program* (Reference 7.2) and described in Section 6 of this Study Plan. Observed failure rates may also be pursued if the criteria generated do not resemble the plotted data.

5.0   DATA REPORTING AND DATA MANAGEMENT

5.1   LABORATORY REPORTING REQUIREMENTS

Each laboratory participating in the study will be required to (1) provide all required information electronically, including raw data; (2) submit summary-level electronic data and sufficient information for Stage 4 data validation to be performed (References 7.7 and 7.9) for the list of forms and data elements required for Stage 4 data validation; (3) deliver an EDD in the format summarized in Attachment 3; and (4) maintain raw data for a period of 5 years and provide it upon request during this 5-year time period. Raw data will include all calibration data; chromatograms; quantitation reports (including quantitation and qualifier peaks, transition ion ratios, peak areas); preparation records for reference standard mixtures (including manufacturer's Certificate of Analysis); bench sheets; and laboratory notebooks showing weights, volumes, manual calculations, and other data that will allow verification of the calculations performed and the final results reported to be traced back to the raw data.

Each laboratory also must adhere to the following rules when reporting data:

- All reports and documentation, including instrument printouts and other raw data, must be sequentially paginated; clearly labeled with the laboratory name; and labeled to provide sufficient identification for method blanks, calibration, and interference checks, etc., necessary to link the raw data with associated summary reports.

- Results from all analyses must be reported, including calibration data and any dilutions or reanalyses performed. The laboratory also must include an explanation of any dilutions or reanalyses performed and identify which of the analyses it considers to be most appropriate for use.

- Concentrations of all qualitatively identifiable peaks must be reported to three significant figures in the appropriate reporting units of parts per trillion or nanograms per liter for aqueous samples and parts per billion or micrograms per kilogram for solid and tissue samples.

- The terms "zero," "trace," or "ND" are not to be used; if a signal is not detected or if the signal produces a concentration < MDL, the value is the MDL and a corresponding "U" flag must be applied to this value.

- If a signal is produced, and the value is above the MDL, it must be reported. If the value is ≥MDL and <LOQ, a "J" flag must be applied to this value.

- Data qualifiers provided in Attachment 3 must be used where appropriate.

- Results must be reported for all Study Samples, including QC samples. Results must include EIS and NIS analytes.

- Data packages must contain all data elements (e.g., forms, standards traceability) needed to conduct a Stage 4 data validation per the study DVGs (Attachment 5).

These rules will be verified through the data management process (Attachment 4). The case narrative will contain detailed descriptions of any difficulties encountered in the generation of the analytical results and QC data. Note that participating laboratories must follow the Study Method (Attachment 1) without modification, even if difficulties are encountered during testing.

All data packages, EDDs, and results will be submitted to HGL and Exa for initial review through the process outlined in Attachment 4. Exa will provide secure access for each laboratory to upload files to the MLV Library SharePoint site hosted both on ©Microsoft Government Community Cloud (GCC) High and DoD environments to meet the unique and evolving requirements of DoD and contractors holding or processing DoD-controlled unclassified information. Access will be strictly controlled to ensure the protection of procurement-sensitive and proprietary data.

5.2   DATA MANAGEMENT AND REPORTING

Exa and HGL will store all submitted electronic data on the MLV Library SharePoint site as described in Attachment 4. The content of the MLV Library will be organized into specific folders that allow different permissions for different users to ensure protection of sensitive and proprietary information. The Library will accommodate storage of the following documents and records:

- This Study Plan (including all draft versions, comments, and revisions);

- Documentation of the procedures used to assess the competency of laboratories participating in this study;

- Documents and records associated with the solicitation and award of participant laboratories, including the SOWs or equivalent that describe participant laboratory requirements;

- Documents and records associated with the procurement of reference standard mixtures and study samples, including SOWs or equivalent that describe the process used to collect and produce study samples;

- The name, address, phone number, and primary contact at the standards vendor and each participating laboratory;

- Copies of all written correspondence (excluding emails) with laboratory staff, sampling personnel, and *MVS Team* staff regarding the study;

- A log (or other record) that documents verbal communication with laboratory staff, sample coordinators, sampling personnel, and *MVS Team* staff regarding study status or problems;

- Records concerning sample shipment and receipt;

- All analytical data resulting from this study, including a database of compiled, validated EDDs;

- All laboratory comments on the method resulting from this study;

- Records of all data review assessments, data validation reports, and statistical analyses submitted to the *MVS Team;* and

- All draft and final reports submitted to the *MVS Team* pertaining to this study.

HGL and the *MVS Team* will develop a schedule for routine communications during the course of the study, based on the specific activities underway at the time. For example, HGL will communicate with the *MVS Team* Project Manager more frequently (e.g., daily) during those periods when samples are being shipped to the laboratories, versus less frequently during the periods when sample analyses are taking place.

Delivery of all data submittals to the MLV Library will be tracked apprise the *MVS Team* of project status. Exa will be responsible for summarizing information from the Tracking System and providing regular updates to the *MVS Team*.

Each laboratory will provide an EDD in the format specified in Attachment 3 as part of the submittal to the Government and IDA for the purpose of conducting planned statistical analyses. Following verification of these data, the EDDs will be posted to the Library folder set up specifically to provide access to the validation entity to upload data validation reports. Laboratory EDDs with embedded validation comments and updated qualifiers will be imported into the project database.

Following validation and review by the *MVS Team*, the validated data will be provided from the project database to IDA to conduct analyses and upload statistical reports. After the *MVS Team* has reviewed these reports, approval documents will also be stored in the MLV Study Library.

Details of the data management process are found in Attachment 4.

6.0 EVALUATION OF METHOD PERFORMANCE

The overall goal of this MLV study is to develop performance data for Method 1633. The results from Phases 3 and 4 will be evaluated using common statistical procedures (References 7.2, 7.10, 7.11, 7.12). The *MVS Team* and IDA will use the results from the samples to develop QC criteria for IPR tests, ongoing precision and recovery tests, labeled compound recoveries, etc. A general description of the derivation of those QC acceptance criteria is based on EPA's method evaluation protocol (Reference 7.2).

Finally, the *MVS Team* and IDA will develop tables of method performance data, including precision and accuracy, as a function of analyte concentration that will provide an indication of expected performance of the procedures under typical conditions. Such tables may be included in the revised procedure as further evidence of its overall capabilities or limitations.

Following completion of the method performance evaluation, IDA and the *MVS Team* will prepare a formal report on the results of the MLV study. The *MVS Team* will submit that draft report to appropriate levels of management review within stakeholder agencies and then revise the report, as needed.

7.0   REFERENCES

7.1   U.S. Environmental Protection Agency (EPA), 2022. *3rd Draft Method 1633, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS*. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division, Washington, DC 20460. EPA 821-D-21-001. December. Available online at https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas.

7.2   U.S. Environmental Protection Agency (EPA), 2018. *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program*. U.S. Environmental Protection Agency. Office of Water, Engineering and Analysis Division. EPA 821-B-18-001. February. Available online at https://www.epa.gov/sites/default/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf.

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7.4   U.S. Environmental Protection Agency (EPA), 2002. *Guidance for Developing Quality Systems for Environmental Programs (QA/G-1)*. EPA/240/R-02/008. November (reissued January 2008).

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7.9   U.S. Department of Defense (DoD). 2019. *United States Department of Defense, General Data Validation Guidelines*. Environmental Data Quality Workgroup. November.

7.10   SAS Institute, Inc., 1994. SAS/STAT User's Guide, Volume 2, GLM-VARCOMP. Version 6, 4th Edition, June.

7.11   Berry, D. A.; Lindgren, B. W. 1990. *Statistics: Theory and Methods*. pp. 286-290, 600-618. Brooks/Cole Publishing Company. Pacific Grove, California.

7.12 Federal Register. *Clean Water Act Methods Update Rule for the Analysis of Effluent*. Vol. 82, No. 165. August 28, 2017. pp. 40939 - 40941.

TABLES

Table 3-1. MVS Team

Name	Agency/ Organization	Project Responsibility	email	Phone Number
Dr. Andrea Leeson	SERDP/ESTCP	Study Supervisor	andrea.leeson.civ@mail.mil	(571) 372-6398
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Dr. Marc Mills	EPA (ORD)	Technical Manager	mills.marc@epa.gov	(513) 595-7322
Dr. Richard Anderson	U.S. Air Force (AFCEC)	Study Evaluation Manager	richard.anderson.55@us.af.mil	(210) 395-0625
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Troy Strock	EPA (OLEM)	Liaison Office of Land and Emergency Management	strock.troy@epa.gov	(703) 308-8637 / (202) 566-0504
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Maggie Radford	Jacobs Engineering Group	Data Validator	maggie.radford@jacobs.com	(919) 749-9479
Jeremy Bishop	Jacobs Engineering Group	Data Validator	1inisa.Bishop@jacobs.com	(541) 768-3299
Kathi Gumpper	Chem Val Consulting	Data Validator	kgumpper@chemval.com	(801) 541-6983
John Gumpper	Chem Val Consulting	Data Validator	jgumpper@chemval.com	(801) 554-9362

Notes:
AFCEC = Air Force Civil Engineer Center
EPA = U.S. Environmental Protection Agency
ESTCP = Environmental Security Technology Certification Program
Exa = Exa Data & Mapping Services Inc.
HGL = HydroGeoLogic, Inc.
IDA = Institute for Defense Analyses
NAVSEA = Naval Sea Systems Command
OLEM = Office of Land and Emergency Management
ORD = Office of Research and Development
OW = Office of Water
SERDP = Strategic Environmental Research and Development Program
USACE = U.S. Army Corps of Engineers

Table 3-2. List of Suppliers and Participating Laboratories

Laboratory/Supplier	Role and Accreditation Details
Alpha Analytical, Inc. Mansfield, New Jersey	MLV Study Participant Laboratory (accredited by ANAB for aqueous and solid matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 05/30/2023)
Battelle Memorial Institute Norwell, Massachusetts	MLV Study Participant Lab (accredited by PJLA for aqueous, solid, and tissue matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 03/31/2023)
California Department of Toxic Substances Control Pasadena, California	MLV Study Participant Laboratory (accredited by A2LA under ISO/IEC 17025:2017; began collecting data for PFAS method validation/verification and plan to add PFAS to accreditation scope in near future; valid until October 31, 2023)
ERA, A Waters Company Golden, Colorado	Study Sample Preparation (accredited by a PTPA under the NEPTP; ISO/IEC 17043:2010 – General requirements for proficiency testing; ISO/IEC 17034:2016 – General requirements for competence of reference material producers; ISO/IEC 17025:2017 – General requirements for the competence of testing and calibration laboratories; and ISO 9001:2015 – Quality management systems-requirements)
Eurofins Lancaster Laboratories Environmental, LLC Lancaster, Pennsylvania	MLV Study Participant Laboratory (accredited by A2LA for aqueous and solid matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 11/30/2024)
Eurofins-TestAmerica, Sacramento West Sacramento, California	MLV Study Participant Laboratory (accredited by ANAB for aqueous, solid, and tissue matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 01/20/2024)
GEL Laboratories, Inc. Charleston, South Carolina	MLV Study Participant Laboratory (accredited by A2LA for AFFF, aqueous, solid, and tissue matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 06/30/2023)
Gulf Coast Analytical Laboratories, LLC dba Pace Analytical Baton Rouge, Louisiana	MLV Study Participant Laboratory (accredited by PJLA for AFFF, aqueous, and solid matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 01/31/2023)
Maryland Department of Health Baltimore, Maryland	MLV Study Participant Laboratory (accredited by A2LA under ISO/IEC 17025:2017 for Determination of PFAS in Aqueous, Solid, Biosolids, and Tissues Samples by Aqueous, Solid, Biosolids, and Tissues [Draft EPA Method 1633] until 05/31/2024)
SGS North America, Inc. Orlando, Florida	MLV Study Participant Laboratory (accredited by ANAB for aqueous and solid matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 12/15/2024)
SGS AXYS Analytical Services, Ltd. Sydney, British Columbia, Canada	Background PFAS Analysis Laboratory (accredited by ANAB for AFFF. Aqueous, solid, and tissue matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 04/23/2024)
TestAmerica, Denver Arvada, Colorado	Chemical Characterization Analyses Laboratory (accredited by A2LA for aqueous and solid matrices for various general, inorganic, and organic chemistry analyses until 10/31/2023)
Vista Analytical Laboratory El Dorado Hills, California	MLV Study Participant Laboratory (accredited by A2LA for aqueous, solid, and tissue matrices for PFAS by LC MS/MS compliant with Table B-15 of QSM 5.1 or latest version under DoD ELAP until 09/30/2023)

Table 3-2. List of Suppliers and Participating Laboratories (Continued)

Laboratory/Supplier	Role and Accreditation Details
Wellington Laboratories, LLC Overland Park, Kansas	PFAS Reference Standard Mixtures Supplier (accredited by ANAB as Reference Material Producer in accordance with ISO 17034:2016 until 02/17/2023)

Notes:
A2LA = American Association of Laboratory Accreditation
AFFF = Aqueous Film-Forming Foam
ANAB = ANSI [American National Standards Institute] National Accreditation Board
DoD ELAP = U.S. Department of Defense Environmental Laboratory Accreditation Program
EPA = U.S. Environmental Protection Agency
IEC = International Electrotechnical Commission
ISO = International Organization for Standardization
LC MS/MS = liquid chromatography mass spectrometry/mass spectrometry
MLV = Multiple Laboratory Validation
NEPTP = National Environmental Proficiency Testing Program
PFAS = per- and polyfluoroalkyl substances
PJLA = Perry Johnson Laboratory Accreditation, Inc.
PTPA = Proficiency Testing Provider Accreditors
QSM = Quality Systems Manual

Table 4-1. Reference Standard Mixtures

Analyte	Acronym	CASRN	Proposed Mixture	Concentration	Ampoule Size
Method Analytes					
Perfluorobutanoic acid	PFBA	375-22-4		4 µg/mL	
Perfluoropentanoic acid	PFPeA	2706-90-3		2 µg/mL	
Perfluorohexanoic acid	PFHxA	307-24-4		1 µg/mL	
Perfluoroheptanoic acid	PFHpA	375-85-9		1 µg/mL	
Perfluorooctanoic acid	PFOA	335-67-1		1 µg/mL	
Perfluorononanoic acid	PFNA	375-95-1		1 µg/mL	
Perfluorodecanoic acid	PFDA	335-76-2		1 µg/mL	
Perfluoroundecanoic acid	PFUnA	2058-94-8		1 µg/mL	
Perfluorododecanoic acid	PFDoA	307-55-1		1 µg/mL	
Perfluorotridecanoic acid	PFTrDA	72629-94-8		1 µg/mL	
Perfluorotetradecanoic acid	PFTeDA	376-06-7		1 µg/mL	
Perfluorobutanesulfonic acid* (K salt)	PFBS	375-73-5		1 µg/mL	
Perfluoropentansulfonic acid* (K salt)	PFPeS	2706-91-4	Custom Mixture	1 µg/mL	3 mL
Perfluorohexanesulfonic acid[1]* (K salt)	PFHxS	355-46-4		1 µg/mL	
Perfluoroheptanesulfonic acid* (K salt)	PFHpS	375-92-8		1 µg/mL	
Perfluorooctanesulfonic acid[1]* (K salt)	PFOS	1763-23-1		1 µg/mL	
Perfluorononanesulfonic acid* (K salt)	PFNS	68259-12-1		1 µg/mL	
Perfluorodecanesulfonic acid* (K salt)	PFDS	335-77-3		1 µg/mL	
Perfluorododecanesulfonic acid* (K salt)	PFDoS	120226-60-0		1 µg/mL	
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid * (K salt)	4:2FTS	757124-72-4		4 µg/mL	
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid * (K salt)	6:2FTS	27619-97-2		4 µg/mL	
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid * (K salt)	8:2FTS	39108-34-4		4 µg/mL	
N-methyl perfluorooctanesulfonamidoacetic acid[1]	NMeFOSAA	2355-31-9		1 µg/mL	
N-ethyl perfluorooctanesulfonamidoacetic acid[1]	NEtFOSAA	2991-50-6		1 µg/mL	
Perfluorooctanesulfonamide	PFOSA	754-91-6		1 µg/mL	
N-methyl perfluorooctanesulfonamide	NMeFOSA	31506-32-8		1 µg/mL	
N-ethyl perfluorooctanesulfonamide	NEtFOSA	4151-50-2	Custom Mixture	1 µg/mL	3 mL
N-methyl perfluorooctanesulfonamidoethanol	MeFOSE	24448-09-7		10 µg/mL	
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2		10 µg/mL	
3-Perfluoropropyl propanoic acid	3:3 FTCA	356-02-5		4 µg/mL	
2H,2H,3H,3H-Perfluorooctanoic acid	5:3 FTCA	914637-49-3	Custom Mixture	20 µg/mL	3 mL
3-Perfluoroheptyl propanoic acid	7:3 FTCA	812-70-4		20 µg/mL	

Table 4-1. Reference Standard Mixtures (Continued)

Analyte	Acronym	CASRN	Proposed Mixture	Concentration	Ampoule Size
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	Existing Mixture (PFAC-MXF)	2 µg/mL	3 mL
4,8-Dioxa-3*H*-perfluorononanoic acid* (K salt)	ADONA	919005-14-4		2 µg/mL	
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid* (K salt)	9Cl-PF3ONS	756426-58-1		2 µg/mL	
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid* (K salt)	11Cl-PF3OudS	763051-92-9		2 µg/mL	
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	Existing Mixture (PFAC-MXG)	2 µg/mL	3 mL
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5		2 µg/mL	
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6		2 µg/mL	
Perfluoro(2-ethoxyethane)sulfonic acid* (K salt)	PFEESA	113507-82-7		2 µg/mL	
EIS Compounds					
Perfluoro-n-[$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA		Custom Mixture	2000 ng/mL	1.2 mL
Perfluoro-n-[$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA			1000 ng/mL	
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA			500 ng/mL	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA			500 ng/mL	
Perfluoro-n-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA			500 ng/mL	
Perfluoro-n-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA			250 ng/mL	
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA			250 ng/mL	
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA			250 ng/mL	
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA			250 ng/mL	
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	$^{13}C_2$-PFTeDA			250 ng/mL	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid* (Na salt)	$^{13}C_3$-PFBS			500 ng/mL	
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid* (Na salt)	$^{13}C_3$-PFHxS			500 ng/mL	
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid* (Na salt)	$^{13}C_8$-PFOS			500 ng/mL	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonic acid* (Na salt)	$^{13}C_2$-4:2 FTS			1000 ng/mL	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]octanesulfonic acid* (Na salt)	$^{13}C_2$-6:2 FTS			1000 ng/mL	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]decanesulfonic acid* (Na salt)	$^{13}C_2$-8:2 FTS			1000 ng/mL	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	$^{13}C_8$-PFOSA			500 ng/mL	
N-methyl-d_3-perfluoro-1-octanesulfonamide	d_3-N-MeFOSA			500 ng/mL	
N-ethyl-d_5-perfluoro-1-octanesulfonamide	d_5-N-EtFOSA			500 ng/mL	
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	d_3-N-MeFOSAA			1000 ng/mL	
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	d_5-N-EtFOSAA			1000 ng/mL	
N-methyl-d_7-perfluorooctanesulfonamidoethanol	d_7-N-MeFOSE			5000 ng/mL	
N-ethyl-d_9-perfluorooctanesulfonamidoethanol	d_9-N-EtFOSE			5000 ng/mL	
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA			2000 ng/mL	

Table 4-1. Reference Standard Mixtures (Continued)

Analyte	Acronym	CASRN	Proposed Mixture	Concentration	Ampoule Size
NIS Compounds					
Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid	$^{13}C_3$-PFBA			1000 ng/mL	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA			500 ng/mL	
Perfluoro-1-hexane[$^{18}O_2$]sulfonic acid* (Na salt)	$^{18}O_2$-PFHxS			500 ng/mL	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA		Custom Mixture	500 ng/mL	1.2 mL
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid	$^{13}C_5$-PFNA			250 ng/mL	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA			250 ng/mL	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanesulfonic acid* (Na salt)	$^{13}C_4$-PFOS			500 ng/mL	

Notes:
*Concentration of the salt is listed
[1]Includes branched and linear isomers
CASRN = Chemical Abstracts Service Registry Number
EIS = extracted internal standard
µg/mL = micrograms per milliliter
mL = milliliters
ng/mL = nanograms per milliliter
NIS = non-extracted internal standard

Table 4-2. Summary of Sample Matrices Included in the Study

Project Phase	Medium	Characteristics	Number of Sample Matrices	Sample Matrix[1]
Phase 3	Purified or Reagent Water	Aqueous medium, no detectable PFAS contamination	19[2]	Provided by each laboratory
	Wetted Ottawa Sand	Solid medium, no detectable PFAS contamination	19[2]	
	Clean Tissue	Tissue medium, no detectable PFAS contamination	19[2]	
Phase 4	Groundwater	No special characteristics, collected from field sites with PFAS in groundwater	3	Matrices collected from multiple sources, with homogenization, spiking, and aliquoting performed by Study Sample vendor.
	Surface Water	River, reservoir, and marine (salt) water, not further characterized	3	
	Wastewater	One or more of the following: TSS > 40 mg/L, TDS > 100 mg/L, Oil and grease > 20 mg/L, $CaCO_3$ > 140 mg/L	7	
	Landfill Leachate	Municipal solid waste and construction debris landfill leachate	3	
	Sediment	Marine, freshwater low TOC, freshwater high TOC	3	
	Biosolids	Municipal biosolids	3	
	Soil	NAPT soils	3	
	Fish/Shellfish Tissue	Low lipid and high lipid samples	3	

Notes:

[1]Detailed sample matrix descriptions provided in Attachment 2.

[2]Includes 14 sample matrices for the Method Detection Limit Study, 1 sample matrix for the limit of quantitation verification, and 4 sample matrices for the on-going precision and recovery.

$CaCO_3$ = calcium carbonate

mg/L = milligram per liter

NAPT = North American Proficiency Testing Program

PFAS = per- and polyfluoroalkyl substances

TDS = total dissolved solids

TOC = total organic carbon

TSS = total suspended solids

Table 4-3. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards, and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
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
Perfluoropentansulfonic 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		
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
1H,1H, 2H, 2H-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-3H-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

Table 4-3. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards, and Non-extracted Internal Standards[1] (Continued)

Target Analyte Name	Abbreviation	CAS Number
Ether sulfonic acids		
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OudS	763051-92-9
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Fluorotelomer carboxylic acids		
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5
2H,2H,3H,3H-Perfluorooctanoic acid	5:3FTCA	914637-49-3
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4
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	
1H,1H,2H,2H-Perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonic acid	$^{13}C_2$-4:2FTS	
1H,1H,2H,2H-Perfluoro-1-[1,2-$^{13}C_2$]octanesulfonic acid	$^{13}C_2$-6:2FTS	
1H,1H,2H,2H-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-ethyl-d$_5$-perfluoro-1-octanesulfonamide	D$_5$-NEtFOSA	
N-methyl-d$_3$-perfluoro-1-octanesulfonamide	D$_3$-NMeFOSA	
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	

Table 4-3. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards, and Non-extracted Internal Standards[1] (Continued)

Notes:

NIS and EIS compounds do not have CASRN.

[1]The target analyte names are for the acid and neutral forms of the analytes. See Table 8 in the Draft Method 1633, Analysis of PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS (Reference 7.1) for the names and CASRN of the corresponding anion forms, where applicable.

CAS = Chemical Abstracts Service

CASRN = Chemical Abstracts Service Registry Number

EIS = Extracted Internal Standard

LC-MS/MS = liquid chromatography mass spectrometry/mass spectrometry

NIS =Non-extracted Internal Standard

PFAS = Per- and Polyfluoroalkyl Substances

Table 4-4. Sample Analysis Method Summary, Sample Containers, Preservation, and Hold Times

Analyte	Analytical Method	Container	Preservation	Minimum Sample Volume/Mass	Holding Time
Aqueous Samples					
Alkalinity (total, carbonate, and bicarbonate)	SM 2320B	250 mL Plastic	$\leq 6°C$	100 mL	14 days
Ammonia	EPA 350.1	250 mL Amber Glass	$\leq 6°C$, H_2SO_4	250 mL	28 days
Calcium, sodium	SW 6010C	250 mL Plastic	$\leq 6°C$, HNO_3	50 mL	6 months
Chloride, sulfate	SW 9056A	250 mL Plastic	$\leq 6°C$	100 mL	28 days
Conductivity	SW 9050A	250 mL Plastic	$\leq 6°C$	50 mL	28 days
Oil and grease	EPA 1664B	250 mL Amber Glass	$\leq 6°C$, H_2SO_4	1 Liter	28 days
pH	SW 9040C	250 mL Plastic	$\leq 6°C$	100 mL	Immediately
Total dissolved solids	SM 2540C	250 mL Plastic	$\leq 6°C$	100 mL	7 days
Total suspended solids	SM 2540D	250 mL Plastic	$\leq 6°C$	100 mL	7 days
Soil and Sediment Samples					
Grain Size*	ASTM D422	16 oz Plastic or Glass	None	500 g	Not defined
Moisture	ASTM D2216	4 oz Glass	$\leq 6°C$	20 g	1 year
pH	SW 9045D	4 oz Glass	$\leq 6°C$	50 g	Immediately
Salinity *(sediment only)*	SM 2520B	4 oz Glass	$\leq 6°C$	50 g	6 months
Total Organic Carbon	SW 9060A	4 oz Glass	$\leq 6°C$	10 g	28 days
Tissue					
Lipids	SM 2540B	4 oz Glass	$\leq 6°C$	20 g	1 year
Biosolids					
pH	SW 9045D	4 oz Glass	$\leq 6°C$	50 g	Immediately

Notes:

*Grain size analysis will be conducted if sufficient mass of natural soil/sediment matrices was provided to ERA. An estimated 757.5 g of soil/sediment is needed to create the Study Samples (spiked and unspiked) for the MLV Study and to include additional soil/sediment for moisture testing.

EPA Methods – USEPA Methods for Chemical Analysis of Water and Wastes (MCAWW) USEPA/600/4-79-020, Revised March 1983.

SW Methods – USEPA Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, Third Edition, 1998.

SM Methods – Standard Methods for the Examination of Water and Wastewater, Revised 2018.

ASTM Methods – ASTM International, Revised 2019.

°C = degrees Celsius	g = grams	L = milliliter	oz = ounce
EPA = U.S. Environmental Protection Agency	HNO_3 = nitric acid	mL = milliliter	
ERA = ERA, A Waters Company	H_2SO_4 = sulfuric acid	MLV = multi-laboratory validation	

Table 4-5. List of Study Samples to Be Provided to Laboratories

Matrix	Individual Samples Collected	Number of Unspiked Samples	Number of Replicates Spiked at Low Level[1]	Number of Replicates Spiked at High Level[1,2]	Total Number of Samples	Spiked Volume/Mass to Be Provided for Each Study Sample
Water						
Wastewater	7	1	3	3	49	500 mL
Groundwater	3	1	3	3	21	500 mL
Surface Water	3	1	3	3	21	500 mL
Landfill Leachate	3	1	3	3	21	100 mL
Soil/Sediment						
Soil	3	1	3	3	21	5 g[3]
Sediment	3	1	3	3	21	5 g[3]
Biosolids	3	1	3	3	21	0.5 g[3]
Tissue						
Fish	3	1	3	3	21	2 g
Total Number					196	

Notes:

[1]Low and high levels for spiking defined in analytical method "Analysis of Analysis of PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS" (Reference 7.2).

[2]An exception applies to fluorotelomer sulfonic acids spiked at 1.5 times the low level.

[3]An additional 10 g of unspiked matrix to be provided for each sample in a separate container for percent moisture determination.

g = grams

LC-MS/MS = liquid chromatography mass spectrometry/mass spectrometry

mL = milliliter

PFAS = Per- and Polyfluoroalkyl Substances

FIGURE
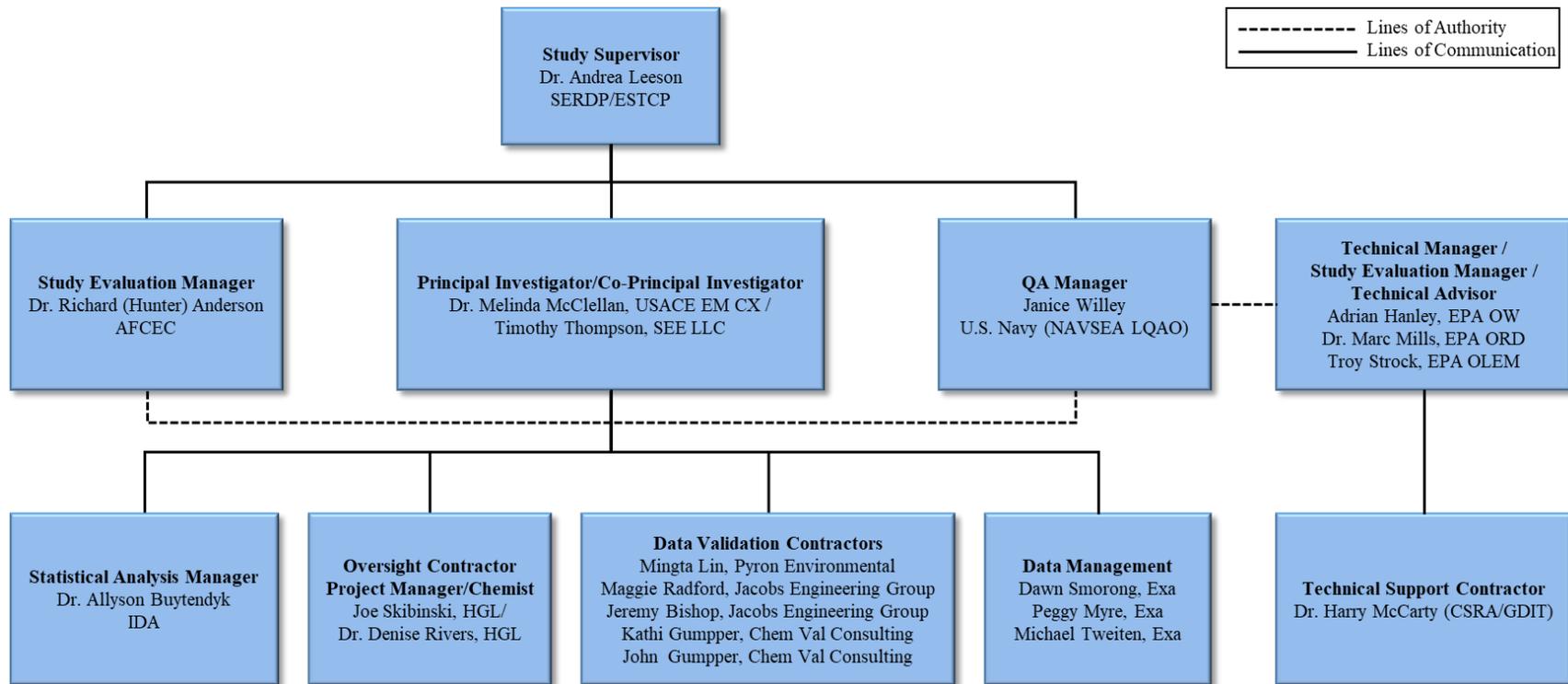
Figure 3-1. Organizational Chart

ATTACHMENT 1

STUDY METHOD (ANNOTATED VERSION OF EPA DRAFT METHOD 1633)

(Dated 10/21/21 and Update Dated 02/11/22)

<u>MLV Study Method Update</u>

On February 8, 2022 the EPA OW released an updated Errata Sheet relating to EPA Draft Method 1633. The changes made to the method as a result of this Errata Sheet apply to the MLV Study Method as well, with one exception. The changes made to Sections 8.2.3 and 8.3.2 relating to laboratory sample storage requirements DO NOT apply to the MLV Study Method. The MLV Study note in Section 8.4.3 of the MLV Study Method still applies; all study samples must be stored at $\leq$ -20°C.

Errata Sheet: https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas

MLV Study Method
Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

October 2021

Notice

This document represents a draft of a PFAS method currently under development by the EPA Office of Water, Engineering and Analysis Division (EAD), in conjunction with the Department of Defense (DOD). **This method is not approved for Clean Water Act compliance monitoring until it has been proposed and promulgated through rulemaking.**

A single-laboratory validation of the procedure has been completed and the report on the results of that study is being prepared. Historically, EAD posts draft methods on the Clean Water Act website after the single-laboratory validation report is completed. However, due to a large number of public and stakeholder requests, this method is being posted on the web before the single-laboratory validation study report is finalized. A revision of this draft method with a later publication date may be issued at that time. No procedural changes are expected as a result of the single-laboratory validation, but some of the performance data (which are presented only as examples) may change once the statistical analysis of the single-laboratory validation data is completed.

This draft method has been subjected to multiple levels of review across several EPA Program Offices. DOD expects to begin a multi-laboratory validation study of the procedure in late 2021, in collaboration with the Office of Water and the Office of Land and Emergency Management.

The Office of Water will use the results of the multi-laboratory validation study to finalize the method and add formal performance criteria. The method validation process may eliminate some of the parameters listed in this draft method.

In the meantime, the Office of Water is releasing this draft on its web site. Laboratories, regulatory authorities, and other interested parties are encouraged to review the method, and where appropriate, utilize it for their own purposes, with the explicit understanding that this is a draft method, subject to revision.

Acknowledgements

This draft method was prepared under the direction of Adrian Hanley of the Engineering and Analysis Division, Office of Science and Technology, within EPA's Office of Water, in collaboration with the Department of Defense.

EPA acknowledges the support of a number of organizations in the development and validation of this draft method, including the developers of the original procedure, the Department of Defense, the members of EPA's workgroup, and EPA's support contractor staff at General Dynamics Information Technology, including:

Disclaimer

See the notice on the title page regarding the status of this method.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Contact

Please address questions, comments, or suggestions to:

CWA Methods Team, Engineering and Analysis Division (4303T)
Office of Science and Technology
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Washington, DC 20460

https://www.epa.gov/cwa-methods
https://www.epa.gov/cwa-methods/forms/contact-us-about-cwa-analytical-methods

Table of Contents

**MLV Study Method - Analysis of Per- and Polyfluoroalkyl Substances (PFAS)
in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS**

1.0   Scope and Application

1.1   Method 1633 is for use in the Clean Water Act (CWA) for the determination of the per- and
polyfluoroalkyl substances (PFAS) in Table 1 in aqueous, solid (soil, biosolids, sediment) and tissue
samples by liquid chromatography/mass spectrometry (LC-MS/MS).

1.2   The method calibrates and quantifies PFAS analytes using isotopically labeled standards. Where
linear and branched isomers exist in the sample and either qualitative or quantitative standards
containing branched and linear isomers are commercially available, the PFAS analyte is reported as
a single analyte consisting of the sum of the linear and branched isomer concentrations.

1.3   The instrumental portion of this method is for use only by analysts experienced with LC-MS/MS or
under the close supervision of such qualified persons. Each laboratory that uses this method must
demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.4   By their very nature, many components of PFAS present analytical challenges unique to this class
of analytes. For example, PFAS analytes readily adhere to the walls of the sample containers and
may also stratify in the container. EPA has included procedures in the method that must be
employed to address such challenges (see Section 11.0 and Appendices A and B).

1.5   This method is "performance-based," which means that modifications may be made without
additional EPA review to improve performance (e.g., overcome interferences, or improve the
sensitivity, accuracy, or precision of the results) *provided that* all performance criteria in this
method are met. Requirements for establishing equivalency are in Section 9.1.2 and include
9.1.2.2c. For CWA uses, additional flexibility is described at 40 CFR 136.6. Changes in
performance, sensitivity, selectivity, precision, recovery, etc., that result from modifications within
the scope of 40 CFR Part 136.6, and Section 9.0 of this method must be documented, as well as
how these modifications compare to the specifications in this method. Changes outside the scope
of 40 CFR Part 136.6 and Section 9.0 of this method may require prior review or approval.

***   MLV Study: Participating laboratories must follow this method without modification.

2.0   Summary of Method

Environmental samples are prepared and extracted using method-specific procedures. Sample extracts
are subjected to cleanup procedures designed to remove interferences. Analyses of the sample extracts
are conducted by LC-MS/MS in the multiple reaction monitoring (MRM) mode. Sample concentrations
are determined by isotope dilution or extracted internal standard quantification (see Section 10.3) using
isotopically labeled compounds added to the samples before extraction.

2.1   Extraction

2.1.1   Aqueous samples are spiked with isotopically labeled standards, extracted using
solid-phase extraction (SPE) cartridges and undergo cleanup using carbon before
analysis.

2.1.2   Solid samples are spiked with isotopically labeled standards, extracted into basic
methanol, and cleaned up by carbon and SPE cartridges before analysis.

2.1.3   Tissue samples are spiked with isotopically labeled standards, extracted in potassium hydroxide and acetonitrile followed by basic methanol, and cleaned up by carbon and SPE cartridges before analysis.

2.2   This method measures the analytes as either their anions or neutral forms. The default approach for Clean Water Act uses of the method is to report the analytes in their acid or neutral forms, using the equations in Section 15.2, although the differences between the anion and acid form concentrations are minimal (See Table 8). Other project-specific reporting schemes may be used where required.

*** MLV Study participants must quantify and report the analytes in their acid form.

2.3   Individual PFAS analytes are identified through peak analysis of the quantification and confirmation ions, where applicable.

2.4   Quantitative determination of target analyte concentrations is made with respect to an isotopically labeled PFAS standard; the concentrations are then used to convert raw peak areas in sample chromatograms to final concentrations.

2.5   Results for target analytes are recovery corrected by the method of quantification (i.e., either isotope dilution or extracted internal standard quantification, see Section 10.3). Isotopically labeled compound recoveries are determined by comparison to the responses of one of seven non-extracted internal standards (a.k.a., the "recovery" standards) and are used as general indicators of overall analytical quality.

2.6   The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and LC-MS/MS systems.

3.0   Definitions

Definitions are provided in the glossary at the end of this method.

4.0   Contamination and interferences

4.1   Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and elevated baselines causing misinterpretation of chromatograms. Specific selection of reagents and solvents may be required.

4.2   Clean all equipment prior to, and after each use to avoid PFAS cross-contamination. Typical cleaning solvents used include water, methanol, and methanolic ammonium hydroxide. The residual PFAS content of disposable plasticware and filters must be verified by batch/lot number and may be used without cleaning if PFAS levels are less than half the Minimum Level (ML, see Table 6).

*** MLV Study: Minimum Level (ML) is equivalent to Limit of Quantitation (LOQ).

4.2.1   All glass equipment that is used in the preparation or storage of reagents is cleaned by washing with detergent and baking in a kiln or furnace (Section 6.2.2). After detergent washing, glassware should be rinsed immediately with reagent water. Prior to use, baked

glassware must be solvent rinsed and then air dried. A solvent rinse procedure using methanolic ammonium hydroxide (1%), toluene, and methanol is recommended.

4.2.2 All parts of the SPE manifold must be cleaned between samples by sonicating in methanolic ammonium hydroxide (1%) and air drying prior to use. Smaller parts, like the needles, adapters, reservoirs, and stopcocks associated with the manifold require rinsing with tap water prior to sonicating in methanolic ammonium hydroxide (1%) and air drying. When in use, after loading the samples but prior to elution procedures, the chamber must be rinsed with methanolic ammonium hydroxide (1%).

4.2.3 All equipment used in the filleting, dissecting, shucking, compositing, and homogenization of tissue must be cleaned with detergent and hot water, then rinsed with ultra-pure water followed by a series of solvent rinses. A typical solvent rinse procedure would be acetone, followed by toluene, and then dichloromethane.

4.3 All materials used in the analysis must be demonstrated to be free from interferences by running method blanks (Section 9.5) at the beginning and with each sample batch (samples started through the extraction process on a given analytical batch to a maximum of 20 field samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix being tested. Ideally, the reference matrix should not contain PFAS in detectable amounts but should contain potential interferents in the concentrations expected to be found in the samples to be analyzed.

4.3.2 For tissue, chicken breast or other similar animal tissue (see Section 7.2.3) may be used as the reference matrix. The laboratory must verify that the source product used does not contain PFAS in detectable amounts.

4.3.3 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.2.1) can be used to simulate water samples and Ottawa sand and/or reagent-grade sand (Section 7.2.2) can be used to simulate soils.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the native PFAS. Because low levels of PFAS are measured by this method, elimination of interferences is essential. The cleanup steps given in Section 12.0 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PFAS at the levels shown in Table 6. The most frequently encountered interferences are fluoropolymers; however, when analyzing whole fish samples, bile salts (e.g., Taurodeoxycholic Acid [TDCA]) can interfere in the chromatography. For this reason, analysis of a standard containing TDCA is required as part of establishing the initial chromatographic conditions (see Sections 10.2.2.5 and 10.3.5).

4.5 Each piece of reusable glassware may be numbered to associate that glassware with the processing of a particular sample. This may assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded.

5.0   Safety

5.1   The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

    5.1.1   PFOA has been described as likely to be carcinogenic to humans. Pure standards should be handled by trained personnel, with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

    5.1.2   It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they must be prepared in a hood, following universal safety measures.

5.2   The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDS) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 1-4. The references and bibliography at the end of Reference 3 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3   Samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling these compounds.

    5.3.1   Facility – When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

    5.3.2   Protective equipment – Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full-face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands.

    5.3.3   Training – Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

    5.3.4   Personal hygiene – Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

    5.3.5   Confinement – Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Waste Handling – Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel should be trained in the safe handling of waste.

5.3.7 Laundry – Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

5.4 Biosolids samples may contain high concentrations of biohazards and must be handled with gloves and opened in a fume hood or biological safety cabinet to prevent exposure. Laboratory staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling biosolids samples.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are for illustration purposes only and no endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here. Meeting the performance requirements of this method is the responsibility of the laboratory.*

6.1 Sampling equipment for discrete or composite sampling

6.1.1 Sample bottles and caps

Note: *Do not use PTFE-lined caps on sample containers.*

6.1.1.1 Liquid samples (waters, sludges, and similar materials containing < 50 mg solids per sample) – Sample bottle, HDPE, with linerless HDPE or polypropylene caps.

Note: *At least two aliquots of aqueous samples are collected to allow sufficient volume for the determination of percent solids and for pre-screening analysis. One aliquot should be collected in a 500-mL container while the second aliquot may be collected in a smaller sample container (e.g., 250-mL or 125-mL).*

*** **MLV Study: Only one sample is received for each sample. Samples are not to be screened by the laboratory. Samples have been prescreened to eliminate excessively high concentrations, where possible. The majority of the analytes will be within the calibration range provided by this method (Table 4). This is one reason why we do not want laboratories to stray significantly from these calibration levels.**

6.1.1.2 Solid samples (soils, sediments, and biosolids that contain more than 50 mg solids) – Sample bottle or jar, wide-mouth, HDPE, 500-mL, with linerless HDPE or polypropylene caps.

*** **MLV Study: Two sample containers will be received for each sample. One will be designated for the determination of % moisture, the other for sample preparation and analysis for PFAS. DO NOT MIX THESE CONTAINERS**

UP. The container designated for the determination of % moisture will not be spiked.

6.1.1.3 Tissue samples – Sample jar, wide-mouth HDPE, 100-mL, with linerless HDPE or polypropylene caps.

6.1.2 Compositing equipment – Automatic or manual compositing system incorporating containers cleaned per bottle cleaning procedure above. Only HDPE tubing must be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing must be thoroughly rinsed with methanol, followed by repeated rinsing with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

6.2 Equipment for glassware cleaning

Note: *If blanks from bottles or other glassware, show no detectable PFAS contamination when using fewer cleaning steps than required above, unnecessary cleaning steps and equipment may be eliminated.*

6.2.1 Laboratory sink with overhead fume hood

6.2.2 Kiln – Capable of reaching 450 ºC within 2 hours and maintaining 450 - 500 ºC ± 10 ºC, with temperature controller and safety switch (Cress Manufacturing Co., Santa Fe Springs, CA, B31H, X31TS, or equivalent). For safety, the kiln or furnace should be vented outside the laboratory, or to a trapping system.

6.3 Equipment for sample preparation

6.3.1 Polyethylene gloves

6.3.2 Laboratory fume hood (of sufficient size to contain the sample preparation equipment listed below)

6.3.3 Glove box (optional)

6.3.4 Tissue homogenizer

6.3.5 Meat grinder – Hobart, or equivalent, with 3- to 5-mm holes in inner plate

6.3.6 Equipment for determining percent moisture

6.3.6.1 Oven – Capable of maintaining a temperature of 110 ± 5 ºC

6.3.6.2 Desiccator

6.3.7 Balances

6.3.7.1 Analytical – Capable of weighing 0.1 mg

6.3.7.2 Top loading – Capable of weighing 10 mg

6.3.8 Aluminum foil

6.3.9 Disposable spoons, 10 mg, polypropylene or stainless steel

6.3.10 Ultrasonic mixer (sonicator)

6.3.11 HDPE bottles, with linerless HDPE or polypropylene caps – 60 mL

6.3.12 pH Paper, range 0-14 - (Whatman® Panpeha™ or equivalent), 0.5-unit readability

6.3.13 Analog or digital vortex mixer, single or multi-tube (Fisher Scientific 02-215-452, or equivalent)

6.3.14 Volumetric flasks, Class A

6.3.15 Disposable polypropylene collection tubes (13 x 100 mm, 8 mL)

6.3.16 Variable speed mixing table (Fisherbrand™ Nutating mixer or equivalent)

6.4 Filtration

6.4.1 Silanized glass wool (Sigma-Aldrich, Cat # 20411 or equivalent) – store in a clean glass jar and rinsed with methanol (2 times) prior to use.

6.4.2 Disposable syringe filter, 25-mm, 0.2-µm Nylon membrane, PALL/Acrodisc or equivalent

6.4.3 Glass fiber filter, 47 mm, 1 µm, PALL A/E or equivalent

6.5 Centrifuge apparatus

6.5.1 Centrifuge (Thermo Scientific Legend RT+, 16 cm rotor, or equivalent), capable of reaching at least 3000 rpm

6.5.2 Centrifuge tubes – Disposable polypropylene centrifuge tubes (50 mL)

6.6 Pipettes

6.6.1 Norm-Ject® syringe (or equivalent), polypropylene/HDPE, 5 mL

6.6.2 Variable volume pipettes with disposable HDPE or polypropylene tips (10 µL to 5 mL) – used for preparation of calibration standards and spiked samples.

6.6.3 Disposable glass pipets

6.6.4 Calibrated mechanical pipettes or Hamilton graduated syringes

6.7 Solid-Phase Extraction

6.7.1 Solid-phase extraction (SPE) cartridges (Waters Oasis WAX 150 mg, Cat # 186002493 or equivalent). The SPE sorbent must have a pKa above 8 so that it remains positively charged during the extraction.

*** MLV Study: Participants must use a 150 mg WAX cartridge.

> *Note:* *SPE cartridges with different bed volume (e.g., 500 mg) may be used; however, the laboratory must demonstrate that the bed volume does not negatively affect analyte absorption and elution, by performing the initial demonstration of capability analyses described in Section 9.2.*

6.7.2 Vacuum manifold for SPE Cartridges (Waters™ extraction manifold #WAT200607 or equivalent)

6.8 Evaporation

6.8.1 Automatic or manual solvent evaporation system (TurboVap® LV or equivalent)

6.8.2 Evaporation/concentrator tubes: 60 mL clear glass vial, 30 x 125 mm, without caps (Wheaton Cat # W226060 or equivalent). Cover with foil if required.

6.9 Vials

6.9.1 Snap cap/crimp top vials, 300 µL, polypropylene (12 x 32 mm) – used in sample pre-screening (DWK Life Sciences Cat # 225180 or equivalent)

6.9.2 Polypropylene crimp/snap vials, 1 mL (Agilent Cat # 5182-0567 or equivalent)

6.9.3 Clear snap cap, PVDC film/white silicone, 11 mm (American Chromatography Supplies Cat # C299-11 or equivalent)

6.9.4 Single step filter vials (Restek Thomson SINGLE StEP® Standard Filter Vials, 0.2 µm Nylon membrane, with Black Preslit caps Cat # 25891 or equivalent) – used in sample pre-screening.

6.10 Instrument

6.10.1 Ultra high-performance liquid chromatograph (UPLC also called UHPLC) or high-performance liquid chromatograph (HPLC) equipped with tandem quadrupole mass spectrometer (Waters Xevo TQ-S Micro or equivalent).

6.10.2 C18 column, 1.7 µm, 50 x 2.1 mm (Waters Acquity UPLC® BEH or equivalent)

6.10.3 Guard column (Phenomenex Kinetex® Evo C18 or equivalent)

6.10.4 Trap/delay column (Purospher Star RP-18 endcapped [3 µm] Hibar® RT 50-4 or equivalent)

6.11 Bottles, HDPE or glass, with linerless HDPE or polypropylene caps. Various sizes. To store prepared reagents.

7.0 **Reagents and standards**

7.1 Reagents

Reagents prepared by the laboratory may be stored in either glass or HDPE containers. Proper cleaning procedures (Section 4.2) must be followed prior to using the containers.

7.1.1 Acetic acid - ACS grade or equivalent, store at room temperature

7.1.2 Acetic acid (0.1%) - dissolve acetic acid (1 mL) in reagent water (1 L), store at room temperature, replace after 3 months.

7.1.3 Acetonitrile – UPLC grade or equivalent, verified before use, store at room temperature

7.1.4 Ammonium acetate - (Caledon Ultra LC/MS grade, or equivalent), store at 2-8° C, replace 2 years after opening date

7.1.5 Ammonium hydroxide - certified ACS+ grade or equivalent, 30% in water, store at room temperature

7.1.6 Aqueous ammonium hydroxide (3%) - add ammonium hydroxide (10 mL, 30%) to reagent water (90 mL), store at room temperature, replace after 3 months

7.1.7 Methanolic ammonium hydroxide

    7.1.7.1 Methanolic ammonium hydroxide (0.3%) - add ammonium hydroxide (1 mL, 30%) to methanol (99 mL), store at room temperature, replace after 1 month

    7.1.7.2 Methanolic ammonium hydroxide (1%) - add ammonium hydroxide (3.3 mL, 30%) to methanol (97 mL), store at room temperature, replace after 1 month

    7.1.7.3 Methanolic ammonium hydroxide (2%) - add ammonium hydroxide (6.6 mL, 30%) to methanol (93.4 mL), store at room temperature, replace after 1 month

7.1.8 Methanolic potassium hydroxide (0.05 M) – add 3.3 g of potassium hydroxide to 1 L of methanol, store at room temperature, replace after 3 months

7.1.9 Methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid - add ammonium hydroxide (3.3 mL, 30%), reagent water (1.7 mL) and acetic acid (0.625 mL) to methanol (92 mL), store at room temperature, replace after 1 month. This solution is used to prepare the instrument blank (Section 7.3.6) and sample dilutions.

7.1.10 Eluent A – Acetonitrile, Caledon Ultra LCMS grade or equivalent

7.1.11 Eluent B - 2 mM ammonium acetate in 95:5 water/acetonitrile. Dissolve 0.154 g of ammonium acetate (Section 7.1.4) in 950 mL of water and 50 mL of acetonitrile (Caledon Ultra LCMS grade, or equivalent). Store at room temperature, shelf life 2 months.

7.1.12 Formic acid - (greater than 96% purity or equivalent), verified by lot number before use, store at room temperature

7.1.13 Formic acid

7.1.13.1 Formic acid (aqueous, 0.1 M) - dissolve formic acid (4.6 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.13.2 Formic acid (aqueous, 0.3 M) - dissolve formic acid (13.8 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.13.3 Formic acid (aqueous, 5% v/v) - mix 5 mL formic acid with 95 mL reagent water, store at room temperature, replace after 2 years

7.1.13.4 Formic acid (aqueous, 50% v/v) - mix 50 mL formic acid with 50 mL reagent water, store at room temperature, replace after 2 years

7.1.13.5 Formic acid (methanolic 1:1, 0.1 M formic acid/methanol) - mix equal volumes of methanol and 0.1 M formic acid, store at room temperature, replace after 2 years

7.1.14 Methanol - (HPLC grade or better, 99.9% purity), verified by lot number before use, store at room temperature

7.1.15 Potassium hydroxide – certified ACS or equivalent, store at room temperature, replace after 2 years

7.1.16 Reagent water – Laboratory reagent water, test by lot/batch number for residual PFAS content

7.1.17 Carbon – EnviCarb® 1-M-USP or equivalent, verified by lot number before use, store at room temperature. Loose carbon allows for better adsorption of interferent organics.

Note: The single-laboratory validation laboratory achieved better performance with loose carbon than carbon cartridges. Loose carbon will be used for the multi-laboratory validation to set statistically based method criteria. Once the method is multi-laboratory validated, laboratories will have the flexibility to use carbon cartridges as long as all method QC criteria are met.

***   MLV Study: Participants must use loose EnviCarb® 1-M-USP or equivalent. The single-laboratory validation laboratory achieved better performance with loose carbon than carbon cartridges. Loose carbon will be used for the multi-laboratory validation to set statistically based method criteria. Once the method is multi-laboratory validated, laboratories will have the flexibility to use carbon cartridges so long as all method QC criteria are met.

7.1.18 Toluene – HPLC grade, verified by lot number before use. Store at room temperature.

7.1.19 Acetone - Pesticide grade, verified by lot number before use in rinsing tissue dissection and processing equipment.

7.1.20 Dichloromethane (methylene chloride), pesticide grade, verified by lot number before use in rinsing tissue dissection and processing equipment.

7.2   Reference matrices – Matrices in which PFAS and interfering compounds are not detected by this method. These matrices are to be used to prepare the batch QC samples (e.g., method blank, and ongoing precision and recovery sample).

    7.2.1   Reagent water – purified water, Type I

    7.2.2   Solids reference matrix – Ottawa or reagent-grade sand

    7.2.3   Tissue reference matrix – chicken breast or similar animal tissue

7.3   Standard solutions – Prepare from materials of known purity and composition or purchase as solutions or mixtures with certification to their purity, concentration, and authenticity. Observe the safety precautions in Section 5.

Purchase of commercial standard solutions or mixtures is highly recommended for this method; however, when these are not available, preparation of stock solutions from neat materials may be necessary. If the chemical purity is 98% or greater, the weight may be used without correction to calculate the concentration of the standard. Dissolve an appropriate amount of assayed reference material in the required solvent. For example, weigh 10 to 20 mg of an individual compound to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with the required solvent. Once the compound is completely dissolved, transfer the solution to a clean vial and cap.

When not being used, store standard solutions in the dark at less than 4 °C unless the vendor recommends otherwise in screw-capped vials with foiled-lined caps. Place a mark on the vial at the level of the solution so that solvent loss by evaporation can be detected. Replace the solution if solvent loss has occurred.

Note: *Native PFAS standards are available from several suppliers. Isotopically labeled compounds are available from Cambridge Isotope Laboratories and Wellington Laboratories, but may also be available from other suppliers. Listing of these suppliers does not constitute a recommendation or endorsement for use. All diluted solutions must be stored in glass or HDPE containers that have been thoroughly rinsed with methanol.*

^{18}O-mass labeled perfluoroalkyl sulfonates may undergo isotopic exchange with water under certain conditions, which lowers the isotopic purity of the standards over time.

The laboratory must maintain records of the certificates for all standards for traceability purposes. Copies of the certificates must be provided as part of the data packages in order to check that proper calculations were performed.

***   **MLV Study: Participating laboratories will receive commercial standard mixtures from the project that must be used for this study when preparing the standards listed in Sections 7.3.1 through 7.3.4. If additional volume is needed, the laboratory must purchase the same standards that were provided by the project.**

    7.3.1   Extracted Internal Standard (EIS) – (a.k.a. isotopically labeled compound) Prepare the EIS solution containing the isotopically labeled compounds listed in Table 3 as extracted internal standards in methanol from prime stocks. An aliquot of EIS solution, typically 50 µL, is added to each sample prior to extraction. Table 3 presents the nominal amounts of EIS compounds added to each sample. The list of isotopically labeled compounds in Table

3 represents the compounds that were available at the time this method was validated. Other isotopically labeled compounds may be used as they become available.

*** **MLV Study: Participating laboratories must use the EIS provided by the project and not change or add any additional isotopically labeled compounds.**

7.3.2 Non-Extracted Internal Standard (NIS) - The NIS solution containing the isotopically labeled compounds listed in Table 3 as non-extracted internal standards is prepared in methanol from prime stock. An aliquot of NIS solution, typically 50 µL, is added to each sample prior to instrumental analysis. Table 3 presents the nominal amounts of NIS compounds added to each sample.

*** **MLV Study: Participating laboratories must use the NIS provided by the project and not change or add any additional isotopically labeled compounds.**

7.3.3 Native Standards Solution - Prepare a spiking solution, containing the method analytes listed in Table 4, in methanol from prime stocks. The solution is used to prepare the calibration standards and to spike the known reference QC samples that are analyzed with every batch. Quantitative standards containing a mixture of branched and linear isomers must be used for method analytes if they are commercially available. Currently, these include PFOS, PFHxS, NMeFOSAA, and NEtFOSAA.

7.3.4 Calibration standard solutions – A series of calibration solutions containing the target analytes and the ^{13}C-, ^{18}O-, and deuterium-labeled extracted internal standards (EIS) and non-extracted internal standards (NIS) is used to establish the initial calibration of the analytical instrument. The concentration of the method analytes in the solutions varies to encompass the working range of the instrument, while the concentrations of the EIS and NIS remain constant. The calibration solutions are prepared using methanol, methanolic ammonium hydroxide (2%), water, acetic acid and the method analyte and isotopically labeled compound standard solutions. After dilution, the final solution will match the solvent mix of sample extracts, which contain methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid (Section 7.1.9). Calibration standard solutions do not undergo solid phase extraction/cleanup.

Concentrations for seven calibration solutions are presented in Table 4. A minimum of six contiguous calibrations standards are required for a valid analysis when using a linear calibration model, with at least five of the six calibration standards being within the quantitation range (e.g., from the LOQ to the highest calibration standard). If a second-order calibration model is used, then a minimum of seven calibration standards are required, with at least six of the seven calibration standards within the quantitation range. The lowest level calibration standard must meet a signal-to-noise ratio of 3:1 and be at a concentration less than or equal to the Limit of Quantitation (LOQ). All initial calibration requirements listed in Table 7 must be met. An instrument sensitivity check (ISC) standard at the concentration of the lowest calibration standard within the quantitation range is required to be analyzed at the beginning of the analytical run (Section 10.3.3.1 and Section 13.3). A mid-level calibration solution is analyzed at least every ten samples or less, on an ongoing basis for the purpose of calibration verification. A mid-level calibration verification (CV) standard must also be analyzed after all sample analyses in order to bracket the analytical batch.

Note: Additional calibration standards, at levels lower than the lowest calibration standard listed in the method, may be added to accommodate a lower limit of quantitation if the instrument

sensitivity allows. Calibration standards at the high end of the calibration may be eliminated if the linearity of the instrument is exceeded or at the low end if those calibration standards do not meet the S/N ratio criterion of 3:1, as long as the required number of calibration points is met. All analytes with commercially available stable isotope analogues must be quantified using isotope dilution.

7.3.5 Qualitative Standards - Standards that contain mixtures of the branched and linear isomers of the method analytes and that are used for comparison against suspected branched isomer peaks in field samples. These qualitative standards are **not** required for those analytes where the quantitative standards in Section 7.3.3 already contain the branched and linear isomers. Qualitative standards that are currently commercially available include PFOA, PFNA, PFOSA, NMeFOSA, NEtFOSA, NEtFOSE, and NMeFOSE.

7.3.6 Instrument Blank – During the analysis of a batch of samples, a solvent blank is analyzed after samples containing high level of target compounds (e.g., calibration, CV) to monitor carryover from the previous injection. The injection blank consists of the solution in Section 7.1.9 fortified with the EIS and NIS for quantitation purposes.

7.3.7 Stability of solutions – Standard solutions used for quantitative purposes (Sections 7.3.1 through 7.3.5) should be assayed periodically (e.g., every 6 months) against certified standard reference materials (SRMs) from the National Institute of Science and Technology (NIST), if available, or certified reference materials from a source that will attest to the authenticity and concentration, to assure that the composition and concentrations have not changed.

7.4 Sodium iodide/cesium iodide mass calibration solution – 2 mg/mL NaI and 50 µg/mL CsI in (1:1) isopropyl alcohol:water (Waters 700000889, or equivalent) or other solution, based on manufacturer's specifications.

7.5 Bile salt interference check standard containing Taurodeoxycholic Acid (TDCA) or Sodium taurodeoxychloate hydrate – (Sigma Aldrich 580221-5GM, or equivalent). This standard is used to evaluate the chromatographic program relative to the risk of an interference from bile salts in tissue samples when using acetonitrile as the mobile phase in the instrument. Prepare solution at a concentration of 1.0 µg/mL in the same solvent as the calibration standards. If using other mobile phases and analyzing tissues, it will be necessary to evaluate taurochenodeoxycholic acid (TCDA) and tauroursodeoxycholic acid (TUDCA) as well.

8.0 Sample collection, preservation, storage, and holding times

8.1 Collect samples in HDPE containers following conventional sampling practices (Reference 5). All sample containers must have linerless HDPE or polypropylene caps. Other sample collection techniques, or sample volumes may be used, if documented.

8.2 Aqueous samples

8.2.1 Samples that flow freely are collected as grab samples or in refrigerated bottles using automatic sampling equipment. Collect 500 mL of sample (other than leachates) in an HDPE bottle.

> *Note: Collect at least two aliquots of all aqueous samples to allow sufficient volume for the determination of percent solids and for pre-screening analysis. That second aliquot may be collected in a smaller sample container (e.g., 250-mL or 125-mL).*
>
> *Because the target analytes are known to bind to the interior surface of the sample container, the entire aqueous sample that is collected must be prepared and analyzed and subsampling avoided whenever possible. Therefore, if a sample volume smaller than 500 mL is to be used for analysis, collect the sample in an appropriately sized HDPE container.*

8.2.2 Leachate samples from landfills can present significant challenges and therefore only 100 mL of sample is collected for the analysis. Collect two 100-mL leachate sample aliquots in a similar manner as described in Section 8.2.1, using appropriately sized containers.

8.2.3 Maintain all aqueous samples protected from light at 0 - 6 ºC from the time of collection until shipped to the laboratory. Samples must be shipped as soon as practical with sufficient ice to maintain the sample temperature below 6 ºC during transport and be received by the laboratory within 48 hours of collection. The laboratory must confirm that the sample temperature is 0 - 6 ºC upon receipt. Once received by the laboratory, the samples must be stored at ≤ -20 ºC until sample preparation.

8.3 Solid (soil, sediment, biosolid), excluding tissue

8.3.1 Collect samples as grab samples using wide-mouth jars and fill no more than ¾ full (see Section 6.1.1.2 for container size and type).

8.3.2 Maintain solid samples protected from light (in HDPE containers) at 0 - 6 ºC from the time of collection until receipt at the laboratory. The laboratory must confirm that the sample temperature is 0 - 6 ºC upon receipt. Once received by the laboratory, the samples must be stored at ≤ -20 ºC until sample preparation.

8.4 Fish and other tissue samples

The nature of the tissues of interest may vary by project. Field sampling plans and protocols should explicitly state the samples to be collected and if any processing will be conducted in the field (e.g., filleting of whole fish or removal of organs). All field procedures must involve materials and equipment that have been shown to be free of PFAS.

8.4.1 Fish may be cleaned, filleted, or processed in other ways in the field, such that the laboratory may expect to receive whole fish, fish fillets, or other tissues for analysis.

8.4.2 If whole fish are collected, wrap the fish in aluminum foil or food-grade polyethylene tubing, and maintain at 0 - 6 ºC from the time of collection until receipt at the laboratory, to a maximum time of 24 hours. If a longer transport time is necessary, freeze the sample before shipping. Ideally, fish should be frozen upon collection and shipped to the laboratory on dry ice.

8.4.3 Once received by the laboratory, the samples must be maintained protected from light at ≤ -20 ºC until prepared. Store unused samples in HDPE containers or wrapped in aluminum foil at ≤ -20 ºC.

*** MLV Study: All study samples will be shipped to the laboratory at ≤ -20 ºC. The laboratories must document the sample temperature upon receipt and note in the case

narrative any samples received at a temperature > 6 ºC (providing their receipt temperatures). When samples are received at >6 ºC, the laboratory must contact HGL before proceeding with analysis. Participating laboratories must store all samples at ≤ -20 ºC (and tissue protected from light) until prepared. THIS APPLIES TO ALL SAMPLES, REGARDLESS OF MATRIX TYPE.

8.5   Holding times

   8.5.1   Aqueous samples (including leachates) should be analyzed as soon as possible; however, samples may be held in the laboratory for up to 90 days from collection, when stored at ≤ -20 ºC and protected from the light. When stored at 0 - 6 ºC and protected from the light, aqueous samples may be held for up to 28 days, with the caveat that issues were observed with certain perfluorooctane sulfonamide ethanols and perfluorooctane sulfonamidoacetic acids after 7 days. These issues are more likely to elevate the observed concentrations of other PFAS compounds via the transformation of these precursors if they are present in the sample.

   8.5.2   Solid samples (soils and sediments) and tissue samples may be held for up to 90 days, if stored by the laboratory in the dark at either 0 - 6 ºC or ≤ -20 ºC, with the caveat that samples may need to be extracted as soon as possible if NFDHA is an important analyte.

***   MLV Study: Given that NFDHA is a target analyte of the study, laboratories must try to extract solid samples as soon as possible.

   8.5.3   Biosolids samples may be held for up to 90 days, if stored by the laboratory in the dark at 0 - 6 ºC or at -20 ºC. Because microbiological activity in biosolids samples at 0 - 6 ºC may lead to production of gases and noxious odors, EPA recommends that samples be frozen if they need to be stored for more than a few days before extraction.

   8.5.4   Store sample extracts in the dark at less than 0 - 4 ºC until analyzed. If stored in the dark at less than 0 - 4 ºC, sample extracts may be stored for up to 90 days, with the caveat that issues were observed for some ether sulfonates after 28 days. These issues may elevate the observed concentrations of the ether sulfonates in the extract over time. Samples may need to be extracted as soon as possible if NFDHA is an important analyte.

***   MLV Study: Laboratories must analyze all extracts within 28 days of preparation. If the 28 day time period is exceeded, the laboratory must discuss the exceedance in the case narrative.

9.0   Quality Control

9.1   Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 6). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with isotopically labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

   If the method is to be applied to a sample matrix other than water (e.g., soils, biosolids, tissue), the appropriate alternative reference matrix (Sections 7.2.2 - 7.2.3) is substituted for the reagent water matrix (Section 7.2.1) in all performance tests.

9.1.1 The laboratory must make an initial demonstration of the ability to generate acceptable precision and recovery with this method. This demonstration is given in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, and to overcome matrix interferences, the laboratory is permitted certain options to improve separations or lower the costs of measurements. These options include alternative extraction, concentration, and cleanup procedures, and changes in sample volumes, columns, and detectors. Alternative determinative techniques and changes that degrade method performance, are *not* allowed without prior review and approval.

*** **MLV Study: Participating laboratories must follow this method without modification.**

Note: For additional flexibility to make modifications without prior EPA review, see 40 CFR Part 136.6.

9.1.2.1 Each time a modification is made to this method, the laboratory is required to repeat the procedure in Section 9.2. If calibration will be affected by the change, the instrument must be recalibrated per Section 10. Once the modification is demonstrated to produce results equivalent or superior to results produced by this method as written, that modification may be used routinely thereafter, so long as the other requirements in this method are met (e.g., isotopically labeled compound recovery).

9.1.2.2 The laboratory is required to maintain records of any modifications made to this method. These records include the following, at a minimum:

a) The names, titles, business addresses, and telephone numbers of the analyst(s) that performed the analyses and modification, and of the quality control officer that witnessed and will verify the analyses and modifications.

b) A listing of pollutant(s) measured, by name and CAS Registry number.

c) A narrative stating reason(s) for the modifications (see Section 1.6).

d) Results from all quality control (QC) tests comparing the modified method to this method, including:

    i. Calibration (Section 10)
    ii. Calibration verification (Section 14.3)
    iii. Initial precision and recovery (Section 9.2.1)
    iv. Isotopically labeled compound recovery (Section 9.3)
    v. Analysis of blanks (Section 9.5)
    vi. Accuracy assessment (Section 9.4)

e) Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

    i. Sample numbers and other identifiers
    ii. Extraction dates
    iii. Analysis dates and times
    iv. Analysis sequence/run chronology

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   v.   Sample weight or volume (Section 11)
   vi.   Extract volume prior to each cleanup step (Section 12)
  vii.   Extract volume after each cleanup step (Section 12)
 viii. Final extract volume prior to injection (Section 12)
   ix.   Injection volume (Section 13.3)
    x.   Dilution data, differentiating between dilution of a sample or extract (Section 15.3)
   xi.   Instrument
  xii.   Column (dimensions, liquid phase, solid support, film thickness, etc.)
 xiii. Operating conditions (temperatures, temperature program, flow rates)
 xiv. Detector (type, operating conditions, etc.)
  xv.   Chromatograms, printer tapes, and other recordings of raw data
 xvi. Quantitation reports, data system outputs, and other data to link the raw data to the results reported

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9.1.2.3 Alternative columns and column systems – If a column or column system other than those specified in this method is used, that column or column system must meet all the requirements of this method.

Note: *The use of alternative columns or programs will likely result in a different elution order.*

9.1.3 Analyses of method blanks are required on an on-going basis to demonstrate the extent of background contamination in any reagents or equipment used to prepare and analyze field samples (Section 4.3). The procedures and criteria for analysis of a method blank are described in Section 9.5.

9.1.4 The laboratory must spike all samples with isotopically labeled compounds to monitor method performance. This test is described in Section 9.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to evaluate whether the performance issue is caused by the sample matrix. Procedures for dilution are given in Section 15.3.

9.1.5 The laboratory must, on an ongoing basis, demonstrate that the analytical system is in control through calibration verification and the analysis of ongoing precision and recovery standards (OPR), spiked at low (LLOPR) and mid-level, and blanks. These procedures are given in Sections 14.1 through 14.7.

9.1.6 The laboratory must maintain records to define the quality of data generated. Development of accuracy statements is described in Section 9.4.

9.2 Initial Demonstration of Capability

9.2.1 Initial precision and recovery (IPR) – To establish the ability to generate acceptable precision and recovery, the laboratory must perform the following operations for each sample matrix type to which the method will be applied by that laboratory.

9.2.1.1 Extract, concentrate, and analyze four aliquots of the matrix type to be tested (Section 7.2.1 through 7.2.3), spiked with 200 µL of the native standard solution (Section 7.3.3), 50 µL of the EIS solution (Section 7.3.1), and 50 µL of NIS solution (Section 7.3.2). At least one method blank, matching the matrix being analyzed, must be prepared with the IPR batch. In the event that more than one MB was prepared and analyzed with the IPR batch, all blank results must be

reported. All sample processing steps that are to be used for processing samples, including preparation and extraction (Sections 11.2 – 11.4), cleanup (Section 12.0) and concentration (Section 12.0), must be included in this test.

9.2.1.2 Using results of the set of four analyses, compute the average percent recovery (R) of the extracts and the relative standard deviation (RSD) of the concentration for each target and EIS compound.

9.2.1.3 For each native and isotopically labeled compound, compare RSD and % recovery with the corresponding limits for initial precision and recovery in Table 5. If RSD and R for all compounds meet the acceptance criteria, system performance is acceptable, and analysis of blanks and samples may begin. If, however, any individual RSD exceeds the precision limit or any individual R falls outside the range for recovery, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 9.2).

*** MLV Study: For this study, the target recovery for method analytes in the IPRs, is 40-150%, the target recovery for EIS compounds is 20-150%, and the target recovery for NIS compounds greater than 30%. If any of the target recoveries are not met in an IPR, rerun the IPR using a fresh aliquot of the extract. If the failure confirms, report the original analysis, if it does not confirm, report the result from the second analysis.

9.2.2 Method detection limit (MDL) - Each laboratory must also establish MDLs for all the analytes using the MDL procedure at 40 CFR Part 136, Appendix B. An MDL determination must be performed for all compounds. The minimum level of quantification (ML) is then calculated by multiplying the MDL by 3.18 and rounding the result to the nearest 1, 2 or 5×10^n, where n is zero or an integer. Example matrix-specific detection limits are listed in Table 6.

9.3 To assess method performance on the sample matrix, the laboratory must spike all samples with the isotopically labeled compound standard solution (Section 7.3.1) and all sample extracts with the NIS spiking solution (Section 7.3.2).

9.3.1 Analyze each sample according to the procedures in Sections 11.0 through 16.0.

9.3.2 Compute the percent recovery of the isotopically labeled compound using the non-extracted internal standard method (Section 15.2) and the equation in Section 14.5.2.

9.3.3 The recovery of each isotopically labeled compound must be within the limits in Table 5. If the recovery of any compound falls outside of these limits, method performance is unacceptable for that compound in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within the normal range after all cleanup procedures have been employed, water samples are diluted, and smaller amounts of soils, biosolids, sediments, and other matrices are prepared and analyzed, per Section 15.3.

*** MLV Study: See MLV Study note in Section 15.3.2 for applicable EIS and NIS criteria and corrective actions.

9.4 Recovery of isotopically labeled compounds from samples must also be assessed and records maintained.

9.4.1 After the analysis of 30 samples of a given matrix type (water, soil, biosolids, tissues, etc.) for which the isotopically labeled compounds pass the tests in Section 9.3, compute the R and the standard deviation of the percent recovery (S_R) for the isotopically labeled compounds only. Express the assessment as a percent recovery interval from R - $2S_R$ to R + $2S_R$ for each matrix. For example, if R = 90% and S_R = 10% for five analyses of soil, the recovery interval is expressed as 70 to 110%.

9.4.2 Update the accuracy assessment for each isotopically labeled compound in each matrix on a regular basis (e.g., after each five to ten new measurements).

9.5 Method blanks – A method blank is analyzed with each sample batch (Section 4.3) to demonstrate freedom from contamination. The matrix for the method blank must be similar to the sample matrix for the batch (e.g., reagent water blank [Section 7.2.1], solids matrix blank [Section 7.2.2], or tissue blank [Section 7.2.3]).

9.5.1 Analyze the cleaned extract (Section 12.0) of the method blank aliquot before the analysis of the OPRs (Section 14.5).

9.5.2 If any PFAS is found in the blank at 1) at a concentration greater than the ML for the analyte, 2) at a concentration greater than one-third the regulatory compliance limit, or 3) at a concentration greater than one-tenth the concentration in a sample in the extraction batch, whichever is greatest, analysis of samples must be halted, and the problem corrected. Other project-specific requirements may apply; therefore, the laboratory may adopt more stringent acceptance limits for the method blank at their discretion. If the contamination is traceable to the extraction batch, samples affected by the blank must be re-extracted and analyzed, provided enough sample volume is available and the sample are still within holding time.

If, continued re-testing results in repeated blank contamination, the laboratory must document and report the failures (e.g., as qualifiers on results), unless the failures are not required to be reported as determined by the regulatory/control authority. Results associated with blank contamination for an analyte regulated in a discharge cannot be used to demonstrate regulatory compliance. QC failures do not relieve a discharger or permittee of reporting timely results.

9.6 The specifications contained in this method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for initial calibration (Section 10.3), calibration verification (Sections 14.2 and 14.3), and for initial (Section 9.2.1) and ongoing (Section 14.5) precision and recovery may be prepared from the same source; however, the use of a secondary source for calibration verification is highly recommended whenever available. If standards from a different vendor are not available, a different lot number from the same vendor can be considered a secondary source. A LC-MS/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for determination of PFAS by this method.

*** MLV Study: If analytes are detected in the blank at concentrations greater than ½ ML (or LOQ) for the analyte, or 2) at concentrations greater than one-tenth the concentration in a sample in the extraction batch, whichever is greatest, a B-flag must be applied to all results for the specific analyte(s)

in the method blank and all affected samples in the associated preparatory batch and discuss the failure in the case narrative.

9.7 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the extracted internal standard method is used.

9.8 Matrix spikes generally are not required for isotope dilution methods because any deleterious effects of the matrix should be evident in the recoveries of the isotopically labeled compounds spiked into every sample. However, because some of the compounds are quantified by a non-analogous isotopically labeled compounds (e.g., PFPeS is quantified by $^{13}C_3$-PFHxS), the analysis of matrix spike samples may help diagnose matrix interferences for specific compounds.

10.0 Calibration and Standardization

10.1 Mass Calibration

The mass spectrometer must undergo mass calibration to ensure accurate assignments of m/z's by the instrument. This mass calibration must be performed at least annually to maintain instrument sensitivity and stability. Mass calibration must be repeated on an as-needed basis (e.g., QC failures, ion masses fall outside of the instrument required mass window, major instrument maintenance, or if the instrument is moved). Mass calibration must be performed using the calibration compounds and procedures prescribed by the manufacturer. The procedures used for mass calibration and mass calibration verification must evaluate an ion range that encompasses the ion range (Q1 and Q2 m/z) of the analytes of interest of this method (Table 2).

Multiple Reaction Monitoring (MRM) analysis is required to achieve better sensitivity than full-scan analysis. The ions to be monitored (Q1 and Q2 m/z) for each native compound, isotopically labeled compound, and NIS are given in Table 2.

10.1.1 During the development of this method, instrumental parameters were optimized for the precursor and product ions listed on Table 2. Product ions other than those listed may be selected; however, the use of ions with lower mass or common ions that may not provide sufficient discrimination between analytes of interest and co-eluting interferences must be avoided.

10.1.2 Optimize the response of the precursor ion [M-H]$^-$ or [M-CO$_2$] for each method analyte following the manufacturer's guidance. MS parameters (e.g., source voltages, source and desolvation temperatures, gas flow, etc.) must be methodically changed until optimal analyte responses are determined. Typically, carboxylic acids have similar MS/MS conditions and sulfonic acids have similar MS/MS conditions. However, since analytes may have different optimal parameters, some compromise on the final operating conditions may be required.

10.1.3 Establish suitable operating conditions using the manufacturer's instructions and use the table below for the MS conditions used during the development of this method as guidance.

Operating Conditions for Waters Acquity UPLC, TQ-S Xevo MS/MS

| Injection volume | 2.0 μL (This is the default volume, and may be changed to improve performance) |

Operating Conditions for Waters Acquity UPLC, TQ-S Xevo MS/MS

	Source Temp (ºC)	140
	Desolvation Temp (°C)	500
MS/MS Conditions	Capillary Voltage (kV)	0.70
	Cone Gas (L/h)	~70
	Desolvation gas (L/h)	~800

10.1.4 In the absence of manufacturer-specific instructions and acceptance criteria, the following procedure may be used for mass calibration.

10.1.4.1 Introduce the NaCsI calibration solution (Section 7.4) to the MS at the flow rate necessary to produce a stable aerosol spray (e.g., 10 µL/min).

10.1.4.2 Scan the MS/MS over the mass range from 20 to 3000 atomic mass units (amu) (or Daltons [Da]). Adjust the source parameters to optimize peak intensity and shape across the mass range. The exact m/z's for NaCsI calibration are:

Calibration Masses (Daltons)

22.9898	922.3552	1971.6149
132.9054	1072.2494	2121.5091
172.8840	1222.1437	2271.4033
322.7782	1372.0379	2421.2976
472.6725	1521.9321	2571.1918
622.5667	1671.8264	2721.0861
772.4610	1821.7206	2870.9803

10.1.4.3 Mass calibration is judged on the basis of the presence or absence of the exact calibration masses (e.g., a limit of the number of masses that are "missed"). Absent vendor-specific instructions, all masses from 22.9898 to 1971.6149 must be present. If peaks in this range are missing or not correctly identified, adjust the MS/MS, and repeat the test. Only after the MS/MS is properly calibrated may standards, blanks, and samples be analyzed.

10.1.4.4 Mass spectrometer optimization – Prior to measurements of a given analyte the mass spectrometer must be separately optimized for that analyte.

10.1.4.5 Using the post-column pump, separately infuse a solution containing 2 - 5 µg/mL of each compound in methanol into the MS.

10.1.4.6 Optimize sensitivity to the product ion m/z for each compound. Precursor-product ion m/z's other than those listed may be used provided requirements in this method are met.

10.1.4.7 After MS calibration and optimization and LC-MS/MS calibration, the same LC-MS/MS conditions must be used for analysis of all standards, blanks, IPR and OPR standards, and samples.

10.1.5 Mass Calibration Verification

A mass calibration verification must be performed following mass calibration, prior to standards and samples analysis. Mass verification checks must also be performed after any subsequent mass calibrations. Each laboratory must follow the instructions for their individual instrument software to confirm the mass calibration, mass resolution and peak relative response. Mass calibration verification must be performed using standards whose mass range brackets the masses of interest (quantitative and qualitative ions).

10.1.5.1 Check the instrument mass resolution to ensure that it is at least unit resolution. Inject a mid-level CAL standard under LC-MS/MS conditions to obtain the retention times of each method analyte. Divide the chromatogram into retention time windows each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M-H]^-$) for the analytes in each window and choose the most abundant product ion. The product ions (also the quantitation ions) chosen during method development are in Table 2, although these will be instrument dependent. Unit resolution is demonstrated when the value of the peak width at half-height is within 0.5 ± 0.1 amu or Da.

10.1.5.2 Check the mass calibration by measuring the amount of peak drift from the expected masses. If the peak apex has shifted more than approximately 0.1 Da, then the instrument will need to be recalibrated following the manufacturer's instructions.

10.2 Chromatographic conditions

10.2.1 The chromatographic conditions should be optimized for compound separation and sensitivity. The same optimized operating conditions must be used for the analysis of all standards, blanks, IPR and OPR standards, and samples. The following table gives the suggested chromatographic conditions for this method using the specified instrument and column. Different instruments may require slightly different operating conditions. **Modification of the solvent composition of the standard or extract by increasing the aqueous content to prevent poor peak shape is not permitted.** The peak shape of early eluting compounds may be improved by increasing the volume of the injection loop or increasing the aqueous content of the initial mobile phase composition.

<div align="center">

General LC Conditions

</div>

Column Temp (°C)	40
Max Pressure (bar)	1100.0

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LC Gradient Program

</div>

Time (min)	Flow mixture [1,2]	Flow Rate Program	Gradient Curve
0.0	2% eluent A, 98% eluent B	0.35 mL/min	Initial
0.2	2% eluent A, 98% eluent B	0.35 mL/min	2
4.0	30% eluent A, 70% eluent B	0.40 mL/min	7
7	55% eluent A, 45% eluent B	0.40 mL/min	8
9	75% eluent A, 25% eluent B	0.40 mL/min	8
10	95% eluent A, 5% eluent B	0.40 mL/min	6
10.4	2% eluent A, 98% eluent B	0.40 mL/min	10
11.8	2% eluent A, 98% eluent B	0.40 mL/min	7
12.0	2% eluent A, 98% eluent B	0.35 mL/min	1

[1] Eluent A = Acetonitrile

[2] Eluent B = 2 mM ammonium acetate in 95:5 water/acetonitrile

> *Note:* *LC system components, as well as the mobile phase constituents, may contain many of the analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration. To minimize the background PFAS peaks and to keep baseline levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times). In addition, priming the mobile phase and flushing the column with at least 90% methanol before initiating a sequence may reduce background contamination.*

10.2.2 Retention time calibration

10.2.2.1 Inject compound solution(s) to determine its retention time. The laboratory may want to inject compounds separately the first time they perform the calibration. All native compounds for which there is an isotopically labeled analog will elute slightly before or with the labeled analog. Store the retention time (RT) for each compound in the data system.

10.2.2.2 Once RT windows have been confirmed for each analyte, once per ICAL and at the beginning of the analytical sequence, the position of each method analyte, EIS analyte, and NIS analyte peaks shall be set using the midpoint standard of the ICAL curve when ICAL is performed. When ICAL is not performed, the initial CV retention times or the midpoint standard of the ICAL curve can be used to establish the RT window position.

10.2.2.3 Method analyte, EIS analyte, and NIS analyte RTs must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CV, whichever was used to establish the RT window position for the analytical batch. All branched isomer peaks identified in either the calibration standard or the qualitative (technical grade) standard must fall within in the retention time window for that analyte.

10.2.2.4 For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within 0.1 minutes of the associated EIS.

10.2.2.5 When establishing the chromatographic conditions, it is important to consider the potential interference of bile salts during analyses of tissue samples. Inject a standard containing TDCA (Section 7.5 if the mobile phase is not acetonitrile) during the retention time calibration process and adjust the conditions to ensure that TDCA (or TCDCA and TUDCA) does not coelute with any of the target analytes, EIS, or NIS standards. Analytical conditions must be set to allow a separation of at least 1 minute between the bile salts and PFOS.

10.3 Initial calibration

Initial calibration is performed using a series of at least six solutions, with at least five of the six calibration standards being within the quantification. (If a second-order calibration model is used, then one additional concentration is required.) The initial calibration solutions contain the entire suite of isotopically labeled compounds, NISs, and target compounds. Calibration is verified with a calibration verification (CV) standard at least once every ten field samples or less, by analysis of a mid-level calibration solution. Calibration verification uses the mean RRs or RFs determined from the initial calibration to calculate the analyte concentrations in the verification standard.

Note: Six calibration standards is the minimum number that must be used in the initial calibration; however, the laboratory may use more standards, as long as the criteria in Section 10.3.3.3 can be met.

Prior to the analysis of samples, and after the mass calibration check has met all criteria in Section 10.1.4, each LC-MS/MS system must be calibrated at a minimum of 6 standard concentrations (Section 7.3.4 and Table 4). This method procedure calibrates and quantifies 40 PFAS target analytes, using the isotopically labeled compounds added to the sample prior to extraction, by one of two approaches:

- True isotope dilution quantification (ID), whereby the response of the target compound is compared to the response of its isotopically labeled analog. Twenty-four target compounds are quantified in this way.

- Extracted internal standard quantification (EIS), whereby the response of the target compound is compared to the response of the isotopically labeled analog of another compound with chemical and retention time similarities. Sixteen target compounds are quantified in this way.

*** MLV Study: Prepare calibration standards containing the native compounds, EISs, and NISs, at the concentrations described in Table 4. If lower LOQs can be achieved, additional lower concentration standards can be added to the calibration. Similarly, standards can be eliminated from the high end of the calibration if the instrument's linear range is exceeded, however, elimination of standards at the high end may result in additional dilutions of samples being required due to quantification range exceedances.

10.3.1   Initial calibration frequency

Each LC-MS/MS system must be calibrated whenever the laboratory takes corrective action that might change or affect the initial calibration criteria, or if either the CV or Instrument Sensitivity Check (ISC) acceptance criteria have not been met.

10.3.2   Initial calibration procedure

Prepare calibration standards containing the native compounds, EISs, and NISs, at the concentrations described in Table 4. Analyze each calibration standard by injecting 2.0 µL (this volume may be changed to improve performance).

Note: The same injection volume must be used for all standards, samples, blanks, and QC samples.

10.3.3   Initial calibration calculations

10.3.3.1   Instrument sensitivity

Sufficient instrument sensitivity is established if a signal-to-noise ratio ≥ 3:1 can be achieved when analyzing the lowest concentration standard within the quantitation range that the laboratory includes in its assessment of calibration linearity (Table 4).

10.3.3.2   Response Ratios (RR) and Response Factors (RF)

The response ratio (RR) for each compound calibrated by isotope dilution is calculated according to the equation below, separately for each of the calibration standards, using the areas of the quantitation ions (Q1) with the m/z shown in Table 2. RR is used for the 24 compounds quantified by true isotope dilution.

$$RR = \frac{Area_n \, M_l}{Area_l \, M_n}$$

where:
$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS
$Area_l$ = The measured area at the Q1 m/z for the corresponding isotopically labeled PFAS added to the sample before extraction
M_l = The mass of the isotopically labeled compound in the calibration standard
M_n = The mass of the native compound in the calibration standard

Similarly, the response factor (RF) for each unlabeled compound calibrated by extracted internal standard is calculated according to the equation below. RF is used for the 16 compounds quantified by extracted internal standard.

$$RF = \frac{Area_s \, M_{EIS}}{Area_{EIS} \, M_s}$$

where:
$Area_s$ = The measured area of the Q1 m/z for the target (unlabeled) PFAS
$Area_{EIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the extracted internal standard (EIS)
M_{EIS} = The mass of the isotopically labeled PFAS used as the extracted internal standard (EIS) in the calibration standard
M_s = The mass of the target (unlabeled) PFAS in the calibration standard

A response factor (RF_s) is calculated for each isotopically labeled compound in the calibration standard using the equation below. RF_s is used for the 24 isotopically labeled compounds quantified by non-extracted internal standard.

$$RF_s = \frac{Area_l \, M_{NIS}}{Area_{NIS} \, M_l}$$

where:
$Area_l$ = The measured area of the Q1 m/z for the isotopically labeled PFAS standard added to the sample before extraction
$Area_{NIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the non-extracted internal standard (NIS)
M_{NIS} = The mass of the isotopically labeled compound used as the non-extracted internal standard (NIS) in the calibration standard
M_l = The mass of the isotopically labeled PFAS standard added to the sample before extraction

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Note: *Other calculation approaches may be used, such as linear regression or non-linear regression based on the capability of the data system used by the laboratory.*

10.3.3.3 Instrument Linearity

One of the following two approaches must be used to evaluate the linearity of the instrument calibration:

Option 1: Calculate the relative standard deviation (RSD) of the RR or RF values of the six initial calibration standards for each native compound and isotopically labeled compound. The RSD must be ≤ 20% to establish instrument linearity.

Option 2: Calculate the relative standard error (RSE) of the six initial calibration standards for each native compound and isotopically labeled compound. The RSE for all method analytes must be ≤ 20% to establish instrument linearity.

10.3.4 Initial calibration corrective actions

If the instrument sensitivity or the instrument linearity criteria for initial calibration are not met, inspect the system for problems and take corrective actions to achieve the criteria. This may require the preparation and analysis of fresh calibration standards. All initial calibration criteria must be met before any samples or required blanks are analyzed.

10.3.5 Bile salts interference check

The laboratory must analyze a bile salt interference check standard (See Section 7.5) after the initial calibration, prior to the analysis of tissue samples, to check for interferences caused by bile salts. If an interference is present, the chromatographic conditions must be modified to eliminate the interference from the bile salts (e.g., changing the retention time of TDCA such that it falls outside the retention window for PFOS by at least one minute), and the initial calibration repeated. If tissue sample analyses are not being conducted, this check may be skipped.

*** MLV Study: Participating laboratories must analyze a bile salt interference check standard after the initial calibration, prior to the analysis of any samples, regardless of media type.

11.0 Sample preparation and extraction

For aqueous samples that contain particles and solid samples, percent solids are determined using the procedures in Section 11.1. This section describes the sample preparation procedures for aqueous samples with < 50 mg solids (Section 11.2), solid (soil, sediment or biosolid) samples (Section 11.3) and tissue samples (Section 11.4).

Note: It is highly recommended that the laboratory pre-screens all samples prior to performing the analysis (see Appendix A). For aqueous samples, use the secondary container provided for percent solids to perform the pre-screening. If high levels of PFAS are present in the sample, a lower volume is required for analysis.

The laboratory may subsample the aqueous samples as described in Appendix B; however, subsampling must meet project-specific requirements. The laboratory must notify the client before proceeding with subsampling. Once the laboratory becomes familiar with the levels of

PFAS in the samples for their clients, the samples should be collected in the appropriate sample container size to avoid subsampling. The sample data report must state when subsampling has been employed.

Do not use any fluoropolymer articles or task wipes in these extraction procedures. Use only HDPE or polypropylene wash bottles and centrifuge tubes. Reagents and solvents for cleaning syringes may be kept in glass containers.

*** MLV Study: Participating laboratories are not to pre-screen aqueous sample. The study samples have been pre-screened to ensure analyte concentrations allow for the method extraction procedures to be followed and have been verified to contain < 50 mg solids.

11.1 Determination of Percent Solids

11.1.1 Determination of percent suspended solids – Aqueous liquids and multi-phase samples consisting of mainly an aqueous phase

11.1.1.1 Desiccate and weigh a glass fiber filter (Section 6.4.3) to three significant figures.

11.1.1.2 Filter 10.0 ± 0.02 mL of well-mixed sample through the filter.

11.1.1.3 Dry the filter a minimum of 12 hours at 110 ± 5 ºC and cool in a desiccator.

11.1.1.4 Calculate percent solids as follows:

$$\% \ solids = \frac{weight \ of \ sample \ aliquot \ after \ drying \ (g) - weight \ of \ filter \ (g)}{10 \ g} \ x \ 100$$

11.1.2 Solids (excluding tissues)

11.1.2.1 Weigh 5 to 10 g of sample to three significant figures in a tared beaker.

11.1.2.2 Dry a minimum of 12 hours at 110 ± 5 ºC, and cool in a desiccator.

11.1.2.3 Calculate percent solids as follows:

$$\% \ solids = \frac{weight \ of \ sample \ aliquot \ after \ drying \ (g)}{weight \ of \ sample \ aliquot \ before \ drying \ (g)} \ x \ 100$$

*** MLV Study: Participating laboratories are to determine % Moisture of solids (excluding tissues) using the Waters/ERA designated container for each sample per Waters/ERA instructions.

11.2 Aqueous Sample Processing

This method is applicable to aqueous samples containing up to 50 mg of suspended solids per sample. The procedure requires the preparation of the entire sample. Smaller sample volumes may be analyzed for samples containing solids greater than specified for this method, or when unavoidable due to high level of PFAS; however, subsampling should be avoided whenever possible. Typical sample size is 500 mL; however, sample size may be up to 1000 mL. The

sample is to be analyzed in its entirety and should not be filtered. Leachate samples are analyzed using a 100-mL sample volume. Therefore, they must not be included in the same sample preparation batch as aqueous samples analyzed which are analyzed using 500-mL sample volumes.

11.2.1 Homogenize the sample by inverting the sample 3 – 4 times and allowing the sample to settle. <u>Do not filter the sample</u>. The standard procedure is to analyze the entire sample, plus a basic methanol rinse of the container.

11.2.2 The volume of the aqueous sample analyzed is determined by weighing the full sample bottle and then the empty sample bottle (see Section 12.2). Weigh each sample bottle (with the lid) to 0.1 g.

*** MLV Study: Participating laboratories will record the volume of sample as indicated by Waters/ERA instructions.

11.2.3 Prepare a method blank and two OPRs using PFAS-free water in HDPE bottles. Select a volume of water that is typical of the samples in the batch. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample bottles designated for use as MS/MSD samples with native standard solution (Section 7.3.3) at a concentration 3 to 5 times the background concentration determined during screening of the unspiked sample. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

*** MLV Study: No MS/MSDs samples are to be prepared for this study.

11.2.4 Spike an aliquot of EIS solution (Section 7.3.1) directly into the sample in the original bottle (or subsampled bottle) as well as to the bottles prepared for the QC samples. Mix by swirling the sample container.

11.2.5 Check that the pH is 6.5 ± 0.5. If necessary, adjust pH with 50% formic acid (Section 7.1.13.4) or ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [Section 7.1.6.2]). The extract is now ready for solid-phase extraction (SPE) and cleanup (Section 12.0).

11.3 Solid Sample (excluding tissues) Processing

Use a stainless spoon to mix the sample in its original jar. If it is impractical to mix the sample within its container transfer the sample to a larger container. Remove rocks, invertebrates, and foreign objects. Vegetation can either be removed from the sample before homogenization or cut into small pieces and included in the sample, based on project requirements. Mix the sample thoroughly, stirring from the bottom to the top and in a circular motion along the sides of the jar, breaking particles to less than 1 mm by pressing against the side of the container. The homogenized sample should be even in colour and have no separate layers. Store the homogenized material in its original container or in multiple smaller containers. Determine the percent solids as per Section 11.1.2.

Note: *The maximum sample weight for sediment or soil is 5 g dry weight. The maximum sample weight for biosolids is 0.5 g dry weight.*

Small amounts of reagent free water used for method blanks (10% of sample weight or less) can be added to unusually dry samples. This is an option, not a requirement.

*** MLV Study: The container designated for the determination of percent solids must be used for this determination. This container has not been spiked with analytes, therefore is NOT interchangeable with the container designated for PFAS analysis. Participating laboratories are NOT to add reagent water to dry samples. Samples have been prepared with the appropriate % moisture content. Laboratories are still to add PFAS-free reagent water to QC samples per section 11.3.2 and document the amount added.

11.3.1 Weigh out an aliquot of solid sample, not dried (aliquot should provide 5 g dry weight for soil and sediment or 0.5 g dry-weight for biosolids) into a 50-mL polypropylene centrifuge tube. Because biosolids samples are analyzed with a 0.5-g sample, they must not be included in the same sample preparation batch as solid samples analyzed with 5-g sample masses.

*** MLV Study: Participating laboratories will follow the instructions provided by Waters/ERA with respect to sample amount and record the weight as directed.

11.3.2 Prepare batch QC samples using 5 g of reference solid (Section 7.2.2) wetted with 2.5 g of reagent water for the method blank and two OPRs (use 0.5 g of reference solid with 0.25 g of reagent water for biosolid sample batches). The addition of reagent water to the sand provides a matrix closer in composition to real-world samples. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample aliquots designated for MS/MSD samples with native standard solution (Section 7.3.3) at the concentration 3 to 5 times the background concentration determined during screening of the unspiked sample. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

*** MLV Study: No MS/MSDs samples will be used in this study.

11.3.3 Spike an aliquot of EIS solution (Section 7.3.1) directly into each centrifuge tube containing the aliquoted field and QC samples. Vortex the sample to disperse the standard and allow to equilibrate for at least 30 minutes.

11.3.4 Add 10 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to each centrifuge tube. Vortex to disperse, then shake for 30 minutes on a variable speed mixing table. Centrifuge at 2800 rpm for 10 minutes and transfer the supernatant to a clean 50-mL polypropylene centrifuge tube.

11.3.5 Add 15 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to the remaining solid sample in each centrifuge tube. Vortex to disperse, then shake for 30 minutes on a variable speed mixing table. Centrifuge at 2800 rpm for 10 minutes and decant the supernatant from the second extraction into the centrifuge tube with the supernatant from the first extraction.

11.3.6 Add another 5 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to the remaining sample in each centrifuge tube. Shake by hand to disperse, centrifuge at 2800 rpm for 10 minutes and decant the supernatant from the third extraction into the centrifuge tube with supernatant from the first and second extractions.

11.3.7 Using a 10-mg scoop, add 10 mg of carbon (Section 7.1.17) to the combined extract, mix by occasional hand shaking for no more than five minutes and then centrifuge at 2800 rpm for 10 minutes. Immediately decant the extract into a 60-mL glass evaporation or concentrator tube.

11.3.8 Dilute to approximately 35 mL with reagent water. A separate concentrator tube marked at the 35-mL level may be kept for a visual reference to get the approximate volume. Samples containing more than 50% water may yield extracts that are greater than 35 mL in volume; therefore, do not add water to these. Determine the water content in the sample as follows (percent moisture is determined from the % solids):

$$Water\ Content\ in\ Sample = \frac{Sample\ Weight\ (g) \times Moisture\ (\%)}{100}$$

11.3.9 Concentrate each extract at approximately 55 °C with a N_2 flow of approximately 1.2 L/min to a final volume that is based on the water content of the sample (*see table below*). Allow extracts to concentrate for 25 minutes, then mix (by vortex if the volume is < 20 mL or using a glass pipette if the volume is > 20 mL). Continue concentrating and mixing every 10 minutes until the extract has been reduced to the required volume as specified in the table below. If the extract volume appears to stop dropping, the concentration must be stopped and the volume at which it was stopped recorded.

Water Content in Sample	Concentrated Final Volume
< 5 g	7 mL
5 – 8 g	8 mL
8 – 9 g	9 mL
9 – 10 g	10 mL

Note: *Slowly concentrating extracts, in 1-mL increments, is necessary to prevent excessive concentration and the loss of neutral compounds (methyl and ethyl FOSEs and FOSAs) and other highly volatile compounds. The extract must be concentrated to remove the methanol as excess methanol during SPE clean-up results in poor recovery of C13 and C14 carboxylic acids and C10 and C12 sulfonates.*

11.3.10 Add 40 - 50 mL of reagent water to the extract and vortex. Check that the pH is 6.5 ±0.5 and adjust as necessary with 50% formic acid (Section 7.1.13.4) or 30% ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [Section 7.1.6.2]). The extracts are ready for SPE and cleanup (Section 12.0).

11.4. Tissue Sample Processing

Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish with the skin on, whole fish with the skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the samples must be prepared and homogenized.

If the laboratory must dissect the whole fish to obtain the appropriate tissue for analysis, cover the benchtop with clean aluminum foil and use clean processing equipment (knives, scalpels, tweezers) to dissect each sample to prevent cross-contamination. Samples should be handled in a semi-thawed state for compositing and/or homogenization. All tissue comprising a sample is collected in a stainless-steel bowl during grinding, then mixed using a stainless-steel spoon. Homogenized samples must be stored in clean HDPE containers and stored frozen for subsequent use.

If using a grinder, after the entire sample has been processed, mix the ground tissue with a spoon, transfer back to the grinder, and repeat the grinding at least two more times until the homogenize tissue has a consistent texture and color.

11.4.1 For each sample, weigh a 2-g aliquot of homogenized tissue into a 15-mL polypropylene centrifuge tube. Reseal the container with the remaining homogenized portion of the sample and return it to frozen storage in the event that it needs to be used for reanalysis.

Note: *The default sample weight for tissue is 2 g wet weight; however, a 1-g sample may be used. Higher sample weights are not recommended for this method.*

*** MLV Study: Participating laboratories will follow the instructions provided by Waters/ERA with respect to sample amount and record the weight as directed.

11.4.2 Prepare the batch QC samples using 2 g of reference tissue matrix (Section 7.2.3) for the method blank and two OPRs. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample aliquots designated as MS/MSD samples with native standard solution (Section 7.3.3) at the concentration 3 to 5 times the background concentration determined during screening of the unspiked sample. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

*** MLV Study: No MS/MSDs samples will be prepared for this study.

11.4.3 Spike an aliquot of EIS solution (Section 7.3.1) directly into each field and QC sample. Vortex and allow to equilibrate for at least 30 minutes.

11.4.4 Add 10 mL of 0.05M KOH in methanol (Section 7.1.8) to each sample. Vortex to disperse the tissue then place tubes on a variable speed mixing table to extract for at least 16 hours. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant in a 50-mL polypropylene centrifuge tube.

11.4.5 Add 10 mL of acetonitrile to remaining tissue in the 15-mL centrifuge tube, vortex to mix and disperse the tissue. Sonicate for 30 minutes. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant, adding it to the 50-mL centrifuge tube containing the initial extract.

11.4.6 Add 5 mL of 0.05M KOH in methanol (Section 7.1.8) to the remaining sample in each centrifuge tube. Vortex to disperse the tissue and hand mix briefly. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant, adding it to the 50-mL centrifuge tube containing the first two extracts.

11.4.7 Using a 10-mg scoop, add 10 mg of carbon (Section 7.1.17) to the combined extract, mix by occasional hand shaking over a period of no more than five minutes and then centrifuge at 2800 rpm for 10 minutes. Immediately decant the extract into a 60-mL glass evaporation or concentrator tube.

11.4.8 Add 1 mL of reagent water to each evaporation/concentrator tube, set the evaporator/concentrator to 55 ºC with a N_2 flow of 1.2 L/min and concentrate the extract to 2.5 mL (only ~1 mL of the methanol should remain).

11.4.9 Add reagent water to each evaporation/concentrator tube to dilute the extracts to 50 mL. Check that the pH = 6.5 ± 0.5 and adjust as needed with 50% formic acid (Section 7.1.13.4) or ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [7.1.6.2]). The extracts are ready for SPE and cleanup (Section 12.0).

12.0 Extraction, Cleanup, and Concentration

All matrices (including batch QC) must undergo SPE and carbon cleanup to remove interferences (Section 12.1). Sample elution as well as any further extract treatment is matrix specific and may be found in Sections 12.2 through 12.4.

Note: Carbon cleanup is required. Carbon cleanup may remove analytes if the sample has a very low organic carbon content (this is unusual for non-drinking water environmental samples). This will be apparent if the isotope dilution standard recoveries are significantly higher on the reanalysis. If the laboratory can demonstrate that the carbon cleanup is detrimental to the sample analysis (by comparing results when skipping the carbon cleanup during reanalysis), then the carbon cleanup may be skipped for that specific sample.

***** MLV Study: Participating laboratories must use carbon cleanup on all samples. Loose carbon must be used (carbon cartridges not permitted.)**

12.1 All sample matrices

12.1.1 Pack clean silanized glass wool to half the height of the WAX SPE cartridge barrel (Section 6.7.1).

12.1.2 Set up the vacuum manifold with one WAX SPE cartridge plus a reservoir and reservoir adaptor for each cartridge for each sample and QC aliquot.

12.1.3 Pre-condition the cartridges by washing them with 15 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2) followed by 5 mL of 0.3M formic acid (Section 7.1.13.2) (do not use the vacuum for this step). Do not allow the WAX SPE to go dry. Discard the wash solvents.

12.1.4 Pour the sample into the reservoir (do not use a pipette), taking care to avoid splashing while loading. Adjust the vacuum and pass the sample through the cartridge at 5 mL/min. Retain the empty sample bottle and allow it to air dry for later rinsing (Section 12.2.2). Discard eluate.

12.1.5 Rinse the walls of the reservoir with 5 mL reagent water (twice) followed by 5 mL of 1:1 0.1M formic acid/methanol (Section 7.1.13.5) and pass those rinses through the cartridge using vacuum. Dry the cartridge by pulling air through for 15 seconds. Discard the rinse solution. Continue to the elution and concentration steps based on the matrix (Section 12.2 – Aqueous, Section 12.3 – Solids and Section 12.4 – Tissue).

12.2 Elution and Extract Concentration of Aqueous Samples

Note: *If two cartridges were used, perform Sections 12.2.1 through 12.2.3 with each cartridge. Filter the eluates through a 25-mm, 0.2-μm syringe filter. Combine both sets of filtered eluates into a clean tube, add the NIS solution, and vortex to mix. Transfer 350 μL of the filtered extract into a 1-mL polypropylene microvial and mark the level. Add another 350-μL portion and using a gentle stream of nitrogen (water bath at 40 ºC), concentrate to the 350-μL mark and submit for LC-MS/MS analysis. This concentration step is only applicable to situations where two SPE cartridges were eluted, each with 5 mL of elution solvent.*

12.2.1 Place clean collection tubes (13 x 100 mm polypropylene) inside the manifold, ensuring that the extract delivery needles do not touch the walls of the tubes. DO NOT add NIS to these collection tubes.

12.2.2 Rinse the inside of the sample bottle with 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the SPE reservoir, washing the walls of the reservoir. Use vacuum to pull the elution solvent through the cartridge and into the collection tubes.

Note: *Air dry the empty sample bottle after the rinse is transferred. Weigh the empty bottle with the cap on and subtract from the weight with the sample determined in Section 11.2.2.*

12.2.3 Add 25 μL of concentrated acetic acid to each sample eluted in the collection tubes and vortex to mix. Add 10 mg of carbon (Section 7.1.17) to each sample and batch QC extract, using a 10-mg scoop. Hand-shake occasionally for no more than 5 minutes. It is important to minimize the time the sample extract is in contact with the carbon. Immediately vortex (30 seconds) and centrifuge at 2800 rpm for 10 minutes.

12.2.4 Add NIS solution (Section 7.3.2) to a clean collection tube. Place a syringe filter (25-mm filter, 0.2-μm nylon membrane) on a 5-mL polypropylene syringe. Take the plunger out and carefully decant the sample supernatant into the syringe barrel. Replace the plunger and filter the entire extract into the new collection tube containing the NIS. Vortex to mix and transfer a portion of the extract into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 4 °C.

12.3 Elution and Extract Concentration of Solid Samples

12.3.1 Add NIS solution (Section 7.3.2) to a clean collection tube (13 x 100 mm polypropylene) for each sample and QC aliquot and place them into the manifold rack, ensuring the extract delivery needles are not touching the walls of the tubes.

12.3.2 Rinse the inside of the evaporation/concentrator tube using 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the reservoir, washing the walls of the reservoir. Use the vacuum to pull the elution solvent through the cartridge and into the collection tubes.

12.3.3 Add 25 µL of concentrated acetic acid to each sample extract in its collection tube and swirl to mix. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5 mL polypropylene syringe. Take the plunger out and carefully decant ~1 mL of sample extract into the syringe barrel. Replace the plunger and filter into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 4 °C.

12.4 Elution and Extract Concentration of Tissue Samples

12.4.1 Add NIS solution (Section 7.3.2) to clean collection tubes (13 x 100 mm, polypropylene) for each sample and QC aliquot. Place the tubes into the manifold rack and ensure the extract delivery needles are not touching the walls of the tubes.

12.4.2 Rinse the inside of the evaporation/concentrator tube using 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the reservoir, washing the walls of the reservoir. Use the vacuum to pull the elution solvent through the cartridge and into the collection tubes.

12.4.3 Add 25 µL of concentrated acetic acid to each sample extract. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5 mL polypropylene syringe. Take the plunger out and carefully decant an aliquot (~1 mL) of the sample extract into the syringe barrel. Replace the plunger and filter into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 4 °C.

13.0 Instrumental Analysis

Analysis of sample extracts for PFAS by LC-MS/MS is performed on an ultrahigh performance liquid chromatograph coupled to a triple quadrupole mass spectrometer, running manufacturer's software. The mass spectrometer is run with unit mass resolution in the multiple reaction monitoring (MRM) mode.

13.1 Perform mass calibration (Section 10.1), establish the operating conditions (Section 10.2), and perform an initial calibration (Section 10.3) prior to analyzing samples. If tissue samples are to be analyzed during the analytical shift, repeat the analysis of the bile salts interference check standard in Section 10.3.5 before analyzing any tissue samples.

*** MLV Study: For each analytical shift (daily sequence), analyze the bile salts interference check standard in Section 10.3.5 before analyzing any field samples, regardless of media type.

13.2 Only after all performance criteria are met may blanks, MDLs, IPRs/OPRs, and samples be analyzed.

13.3 After a successful initial calibration has been completed, the analytical sequence for a batch of samples analyzed during the same time period is as follows. The volume injected for samples and QCs must be identical to the volume used for calibration (Section 10.3). Standards and sample

extracts must be brought to room temperature and vortexed prior to aliquoting into an instrument vial in order to ensure homogeneity of the extract.

1. Instrument Blank
2. Instrument Sensitivity Check (see Section 10.3.3.1)
3. Calibration Verification Standard
4. Qualitative Identification Standards
5. Instrument Blank
6. Method Blank
7. Low-level OPR (LLOPR)
8. OPR
9. Bile salts interference check standard (Section 7.5)
10. Samples (10 or fewer)
11. Calibration Verification Standard
12. Instrument Blank
13. Samples (10 or fewer)
14. Calibration Verification Standard
15. Instrument Blank

If the results are acceptable, the closing calibration verification solution (#13 above) may be used as the opening solution for the next analytical sequence.

13.4 If the response exceeds the calibration range for any sample, extracts are diluted as per Section 15.3 to bring all target responses within the calibration range.

Note: *If the analytes that exceed the calibration range in the original analysis are known to not be of concern for the specific project (e.g., are not listed in a discharge permit), then the laboratory may consult with the client regarding the possibility of reporting that sample from the undiluted analysis.*

*** MLV Study: If the response for any analyte exceeds the calibration range for any sample, extracts must be diluted as per Section 15.3 to bring the exceeding analyte(s) response(s) within the calibration range. Method analytes reported from a dilution must be qualified with a "D" data qualifier.

14.0 Performance Tests during Routine Operations

The following performance tests must be successfully completed as part of each routine instrumental analysis shift described in Section 13.3 above.

14.1 MS resolution – A mass calibration must be performed prior to analysis of the calibration curve. LC-MS/MS system performance is checked by performing an MS resolution verification after the mass calibration. MS resolution must be verified prior to any samples or QC as per Section 10.1. If the requirements in Section 10.1 cannot be met, the problem must be corrected before analyses can proceed. If any of the samples in the previous shift may be affected by poor mass resolution, the extracts of those samples must be re-analyzed.

14.2 Instrument sensitivity check

The signal-to-noise ratio of the ISC standard (Section 7.3.4) must be greater than or equal to 3:1. If the requirements cannot be met, the problem must be corrected before analyses can proceed.

Note: *An interim limit of 70-130% for 90% of the native and isotopically labeled compounds should be used, with the other recoveries achieving 50-150%.*

*** MLV Study: Method analytes and EIS compound recoveries must be within 70-130% in the ISC standard.

14.3   Calibration verification (CV)

After a passing MS resolution (Section 14.1) and a successful initial calibration (Section 10.3.3.3) is achieved, prior to the analysis of any samples, analyze a mid-level calibration standard (Section 7.3.4).

14.3.1   The calibration is verified by analyzing a CV standard at the beginning of each analytical sequence, every ten samples or less, and at the end of the analytical sequence.

14.3.2   Calculate concentration for each native and isotopically labeled compound in the CV using the equation in Section 15.2.

14.3.3   The recovery of native and isotopically labeled compounds for the CVs must be within 70 - 130%.

14.3.4   If the CV criterion in Section 14.3.3 is not met, recalibrate the LC-MS/MS instrument according to Section 10.3.

14.4   Retention times and resolution

14.4.1   For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within ± 0.1 minutes of the associated EIS.

14.4.2   The retention times of each native and isotopically labeled compound must be within ± 0.4 minutes of the ICAL or CV used to establish the RT windows for the samples and batch QC.

14.5   Ongoing precision and recovery (OPR)

14.5.1   After verification, analyze the extract of the OPR (Sections 12.2.4, 12.3.3, and 12.4.3) prior to analysis of samples from the same batch to ensure the analytical process is under control.

14.5.2   Compute the percent recovery of the native compounds by the appropriate quantification method depending on the compound (Section 10.3). Compute the percent recovery of each isotopically labeled compound by the non-extracted internal standard method (Sections 1.2 and 10.3).

$$Recovery\ (\%) = \frac{Concentration\ found\ (ng/mL)}{Concentration\ spiked\ (ng/mL)}\ x\ 100$$

14.5.3   For the native compounds and isotopically labeled compounds, compare the recovery to the OPR limits given in Table 5. If all compounds meet the acceptance criteria, system performance is acceptable, and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the given range, the extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-prepare, extract, and clean up the sample batch and repeat the ongoing precision and recovery test.

*** MLV Study: **For this study, the target recovery for method analytes in the OPRs, is 40-150%, the target recovery for EIS compounds is 20-150%, and the target recovery for NIS compounds is greater than 30%. If any of these criteria are not met in an OPR, rerun the OPR using a fresh aliquot of the extract. If the failure confirms, report the original analysis, if it does not confirm, report the result from the second analysis.**

14.5.4 If desired, add results that pass the specifications in Section 14.5.3 to initial and previous ongoing data for each compound in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each compound in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (SR). Express the accuracy as a recovery interval from R - 2SR to R + 2SR. For example, if R = 95% and SR = 5%, the accuracy is 85 to 105%.

14.6 Instrument blank – At the beginning of the analytical sequence and after the analysis of high concentration samples (e.g., highest calibration standard, CV), analyze an instrument blank to ensure no instrument contamination has occurred.

14.7 Method blank – After the analysis of the solvent blank and prior to the analysis of samples, analyze a method blank (Section 9.5).

14.8 A qualitative identification standard (Section 7.3.5) containing all available isomers (branched and linear) is analyzed once daily at the beginning of the analytical sequence, to confirm the retention time of each linear and known branched isomer or isomer group.

14.9 Instrument sensitivity (optional)

This step is recommended as a follow-up step if the ISC does not meet criteria. Compare the NIS peak areas from the QC and field samples to the average area of the corresponding NIS on the calibration standards to check for possible bad injections of NIS solution or loss of instrument sensitivity. The QC and field sample NIS areas should be within 50 – 200% of that in the standards. If the areas are low for all the samples and QC in the batch, it suggests a loss of instrument sensitivity, while low areas on only some QC or field samples suggests a possible bad injection.

*** **MLV Study: If the NIS criteria (>30% of the average area of the corresponding NIS on the calibration standards) is not met for any field or QC sample, reanalyze the sample using a fresh aliquot of the extract. If the failure is not confirmed, report results from the second analysis. If the failure is confirmed, report the original analysis.**

15.0 Data Analysis and Calculations

15.1 Qualitative determination and peak identification

A native or isotopically labeled compound is identified in a standard, blank, sample, or QC sample when all of the criteria in Sections 15.1.1 through 15.1.5 are met.

15.1.1 Peak responses must be at least three times the background noise level (S/N 3:1). If the S/N ratio is not met due to high background noise, the laboratory must correct the issue (e.g., perform instrument troubleshooting to check and if needed, replace, the transfer line,

column, detector, liner, filament, etc.). If the S/N ratio is not met but the background is low, then the analyte is to be considered a non-detect.

15.1.2 Target analyte, EIS analyte, and NIS analyte RTs must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CV, whichever was used to establish the RT window position for the analytical batch. The retention time window used must be of sufficient width to detect earlier-eluting branched isomers. For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within ± 0.1 minutes of the associated EIS.

15.1.3 The laboratory must follow the identification requirements specified by the client for the project. In the event there are no project-specific requirements, the following general requirements apply. For concentrations at or above the method LOQ, the total (branched and linear isomer) quantification ion response to the total (branched and linear isomer) confirmation ion response ratio must fall within ± 50% of the ratio observed in the mid-point initial calibration standard. If project-specific requirements involve reporting sample concentrations below the LOQ or ML, the response ratio must also fall within ± 50% of the ratio observed in the initial daily CV.

***   MLV Study: Since results are required to be reported down to the MDL for this study, the requirement contained in 15.1.3 (project-specific requirements) applies. If ion response ratios fail to meet the any of the criteria stated in Section 15.1.3, the failure must be confirmed through re-analysis. If the failure confirms, the analyte concentration must be qualified with an "I" data qualifier and discussed in the case narrative (providing the % response for each failure).

The response of all isomers in the quantitative standards should be used to define ratio. In samples, the total response should include only the branched isomer peaks that have been identified in either the quantitative or qualitative standard (see Section 7.3 regarding records of traceability of all standards). If standards (either quantitative or qualitative) are not available for purchase, only the linear isomer can be identified and quantitated in samples. The ratio requirement does not apply for PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA because suitable (not detectable or inadequate S/N) secondary transitions are unavailable.

15.1.4 If the field sample result does not all meet the criteria stated in Sections 15.1.2 through 15.1.3, and all sample preparation avenues (e.g., extract cleanup, sample dilution, etc.) have been exhausted, the result may only be reported with a data qualifier alerting the data user that the result could not be confirmed because it did not meet the method-required criteria and therefore should be considered an estimated value. If the criteria listed above are not met for the standards, the laboratory must stop analysis of samples and correct the issue.

15.2 Quantitative determination

Concentrations of the target analytes are determined with respect to the extracted internal standard (EIS) which is added to the sample prior to extraction. The EIS is quantitated with respect to a non-extracted internal standard (NIS), as shown in Table 2, using the response ratios or response factors from the most recent multi-level initial calibration (Section 10.3). Other equations may be used if the laboratory demonstrates that those equations produce the same numerical result as produced by the equations below.

For the native analytes:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_n\ M_l}{Area_l\ (\overline{RR}\ or\ \overline{RF})} \times \frac{1}{W_S}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS). *See note below.*

M_l = The mass of the isotopically labeled compound added (ng)

$\overline{RR}$ = Average response ratio used to quantify target compounds by the isotope dilution method

$\overline{RF}$ = Average response factor used to quantify target compounds by the extracted internal standard method

W_S = Sample volume (L) or weight (g)

Note: *For better accuracy, PFTrDA is quantitated using the average of the areas of labeled compounds $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA.*

And for the EIS analytes:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_l\ M_{nis}}{Area_{nis}\ \overline{RF_s}} \times \frac{1}{W_S}$$

where:

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS)

$Area_{nis}$ = The measured area of the Q1 m/z for the non-extracted internal standard (NIS)

M_{nis} = The mass of the added non-extracted internal standard (NIS) compound (ng)

W_S = Sample volume (L) or weight (g)

$\overline{RF_s}$ = Average response factor used to quantify the isotopically labeled compound by the non-extracted internal standard method

Results for native compounds are recovery corrected by the method of quantification. Extracted internal standard (EIS) recoveries are determined similarly against the non-extracted internal standard (NIS) and are used as general indicators of overall analytical quality.

The instrument measures the target analytes as either their anions or neutral forms. **The default approach for Clean Water Act uses of the method is to report the analytes in their acid or neutral forms,** using the following equation to convert the concentrations:

$$C_{Acid} = C_{Anion} \times \frac{MW_{Acid}}{MW_{Anion}}$$

where:

C_{Anion} = The analyte concentration in anion form

MW_{Acid} = The molecular weight of the acid form

MW_{Anion} = The molecular weight of the anion form

*** MLV Study: MLV Target analytes must be quantified and reported in their acid form.

15.3 Sample dilutions

15.3.1 If the Q1 area for any compound exceeds the calibration range of the system, dilute a subsample of the sample extract with methanol containing 4% water, 1% ammonium

hydroxide, and 0.625% acetic acid (Section 7.1.9) by a factor no greater than 10x adjust the amount of the NIS in the diluted extract, then analyze the diluted extract using the percent recovery of the EIS from the original analysis. If the compound cannot be measured reliably by isotope dilution, dilute and analyze aqueous sample, or analyze a smaller aliquot of soil, biosolid, sediment, or tissue sample. Adjust the compound concentrations, detection limits, and minimum levels to account for the dilution.

15.3.2 If the recovery of any isotopically labeled compound is outside of the acceptance limits (Table 5), a diluted aqueous sample or smaller aliquot (for solids and tissue) must be analyzed (Section 15.3.1). If the recovery of any isotopically labeled compound in the diluted sample is outside of the normal range, the method does not apply to the sample being analyzed and the result may not be reported or used for permitting or regulatory compliance purposes. In this case, an alternative column could be employed to resolve the interference. If all cleanup procedures in this method and an alternative column have been employed and isotopically labeled compound recovery remains outside of the normal range, extraction and/or cleanup procedures that are beyond this scope of this method will be required to analyze the sample.

***   MLV Study: For this study, the target recovery for EIS compounds is 20-150%, and the target recovery for NIS compounds is greater than 30%. If any of these criteria are not met in a sample, rerun the sample using a fresh aliquot of the extract. If the rerun does not confirm the failure, report the second analysis. If the failure confirms, dilute the sample as instructed in Section 15.3.2. If the diluted sample meets criteria, report the diluted sample results. If the failure confirms, report the original analysis.

15.4 Reporting of analytical results (acid/neutral forms)

The data reporting practices described here are focused on NPDES monitoring needs and may not be relevant to other uses of the method. For analytes reported in their acid form, use the equations in Section 15.2 and the analyte names Table 1. For analytes reported in their anion form, see Table 8 for the appropriate names and CAS Registry Numbers.

15.4.1 Report results for aqueous samples in ng/L. Report results for solid samples in ng/g, on a dry-weight basis, and report the percent solids for each sample separately. Report results for tissue samples in ng/g, on a wet-weight basis. Other units may be used if required in a permit or for a project. Report all QC data with the sample results.

15.4.2 Reporting level

Unless specified otherwise by a regulatory authority or in a discharge permit, results for analytes that meet the identification criteria are reported down to the concentration of the ML established by the laboratory through calibration of the instrument (see the glossary for the derivation of the ML). EPA considers the terms "reporting limit," "quantitation limit," "limit of quantitation," and "minimum level" to be synonymous.

***   MLV Study: Results for analytes that meet the identification criteria and are at or above the MDL concentration must be reported.

    15.4.2.1 Report a result for each analyte in each field sample or QC standard at or above the ML to 3 significant figures. Report a result for each analyte found in each field sample or QC standard below the ML as "<ML," where ML is the

concentration of the analyte at the ML, or as required by the regulatory/control authority or permit.

***   **MLV Study:**   **Report a result for each analyte in each field sample or QC standard at or above the MDL to 3 significant figures. For analytes that are not detected, report the laboratory's sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account) and qualify the concentration with a "U" data qualifier. Report a result for each analyte found in each field sample or QC standard between the MDL and ML (LOQ) and qualify the concentration with a "J" data qualifier.**

15.4.2.2   Report a result for each analyte in a blank at or above the MDL to 2 significant figures. Report a result for each analyte found in a blank below the MDL as "<MDL," where MDL is the concentration of the analyte at the MDL, or as required by the regulatory/control authority or permit.

***   **MLV Study:**   **MLV Study note in Section 15.4.2.1 applies.**

15.4.2.3   Report a result for an analyte found in a sample or extract that has been diluted at the least dilute level at which the area at the quantitation m/z is within the calibration range (e.g., above the ML for the analyte and below the highest calibration standard) and with isotopically labeled compound recoveries within their respective QC acceptance criteria. This may require reporting results for some analytes from different analyses.

15.4.2.4   Report recoveries of all associated EIS compounds for all field samples and QC standards.

***   **MLV Study:**   **Report recoveries of all associated EIS and NIS compounds for all study samples, blanks, and QC samples.**

15.4.3   Results from tests performed with an analytical system that is not in control (i.e., that does not meet acceptance criteria for any QC tests in this method) must be documented and reported (e.g., as a qualifier on results), unless the failure is not required to be reported as determined by the regulatory/control authority. Results associated with a QC failure cannot be used to demonstrate regulatory compliance. QC failures do not relieve a discharger or permittee of reporting timely results. If the holding time would be exceeded for a reanalysis of the sample, the regulatory/control authority should be consulted for disposition.

***   **MLV Study:**   **Results associated with QC elements not meeting the targeted criteria (OPR, EIS, and NIS recoveries) are to be reported without qualification if the failure is confirmed. The only data qualifiers to be utilized are "U", "I", "D", and "B".**

16.0 Method Performance

Routine method performance is validated through analysis of matrix-specific reference samples, including spikes and certified reference materials. Ongoing method performance is monitored through QC samples analyzed alongside samples. The parameters monitored include percent recovery of isotopically labeled compounds, blank concentrations, and native compound recoveries.

This method is being validated, and performance specifications will be developed using data from DOD's interlaboratory validation study (Reference 10). A summary of the single-laboratory performance is presented in Table 5.

17.0 Pollution Prevention

17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot be reduced feasibly at the source, EPA recommends recycling as the next best option.

17.2 The compounds in this method are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

17.3 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 7).

18.0 Waste Management

18.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (Reference 8).

18.2 Samples at pH < 2 or pH > 12, are hazardous and must be handled and disposed of as hazardous waste or neutralized and disposed of in accordance with all federal, state, and local regulations. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions.

18.3 For further information on waste management, consult The Waste Management Manual for Laboratory Personnel and Less is Better-Laboratory Chemical Management for Waste Reduction, (Reference 9).

19.0 References

1. "Working with Carcinogens," Department of Health, Education, & Welfare, Public Health Service, Centers for Disease Control, NIOSH, Publication 77-206, August 1977, NTIS PB-277256.

2. "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910.

3. "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

4. "Standard Methods for the Examination of Water and Wastewater," 18th edition and later revisions, American Public Health Association, 1015 15th St, NW, Washington, DC 20005, 1-35: Section 1090 (Safety), 1992.

5. "Standard Practice for Sampling Water," ASTM Annual Book of Standards, ASTM, 1916 Race Street, Philadelphia, PA 19103-1187, 1980.

6. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA EMSL, Cincinnati, OH 45268, EPA 600/4-79-019, April 1979.

7. "Less is Better: Laboratory Chemical Management for Waste Reduction," American Chemical Society, 1993. Available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

8. "Environmental Management Guide for Small Laboratories," USEPA, Small Business Division, Washington DC, EPA 233-B-00-001, May 2000.

9. "The Waste Management Manual for Laboratory Personnel," American Chemical Society, 1990. Available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

10. *DOD single- laboratory study reference will be added here.*

11. *DOD interlaboratory study reference will be added here.*

12. DoD QSM (US Department of Defense Quality Systems Manual for Environmental Laboratories, version 5.3, 2019)

13. Woudneh, Million B., Bharat Chandramouli, Coreen Hamilton, Richard Grace, 2019, "Effects of Sample Storage on the Quantitative Determination of 29 PFAS: Observation of Analyte Interconversions during Storage", Environmental Science and Technology 53(21): 12576-12585.

20.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1.   Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
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
Perfluoropentansulfonic 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		
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
1H,1H, 2H, 2H-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-3H-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

Table 1.   Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
Ether sulfonic acids		
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Fluorotelomer carboxylic acids		
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5
2H,2H,3H,3H-Perfluorooctanoic acid	5:3FTCA	914637-49-3
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4
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	
1H,1H,2H,2H-Perfluoro-1-[1,2-$^{13}C_2$]hexan sulfonic acid	$^{13}C_2$-4:2FTS	
1H,1H,2H,2H-Perfluoro-1-[1,2-$^{13}C_2$]octanesulfonic acid	$^{13}C_2$-6:2FTS	
1H,1H,2H,2H-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-ethyl-d$_5$-perfluoro-1-octanesulfonamide	D$_5$-NEtFOSA	
N-methyl-d$_3$-perfluoro-1-octanesulfonamide	D$_3$-NMeFOSA	
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	

[1]   The target analyte names are for the acid and neutral forms of the analytes. See Table 8 for the names and CASRN of the corresponding anion forms, where applicable.

NA   Not assigned a CASRN

Table 2. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	Example Retention Time [1]	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass	Typical Ion Ratio	Quantification Reference Compound
Target Analytes						
PFBA	1.96	212.8	168.9	NA	NA	$^{13}C_4$-PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	$^{13}C_5$-PFPeA
PFHxA	4.81	313.0	269.0	118.9	13	$^{13}C_5$-PFHxA
PFHpA	5.32	363.1	319.0	169.0	3.5	$^{13}C_4$-PFHpA
PFOA	6.16	413.0	369.0	169.0	3.0	$^{13}C_8$-PFOA
PFNA	6.99	463.0	419.0	219.0	4.9	$^{13}C_9$-PFNA
PFDA	7.47	512.9	469.0	219.0	5.5	$^{13}C_6$-PFDA
PFUnA	7.81	563.1	519.0	269.1	6.9	$^{13}C_7$-PFUnA
PFDoA	8.13	613.1	569.0	319.0	10	$^{13}C_2$-PFDoA
PFTrDA[2]	8.53	663.0	619.0	168.9	6.7	avg.$^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA
PFTeDA	8.96	713.1	669.0	168.9	6.0	$^{13}C_2$-PFTeDA
PFBS	4.79	298.7	79.9	98.8	2.1	$^{13}C_3$-PFBS
PFPeS	5.38	349.1	79.9	98.9	1.8	$^{13}C_3$-PFHxS
PFHxS	6.31	398.7	79.9	98.9	1.9	$^{13}C_3$-PFHxS
PFHpS	7.11	449.0	79.9	98.8	1.7	$^{13}C_8$-PFOS
PFOS	7.59	498.9	79.9	98.8	2.3	$^{13}C_8$-PFOS
PFNS	7.92	548.8	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDS	8.28	599.0	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDoS	9.14	699.1	79.9	98.8	1.9	$^{13}C_8$-PFOS
4:2FTS	4.67	327.1	307.0	80.9	1.7	$^{13}C_2$-4:2FTS
6:2FTS	5.81	427.1	407.0	80.9	1.9	$^{13}C_2$-6:2FTS
8:2FTS	7.28	527.1	507.0	80.8	3.0	$^{13}C_2$-8:2FTS
PFOSA	8.41	498.1	77.9	478.0	47	$^{13}C_8$-PFOSA
NMeFOSA	9.70	511.9	219.0	169.0	0.66	D_3-NMeFOSA
NEtFOSA	9.94	526.0	219.0	169.0	0.63	D_5-NEtFOSA
NMeFOSAA	7.51	570.1	419.0	483.0	2.0	D_3-NMeFOSAA
NEtFOSAA	7.65	584.2	419.1	526.0	1.2	D_5-N-EtFOSAA
NMeFOSE	9.57	616.1	58.9	NA	NA	D_7-NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D_9-NEtFOSE
HFPO-DA	4.97	284.9	168.9	184.9	1.95	$^{13}C_3$-HFPO-DA
ADONA	5.79	376.9	250.9	84.8	2.8	$^{13}C_3$-HFPO-DA
9Cl-PF3ONS	7.82	530.8	351.0	532.8→353.0	3.2	$^{13}C_3$-HFPO-DA
11Cl-PF3OUdS	8.62	630.9	450.9	632.9→452.9	3.0	$^{13}C_3$-HFPO-DA
3:3FTCA	3.89	241.0	177.0	117.0	1.70	$^{13}C_5$-PFPeA
5:3FTCA	5.14	341.0	237.1	217.0	1.16	$^{13}C_5$-PFHxA
7:3FTCA	6.76	441.0	316.9	336.9	0.69	$^{13}C_5$-PFHxA
PFEESA	5.08	314.8	134.9	82.9	9.22	$^{13}C_5$-PFHxA
PFMPA	3.21	229.0	84.9	NA	NA	$^{13}C_5$-PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	$^{13}C_5$-PFPeA
NFDHA	4.84	295.0	201.0	84.9	1.46	$^{13}C_5$-PFHxA
Extracted Internal Standards						
$^{13}C_4$-PFBA	1.95	216.8	171.9	NA		$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	4.18	268.3	223.0	NA		$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	4.80	318.0	273.0	120.3		$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	5.32	367.1	322.0	NA		$^{13}C_2$-PFHxA
$^{13}C_8$-PFOA	6.16	421.1	376.0	NA		$^{13}C_4$-PFOA

Table 2. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	Example Retention Time [1]	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass	Typical Ion Ratio	Quantification Reference Compound
$^{13}C_9$-PFNA	6.99	472.1	427.0	NA		$^{13}C_5$-PFNA
$^{13}C_6$-PFDA	7.47	519.1	474.1	NA		$^{13}C_2$-PFDA
$^{13}C_7$-PFUnA	7.81	570.0	525.1	NA		$^{13}C_2$-PFDA
$^{13}C_2$-PFDoA	8.13	615.1	570.0	NA		$^{13}C_2$-PFDA
$^{13}C_2$-PFTeDA	8.96	715.2	670.0	NA		$^{13}C_2$-PFDA
$^{13}C_3$-PFBS	4.78	302.1	79.9	98.9		$^{18}O_2$-PFHxS
$^{13}C_3$-PFHxS	6.30	402.1	79.9	98.8		$^{18}O_2$-PFHxS
$^{13}C_8$-PFOS	7.59	507.1	79.9	98.9		$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	4.67	329.1	80.9	309.0		$^{18}O_2$-PFHxS
$^{13}C_2$-6:2FTS	5.82	429.1	80.9	409.0		$^{18}O_2$-PFHxS
$^{13}C_2$-8:2FTS	7.28	529.1	80.9	509.0		$^{18}O_2$-PFHxS
$^{13}C_8$-PFOSA	8.41	506.1	77.8	NA		$^{13}C_4$-PFOS
D_3-NMeFOSA	9.70	515.0	219.0	NA		$^{13}C_4$-PFOS
D_5-NEtFOSA	9.94	531.1	219.0	NA		$^{13}C_4$-PFOS
D_3-NMeFOSAA	7.51	573.2	419.0	NA		$^{13}C_4$-PFOS
D_5-NEtFOSAA	7.65	589.2	419.0	NA		$^{13}C_4$-PFOS
D_7-NMeFOSE	9.56	623.2	58.9	NA		$^{13}C_4$-PFOS
D_9-NEtFOSE	9.83	639.2	58.9	NA		$^{13}C_4$-PFOS
$^{13}C_3$-HFPO-DA	4.97	286.9	168.9	184.9		$^{13}C_2$-PFHxA
Non-Extracted Internal Standards						
$^{13}C_3$-PFBA	1.95	216.0	172.0	NA		
$^{13}C_2$-PFHxA	4.80	315.1	270.0	119.4		
$^{13}C_4$-PFOA	6.16	417.1	172.0	NA		
$^{13}C_5$-PFNA	6.99	468.0	423.0	NA		
$^{13}C_2$-PFDA	7.47	515.1	470.1	NA		
$^{18}O_2$-PFHxS	6.30	403.0	83.9	NA		
$^{13}C_4$-PFOS	7.59	502.8	79.9	98.9		

[1] Times shown are in decimal minute units. Example retention times are based on the instrument operating conditions and column specified in Section 10.2.

[2] For improved accuracy, PFTrDA is quantitated using the average areas of the labeled compounds $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA.

Table 3. Nominal Masses of Spike Added to Samples or Extracts

Analyte	Amount Added (ng)
Extracted Internal Standards	
$^{13}C_4$-PFBA	40
$^{13}C_5$-PFPeA	20
$^{13}C_5$-PFHxA	10
$^{13}C_4$-PFHpA	10
$^{13}C_8$-PFOA	10
$^{13}C_9$-PFNA	5
$^{13}C_6$-PFDA	5
$^{13}C_7$-PFUnA	5
$^{13}C_2$-PFDoA	5
$^{13}C_2$-PFTeDA	5
$^{13}C_3$-PFBS	10
$^{13}C_3$-PFHxS	10
$^{13}C_8$-PFOS	10
$^{13}C_2$-4:2FTS	20
$^{13}C_2$-6:2FTS	20
$^{13}C_2$-8:2FTS	20
$^{13}C_8$-PFOSA	10
D_3-NMeFOSA	10
D_5-NEtFOSA	10
D_3-NMeFOSAA	20
D_5-NEtFOSAA	20
D_7-NMeFOSE	100
D_9-NEtFOSE	100
$^{13}C_3$-HFPO-DA	40
Non-extracted Internal Standards	
$^{13}C_3$-PFBA	20
$^{13}C_2$-PFHxA	10
$^{13}C_4$-PFOA	10
$^{13}C_5$-PFNA	5
$^{13}C_2$-PFDA	5
$^{18}O_2$-PFHxS	10
$^{13}C_4$-PFOS	10

Table 4.   Calibration Solutions (ng/mL)

Compound	CS1 (LOQ)	CS2	CS3	CS4 (CV[1])	CS5	CS6	CS7[2]
Perfluoroalkyl carboxylic acids							
PFBA	0.8	2	5	10	20	50	250
PFPeA	0.4	1	2.5	5	10	25	125
PFHxA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHpA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFOA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFNA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFUnA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDoA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFTrDA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFTeDA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluoroalkyl sulfonic acids							
PFBS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFPeS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHxS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHpS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFOS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFNS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDoS	0.2	0.5	1.25	2.5	5	12.5	62.5
Fluorotelomer sulfonic acids							
4:2FTS	0.8	2	5	10	20	50	NA
6:2FTS	0.8	2	5	10	20	50	NA
8:2FTS	0.8	2	5	10	20	50	NA
Perfluorooctane sulfonamides							
PFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
NMeFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
NEtFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluorooctane sulfonamidoacetic acids							
NMeFOSAA	0.2	0.5	1.25	2.5	5	12.5	62.5
NEtFOSAA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluorooctane sulfonamide ethanols							
NMeFOSE	2	5	12.5	25	50	125	625
NEtFOSE	2	5	12.5	25	50	125	625
Per- and polyfluoroether carboxylic acids							
HFPO-DA	0.8	2	5	10	20	50	250
ADONA	0.8	2	5	10	20	50	250
PFMPA	0.4	1	2.5	5	10	25	125
PFMBA	0.4	1	2.5	5	10	25	125
NFDHA	0.4	1	2.5	5	10	25	125
Ether sulfonic acids							
9Cl-PF3ONS	0.8	2	5	10	20	50	250
11Cl-PF3OUdS	0.8	2	5	10	20	50	250
PFEESA	0.4	1	2.5	5	10	25	125

Table 4. Calibration Solutions (ng/mL)

Compound	CS1 (LOQ)	CS2	CS3	CS4 (CV[1])	CS5	CS6	CS7[2]
Fluorotelomer carboxylic acids							
3:3FTCA	1.0	2.5	6.26	12.5	25	62.4	312
5:3FTCA	5.0	12.5	31.3	62.5	125	312	1560
7:3FTCA	5.0	12.5	31.3	62.5	125	312	1560
Extracted Internal Standard (EIS) Analytes							
$^{13}C_4$-PFBA	10	10	10	10	10	10	10
$^{13}C_5$-PFPeA	5	5	5	5	5	5	5
$^{13}C_5$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFHpA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_9$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_6$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_7$-PFUnA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFDoA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFTeDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_3$-PFBS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_3$-PFHxS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_2$-4:2 FTS	5	5	5	5	5	5	5
$^{13}C_2$-6:2 FTS	5	5	5	5	5	5	5
$^{13}C_2$-8:2 FTS	5	5	5	5	5	5	5
$^{13}C_8$-PFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_5-NEtFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSAA	5	5	5	5	5	5	5
D_5-NEtFOSAA	5	5	5	5	5	5	5
D_7-NMeFOSE	25	25	25	25	25	25	25
D_9-NEtFOSE	25	25	25	25	25	25	25
$^{13}C_3$-HFPO-DA	10	10	10	10	10	10	10
Non-extracted Internal Standard (NIS) Analytes							
$^{13}C_3$-PFBA	5	5	5	5	5	5	5
$^{13}C_2$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_5$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{18}O_2$-PFHxS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5

[1] This calibration point is used as the calibration verification (CV)
[2] A minimum of six contiguous calibrations standards are required for linear models and a minimum of seven calibration standards are required for second-order models.

Table 5. Single-Laboratory Validation Performance Summary for Target Compounds and Extracted Internal Standards

Compounds	Blank (ng/mL)	Aqueous Matrices[1]			Solid Matrices[1]			Tissue Matrices[1]		
		IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)
Target Compounds										
PFBA	< 0.4	89 - 107	4.8	89 – 113	95 – 99	1.0	92 – 108	89 – 104	3.9	90 – 110
PFPeA	< 0.2	85 - 106	5.5	89 – 121	92 – 105	3.4	94 – 115	80 – 98	5.0	96 – 114
PFHxA	< 0.1	75 - 109	9.1	89 – 111	93 – 101	2.2	89 – 107	72 – 110	10.2	90 – 111
PFHpA	< 0.1	87 - 102	4.1	90 – 110	94 – 102	2.2	89 – 107	87 – 102	4.0	87 – 118
PFOA	< 0.1	88 - 98	2.8	87 – 112	92 – 100	2.0	90 – 106	78 – 85	2.4	82 – 114
PFNA	< 0.1	88 - 104	4.1	90 – 111	91 – 102	2.7	88 – 112	85 – 110	6.3	87 – 119
PFDA	< 0.1	82 - 115	8.3	92 – 115	97 – 103	1.5	89 – 118	76 – 115	10.2	84 – 112
PFUnA	< 0.1	83 - 98	4.2	89 – 112	91 – 107	4.0	92 – 111	83 – 102	5.1	91 – 117
PFDoA	< 0.1	58 - 111	15.7	84 – 123	73 – 120	12.1	88 – 119	83 – 105	5.7	77 – 141
PFTrDA	< 0.1	80 - 111	8.1	92 – 119	91 – 112	5.2	89 – 125	92 – 114	5.3	106 – 133
PFTeDA	< 0.1	88 - 103	4.1	89 – 116	94 – 104	2.5	92 – 110	76 – 103	7.4	91 – 111
PFBS	< 0.1	85 - 111	6.6	87 – 116	91 – 103	3.2	91 – 111	69 – 105	10.3	89 – 117
PFPeS	< 0.1	87 - 115	6.9	87 – 115	87 – 103	4.3	89 – 112	77 – 96	5.4	89 – 112
PFHxS	< 0.1	90 - 107	4.4	97 – 119	98 – 106	2.0	96 – 113	81 – 101	5.3	91 – 123
PFHpS	< 0.1	84 - 126	10.2	86 – 114	87 – 104	4.4	88 – 104	77 – 108	8.4	86 – 108
PFOS	< 0.1	93 - 122	6.7	91 – 120	95 – 108	3.4	94 – 115	98 – 112	3.2	97 – 124
PFNS	< 0.1	64 - 141	18.8	86 – 123	98 – 111	3.0	76 – 117	65 – 88	7.5	85 – 114
PFDS	< 0.1	75 - 121	11.7	84 – 107	83 – 102	5.2	84 – 107	82 – 94	3.6	78 – 110
PFDoS	< 0.1	74 - 114	10.6	78 – 102	76 – 99	6.5	77 – 100	73 – 96	6.9	29 – 108
4:2FTS	< 0.4	76 - 123	12.0	91 – 119	98 – 100	0.5	87 – 113	66 – 126	15.6	90 – 103
6:2FTS	< 0.4	71 - 148	17.5	81 – 129	94 – 123	6.5	60 – 166	77 – 105	7.8	92 – 119
8:2FTS	< 0.4	85 - 109	6.1	99 – 124	109 – 128	3.8	104 – 127	66 – 148	19.3	102 – 136
PFOSA	< 0.1	90 - 107	4.4	91 – 122	92 – 106	3.4	94 – 114	92 – 116	5.7	96 – 121
NMeFOSA	< 0.1	78 - 90	3.6	84 – 112	87 – 104	4.4	91 – 117	81 – 100	5.5	86 – 117
NEtFOSA	< 0.1	79 - 97	5.0	83 – 108	98 – 102	1.0	96 – 115	74 – 114	10.7	90 – 127
NMeFOSAA	< 0.1	82 - 115	8.2	81 – 120	91 – 107	4.0	90 – 113	89 – 136	10.4	93 – 117
NEtFOSAA	< 0.1	79 - 120	10.3	85 – 124	102 – 108	1.6	87 – 117	53 – 115	18.3	90 – 117
NMeFOSE	< 1	87 - 102	3.9	92 – 115	98 – 103	1.3	94 – 112	71 – 292	30.3	118 – 344
NEtFOSE	< 1	87 - 104	4.7	91 – 118	97 – 104	1.9	96 – 115	97 – 133	8.0	61 – 159
HFPO-DA	< 0.4	88 - 114	6.5	84 – 118	83 – 105	5.9	80 – 120	73 – 100	7.8	86 – 114
ADONA	< 0.4	77 - 106	7.9	77 – 117	85 – 96	3.2	76 – 124	82 – 95	3.8	86 – 132
PFMPA	< 0.2	86 - 106	6.6	83 – 120	91 – 98	1.8	85 – 117	78 – 93	4.2	86 – 109
PFMBA	< 0.2	62 - 122	5.2	81 – 115	88 – 97	2.6	85 – 120	74 – 104	8.4	84 – 117
NFDHA	< 0.2	44 - 149	16.3	56 – 138	53 – 103	16.2	58 – 136	49 – 86	13.8	56 – 115
9Cl-PF3ONS	< 0.4	84 - 101	27.4	80 – 120	84 – 100	4.4	79 – 131	69 – 98	8.7	95 – 126
11Cl-PF3OUdS	< 0.4	80 - 95	4.5	76 – 116	84 – 96	3.3	77 – 127	85 – 100	4.3	94 – 138
PFEESA	< 0.2	80 - 104	4.4	85 – 115	80 – 93	3.8	89 – 109	68 – 99	9.3	88 – 107
3:3FTCA	< 0.5	84 - 103	5.0	66 – 127	86 – 98	3.3	76 – 116	66 – 94	9.0	41 – 126
5:3FTCA	< 2.5	84 - 101	4.6	84 – 113	83 – 94	3.1	80 – 101	95 – 131	7.9	78 – 199
7:3FTCA	< 2.5	78 - 103	7.0	82 – 116	90 – 106	4.1	75 – 104	84 – 111	6.7	99 – 139

Table 5. Single-Laboratory Validation Performance Summary for Target Compounds and Extracted Internal Standards

Compounds	Blank (ng/mL)	Aqueous Matrices[1]			Solid Matrices[1]			Tissue Matrices[1]		
		IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)
Extracted Internal Standard (EIS)										
$^{13}C_4$-PFBA	N/A	85 - 91	1.6	88 – 108	92 – 99	1.6	95 – 109	93 – 97	1.0	95 – 105
$^{13}C_5$-PFPeA	N/A	87 - 95	2.4	84 – 111	86 – 106	5.3	80 – 110	85 – 108	6.0	89 – 103
$^{13}C_5$-PFHxA	N/A	85 - 92	1.9	83 – 108	83 – 101	4.8	92 – 106	79 – 111	8.5	88 – 98
$^{13}C_4$-PFHpA	N/A	78 - 100	6.2	83 – 106	87 – 102	4.1	90 – 100	88 – 93	1.3	80 – 102
$^{13}C_8$-PFOA	N/A	77 - 98	6.0	84 – 107	89 – 101	3.2	92 – 104	91 – 98	1.7	86 – 102
$^{13}C_9$-PFNA	N/A	82 - 96	3.8	84 – 107	86 – 101	4.1	90 – 106	91 – 104	3.3	89 – 101
$^{13}C_6$-PFDA	N/A	81 - 98	4.7	84 – 106	79 – 101	6.0	86 – 109	89 – 104	4.0	90 – 104
$^{13}C_7$-PFUnA	N/A	84 - 100	4.4	84 – 109	84 – 104	5.4	91 – 116	84 – 118	8.4	88 – 109
$^{13}C_2$-PFDoA	N/A	61 - 103	12.9	73 – 101	70 – 93	7.1	73 – 106	95 – 125	6.8	70 – 108
$^{13}C_2$-PFTeDA	N/A	72 - 89	5.4	74 – 97	83 – 88	1.5	74 – 107	81 – 114	8.5	10 – 110
$^{13}C_3$-PFBS	N/A	87 - 94	2.0	88 – 110	97 – 105	1.8	96 – 109	87 – 114	6.5	95 – 106
$^{13}C_3$-PFHxS	N/A	83 - 89	1.9	85 – 103	92 – 97	1.4	92 – 106	92 – 97	1.4	91 – 103
$^{13}C_8$-PFOS	N/A	78 - 92	3.9	86 – 110	87 – 107	4.9	95 – 109	87 – 93	1.6	95 – 103
$^{13}C_2$-4:2 FTS	N/A	64 - 106	12.1	87 – 137	132 – 135	0.6	123 – 145	106 – 221	17.6	155 – 291
$^{13}C_2$-6:2 FTS	N/A	93 - 102	2.2	67 – 149	118 – 129	2.3	104 – 138	87 – 135	10.8	117 – 149
$^{13}C_2$-8:2 FTS	N/A	99 - 109	2.5	71 – 137	96 – 122	6.1	93 – 123	179 – 299	12.5	79 – 304
$^{13}C_8$-PFOSA	N/A	60 - 107	14.2	57 – 109	69 – 86	5.4	66 – 100	104 – 153	9.4	88 – 120
D_3-NMeFOSA	N/A	55 - 85	10.8	39 – 84	47 – 59	5.4	25 – 64	20 – 58	24.5	3 – 34
D_5-NEtFOSA	N/A	54 - 91	12.9	43 – 84	43 – 51	4.5	18 – 58	30 – 56	15.2	0 – 56*
D_3-NMeFOSAA	N/A	63 - 117	14.9	66 – 117	98 – 107	2.1	86 – 109	102 – 187	14.7	144 – 196
D_5-NEtFOSAA	N/A	66 - 115	13.7	63 – 115	98 – 104	1.3	85 – 109	178 – 216	4.9	175 – 223
D_7-NMeFOSE	N/A	61 - 106	13.6	42 – 99	50 – 61	5.1	35 – 76	3 – 5	11.6	0 – 8*
D_9-NEtFOSE	N/A	63 - 108	13.2	44 – 90	46 – 57	5.5	32 – 72	8 – 33	30.0	0 – 33*
$^{13}C_3$-HFPO-DA	N/A	89 - 106	4.5	88 – 121	98 – 108	2.4	83 – 125	87 – 106	4.9	81 – 106

[1] The recovery limits are applied to all samples, method blanks, IPR, OPR samples for all matrix types.

* Ranges were determined at ± 2 standard deviations from the mean. Because of the low recoveries for these EIS, the calculated lower limits were negative values. Therefore, the lower limits have been set to 0 for these analytes.

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated to reflect the interlaboratory study results in a subsequent revision. Therefore, these criteria will change after interlaboratory validation. Several sections of this method state that Table 5 criteria are required, this is standard language that will be applicable when the method is finalized.

Table 6. Pooled MDL$_s$ and ML values from the Single-laboratory Validation Study, by Matrix[1]

Compound	Aqueous (ng/L)		Solid (ng/g)		Tissue (ng/g)	
	MDL$_s$	ML	MDL$_s$	ML	MDL$_s$	ML
PFBA	0.330	6.4	0.401	0.8	0.593	2.0
PFPeA	0.196	3.2	0.021	0.4	0.083	1.0
PFHxA	0.318	1.6	0.020	0.2	0.096	0.5
PFHpA	0.221	1.6	0.029	0.2	0.088	0.5
PFOA	0.302	1.6	0.037	0.2	0.086	0.5
PFNA	0.221	1.6	0.086	0.2	0.160	0.5
PFDA	0.333	1.6	0.031	0.2	0.124	0.5
PFUnA	0.264	1.6	0.033	0.2	0.152	0.5
PFDoA	0.379	1.6	0.059	0.2	0.130	0.5
PFTrDA	0.238	1.6	0.038	0.2	0.086	0.5
PFTeDA	0.264	1.6	0.032	0.2	0.185	0.5
PFBS	0.245	1.6	0.014	0.2	0.070	0.5
PFPeS	0.204	1.6	0.015	0.2	0.032	0.5
PFHxS[1]	0.217	1.6	0.018	0.2	0.083	0.5
PFHpS	0.137	1.6	0.057	0.2	0.043	0.5
PFOS[1]	0.327	1.6	0.067	0.2	0.294	0.5
PFNS	0.303	1.6	0.046	0.2	0.114	0.5
PFDS	0.334	1.6	0.040	0.2	0.101	0.5
PFDoS	0.179	1.6	0.038	0.2	0.177	0.5
4:2 FTS	2.281	6.4	0.282	0.8	0.740	2.0
6:2 FTS	3.973	6.4	0.116	0.8	1.149	2.0
8:2 FTS	1.566	6.4	0.225	0.8	0.373	2.0
PFOSA	0.227	1.6	0.068	0.2	0.094	0.5
NMeFOSA	0.196	1.6	0.049	0.2	0.161	0.5
NEtFOSA	0.585	1.6	0.038	0.2	0.169	0.5
NMeFOSAA[1]	0.586	1.6	0.030	0.2	0.093	0.5
NEtFOSAA[1]	0.324	1.6	0.044	0.2	0.138	0.5
NMeFOSE	1.191	16	0.203	2.0	9.978	5.0
NEtFOSE	1.022	16	0.247	2.0	1.501	5.0
HFPO-DA	0.406	6.4	0.136	0.8	0.161	2.0
ADONA	0.779	6.4	0.057	0.8	0.082	2.0
PFEESA	0.137	3.2	0.018	0.4	0.045	1.0
PFMPA	0.177	3.2	0.033	0.4	0.070	1.0
PFMBA	0.117	3.2	0.029	0.4	0.069	1.0
NFDHA	1.384	3.2	0.084	0.4	0.294	1.0
9CL-PF3ONS	0.871	6.4	0.038	0.8	0.152	2.0
11CL-PF3OUDS	0.819	6.4	0.071	0.8	0.312	2.0
3:3 FTCA	0.721	8.0	0.060	1.0	0.247	2.5
5:3 FTCA	5.066	40	0.363	5.0	1.537	12.5
7:3 FTCA	5.942	40	0.308	5.0	0.845	12.5

[1] A standard containing a mixture of branched and linear isomer of suitable quality to be used for quantitation is currently available and required to be used for all calibration, calibration verifications, and QC samples. If more become commercially available for other target analytes, they must be utilized in the same manner.

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the pooled MDLs from the interlaboratory study results in a subsequent revision.

Table 7. Summary of Quality Control

Method Reference	Requirement	Specification and Frequency
Section 10.1	Mass Calibration	Annually and on as-needed basis
Section 10.1.5	Mass Calibration Verification	After mass calibration
Section 10.3	Initial Calibration (ICAL)	Minimum 6 calibration standards for linear model and 7 calibration standards for non-linear models.
Sections 10.2.2, 14.4	Retention Time (RT) window	After ICAL and at the beginning of analytical sequence
Sections 7.3.1, 9.4	Extracted Internal Standard (EIS) Analytes	All CAL standards, batch QC and field samples
Sections 7.3.2	Non-extracted Internal Standards (NIS)	All CAL standards, batch QC and field samples
Sections 7.3.4, 10.3.1, 13.3	Instrument Sensitivity Check (ISC)	Daily, prior to analysis
Section 14.2	Calibration Verification (CV)	At the beginning and every 10 samples
Section 14.6	Instrument Blank	Daily prior to analysis and after high standards
Sections 9.1.3, 9.5, 14.7	Method Blank (MB)	One per preparation batch
Section 14.5	Ongoing Precision Recovery (OPR)	One per preparation batch
Section 11.0	Limit of Quantitation Verification (LLOPR)	Prior to analyzing samples
Section 11.0	Matrix Spike (MS/MSD)	One per preparation batch (if required)

Table 8. Cross-reference of Abbreviations, Analyte Names, CAS Numbers for the Acid and Anion Forms of the Perfluoroalkyl carboxylates and Perfluoroalkyl sulfonates

Abbreviation	Acid Name	CASRN	Anion Name	CASRN
Perfluoroalkyl carboxylic acids/anions				
PFBA	Perfluorobutanoic acid	375-22-4	Perfluorobutanoate	45048-62-2
PFPeA	Perfluoropentanoic acid	2706-90-3	Perfluoropentanoate	45167-47-3
PFHxA	Perfluorohexanoic acid	307-24-4	Perfluorohexanoate	92612-52-7
PFHpA	Perfluoroheptanoic acid	375-85-9	Perflluoroheptanoate	120885-29-2
PFOA	Perfluorooctanoic acid	335-67-1	Pefluorooctanoate	45285-51-6
PFNA	Perfluorononanoic acid	375-95-1	Perfluorononanoate	72007-68-2
PFDA	Perfluorodecanoic acid	335-76-2	Perfluorodecanoate	73829-36-4
PFUnA	Perfluoroundecanoic acid	2058-94-8	Perfluoroundecanoate	196859-54-8
PFDoA	Perfluorododecanoic acid	307-55-1	Perfluorododecanoate	171978-95-3
PFTrDA	Perfluorotridecanoic acid	72629-94-8	Perfluorotridecanoate	862374-87-6
PFTeDA	Perfluorotetradecanoic acid	376-06-7	Perfluorotetradecanoate	365971-87-5
Perfluoroalkyl sulfonic acids/anions				
PFBS	Perfluorobutanesulfonic acid	375-73-5	Perfluorobutane sulfonate	45187-15-3
PFPeS	Perfluoropentansulfonic acid	2706-91-4	Perfluoropentane sulfonate	175905-36-9
PFHxS	Perfluorohexanesulfonic acid	355-46-4	Perfluorohexane sulfonate	108427-53-8
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	Perfluoroheptane sulfonate	146689-46-5
PFOS	Perfluorooctanesulfonic acid	1763-23-1	Perfluorooctane sulfonate	45298-90-6
PFNS	Perfluorononanesulfonic acid	68259-12-1	Perfluorononane sulfonate	474511-07-4
PFDS	Perfluorodecanesulfonic acid	335-77-3	Perfluorodecane sulfonate	126105-34-8
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	Perfluorododecane sulfonate	343629-43-6

Table 9. Range of Recoveries for Extracted Internal Standards (EIS) in the Single-laboratory Validation Study, by Matrix

EIS Compounds	Aqueous			Solid			Tissue		
	% Recovery		RSD	% Recovery		RSD	% Recovery		RSD
	Min	Max	(%)	Min	Max	(%)	Min	Max	(%)
$^{13}C_4$-PFBA	9	97	15.9	3	113	37.4	84	99	8.0
$^{13}C_5$-PFPeA	39	103	13.3	28	112	17.2	86	107	11.1
$^{13}C_5$-PFHxA	73	97	2.7	79	110	5.5	92	95	1.6
$^{13}C_4$-PFHpA	77	95	2.4	73	111	6.0	80	93	8.2
$^{13}C_8$-PFOA	87	95	0.8	86	115	4.4	90	95	2.8
$^{13}C_9$-PFNA	82	95	1.6	87	110	4.2	90	98	4.3
$^{13}C_6$-PFDA	71	93	3.3	87	112	4.9	83	97	7.7
$^{13}C_7$-PFUnA	56	94	6.5	66	124	11.6	71	91	12.9
$^{13}C_2$-PFDoA	34	87	13.7	26	109	24.3	54	96	29.2
$^{13}C_2$-PFTeDA	17	153	26.2	18	110	30.1	31	102	67.8
$^{13}C_3$-PFBS	72	100	4.7	89	120	5.4	89	98	5.1
$^{13}C_3$-PFHxS	79	95	1.6	87	110	4.4	98	99	0.1
$^{13}C_8$-PFOS	67	96	3.6	79	113	5.7	92	103	6.0
$^{13}C_2$-4:2FTS	81	199	14.8	95	248	17.0	192	215	6.2
$^{13}C_2$-6:2FTS	64	183	16.4	76	127	9.4	145	230	27.2
$^{13}C_2$-8:2FTS	65	139	8.4	86	173	15.2	136	220	24.6
$^{13}C_8$-PFOSA	27	93	15.4	61	123	10.0	87	96	4.5
D_3-NMeFOSA	14	74	16.4	28	86	22.7	8	38	61.9
D_5-NEtFOSA	12	70	16.5	21	70	25.5	8	30	57.8
D_3-NMeFOSAA	21	113	7.3	52	142	14.8	106	139	13.1
D_5-NEtFOSAA	12	106	8.2	68	151	16.9	79	151	31.8
D_7-NMeFOSE	11	77	18.6	13	107	27.9	5	30	81.1
D_9-NEtFOSE	8	73	19.6	16	97	30.4	0	29	103.1
$^{13}C_3$-HFPO-DA	92	113	2.0	70	119	10.4	93	102	5.1

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the interlaboratory study results in a subsequent revision.

Table 10. Range of Recoveries for Non-Extracted Internal Standards in the Single-laboratory Validation Study, by Matrix

	Aqueous			Solid			Tissue		
	% Recovery		RSD	% Recovery		RSD	% Recovery		RSD
NIS Compounds	Min	Max	(%)	Min	Max	(%)	Min	Max	(%)
$^{13}C_3$-PFBA	60	91	10.3	54	89	6.4	51	82	7.0
$^{13}C_2$-PFHxA	43	94	18.6	52	90	7.4	41	80	19.3
$^{13}C_4$-PFOA	59	87	9.7	54	89	6.4	51	82	9.5
$^{13}C_5$-PFNA	64	87	7.5	59	94	7.1	52	88	11.2
$^{13}C_2$-PFDA	57	86	10.0	55	91	8.6	47	85	19.4
$^{18}O_2$-PFHxS	59	87	9.6	53	87	7.1	51	80	8.1
$^{13}C_4$-PFOS	60	82	7.5	58	86	7.0	52	85	10.3

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the interlaboratory study results in a subsequent revision.

21.0 Glossary

These definitions and purposes are specific to this method, but have been conformed to common usage to the extent possible.

21.1 Units of weight and measure and their abbreviations

21.1.1 Symbols

ºC	degrees Celsius
Da	Dalton (equivalent to "amu" below)
µg	microgram
µL	microliter
µm	micrometer
<	less than
≤	less than or equal
>	greater than
≥	greater than or equal
%	percent
±	plus or minus

21.1.2 Alphabetical abbreviations

amu	atomic mass unit (equivalent to Dalton)
cm	centimeter
g	gram
h	hour
L	liter
M	molar
mg	milligram
min	minute
mL	milliliter
mm	millimeter
cm	centimeter
m/z	mass-to-charge ratio
ng	nanogram
Q1	quantitation ion
Q2	confirmation ion
rpm	revolutions per minute
v/v	percent volume per volume

21.2 Definitions and acronyms (in alphabetical order)

Analyte – A PFAS compound included in this method. The analytes are listed in Table 1.

Calibration standard (CS) – A solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the LC-MS/MS instrument.

Calibration verification standard (CV) – The mid-point calibration standard (CS-4) that is used to verify calibration. See Table 6.

CFR – Code of Federal Regulations

Compound - One of many variants or configurations of a common chemical structure. Individual compounds are identified by the number of carbon atoms and functional group attached at the end of the chain.

Class A glassware – Volumetric glassware that provides the highest accuracy. Class A volumetric glassware complies with the Class A tolerances defined in ASTM E694, must be permanently labeled as Class A, and is supplied with a serialized certificate of precision.

CWA – Clean Water Act

Extracted internal standard (EIS) quantification – The response of the target compound is compared to the response of the labeled analog of another compound in the same LOC.

LC – Liquid chromatograph or liquid chromatography

Internal standard – A labeled compound used as a reference for quantitation of other labeled compounds and for quantitation of native PFAS compounds other than the compound of which it is a labeled analog. See Internal standard quantitation.

Instrument sensitivity check – solution used to check the sensitivity of the instrument. The solution contains the native compounds at the concentration of the LOQ.

Internal standard quantitation – A means of determining the concentration of (1) a naturally occurring (native) compound by reference to a compound other than its labeled analog and (2) a labeled compound by reference to another labeled compound

IPR – Initial precision and recovery; four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

Isotope dilution (ID) quantitation – A means of determining a naturally occurring (native) compound by reference to the same compound in which one or more atoms has been isotopically enriched. The labeled PFAS are spiked into each sample and allow identification and correction of the concentration of the native compounds in the analytical process.

Isotopically labeled compound – An analog of a target analyte in the method which has been synthesized with one or more atoms in the structure replaced by a stable (non-radioactive) isotope of that atom. Common stable isotopes used are ^{13}C (Carbon-13) or Deuterium (D or ^2H). These labeled compounds do not occur in nature, so they can be used for isotope dilution quantitation or other method-specific purposes.

Limit of Quantitation (LOQ) – The smallest concentration that produces a quantitative result with known and recorded precision and bias. The LOQ shall be set at or above the concentration of the lowest initial calibration standard (the lowest calibration standard must fall within the linear range).

Method blank – An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and labeled compounds that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Method Detection Limit (MDL) – The minimum measured concentration of a substance that can be reported with 99% confidence that the measured analyte concentration is distinguishable from method blank results (40 CFR 136, Appendix B).

MESA – Mining Enforcement and Safety Administration

Minimum level of quantitation (ML) – The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. The ML represents the lowest concentration at which an analyte can be measured with a known level of confidence. It may be equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. Alternatively, the ML may be established by multiplying the MDL (pooled or unpooled, as appropriate) by 3.18 and rounding the result to the number nearest to 1, 2, or 5 x 10^n, where n is zero or an integer (see 68 FR 11770).

MS – Mass spectrometer or mass spectrometry

Matrix Spike/Matrix Spike Duplicate (MS/MSD) – Aliquots of field samples that have been fortified with a known concentration of target compounds, prior to sample preparation and extraction, and analyzed to measure the effect of matrix interferences. The use of MS/MSD samples is generally not required in isotope dilution methods because the labeled compounds added to every sample provide more performance data than spiking a single sample in each preparation batch.

Multiple reaction monitoring (MRM) – Also known as selected reaction monitoring (SRM). A type of mass spectrometry where a parent mass of the compound is fragmented through MS/MS and then specifically monitored for a single fragment ion.

Must – This action, activity, or procedural step is required.

NIOSH – The National Institute of Occupational Safety and Health

Non-extracted internal standard (NIS) –Labeled PFAS compounds spiked into the concentrated extract immediately prior to injection of an aliquot of the extract into the LC-MS/MS.

OPR – Ongoing precision and recovery standard (OPR); a method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Precursor Ion – For the purpose of this method, the precursor ion is the deprotonated molecule ([M-H]-) of the method analyte. In MS/MS, the precursor ion is mass selected and fragmented by collisionally activated dissociation to produce distinctive product ions of smaller *m/z*.

PFAS – Per- and Polyfluoroalkyl substances –A group of man-made fluorinated compounds that are hydrophobic and lipophobic, manufactured and used in a variety of industries globally. These compounds are persistent in the environment as well as in the human body. This method analyzes for the PFAS listed in Table 1.

Reagent water – Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD) – The standard deviation multiplied by 100 and divided by the mean. Also termed "coefficient of variation."

Relative Standard Error (RSE) – The standard error of the mean divided by the mean and multiplied by 100.

RF – Response factor. See Section 10.3.3.2.

RR – Relative response. See Section 10.3.3.2.

RT – Retention time; the time it takes for an analyte or labeled compound to elute off the HPLC/UPLC column

Should – This action, activity, or procedural step is suggested but not required.

Signal-to-noise ratio (S/N) – The height of the signal as measured from the mean (average) of the noise to the peak maximum divided by the width of the noise.

SPE – Solid-phase extraction; a technique in which an analyte is extracted from an aqueous solution or a solid/tissue extract by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction.

Stock solution – A solution containing an analyte that is prepared using a reference material traceable to EPA, NIST, or a source that will attest to the purity and authenticity of the reference material.

Appendix A - Sample Pre-screening Instructions

Samples that are known or suspected to contain high levels of analytes may be pre-screened using the following procedure. These are example procedures using smaller sample aliquots spiked with EIS and NIS and no clean up procedures. Other pre-screening procedures may be used.

Aqueous Samples

1. Weight out 10 (±0.1) g of sample into a 50-mL centrifuge tube.
2. Add 50 µL of EIS and NIS to the sample and vortex to mix.
3. Filter 1 mL of the sample through 0.2-µm membrane filter into a microvial. Sample is ready for instrumental analysis.

Solid and Tissue Samples

1. Weigh 1.0 (±0.1) g sample into 50-mL polypropylene centrifuge tubes.
2. Add 20 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1). Vortex and mix on a shaker table (or equivalent) for 10 min. Allow to settle and/or centrifuge to produce a clear extract.
3. Filter using a Single Step® filter vial:
   a. Add 20 µL of EIS to a clean Single Step® filter vial (chamber).
   b. Add 400 µL of clear extract from step 2 (e.g., by adding extract until it reaches the fill line), carefully vortex to mix.
   c. Use filter/plunger part and filter.
4. Transfer 30 µL of filtrate to a ~300-µL polypropylene micro-vial and dilute to 300 µL with 0.3% methanolic ammonium hydroxide (Section 7.1.7.1). Add NIS to the filtrate.
5. The extract is now a 10x dilution.
6. Sample is ready for instrumental analysis.

Calculate results using the equivalent sample weight computed as follows:

$$Equivalent\ Weight = Sample\ weight\ (g) \times \frac{0.4\ mL}{20\ mL}$$

Note that the EIS concentration in the diluted portion is 0.5x the level in the regular analysis of solid samples.

Appendix B - Aqueous Sample Subsampling Instructions

Warning: **Because some target analytes may be stratified within the sample (e.g., AFFF-contaminated media, surfactants), or adhere to the walls of the sample container, subsampling may only be done on a project-specific basis. Subsampling has been shown to increase uncertainty in PFAS analysis, especially on foaming samples.**

If a reduced sample size is required, transfer a weighed subsample using the following subsampling procedure to a 60-mL HDPE bottle and dilute to approximately 60 mL using reagent water. This container is now considered the "sample bottle."

1. Gently invert sample 3-4 times being careful to avoid foam formation and subsample immediately (do not let stand).

2. If foam forms and more than 5 mL is required – pour sample, avoiding any foam.

3. If foaming forms and a volume less than 5 mL is required – pipette from ½ cm below the foam.

4. If no foam forms – pour or pipette based on volume required.

ATTACHMENT 2

REQUIRED SAMPLE NOMENCLATURE AND MATRIX TYPES FOR THE MULTI-LABORATORY VALIDATION STUDY

Attachment 2. Required Sample Nomenclature and Matrix Types for the Multi-Laboratory Validation Study

Matrix Type	Requested Name	Description	Matrix Code	Sample Identifier	Characterization Pre-spike	Unspiked	Low Replicate 1	Low Replicate 2	Low Replicate 3	High Replicate 1	High Replicate 2	High Replicate 3
Groundwater	USACE	GW #1, midwest	GW	A	GWA0	GWA1	GWA2	GWA3	GWA4	GWA5	GWA6	GWA7
Groundwater	LRPCD	GW #2, southwest	GW	B	GWB0	GWB1	GWB2	GWB3	GWB4	GWB5	GWB6	GWB7
Groundwater	USACE	GW #13	GW	C	GWC0	GWC1	GWC2	GWC3	GWC4	GWC5	GWC6	GWC7
Surface Water	Lake Harsha, OH	SW OH 9/10	SW	D	SWD0	SWD1	SWD2	SWD3	SWD4	SWD5	SWD6	SWD7
Surface Water	Norwell, MA	SW MA 9/24	SW	E	SWE0	SWE1	SWE2	SWE3	SWE4	SWE5	SWE6	SWE7
Surface Water	Burley Creek, WA	Burley Creek	SW	F	SWF0	SWF1	SWF2	SWF3	SWF4	SWF5	SWF6	SWF7
Surface Water	Sequim Bay, WA	Sequim Seawater	SW	G	SWG0	SWG1	SWG2	SWG3	SWG4	SWG5	SWG6	SWG7
Wastewater	Metal Finisher	Metal Finisher	WW	H	WWH0	WWH1	WWH2	WWH3	WWH4	WWH5	WWH6	WWH7
Wastewater	Hospital	Hospital	WW	I	WWI0	WWI1	WWI2	WWI3	WWI4	WWI5	WWI6	WWI7
Wastewater	POTW Influent	POTW Influent	WW	J	WWJ0	WWJ1	WWJ2	WWJ3	WWJ4	WWJ5	WWJ6	WWJ7
Wastewater	ASTM Substitute	ASTM Substitute	WW	K	WWK0	WWK1	WWK2	WWK3	WWK4	WWK5	WWK6	WWK7
Wastewater	WW Bus Washing Station	WW Bus Wash	WW	L	WWL0	WWL1	WWL2	WWL3	WWL4	WWL5	WWL6	WWL7
Wastewater	Playa Del Ray, CA	Plant Effluent	WW	M	WWM0	WWM1	WWM2	WWM3	WWM4	WWM5	WWM6	WWM7
Wastewater	Pulp & Paper WW	#1- 28	WW	N	WWN0	WWN1	WWN2	WWN3	WWN4	WWN5	WWN6	WWN7
Wastewater	POTW Effluent	POTW Effluent	WW	O	WWO0	WWO1	WWO2	WWO3	WWO4	WWO5	WWO6	WWO7
Soil	Musselshell, Clark Co. MT	AA (2016-106), L32547-2	SS	R	SSR0	SSR1	SSR2	SSR3	SSR4	SSR5	SSR6	SSR7
Soil	Ivy, Cashe Co. UT	BB (2017-111), L32547-3	SS	S	SSS0	SSS1	SSS2	SSS3	SSS4	SSS5	SSS6	SSS7
Soil	Fruitland, San Juan Co. NM	CC (2018-105), L32547-4	SS	T	SST0	SST1	SST2	SST3	SST4	SST5	SST6	SST7
Soil	Armijo, Dona Ana Co. NM	DD (2018-116), L32547-5	SS	U	SSU0	SSU1	SSU2	SSU3	SSU4	SSU5	SSU6	SSU7
Soil	Drummer, Dekalb Co. IL	EE (2019-107), L32547-6	SS	V	SSV0	SSV1	SSV2	SSV3	SSV4	SSV5	SSV6	SSV7
Soil	Brock, Wheatley Co. TN	FF (2019-110), L32547-7	SS	W	SSW0	SSW1	SSW2	SSW3	SSW4	SSW5	SSW6	SSW7
Soil	Delhi, Fresno County. CA	2014-107	SS	X	SSX0	SSX1	SSX2	SSX3	SSX4	SSX5	SSX6	SSX7
Sediment	Burley 1 Sed. Burley Creek, WA	Burley 1 Sed.	SD	Y	SDY0	SDY1	SDY2	SDY3	SDY4	SDY5	SDY6	SDY7
Sediment	Burley 2 Sed. Burley Creek, WA	Burley 2 Sed.	SD	Z	SDZ0	SDZ1	SDZ2	SDZ3	SDZ4	SDZ5	SDZ6	SDZ7
Sediment	Sequim Bay Sediment	Sequim Bay Sediment	SD	AA	SDAA0	SDAA1	SDAA2	SDAA3	SDAA4	SDAA5	SDAA6	SDAA7
Fish Tissue	Walleye (low lipid fish)	Walleye	TS	AB	TSAB0	TSAB1	TSAB2	TSAB3	TSAB4	TSAB5	TSAB6	TSAB7
Fish Tissue	Salmon (high lipid fish)	Salmon	TS	AC	TSAC0	TSAC1	TSAC2	TSAC3	TSAC4	TSAC5	TSAC6	TSAC7
Fish Tissue	Clams	Clams	TS	AD	TSAD0	TSAD1	TSAD2	TSAD3	TSAD4	TSAD5	TSAD6	TSAD7
Leachate	MSW LF Leachate Sample	MSW LF Leachate Sample	LC	AE	LCAE0	LCAE1	LCAE2	LCAE3	LCAE4	LCAE5	LCAE6	LCAE7
Leachate	CDD Landfill	CDD	LC	AF	LCAF0	LCAF1	LCAF2	LCAF3	LCAF4	LCAF5	LCAF6	LCAF7
Leachate	Ash leachate	Ash leachate	LC	AG	LCAG0	LCAG1	LCAG2	LCAG3	LCAG4	LCAG5	LCAG6	LCAG7
Biosolids	Playa Del Ray, CA	Wetcake	BS	AH	BSAH0	BSAH1	BSAH2	BSAH3	BSAH4	BSAH5	BSAH6	BSAH7
Biosolids	Biosolids	Biosolids	BS	AI	BSAI0	BSAI1	BSAI2	BSAI3	BSAI4	BSAI5	BSAI6	BSAI7
Biosolids	Renton, WA	Renton, WA	BS	AJ	BSAJ0	BSAJ1	BSAJ2	BSAJ3	BSAJ4	BSAJ5	BSAJ6	BSAJ7

ATTACHMENT 3

ELECTRONIC DATA DELIVERABLE INSTRUCTIONS

(Instructions Dated 02/18/22)

Following is the description of data fields requested for electronic data deliverables (EDDs) for the PFAS Multi-laboratory Validation Study. The format of data in each field is indicated in brackets (e.g. [text string string]). [*Note: The format of the EDD will be finalized when spiked matrices are shipped to the laboratories participating in the MLV study*].

Data Fields:

1) **Lab_ID**: [text string] Laboratory Name.

2) **Sample_No**: [text string] For samples, these are the sample identification names (IDs) from the Chain of Custody. The Sample_No is the same, regardless of whether or not the sample is diluted or reanalyzed. For preparation batch QC, these are "MB" for the Method Blank, "OPR" for the OPR, and "LLOPR" for the LLOPR. For Initial Demonstration of Capability samples, "IPR" for the IPR samples, "MDLB" for the MDL_b samples, "MDLS" for the MDLs samples, and "LOQVER" for the LOQVER samples.

   Lab_Sample_ID: [text string] The ID the laboratory assigns to the sample (which identifies the sample on the associated data files and reports). For samples that need to be re-analyzed for issues other than dilution, attach the following identifiers to the end of the lab sample identifier without a space between them (e.g., 02082022-01R):

   o "R" for analytes, EISs and NISs reported from first re-analysis not due to dilution;

   o "R1" for analytes, EISs and NISs reported from second re-analysis not due to dilution; and

   o "R2" for analytes, EISs and NISs reported from second re-analysis not due to dilution

   If more re-analyses not due to dilution are needed to be reported beyond three for a sample, continue on with the numbering (e.g., R3, R4, R5, etc.).

3) **Analysis_Date**: [short date] Use format mm/dd/yyyy (e.g., 11/20/2019) – do not include time stamp.

4) **Analysis**: [text string] fill in "PFAS"

5) **Compound**: [text string] Use the names included in the example EDD. DO NOT CHANGE. Method analytes, and EIS and NIS compounds must be reported for each sample.

6) **CAS_No**: [text string] Use the Chemical Abstract Service Registration Number (CASRN) included in the example EDD. DO NOT CHANGE. For compounds with no CASRN, leave blank.

7) **PFAS_Acronym**: [text string] Use acronyms included in the example EDD. DO NOT CHANGE.

8) **Dilution**: [number integer] Dilution made post extraction (e.g., extract diluted 1:10 is entered as "10"). If analyzed without dilution, enter "1."

9) **Conc_Found**: [number, double] Enter numeric quantitative result value only. Report to three significant figures. Do NOT enter any text string strings or symbols (e.g., "ND", "<"). For analytes that are not detected, the laboratory's sample specific MDL (i.e., with extract dilution factor, sample volume/weight and final volume taken into account) is entered. Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. Report result units in "Unit" field, consistent for all sample fields.

10) **Lab_Flag**: [text string] Laboratory qualifiers

   "U" for analytes that were not <u>detected</u> or were detected at a concentration less than the MDL.

   "J" for analytes that were at a concentration between the MDL and LOQ.

   "B" for analytes that were detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than $1/10^{th}$ the concentration in the sample, whichever is greatest. The MB must also be flagged with a "B" for all concentrations greater than ½ the LOQ.

   "I" for analytes that fail to meet ion ratio criteria.

   "D" for analytes, EISs, and NISs reported from a dilution.

   These flags apply to all samples (field and QC).

11) If you have multiple flags assigned to a result, do not include any spacing between the flags. **Conc_Spike**: [number, double] For unspiked samples enter "0" for method analytes. For spiked samples, enter the spike concentration representing the estimated concentration in the final extract (i.e., with extract dilution factor, sample volume/weight and final volume taken into account). Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. For EIS and NIS, enter the spike concentration representing the concentration in the final extract in units consistent with sample result units. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to three significant figures.

12) **Percent_Rec**: [number, double] For unspiked samples, leave blank. No text should be included in this field (e.g., N/A). For spiked samples (OPR, LLOPR, MDL$_s$ and LOQVER), enter the spike percentage recovery as a whole number (e.g., 95 versus 0.95). Do NOT include "%" symbol. For EIS and NIS recoveries, enter the spike % recovery as a whole number (e.g., 95 versus 0.95). Report to three significant figures. Do NOT include "%".

13) **MDL** (Method Detection Limit): [number, double] Enter the sample specific MDL (i.e., with extract dilution factor, sample volume/weight and final volume taken into account). The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to three significant figures.

14) **LOQ (**Limit of Quantitation): [number, double] Enter the sample specific LOQ (i.e., with extract dilution factor, sample volume/weight and final volume taken into account).

Report to 3 significant figures. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to three significant figures.

15) **Unit**: [text string] The reporting units must be consistent for the sample record including Conc_Found, MDL, LOQ etc. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Ensure that all values for the sample record are reported in the same units.

16) **Sample_Transition_Ratio**: [text string] Enter the calculated Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte in the sample. Report to three significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g., N/A).

17) **Expected_Transition_Ratio**: [text string] Enter the expected Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte per the method. Report to three significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g., N/A).

18) **RRT**: [text string] Enter relative retention time

19) **Sample_Size**: [number, double] Enter volume (aqueous samples) or weight (solid samples) of sample extracted (in liters for aqueous samples, in kilograms for solids).

20) **Sample_size_unit:** [text string] Will be liters (L) for aqueous samples or kilograms (Kg) for solid samples

21) **Extraction_date**: [short date] Use format mm/dd/yyyy (e.g., 11/20/2019) – do not include time stamp.

22) **Perc_Moisture** [number double] Percent moisture in soil, solid, and biosolid samples only. Enter the percent moisture as a whole number (e.g., 73 versus 0.73). Do NOT include "%" symbol.

23) **Matrix**: [text string]

For method analytes, EIS, and NIS in field samples only:

- **GW** = Groundwater
- **SW** = Surface Water
- **SD** = Sediment
- **SS** = Soil
- **TS** = Tissue
- **WW** = Wastewater
- **LC** = Leachate

- **BS** = Biosolid

For method analytes, EIS, and NIS in Method Blanks and MDLBs:

- **RW** = Reagent water for all aqueous MBs
- **OS** = Ottawa sand for all soil, sediment, and biosolid MBs
- **RT** = Reference Tissue for tissue MBs

For method analytes, EIS, and NIS in OPRs, IPRs, LOQVERs, MDLSs, and LLOPRs:

- QC = quality control sample

24) **Method**: [text string] Laboratory SOP Name in format of "name(space)revision number."

25) **Study_Phase** – [text string] Multi-Lab Validation Study Phase:

- Enter "**Phase 3**" for Initial Demonstration of Capabilities (IDC), MDL Study, IPRs, and LOQ Verification
- Enter "**Phase 4.4.1**" for GW, SW, and WW matrices
- Enter "**Phase 4.4.2**" = SS and SD matrices
- Enter "**Phase 4.4.3**" = TS matrices
- Enter "**Phase 4.4.4**" = LC and BS matrices

26) **Sample_Type** [text string]:

- For method analytes in MDL Blank IDC samples enter "**MDLB**"
- For method analytes in MDL Spike IDC samples enter "**MDLS**"
- For method analytes in IPR IDC samples enter "**IPR**"
- For method analytes in LOQVER IDC samples enter "**LOQVER**"
- For method analytes in field samples enter "**NORMAL**"
- For method analytes in MBs enter "**BLANK**"
- For method analytes in OPRs enter "**OPR**"
- For method analytes in LLOPRs enter "**LLOPR**"
- For EISs in all samples enter "**EIS**"
- For NISs in all samples enter "**NIS**"

ATTACHMENT 4

DATA MANAGEMENT PLAN, QA/QC AND DATA PROCESSING PROCEDURES, AND DATA MANAGEMENT PLAN ADDENDUM

(Dated 03/01/22, 05/04/22, and 01/23/23, respectively)

Data Management Plan for the

Multi-Laboratory Validation Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

Prepared for:

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LIST OF ACRONYMS AND ABBREVIATIONS

AFFF   Aqueous Film-Forming Foams
DoD   US Department of Defense
DMP   Data Management Plan
EDD   electronic data deliverable
EIS   extracted internal standard
EPA   US Environmental Protection Agency
ESTCP   Environmental Security Technology Certification Program
ETL   Extract, Transform, Load
GCC   Government Community Cloud
HGL   HydroGeoLogic, Inc.
IDA   Institute for Defense Analysis
MLV   Multi-Laboratory Validation
MVS Team   Method Validation Study Team
NIS   Non-extracted internal standard
PFAS   per- and polyfluoroalkyl substances
QA   quality assurance
QC   quality control
SEE   Science and Engineering for the Environment
SERDP   Strategic Environmental Research and Development Program
SLV   Single-Laboratory Validation
USACE   US Army Corps of Engineers

1.0   INTRODUCTION

This document describes data management processes and procedures for the Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. EPA Method 1633 is an interim draft method for analyzing per- and polyfluoroalkyl substances (PFAS), and now requires a Multiple-Laboratory Validation (MLV) Study. The Data Management Plan (DMP) includes the processes and procedures for the transmission, tracking, verification, review, storage, and delivery of laboratory data and associated validation and analyses data collected in support of the MLV Study. During the course of the project, the intended design of certain elements of the DMP may be adjusted; the final processes and procedures used during the project will be documented in the Final Data Management Summary Report.

To meet study requirements for the acquisition of technically sound and legally admissible data, a traceable audit trail will be established from the shipment of sample matrices to each participating laboratory through the archiving of information and data. Each step will be conducted in accordance with the MLV Study Work Plan (SERDP/ESCTP 2022). All potential variations in the analytical and reporting process will be documented and retained with other laboratory data and digital information generated during the MLV Study.

1.1   Background

The MLV Study is currently being conducted by the US Department of Defense's (DoD) Strategic Environmental Research and Development Program (SERDP) in cooperation with the US Environmental Protection Agency (EPA), the US Navy (Navy), the US Air Force (Air Force), and the US Army Corps of Engineers (USACE). Members from each of these agencies comprises the advisory Method Validation Study (MVS) Team. The study is being conducted as SERDP Project ER19-1409. The end goal of the MLV Study is to use the findings to revise, as necessary, draft Method 1633, and to submit the supporting data packages to the EPA Office of Water for consideration as a final method under the Clean Water Act.

As part of the method validation, the MVS Team also worked with Federal, municipal, state, and regional contacts to obtain sufficient volumes/masses of samples from eight different environmental matrices, including wastewater, landfill leachate, groundwater, surface water, fish tissue/clams, biosolids, sediment, and soil. Sample matrices were collected and transferred under chain of custody between September and December 2020. A replacement biosolids sample was collected in October 2021 and an ASTM substitute wastewater sample was developed in December 2021.

Specific steps of the MLV Study are to (a) develop the analytical method, (b) conduct single and multi-laboratory validation studies, and (c) perform statistical analyses of the resultant analytical data to develop appropriate Quality Assurance (QA) and Quality Control (QC) criteria for the method. The draft EPA Office of Water Method 1633 for PFAS has been demonstrated in the Single Laboratory Validation (SLV) Study conducted under ER19-1409 (Willey *et al.* 2021). The Method was evaluated and determined to be sufficiently robust to proceed to the Multi-Laboratory Validation Study. A Final EPA Office of Water Method 1633 for PFAS is critical to DoD Remedial Project Managers working at aqueous film-forming foams (AFFF)-impacted sites. The method is also of critical importance nationally to wastewater permit writers, ecological and human health risk assessments.

1.2   Phases of Data Management

The DMP processes and procedures described herein are applicable to Phases 3-6 of the MLV Study Work Plan (SERDP/ESTCP 2022). The six phases of the plan include:

- Phase 1 – Soliciting Laboratories: This phase involved soliciting proposals and awarding subcontracts to laboratories and suppliers to participate in the Study.

- Phase 2 – Procuring Standards and Study Samples: This phase involved procuring the standards, acquiring and characterizing sample media, and creating the Study Samples.

- Phase 3 – Calibration and Demonstration of Capability: This phase requires each laboratory to (1) develop and submit a Standard Operating Procedure (SOP), (2) perform a minimum of three initial multi-point calibrations, and (3) conduct an initial demonstration of capabilities (IDC) for 'clean' sample matrices. Data/information for this phase includes laboratory-specific SOPs, calibration data, and results from the IDC as well as records related to document reviews, corrections, and approvals.

- Phase 4 – Analyses of Study Samples: This phase involves all participant laboratories using the Study Method to analyze the Study Samples. Data/information for this phase includes laboratory-specific data for each Study Sample (electronic data deliverables [EDDs] and Data Packages).

- Phase 5 – Data Validation: An independent third-party will conduct data validation for all study results. Data/information for this phase includes data reviews, updated/corrected EDDs and Data Packages, correspondence related to corrections, and approvals.

- Phase 6 – Development of QC Acceptance Criteria: Data/information for this phase includes results from the statistical analysis of data from the MLV Study, quality control (QC) acceptance criteria, recommendations for revisions to draft Method 1633, and the MLV Study Report that will be submitted to EPA.

1.3 Data Management Objectives

The primary objective of the DMP is to provide an efficient and organized method of data management to streamline data flow and ensure the highest quality data are compiled. Specific objectives are:

- To facilitate and coordinate with the MVS Team members to ensure that data management system meets overall project objectives;
- To ensure high quality data that provides an accurate representation of all data produced during the study;
- To standardize and store the data in a structured format to allow for accurate querying and statistical analyses;
- To ensure efficient and timely data processing;
- To store the data produced during the MLV Study in a secure location that restricts access to team members with appropriate credentials;
- To allow easy access to the data by project stakeholders; and,
- To implement documentation procedures that ensure the data is technically defensible and legally admissible.

The data management methodology is critical to ensure that laboratory analytical data, validation information, and final statistical calculations are of the highest quality to support and defend the publication of the final method.

2.0 ROLES AND RESPONSIBILITIES

Under the leadership and guidance of the MVS Team, the Data Management Team will work to properly execute the DMP and ensure that the project objectives and scope are achieved. The Data Management Team consists of Exa Data & Management, Inc. (Exa) and HydroGeoLogic, Inc. (HGL; Figure 1). The Data Management Team will coordinate with the data providers, including the analytical laboratories, the validator team, and the statistics and analysis team (Institute for Defense Analysis [IDA]). HGL will manage the laboratories and provide an initial review of the laboratory data to ensure contractual compliance, and Exa will be responsible for all other aspects of data management as described in Section 4.0.
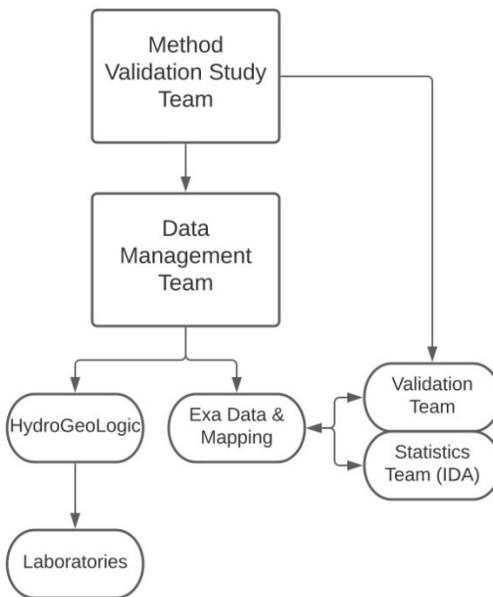
Figure 1. Organization of the Multi-Laboratory Validation Study teams relative to their roles in managing data

Specific roles of individual team members are provided in Table 1. Ms. Dawn Smorong, Exa's Project/Database Manager, has overall responsibility for ensuring the data are managed in accordance with the approved MLV Study Work Plan (SERDP/ESTCP 2022) and other related documents. Other Exa team roles include that of Ms. Peggy Myre, who will serve an oversight role to ensure that project data management goals and target schedule milestones are met. Dr. Michael Tweiten of Exa will bear primary responsibility for management of centralized file sharing system discussed in in Section 3.0.

Table 1. MLV Study Data Management Team Member Roles and Responsibilities

Organization	Team Member	Role	Data Management Responsibility
Exa Data & Management	Dawn Smorong	Exa PM; Data Manager	Exa project completion and database management
	Peggy Myre	Exa Data Quality Officer	Ensure compliance with project goals and the DMP
	Michael Tweiten	Exa Data Library Manager	Setup and manage MLV Study Library storage and users
HydroGeoLogic	Joe Skibinski	HGL PM	HGL project completion, lab coordination
	John Powell	HGL Program Chemist	Laboratory coordination, chemistry review
	Denise Rivers	HGL Project Chemist	Laboratory data compliance, chemistry review
	Ken Rapuano	HGL Project Chemist	Laboratory data compliance, chemistry review
	Andrea Fletcher	HGL Data Manager	Laboratory EDD and data package tracking and coordination

The HGL data management role will be to coordinate incoming data from the laboratories, and to perform initial checks of data acceptability as described in Section 4.1.2.

3.0 DATA SHARING PLAN AND FILE TRACKING

A critical element to ensure proper organization of the data collected for the MLV Study will be managing the files generated to support the project. A file storage server will be deployed to serve as a repository for all documents and data for the project, termed the MLV Study Library (Section 3.1). As part of the file organization strategy, a File Tracking System was developed (Section 3.2), including strict rules for file-naming (Section 3.3).

3.1 MLV Study Library: Host and Software

All project data and information will be stored on a centralized, secure server managed by the Exa team. Table 2 provides a listing of the general data types stored on the server, as well as the MVS Team member responsible for upload and maintenance of the associated files.

Table 2. MLV Study General Data Types

Data Type	Example Data	Team Member Responsible for Upload/Maintenance
Project Documents	Background Documents - UFP-QAPP and PMP, Study Work Plan, MLV Study Report	HGL/Exa
	Correspondence	All
	Meetings and Schedules - Schedule, Contact list, Meeting Minutes	HGL, SEE
	Samples and Shipments - Sample Shipments, Standards and Study Samples	HGL
	Project Reports - working and final versions of reports generated for the MLV Study	MVS Team
Laboratory Data	EDDs (csv)	Individual labs
	Data packages (pdf)	Individual labs
	ICAL Data Packages	Individual labs
	IDC (EDD and Data Packages for aqueous, solid and tissue matrices)	Individual labs
	Standard Operating Procedures (SOP)	Individual labs
	Spike Levels and Background Analytical Data	HGL
Validator Data	Amended EDDs (xlsx), Lab Data Packages	Exa
	DV Report (pdf)	Individual validators
	Amended EDDs with validator fields populated (xlsx)	Individual validators
	Evidence of 10% verification (xlsx)	Individual validators
Statistics Data	Database exports (xlsx)	Exa
	Report with appendices (pdf) and supporting calculations (xlsx)	IDA
Database	Database (accdb), documentation (pdf)	Exa
Tracking	File Tracking system	Exa/HGL

Access will be strictly controlled to ensure the protection of all proprietary data. The selected platform is ©Microsoft (MS) Office 365 Enterprise software; the SharePoint application will be used for the central storage and accessing of documents, data, and other information related to the MLV Study. This section details server specifications, the folder structure, as well as the list of users and their access level (permissions).

The MLV Study Library will be hosted on the Microsoft Azure Government Community Cloud (GCC) High and DoD environments to ensure cloud-service compliance, including Federal Risk and Authorization Management Program (FedRAMP) High, Department of Defense Security Requirements Guidelines, Defense Federal Acquisition Regulations Supplement (DFARS), and International Traffic in Arms Regulations (ITAR). The MLV Study Library will utilize a Microsoft Office 365 E1 Enterprise environment, including Microsoft Office 365 software tools to enable file sharing, editing and team communications and identity and access management.

The MLV Study Library will include access restrictions with requirements for authentication and user credentials to gain access. Exa's Michael Tweiten will be responsible for setting up the system and assigning users and user privileges based on assigned project roles and responsibilities. The laboratory, validator and statistics participants will only be allowed to upload/view their own data. Additional details regarding users and defined privileges are provided in Section 3.1.2.

3.1.1 Folder Structure

The MLV Study Library will employ a strict, hierarchal folder structure, and will display a list of files and key information about the files, such as who was the last person to modify the file. The folder and sub-folder structure will support access permissions as described in Section 3.1.2. The top-level folders will clearly indicate the type of data and other content available in each folder (Figure 2).
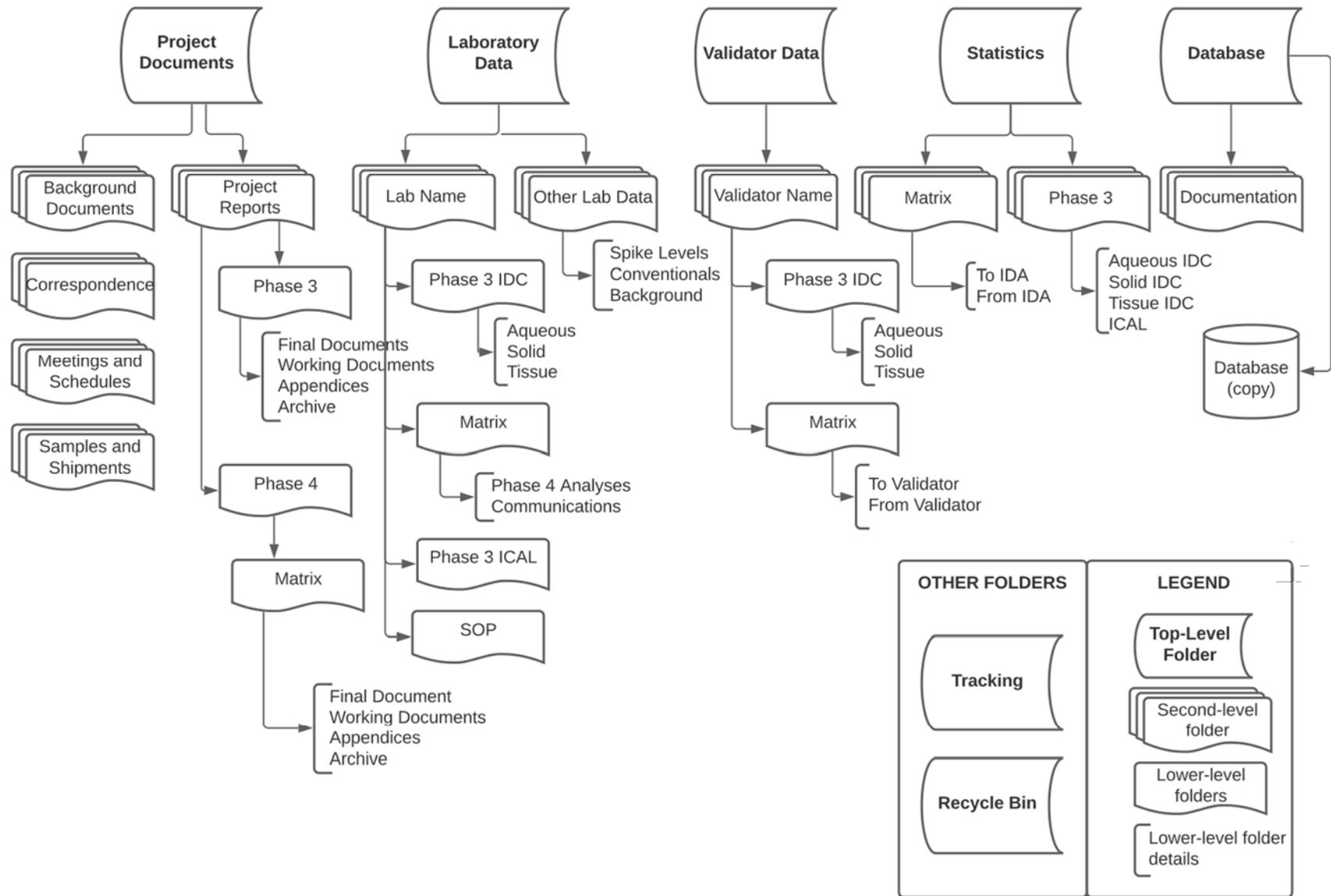
Figure 2. Folder structure for the MLV Study Library

Each top-level folder contains sub-folders for different types of data, from different sources:

Project Documents
- **Background Documents** – This folder will include reports and documentation that guide the MLV Study (e.g., Study Work Plan, UFP-QAPP).
- **Correspondence** – MVS Team members will ensure that project communication (including email) is backed up. Correspondence to include on the MLV Study Library includes: any written communication (including emails) that document major decisions and information regarding study status and/or problems; a log documenting verbal communication with team participants regarding study status or issues.
- **Meetings and Schedules** - The project schedule (MS Project) will be a shared document updated regularly by HGL and Science and Engineering for the Environment (SEE); a method for sharing key milestones with all team members will be developed.
- **Samples and Shipments** - This folder will include records concerning sample shipments and receipts, as well as records and documents associated with the procurement of standards from Wellington, and the creation and production of ERA Study Samples.
- **Project Reports** – MVS Team members with appropriate permissions (Section 3.1.2) will maintain working and final versions of the reports generated during the MLV Study in this folder by Phase and Matrix.

Laboratory Data
- **Lab Name** – Each laboratory will have their own folder including the same structure of sub-folders.
    - **Phase 3 IDC** – This folder will include the Phase 3 Initial Demonstration of Capability (IDC) EDDs and Data Packages for aqueous, solid and tissue matrices.
    - **Phase 3 ICAL** – This folder will include the Phase 3 Initial Calibration (ICAL) Data Package.
    - **Matrix** – There will be one sub-folder for each of eight matrices.
        - **Phase 4 Analyses –** This folder will include the EDDs and Data Packages for the relevant matrix.
        - **Communications** – This folder will contain documents provided to the laboratories (e.g., EDDs with comments incorporated, resubmission requests).
    - **SOP** – This folder will contain each laboratory's Standard Operating Procedures.
- **Other Lab Data -** This folder will include the files provided by AXYS, Test America and ERA/Waters with data for spike levels, conventional results and background analytical data.

Validator Data
- **Validator Name** – Each validator will have their own folder including the same structure of sub-folders.

- o **Phase 3 IDC** – There will be one folder for each of the three IDC matrices (aqueous, solid and tissue).
  - ▪ **To Validator** - Phase 3 IDC Amended EDDs will be provided in this folder (by Exa) for each laboratory (not shown on Figure 2).
  - ▪ **From Validator** – Phase 3 IDC results provided by the data validator (Amended EDD with validator fields populated) will be included in this folder, for each laboratory (not shown on Figure 2).
- o **Matrix** – There will be one sub-folder for each of eight matrices.
  - ▪ **To Validator** – This folder will include Amended EDDs (from Exa) and laboratory Data Packages, for each laboratory.
  - ▪ **From Validator** – This folder will include the DV Report, Amended EDD with validator fields populated, and evidence of 10% verification, for each laboratory.

Statistics
- **Phase 3** – There will be one folder for each of the three IDC matrices and the ICAL data.
  - o **Matrix** – There will be one folder for each of the three IDC matrices (aqueous, solid and tissue).
    - ▪ **To IDA** – This folder will contain IDA Database Exports (from Exa) (not shown on Figure 2).
    - ▪ **From IDA** – This folder will include IDA's report with appendices (pdf), along with supporting calculations (xlsx) (not shown on Figure 2).
  - o **ICAL**
    - ▪ **To IDA** – This folder will contain the tabular version of the ICAL results (from SEE) (not shown on Figure 2).
    - ▪ **From IDA** – This folder will include IDA's report with appendices (pdf), along with supporting calculations (xlsx) (not shown on Figure 2).
- **Matrix** – There will be one sub-folder for each of eight matrices.
  - o **To IDA** – This folder will contain Database Exports (from Exa).
  - o **From IDA** – This folder will include IDA's report with appendices (pdf), along with supporting calculations (xlsx).

Database
- **Database** – A copy of the Project Database (MS Access) will be posted regularly throughout the program; version will be indicated by the date in the filename (e.g., MLVS_Database_20220203). Older versions of the database copies will be moved to an Archive within the Documentation folder.
- **Documentation** – This folder will contain database documentation files (e.g., database dictionary, valid value lists, QA/QC application, scripts, archived database copies).

Tracking

- The File Tracking System will be a shared document that will be updated by Exa and HGL; Exa will have responsibility for the overall management of the File Tracking System.

Laboratory, validator and statisticians will be given access to their specific folders ONLY as described in Section 3.1.2. The Project Documents folder will also have limited access as described below. Exa is responsible for reviewing the files uploaded to the site by the laboratory, validator, and statistician team members, ensuring their documents are properly filed and the file-naming protocols are adhered to (described in Section 3.3).

3.1.2 Access and Permissions

There will be a strict permission structure limiting access to certain folders to specific users. Staff at Exa will be the only organization with Administrator permission, with full control to audit all site content and receive administrative messages. All other permissions will be "Owner" status, which allows full control permissions (upload/download/edit) to the folders specified in Table 3.

Table 3. MLV Study Library Permission Structure

Team Member Organization	Team Member Role	Team Member	MLV Study Library Folder					
			Project Documents /Project Reports	Project Documents /Other Folders	Laboratory Data	Validator Data	Statistics	Database
Exa*	Data Management	See Table 1	X	X	X	X	X	X
SEE	Co-Principal Investigator	Tim Thompson	X	X	X	X	X	X
NAVSEA LQAO	QA Manager	Janice Willey	X	X	X	X	X	X
SERDP/ESTCP	Study Supervisor	Dr. Andrea Leeson	X	X				
AFCEC	Study Evaluation Manager	Dr. Hunter Anderson	X	X			X	X
EPA OW	Senior Chemist	Adrian Hanley	X	X			X	
HGL	Laboratory / Data Management	See Table 1		X	X	X	X	
Multiple	Laboratory Analyses	See Table 4			X			
Multiple	3rd-Party Data Validation	See Table 4				X		
IDA	Statistical Analyses	Allyson Buytendyk		X			X	

*All team members have full control permisions for the folders to which they have designated access; Exa has Administrator permision level.

Library information is permissions-trimmed, meaning that individuals will only have access to designated folders within the MLV Study Library folder structure. In other words, members from each individual laboratory can only access their own EDDs and Data Packages in

order to maintain control of proprietary data. Similarly, individuals from the validation or statistics groups will only be able to access the folders designated to them.

Table 4. Project Participants with Access to the MLV Study Library

Team / Organization	Team Member	Phone	Email
Method Validation Study Team			
NAVSEA	Janice Willey	843-327-1152	janice.willey@navy.mil
SEE LLC	Tim Thompson	206-418-6173	tthompson@seellc.onmicrosoft.com
AFCEC	Hunter Anderson	210-395-0625	Richard.anderson.55@us.af.mil
SERDP/ESTCP	Andrea Leeson	571-372-6398	andrea.leeson.civ@mail.mil
EPA (OW)	Adrian Hanley	202-564-1564	hanley.adrian@epa.gov
Data Management Team			
Exa	Dawn Smorong	250-713-8601	dawn@exadata.net
	Michael Tweiten	360-930-8530	michael@exadata.net
	Peggy Myre	360-774-0380	peggy.myre@exadata.net
HGL	John Powell	913-378-2315	jpowell@hgl.com
	Joe Skibinski	703-853-5083	jskibinski@hgl.com
	Denise Rivers	910-233-8460	drivers@hgl.com
	Ken Rapuano	703-736-4546	krapuano@hgl.com
	Andrea Fletcher	913-317-8860	afletcher@hgl.com
Laboratories			
California DTSC	Katie Hamblin	626-344-1220	Katherine.Castor@dtsc.ca.gov
Pace	Stephen Somerville	804-516-5887	Stephen.somerville@pacelabs.com
SGS	Andrea Colby	609-495-5231	andrea.colby@sgs.com
Battelle	Jon Thorn	781-681-5565	thorn@battelle.org
GEL	Vonda Fields	843-556-8171 x4262	Vonda.Fields@gel.com
Vista Analytical	Anne Wilhoit	916-673-1520	awilhoit@vista-analytical.com
Maryland DOH	Sin Urban	443-681-3852	sinisa.urban@maryland.gov
Alpha Analytical	Alycia Mogayzel	508-844-4120	amogayzel@alphalab.com
Eurofins Lancaster	Bradley Ayars	717-556-7265	Bradley.Ayars@EurofinsET.com
ETA - Sacramento	Jill Kellman	916-374-4402	jill.kellmann@eurofinsET.com
Validators			
Pyron	Mingta Lin	360-556-5952	mingta_lin@comcast.net
Jacobs	Maggie Radford, PE	919-749-9479	maggie.radford@jacobs.com
	Jeremy Bishop	541-768-3299	jeremy.Bishop@jacobs.com
Chem Val	Kathi Gumpper	801-541-6983	kgumpper@chemval.com
	John Gumpper	801-554-9362	jgumpper@chemval.com
	Gumpper (home)	231-723-4043	--
Statisticians (IDA)	Allyson Buytendyk	703-845-6806	abuytend@ida.org

Specific members of the MVS Team will have access to different folders. For example, under the primary folder called "Project Documents," most team members will have access to Background Documents, Meetings and Schedules, Samples and Shipments, and Correspondence folders. A smaller group will have access to the Project Reports folder (Table 3). Table 4

includes the list of project participants that will have access to the MLV Study Library site, as well as their contact information.

3.2　File Tracking System

A File Tracking System ("Tracking System") was developed to ensure that the flow of laboratory data is logged at each stage of the project. The workflow for handling data from the laboratories, through the validators and the statisticians is discussed in Section 4.1. In this section, the format of the Tracking System is defined and described.

The Tracking System includes four stages of laboratory data tracking:

- General – Defines the basis for a unique set of files from the laboratory, including the EDD and the Data Package, linked to the laboratory of origin and the matrix analyzed. If an EDD is rejected and resubmitted, then the resubmitted EDD/Data Package receives a new version number and are tracked separately from the original.
- Laboratory – This element of the Tracking System defines the status of laboratory data receipt and review by the Data Management Team.
- Validator – This element of the Tracking System defines the status of receipt, processing, and return of the laboratory data to and from the Validators.
- Dbase – This element of the Tracking System defines the status of laboratory and validation information compiled into the Project Database.

The status of data provided to the statistics team (IDA) will be tracked separately, since they will receive Database Exports for each matrix (i.e., not on an EDD-specific basis).

A summary of the tracking fields is provided in Appendix A1. Several Tracking System fields will be limited to specific content ("valid values"); the list of acceptable entries for the valid value fields is provided in Appendix A2.

Tracking information will be imported into the Project Database and used to build regular status reports for the MVS Team partners.

3.3　File-Naming Protocols

As part of the File Tracking System, a strict file-naming protocol has been devised and guidance produced for the laboratories, validators, and statisticians. Each laboratory EDD and accompanying Data Package (DP) will be named according to the laboratory, the matrix, and the version of the data. If the delivered data is a resubmission (Section 4.1.6), then the file name will reflect that the data are of a new version (Table 5). Importantly, the laboratory must resubmit BOTH the EDD and the Data Package with a new version number, even if only one or

the other was revised. Similar file-naming protocols have been developed for the validators and statisticians.

The Exa team will be responsible for reviewing the names of submitted files and if the file-naming protocols are not adhered to the participant will be asked to resubmit the files. Details of the file-naming protocols are provided in Appendices B1 – B3. A Quick Start Guide will be provided to the participating laboratories, validators and statisticians to provide instructions on file-naming protocols and using the MLV Study Library site.

Table 5. Laboratory Data File-Naming Protocol Examples

Tracking ID	EDD File Name	Data Package File Name	Laboratory Name Code	Matrix Code	Description
ALPHA_GW_ver0	ALPHA_GW_ver0.csv	ALPHA_GW_ver0.pdf	ALPHA	GW	First EDD/DP submitted by Alpha for groundwater
ETA_SD_ver0	ETA_SD_ver0.csv	ETA_SD_ver0.pdf	ETA	SD	First EDD/DP submitted by ETA for sediment
ETA_SD_ver1	ETA_SD_ver1.csv	ETA_SD_ver1.pdf	ETA	SD	First revision of ETA EDD/DP for sediment
ALPHA_GW_ver1	ALPHA_GW_ver1.csv	ALPHA_GW_ver1.pdf	ALPHA	GW	First revision of Alpha EDD/DP for groundwater
ALPHA_GW_ver2	ALPHA_GW_ver2.csv	ALPHA_GW_ver1.pdf	ALPHA	GW	Second revision of Alpha EDD/DP for groundwater

4.0 DATA MANAGEMENT PROCESSES AND PROCEDURES

In addition to the MLV Study Library, a Project Database ("database") will be developed to incorporate the laboratory EDD data generated for the project, as well as the data validation results. This section of the DMP provides an overview of the main components of the workflow (Section 4.1.1 – 4.1.5), a description of the rejection criteria and resubmission process (Section 4.1.6), and a description of the database and related tools for processing data (Section 4.2).

4.1 Workflow

One important element to meet project goals is a specific, rigorous, and well-documented workflow for the data generated during the project. This section provides detailed descriptions of every step of that workflow. At each step, dates of actions and descriptions of decisions will be logged in the Tracking System.

A diagram of the MLV Study workflow is provided in Figures 3a and 3b. The workflow outlines the sequence of processes that will be adhered to by all team members, including the Data Management Team (Exa/HGL), the MVS Team, laboratories, the validation team, and the statistical analysis team (IDA).

One of the key elements of workflow is the multiple stages of data QA/QC by the Data Management Team, the validators, and the MVS Team. At each stage, the Exa Data Manager will ensure that the review information is captured in the Tracking System so that the MVS Team will always know the status of the laboratory data.

4.1.1 Receipt of Data Sets

As shown in Figure 3a, the first component of the workflow is the receipt of data sets, where the laboratories upload an EDD/Data Package to the MLV Study Library site. The initial QA/QC checks and tracking steps to be conducted immediately upon receipt of the EDD and Data Package by the Exa Data Manager and include:

- Verify EDD and Data Package match;
- Enter EDD in File Tracking System; and,
- Confirm file-naming protocol was followed.

In addition, the MVS Team are notified that the laboratory has uploaded a submission. If errors or omissions are found, the issues are documented in the Tracking System and the EDD and Data Package will be rejected. Exa will inform HGL of the issues, who will subsequently inform the laboratories that they must address the issue(s) and resubmit the data.

Details on the rejection criteria and resubmission process is described in Section 4.1.6.
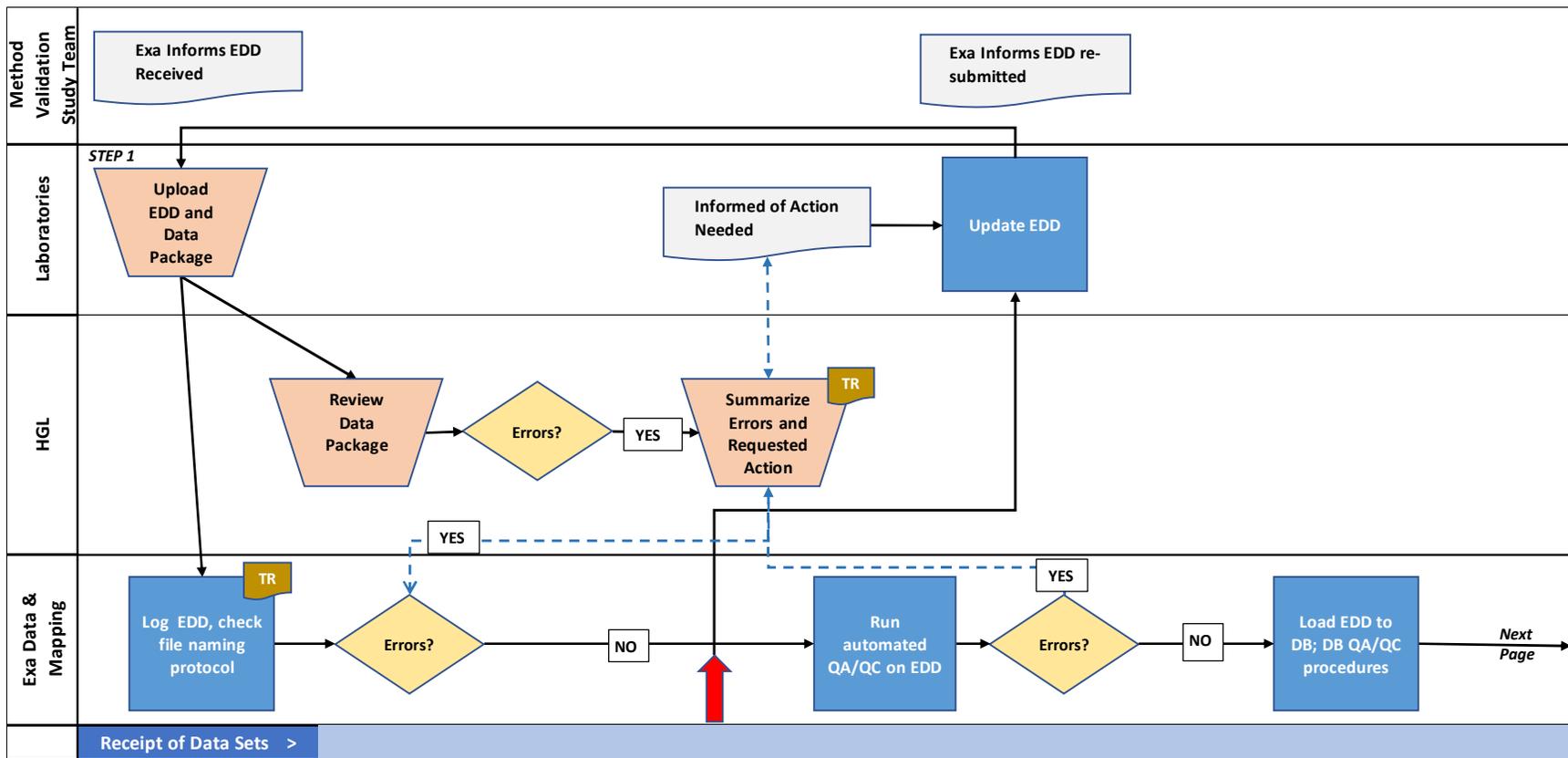
Figure 3a. Workflow for the PFAS Multi-Laboratory Validation Study
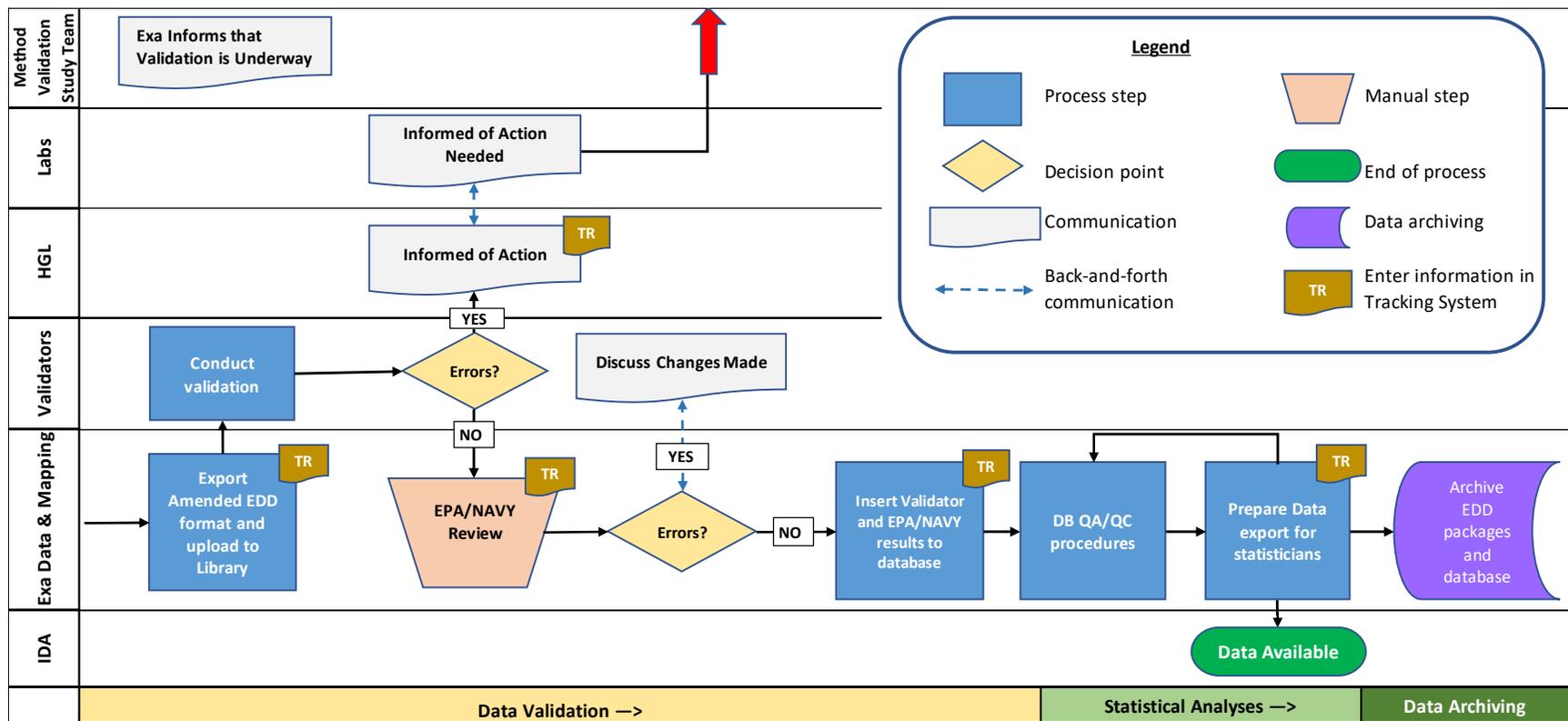
Figure 3b. Workflow for the PFAS Multi-Laboratory Validation Study (continued)

4.1.2 Review Laboratory EDD/Data Package Submissions

The next step in the workflow (Figure 3a) is to conduct a detailed review of the data submitted from the laboratory. This involves two major steps. First, the HGL Project Chemist will review the Data Package. These checks include:

- Verify each element is reported and no gross contamination in blanks;
- Ensure all mandatory elements are present in Data Packages for validation;
- Confirm all data for samples and QC samples reported in the Data Packages have been included and that all fields are completed.

If errors or omissions are found, the issues are documented in the Tracking System and the EDD and Data Package will be rejected. HGL will inform the laboratories that they must address the issue(s) and resubmit the data. A timetable for receipt of the resubmitted data will be established and logged into the Tracking System.

Details on the rejection criteria and resubmission process is described in Section 4.1.6.

The second step of this part of the workflow involves Exa conducting automated QA/QC checks on the EDD using a customized application. If errors are found, the file will be noted as rejected in the Tracking System, with the reasons for rejection. HGL will then notify the laboratory that they must address the issue(s) and resubmit the data.

These automated QA/QC checks will ensure that each EDD contains all information required by the template guidance (SERDP/ESTCP 2022, Attachment 3), and each data field in each EDD is completed in accordance with those instructions. The initial list of automated QA/QC checks will require that:

- Required fields are populated;
- Valid value fields match required content;
- Significant figures are compliant (more than three significant figures are not allowed);
- The full list of required analytes are reported;
- Records are unique, based on ensuring a single result for each method analyte, Extracted Internal Standard (EIS) compound, and non-extracted internal standard (NIS) compound is present for each sample (batch QC and study samples);
- The NIS/EIS result can be linked to the associated target analyte for the same sample by SDG;
- Specific cell formats are correct:
  - Dates use the correct format, and does not include time stamp
  - Numbers reported in number field (e.g., no text content like 'trace' or 'ND')
  - Spike percentage recoveries are entered as whole numbers;
- Units are appropriate for the matrix (ppt or ng/L for aqueous samples; ppb or mg/kg for solid and tissue samples);

- Check for reported value of zero (0) in number fields.

There are other logic checks comparing different fields that will be generated. For example, concentration checks:

- If Conc_found = MDL, then Lab_Flag must contain *U*
- If Conc_found < MDL, then flag as error (if a signal is not detected or if the signal produces a concentration < MDL the value must be set to the MDL)
- If Lab_Flag contains *U* then Conc_found = MDL
- If the Conc_found is >/= MDL and <LOQ, then Lab_Flag must contain *J*
- If Sample_No is "MB", then Sample_Type should be "BLANK"
- If Sample_No is "OPR", "LLOPR", "IPR", "MDLB", "MDLS", or "LOQVER", then Sample_Type should also be the same

Other checks will be generated as needed. The automated QA/QC checking application will be developed by the Exa team and then tested. Testing will involve multiple Exa team members running the checks on multiple test data sets to identify bugs and inconsistencies in the QA/QC checks. Fixes will then be incorporated into the checking routines.

The final step of this component of the workflow is to load the EDD data into the Project Database. As shown in Figure 3a, this only occurs if the laboratory EDD and Data Package has passed all the review steps with no errors or omissions.

4.1.3 Data Validation

The next component of the workflow is Data Validation and is shown in Figure 3b. Three independent third-party validators are responsible for the validation of Data Packages and EDDs in accordance with the study data validation guidelines (SERDP/ESTCP 2022, Attachment 5). Following review and acceptance of the EDD and Data Packages by the Data Management Team, an Amended EDD file will be generated from the database for the selected validator, which will include the laboratory EDD fields, as well as several additional fields incorporated for the validator to populate. The format of the validator Amended EDD is described in Section 4.2.2 and Appendix C1. The Amended EDD, as well as the original Data Package, will be provided to the validator in the appropriate folder on the MLV Study Library. In addition, Exa will inform the MVS Team that the data validation is underway.

The validator then conducts the data validation procedures. If the validator finds errors in the laboratory data that require the laboratory to revise the information and data they have submitted, the validator will inform Janice Willey (NAVSEA) who will determine the course of action to be followed. If a laboratory resubmission is warranted, HGL will enter the issues found in the Tracking System and communicate with the laboratory regarding the requirement for a

resubmission. A timetable for receipt of the resubmitted data will be established and logged into the Tracking System.

If no errors are found in the laboratory data, the validator will complete the data validation procedures and provide a data validation report and the associated Amended EDD with the validator fields populated. The Exa team will be responsible for logging the receipt of the submitted files into the Tracking System, reviewing the names of submitted files and if the file-naming protocols are not adhered to the validator will be asked to resubmit the files.

The next step in the workflow is for Navy and EPA members of the MVS Team to review the validator results. If the Navy/EPA reviewers disagree with the validator qualifiers, they will enter qualifiers and comments into the valReviewer_qualifier and valReviewer_notes fields of the Amended EDD; these changes will be communicated to the data validator.

The NAVY/EPA reviewers will upload the revised Amended EDD (including the valReviewer fields populated) with a '_valreviewed' suffix on the file name, to the appropriate folder in the MLV Study Library. For example, if the file name submitted by the validator was 'CHEMVAL_ALPHA_GW_results_v0.xlsx', the file re-uploaded by the NAVY/EPA review should be 'CHEMVAL_ALPHA_GW_results_v0_valreviewed.xlsx.

When the review of the validation results has been completed by the NAVY/EPA reviewers, they will notify the Exa Data Manager and inform them whether they uploaded a revised Amended EDD file to the MLV Study Library, or if the original file submitted by the validator is the final version. The Exa Database Manager will then run a routine to link the Project Database to the appropriate file to incorporate validator and validator reviewer qualifiers and comments.

Exa will be responsible for logging the receipt of the submitted files in the Tracking System, checking that the file-naming protocols are followed.

4.1.4 Statistical Analyses

The next step of the workflow is Statistical Analyses and is shown in Figure 3b. Once the database is complete for one matrix, the Exa Data Manager will execute automated database-level checks to ensure results are consistent for the given matrix, and then export the complete dataset for that matrix and provide it to the statistics team member (IDA) in their folder in the MLV Study Library. The format of this Database Export is described in Section 4.2.2 and Appendix C1. Upon completion of the statistical analysis, IDA will upload all files discussing the results to the appropriate MLV Study Library folder [for each matrix, this will include a report with appendices (pdf), along with supporting calculations (xlsx)]. The Exa team will be responsible for reviewing the names of submitted files and if the file-naming protocols are not adhered to the participant will be asked to resubmit the files.

The Phase 3 ICAL results have a slightly different workflow – these data will be compiled from the laboratory Data Packages by SEE and then provided to Exa for QA/QC review. Subsequently, Exa will upload the final file to the appropriate folder in the MLV Study Library (Phase 3 ICAL/To IDA). After statistical analyses are complete, IDA will supply the results in the Phase 3 ICAL/From IDA folder. Note that Phase 3 ICAL data is not stored in the Project Database.

If the statisticians encounter issues with the Database Export provided to them by Exa, the issues will be recorded in the Tracking System, the issues will be resolved and a new Database Export will be provided, with a revised version number.

4.1.5 Data Archiving

The final step of the workflow (Figure 3b) is to archive the data, both during the project and at project completion. The master version of the Project Database will be backed up regularly on Exa's servers and copies will also be posted on the MLV Study Library with the date of posting, along with current versions of the QA/QC application and other database routines and scripts. The MLV Study Library has several features as a part of the Microsoft Enterprise environment, to ensure the information stored on the SharePoint site is always recoverable (Microsoft 2022). For example, the Microsoft datacenters are geo-distributed to mitigate the impact of a natural disaster or local power outage; backups are retained for 14 days and can be restored to any point in time.

At the completion of the project, an archive of the database, all related templates, tools, and documentation will be compiled for delivery, along with the final data management report. The data archive information package will be prepared that describes the data system, file format, and method of archival. Sufficient documentation will accompany the archived data to fully describe the source, contents, and structure of the data to ensure future usability.

A final archive of the MLV Study Library will be transmitted to the SERDP upon project completion as requested. Prior to archiving the MLV Study Library, the folder structure under the following top-level folders will be condensed to separate the final versions of the raw data files from preliminary versions: Laboratory Data, Validator Data, Statistics.

4.1.6 Rejection and Resubmission Process

There are several steps along the workflow where laboratory EDDs/Data Packages can be rejected, as shown in Figure 3a and 3b and described in Sections 4.1.1 through 4.1.4. If the laboratory EDD and/or Data Package includes any inconsistencies with the instructions provided in their contract, or they have not followed the instructions for populating the EDD template, the submission will be rejected. Importantly, the Exa data managers will not conduct any

editing or data cleaning procedures to amend the data provided by the laboratories. In addition, the laboratory EDD/Data Package may be rejected if the data validators find issues with the data that require re-analysis. If the EDD/Data Package is rejected, the laboratory will be informed that they must address the errors and resubmit the data. A timetable for receipt of the resubmitted data will be established and logged into the Tracking System. The resubmittal must be given a revised version number as described above and shown on Table 5.

4.2   Database and Tools

This section describes the structure and associated tools for compiling the EDDs into the Project Database. The Project Database will be a relational database using MS Access as the selected database software. Access was chosen due to its common usage and ease of transforming the data to other formats, as necessary. The master version of the Project Database will be stored on Exa's local server, ensuring that access to the 'working' database is limited to the Database Managers. If project participants request access to the Project Database, they will be given permissions to access the current copy of the database posted on the MLV Study Library site. Alternatively, Exa can generate customized data exports for specific purposes, if requested. During the project, there will only a portion of the EDDs loaded into the database, and not all EDDs will have the data validation information loaded; therefore, prior to uploading the current version of the Project Database to the MLV Study Library, Exa will devise a method for identifying laboratory EDDs that are not complete and finalized (i.e., may still be undergoing review and/or may not yet have data validation results incorporated).

The goals of the Project Database and associated toolsets are as follows:

- Maximize the reliability of the database by designing and implementing automated QA/QC and verification checks;
- Store the data in a structured database with rules that restrict data import to specific valid values, and that follow relational database rules such as primary keys and inter-table relationships;
- Promote accurate and rapid transfer of data to a variety of export and imports formats for use by team members (validators, IDA) and reporting to the MVS Team.

4.2.1 Database Structure

The database will be managed in MS Access and copies will be posted regularly on the MLV Study Library. The structure of the database is provided in Figure 4 as an entity-relationship diagram (ERD), which describes the tables and fields in the database and how they are related. The field definitions are compiled in the database dictionary as seen in Appendix C1.

TR2_LABORATORY
- Tracking ID
- Laboratory Name
- Matrix
- EDD/DP Due Date
- EDD/DP Date Received
- HGL Reviewer
- Date HGL Review Complete
- EDD Rejected or Approved - HGL
- Summary of Errors - HGL
- Exa Reviewer
- Date Exa Review Complete
- EDD Rejected or Approved - Exa
- Summary of Errors - Exa
- Report/EDD Resubmission Request Date

TR1_GENERAL
- Tracking ID
- Project Phase
- EDD File Name
- Data Package File Name
- Laboratory Name
- Matrix

Lab_EDD_Results
- TrackingID
- LAB_ID
- lab_num
- sdg_num
- SAMPLE_NO
- LAB_SAMPLE_ID
- ANALYSIS_DATE
- ANALYSIS
- PFAS_ACRONYM
- DILUTION
- CONC_FOUND
- LAB_FLAG
- CONC_SPIKE
- PERCENT _REC
- MDL
- LOQ
- UNIT
- SAMPLE_TRANSITION_RATIO
- EXPECTED_TRANSITION_RATIO
- RRT
- SAMPLE_SIZE
- SAMPLE_SIZE_UNIT
- EXTRACTION_DATE
- PERC_MOISTURE
- MATRIX
- METHOD
- STUDY_PHASE
- SAMPLE_TYPE
- result_type
- spike_cat
- validation_level
- validator
- dv_qualifier
- final_qualifier
- dv_qualifier_reason
- valnotes
- validator_result
- final_result
- valResultChange_yn
- valResultChange_desc
- valReviewer_qualifier
- valReviewer_notes

Convent
- LAB_ID
- SAMPLE_NO
- LAB_SAMPLE_ID
- ANALYSIS_DATE
- COMPOUND
- DILUTION

LU_compound
- PFAS_ACRONYM
- Compound
- CAS_NO

LU_Spike_Levels
- PFAS_ACRONYM
- Matrix
- PREP
- Low_Spike_ngL
- High_Spike_ngL

dicValidValues
- VVLField
- ValidValue
- VV_Description
- VVL_match_alt_field
- VVL_match_alt_code

TR3_VALIDATOR
- Tracking ID
- Laboratory Name
- Matrix
- Data Validator
- Amended EDD File Name to DV
- Date Amended EDD to DV
- DV Amended EDD File Name
- DV Other File Names
- Date DV Report/Files Received
- EDD Rejected or Approved - DV
- Summary of Errors - DV
- DV Report/Files Resubmission Request Date
- EPA/NAVY Reviewer
- DV Amended EDD Revised or Accepted?
- EPA/NAVY Reviewer File Name

TR4_DBASE
- Tracking ID
- Laboratory Name
- Matrix
- EDD Upload Date
- EDD Upload Initials
- DV Upload Date
- DV Upload Initials
- Date Removed From DB

TR5_STATS
- Matrix
- Stats DB Export File Name
- Date DB Export to IDA
- Date Stats Results Received
- Summary of Errors - IDA

Figure 4. Entity-Relationship Diagram (ERD) for the Project Database

The main EDD data table ("Lab_EDD_Results") parallels the format of the laboratory EDD (SERDP/ESTCP 2022, Attachment 3), with EDD field names shown in all capital letters in Figure 4. In addition to the EDD fields, there are several additional fields that will be added to the main EDD table, shown in lower case, including the Tracking ID, validation information, a result type and the spike level (Appendix C1).

The valid value tables (dicValidValues, LU_Compound) will be maintained separately but linked to the main EDD table to enforce those valid values and are shown in Appendix C2 and C3, respectively. Retaining strict valid values will enable both the validators and IDA to accurately filter and analyze the output data.

The database structure includes the LU_SpikeLevels table to allow this information to be included in the Database Exports for the statisticians, and the CONVENT table to store conventional results measured in select samples.

Finally, the database structure includes all tables from the Tracking System in order to support summary status reports for the project.

4.2.2 Import and Export File Structures

The primary import structure for the Project Database is the laboratory EDD, provided as Attachment 3 in the Study Plan (SERDP/ESTCP 2022).

There are several other import and export routines that will be used in the overall workflow of the MLV Study using queries in the database:

- Export of the Amended EDD for the validators – Includes the laboratory EDD results, and additional fields to be populated by the validator when reviewing the results provided by one laboratory for one matrix.
- Import of the Amended EDD, with validation fields populated – Used to update the Project Database with the results from the validator and the data validation reviewers.
- Database Export for the statistics team – Used to create a dataset for for a single matrix for IDA in generating statistics and analysis for the project. This database export includes final results and qualifiers, considering laboratory, data validator and data validator reviewer results.

The output formats to be provided to the validators and IDA are provided in Appendix C1 (see columns named 'Include in Amended EDD for DV' and 'Include in Exports for IDA', respectively).

4.2.3 Data Processing Tools

A variety of database scripts, queries, and routines will be developed in order to automate workflow processes. These tools are described in this section.

- EDD Import – The import script will automatically import the EDD into a customized QA/QC application in preparation for the initial QA/QC checks on the EDD.
- EDD QA/QC Checks – The code in the customized QA/QC application will runa series of automated data verification checks as described in 4.1.2. If any of the checks fail, a report of those failed items will be provided to HGL and the laboratory for addressing.
- Extract, Transform, and Load (ETL) routine – Once the EDD has passed the QA/QC screening checks, the ETL database code will extract the fields from the EDD, add the additional related fields as described in Section 4.2.1, and then append the new rows to the master EDD table. The code will also include a check to ensure accuracy of the number of rows appended.
- Database QA/QC Checks – Automated routines to check for internal consistency within the Project Database.
- Tracking System – An automated routine will be developed to import the Tracking System tables into the Project Database and then generate summary reports for the MVS Team.
- Generation of files for validators and statisticians – Queries will be developed to generate the Amended EDD for the Validators and the Database Export forthe Statisticians.
- Validation Import – A routine will be generated to import the Amended EDD with validator fields populated, link to the Project Database, and update the validator fields including the final qualifier code.
- EDD archiving – Procedures will be developed to extract and archive EDDs loaded into the Project Database and subsequently rejected (i.e., EDDs that passed initial QA/QC checks but were then rejected by the data validators; these EDDs will be replaced by re-submissions).

The automated data processing procedures will be developed by the Exa team and then tested. Testing will involve multiple Exa team members running the procedures on multiple test data sets to identify bugs and inconsistencies. Fixes will then be incorporated into the automated routines.

5.0 REFERENCES

SERDP/ESTCP PFAS Method Validation Study Team. 2022. Study Plan for Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC- MS/MS. Prepared for Program Manager for Environmental Restoration, Strategic Environmental Research and Development Program (SERDP).

Microsoft. 2022. How SharePoint and OneDrive safeguard your data in the cloud. https://docs.microsoft.com/en-us/sharepoint/safeguarding-your-data

Willey, J., R. Anderson, A. Hanley, M. Mills, C. Hamilton, T. Thompson, and A. Leeson. 2021. Report on the Single-Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS. Strategic Environmental Research and Development Program (SERDP) Project ER19-1409. https://serdp-estcp.org/content/download/54966/539631/file/Single-Laboratory%20Validation%20Study%20Report.pdf

Appendix A1 - Description of File Tracking System

TRACKING WORKSHEET	TRACKING FIELD	VV Field	TRACKING FIELD DESCRIPTION
GENERAL	Tracking ID		Root file name
	Project Phase		Project Phase. See 'ValidValues'
	EDD File Name		EDD File name
	Data Package File Name		Data Package File Name
	Laboratory Name	Yes	Laboratory Name. See 'ValidValues'
	Matrix	Yes	Matrix. See 'ValidValues'
LABORATORY	EDD/DP Due Date		Due date for the Lab EDD/Data Package (mm/dd/yyyy)
	EDD/DP Date Received		Date Lab EDD/Data Package received (mm/dd/yyyy; uploaded to Sharepoint)
	HGL Reviewer		Initials of HGL staff conducting the Data Package review
	Date HGL Review Complete		Date HGL review complete (mm/dd/yyyy)
	EDD Rejected or Approved - HGL	Yes	Indicate whether EDD/Data Package was rejected by HGL. See 'ValidValues'
	Summary of Errors - HGL		Brief summary of issues found during HGL Data Package review
	Exa Reviewer		Initials of Exa staff conducting the automated EDD review
	Date Exa Review Complete		Date Exa review complete (mm/dd/yyyy)
	EDD Rejected or Approved - Exa	Yes	Indicate whether EDD/Data Package was rejected by Exa. See 'ValidValues'
	Summary of Errors - Exa		Brief summary of issues found during Exa EDD review
	Report/EDD Resubmission Request Date		Date Lab EDD/Data Package re-submission requested by HGL (mm/dd/yyyy). If a re-submission is requested, these will be entered on new rows when they are received. Remaining columns for the original submission should be left blank.
VALIDATOR	Data Validator	Yes	Data Validator. See 'ValidValues'. Get this from the table tracking which Data Validator will get which EDD (from Tim).
	Amended EDD File Name to DV		Name of file provided to Data Validator (Amended EDD prepared by Exa)
	Date Amended EDD to DV		Date the Amended EDD was provided to Data Validator (mm/dd/yyyy)
	DV Amended EDD File Name		Name of the Amended EDD file provided by the Data Validator (with validator fields populated)
	DV Other File Names		Names of other files provided by the Data Validator (verification file, report file)
	Date DV Report/Files Received		Date Data Validator report/files received (mm/dd/yyyy; uploaded to Sharepoint).
	EDD Rejected or Approved - DV	Yes	Indicate whether EDD/Data Package was rejected by the Data Validator. See 'ValidValues'
	Summary of Errors - DV		Brief summary of issues found during data validation that require additional communication with lab and/or a re-submission.
	DV Report/Files Resubmission Request Date		Date Lab EDD/Data Package re-submission requested by HGL (mm/dd/yyyy). If a re-submission is requested, these will be entered on new rows when they are received. Remaining columns for the original submission should be left blank.
	EPA/NAVY Reviewer		Initials of EPA/NAVY staff conducting the review of the Data Validation report
	DV Amended EDD Revised or Accepted?	Yes	Indicate whether the Data Validator Amended EDD was revised by the EPA/NAVY reviewers, or whether it was accepted with no revisions.
	Summary of Revisions - EPA/NAVY		Brief summary of issues found during EPA/NAVY EDD review that require additional communication with the data validator.

Appendix A1 - Description of File Tracking System

TRACKING WORKSHEET	TRACKING FIELD	VV Field	TRACKING FIELD DESCRIPTION
	EPA/NAVY Reviewer File Name		Name of the Amended EDD file provided by the NAVY/EPA Reviewer
DBASE	EDD Upload Date		Date the Lab EDD was uploaded to the database (mm/dd/yyyy)
	EDD Upload Initials		Initials of Exa staff conducting EDD upload to the database
	DV Upload Date		Date the Data Validator results were uploaded to the database (mm/dd/yyyy)
	DV Upload Initials		Initials of Exa staff conducting DV results upload to the database
	Date Removed From DB		Data the EDD was removed from the database (mm/dd/yyyy). EDDs loaded into the database and subsequently rejected will be removed from the main database and archived (to be replaced by re-submissions).
STATS**	Stats DB Export File Name		Name of file provided to IDA (Database Export prepared by Exa)
	Date DB Export to IDA		Date the DB export was provided to IDA (mm/dd/yyyy)
	Stats File Name		Name of the files provided by IDA
	Date Stats Results Received		Date IDA report/files received (mm/dd/yyyy; uploaded to Sharepoint)
	Summary of Errors - IDA		Brief summary of issues found during statistical analysis that require a re-submission of the DB Export from Exa and/or additional communication with other team members.

**STATS table will not be linked to the other Tracking tables directly because statistics are conducted on a
   matrix-basis (not a Tracking ID basis).

Acronyms
DB - Project Database
DP - Data Package
DV - Data Validator
EDD - Electronic Data Deliverable

Appendix A2 - File Tracking System - ValidValues

Worksheet	Field	Valid Value Code	Valid Value Code Description
GENERAL	Laboratory Name	ALPHA	Alpha Analytical
GENERAL	Laboratory Name	BATTELLE	Battelle
GENERAL	Laboratory Name	CALEPA	CalEPA DTSC
GENERAL	Laboratory Name	ELLET	Eurofins Lancaster Labs
GENERAL	Laboratory Name	ETA	ETA, Sacramento
GENERAL	Laboratory Name	GEL	GEL Laboratories
GENERAL	Laboratory Name	MDH	Maryland Department of Health
GENERAL	Laboratory Name	PACE	GCAL/Pace
GENERAL	Laboratory Name	SGSNA	SGS North America
GENERAL	Laboratory Name	VISTA	Vista Analytical
GENERAL	Matrix	GW	Groundwater
GENERAL	Matrix	SW	Surface water
GENERAL	Matrix	SD	Sediment
GENERAL	Matrix	SS	Soil
GENERAL	Matrix	TS	Tissue
GENERAL	Matrix	WW	Wastewater
GENERAL	Matrix	LC	Landfill Leachate
GENERAL	Matrix	BS	Biosolids
GENERAL	Project Phase	Phase 3	Initial Demonstration of Capabilities (IDC)
GENERAL	Project Phase	Phase 4.4.1	GW, SW, LC, and WW matrices
GENERAL	Project Phase	Phase 4.4.2	SS, SD, and BS matrices
GENERAL	Project Phase	Phase 4.4.3	Tissue matrices
LABORATORY	EDD Rejected or Approved - HGL	Approved	Passed review
LABORATORY	EDD Rejected or Approved - HGL	Rejected	Did not pass review
LABORATORY	EDD Rejected or Approved - Exa	Approved	Passed review
LABORATORY	EDD Rejected or Approved - Exa	Rejected	Did not pass review
VALIDATOR	Data Validator	CHEMVAL	ChemVal
VALIDATOR	Data Validator	PYRON	Pyron Environmental
VALIDATOR	Data Validator	JACOBS	Jacobs Engineering
VALIDATOR	EDD Rejected or Approved - DV	Approved	Passed review
VALIDATOR	EDD Rejected or Approved - DV	Rejected	Did not pass review
VALIDATOR	DV Amended EDD Revised or Accepted?	Accepted	Amended EDD accepted with no revisions
VALIDATOR	DV Amended EDD Revised or Accepted?	Revised	Amended EDD revised by NAVY/EPA reviewers

Appendix B1 - Quick Start Guide for LABORATORIES

Lines of Communication

Michael Tweiten (Exa) - Questions/issues with the technical aspects of the SharePoint site (e.g., log in credentials).

HGL Team - Questions/issues with data provided in the EDD/Data Packages.

Dawn Smorong (Exa) - Questions/issues regarding naming of files submitted and/or which folder they were uploaded to.

Contact information:

John Powell	913-378-2315	jpowell@hgl.com	HGL
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Denise Rivers	910-233-8460	drivers@hgl.com	HGL
Ken Rapuano	703-736-4546	krapuano@hgl.com	HGL
Andrea Fletcher	913-317-8860	afletcher@hgl.com	HGL
Dawn Smorong	250-713-8601	dawn@exadata.net	Exa
Michael Tweiten	360-930-8530	michael@exadata.net	Exa

File-naming Protocols

Instructions:

Data package (pdf) must have the EXACT sample file name as the EDD (xlsx or csv).

If either the EDD or the Data Package is revised, the laboratory must resubmit both with the same file name (even if one or the other hasn't been revised).

Files submitted that do not adhere to these file naming protocols will require that they are renamed and resubmitted.

Format:	LabName_matrix_version
Phase 3 example - EDD:	BATTELLE_IDC-solid_ver1.csv
Phase 3 example - data package:	BATTELLE_IDC-solid_ver1.pdf
Phase 4 example - EDD:	ALPHA_GW_ver1.csv
Phase 4 example - data package:	ALPHA_GW_ver1.pdf

LabName codes:

Alpha Analytical	ALPHA
Battelle	BATTELLE
CalEPA DTSC	CALEPA
Eurofins Lancaster Labs	ELLET
ETA, Sacramento	ETA
GEL Laboratories	GEL
Maryland Department of Health	MDH
GCAL/Pace	PACE
SGS North America	SGSNA
Vista Analytical	VISTA

Matrix Codes:

Phase 3:

IDC-aqueous	Initial Demonstration of Capability - aqueous matrix
IDC-solid	Initial Demonstration of Capability - solid matrix
IDC-tissue	Initial Demonstration of Capability - tissue matrix

Phase 4:

GW	Groundwater
SW	Surface water
SD	Sediment
SS	Soil
TS	Tissue
WW	Wastewater
LC	Landfill Leachate
BS	Biosolids

Version codes:

ver0	version 0 (original submission)
ver1	version 1 (re-submission)
ver2	version 2 (re-submission)
etc…	

Appendix B1 - Quick Start Guide for LABORATORIES

SharePoint instructions

Michael Tweiten will be in contact with you to provide credentials for accessing the MLV Study SharePoint site.

Your folder can only be viewed by your team and select members of the MVS Team.

Original and resubmitted files will be retained - do not delete any files from the SharePoint site.

Please ensure you upload your files into the appropriate sub-folder within your main folder, as follows:

Phase 3 IDC

Aqueous	Upload the Phase 3 Initial Demonstration of Capability EDD/Data Package for the aqueous matrix into this folder.
Solids	Upload the Phase 3 Initial Demonstration of Capability EDD/Data Package for the solids matrix into this folder.
Tissue	Upload the Phase 3 Initial Demonstration of Capability EDD/Data Package for the tissue matrix into this folder.

Phase 3 ICAL   Upload the Phase 3 initial calibration data package into this folder.

SOP   Upload the Standard Operating Procedure developed for the MLV Study.

Groundwater

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the groundwater matrix into this folder.
*Communications**	Will contain documents provided to you by the Data Management Team relevant to the groundwater matrix.

Surface water

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the surface water matrix into this folder.
Communications	Will contain documents provided to you by the Data Management Team relevant to the surface water matrix.

Sediment

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the sediment matrix into this folder.
Communications	Will contain documents provided to you by the Data Management Team relevant to the sediment matrix.

Soil

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the soil matrix into this folder.
Communications	Will contain documents provided to you by the Data Management Team relevant to the soil matrix.

Tissue

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the tissue matrix into this folder.
Communications	Will contain documents provided to you by the Data Management Team relevant to the tissue matrix.

Wastewater

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the wastewater matrix into this folder.
Communications	Will contain documents provided to you by the Data Management Team relevant to the wastewater matrix.

Landfill Leachate

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the landfill leachate matrix into this folder.
Communications	Will contain documents provided to you by the Data Management Team relevant to the landfill leachate matrix.

Biosolids

Phase 4 Analysis	Upload the Phase 4 EDDs and Data Packages for the biosolids matrix into this folder.
Communications	Will contain documents provided to you by the Data Management Team relevant to the biosolids matrix.

* Examples of files to be provided in the Communications folder: EDDs with comments incorporated, details regarding resubmission requests.

Appendix B2 - Quick Start Guide for VALIDATORS

Lines of Communication
Michael Tweiten (Exa) - Questions/issues with the technical aspects of the SharePoint site (e.g., log in credentials).
Dawn Smorong (Exa) - Questions/issues regarding Amended EDDs, naming of files submitted and/or which folder they were uploaded to.
Janice Willey (NAVYSEA) - Questions/issues with the laboratory data and/or the validation results.

Contact information:

Janice Willey	843-327-1152	janice.willey@navy.mil	NAVSEA
Dawn Smorong	250-713-8601	dawn@exadata.net	Exa
Michael Tweiten	360-930-8530	michael@exadata.net	Exa

File-naming Protocols

Instructions:

If a resubmission is required, resubmit all files in the package with the same revision number.

Files submitted that do not adhere to these file naming protocols will require that they are renamed and resubmitted.

Amended EDDs from Exa

Format:	**Validator_LabName_matrix_filetype_version**
Phase 3 example:	PYRON_ELLET_IDC-tissue_amended_EDD_V0.xlsx
Phase 4 example:	CHEMVAL_ALPHA_GW_amended_EDD_V0.xlsx

Results from Validators

Format:	**Validator_LabName_matrix_filetype_version**
Phase 3 example - DV Results:	PYRON_ELLET_IDC-tissue_results_v0.xlsx
Phase 3 example - report:	PYRON_ELLET_IDC-tissue_report_v0.pdf
Phase 3 example - verification file:	PYRON_ELLET_IDC-tissue_verification_v0.xlsx
Phase 4 example - DV Results:	CHEMVAL_ALPHA_GW_results_v0.xlsx
Phase 4 example - report:	CHEMVAL_ALPHA_GW_report_v0.pdf
Phase 4 example - verification file:	CHEMVAL_ALPHA_GW_verification_v0.xlsx

Validator codes:

ChemVal	CHEMVAL
Pyron Environmental	PYRON
Jacobs Engineering	JACOBS

LabName codes:

Alpha Analytical	ALPHA
Battelle	BATTELLE
CalEPA DTSC	CALEPA
Eurofins Lancaster Labs	ELLET
ETA, Sacramento	ETA
GEL Laboratories	GEL
Maryland Department of Health	MDH
GCAL/Pace	PACE
SGS North America	SGSNA
Vista Analytical	VISTA

Matrix Codes:

Phase 3:

IDC-aqueous	Initial Demonstration of Capability - aqueous matrix
IDC-solid	Initial Demonstration of Capability - solid matrix
IDC-tissue	Initial Demonstration of Capability - tissue matrix

Appendix B2 - Quick Start Guide for VALIDATORS

File-naming Protocols (continued)	
Matrix Codes:	
<u>Phase 4:</u>	
GW	Groundwater
SW	Surface water
SD	Sediment
SS	Soil
TS	Tissue
WW	Wastewater
LC	Landfill Leachate
BS	Biosolids
Version codes:	
v0	version 0 (original submission)
v1	version 1 (re-submission)
v2	version 2 (re-submission)
etc..	
Filetype codes:	
amended_EDD	Amended EDD including lab EDD results and fields to capture validator results
results	Amended EDD with validator results incorporated
report	Associated validator narrative report
verification	Evidence of 10% verification

Appendix B2 - Quick Start Guide for VALIDATORS

SharePoint instructions	
Michael Tweiten will be in contact with you to provide credentials for accessing the MLV Study SharePoint site.	
Your folder can only be viewed by your team and select members of the MVS Team.	
Original and resubmitted files will be retained - do not delete any files from the SharePoint site.	
Please ensure you upload your files into the appropriate sub-folder within your main folder, as follows:	
Phase 3 IDC	
Aqueous	
To Validator	Phase 3 IDC Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the aqueous matrix.
*From Validator**	Upload the validation results for the Phase 3 IDC aqueous data for each laboratory into this folder.
Solids	
To Validator	Phase 3 IDC Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the solids matrix.
From Validator	Upload the validation results for the Phase 3 IDC solids data for each laboratory into this folder.
Tissue	
To Validator	Phase 3 IDC Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the tissue matrix.
From Validator	Upload the validation results for the Phase 3 IDC tissue data for each laboratory into this folder.
Groundwater	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the groundwater matrix.
From Validator	Upload the validation results for each laboratory for the groundwater matrix.
Surface water	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the surface water matrix.
From Validator	Upload the validation results for each laboratory for the surface water matrix.
Sediment	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the sediment matrix.
From Validator	Upload the validation results for each laboratory for the sediment matrix.
Soil	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the soil matrix.
From Validator	Upload the validation results for each laboratory for the soil matrix.
Tissue	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the tissue matrix.
From Validator	Upload the validation results for each laboratory for the tissue matrix.
Wastewater	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the wastewater matrix.
From Validator	Upload the validation results for each laboratory for the wastewater matrix.
Landfill Leachate	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the landfill leachate matrix.
From Validator	Upload the validation results for each laboratory for the landfill leachate matrix.
Biosolids	
To Validator	Amended EDDs will be provided in this folder (by Exa) for each laboratory, for the biosolids matrix.
From Validator	Upload the validation results for each laboratory for the biosolids matrix.
* Results expected from the validator are: DV Report, Amended EDD with validator fields populated, and evidence of 10% verification.	

Appendix B3 - Quick Start Guide for IDA

Lines of Communication
Michael Tweiten (Exa) - Questions/issues with the technical aspects of the SharePoint site (e.g., log in credentials).
Dawn Smorong (Exa) - Questions/issues regarding Database Exports, naming of files submitted and/or which folder they were uploaded to.
Janice Willey (NAVYSEA) - Questions/issues with the data and statistical analyses.

	Contact information:			
	Janice Willey	843-327-1152	janice.willey@navy.mil	NAVSEA
	Dawn Smorong	250-713-8601	dawn@exadata.net	Exa
	Michael Tweiten	360-930-8530	michael@exadata.net	Exa

File-naming Protocols

Instructions:

If a resubmission is required, resubmit all files in the package with the same revision number.

Phase 3 ICAL Database Export from Exa will be named 'ICAL_Dbexport_V0.xlsx'; the files with the ICAL statistical results should be named 'ICAL_report_V0.xlsx' and 'ICAL_results_V0.xlsx'. If additional submissions are necessary change the prefix to _V1, _V2, etc.

Files submitted that do not adhere to these file naming protocols will require that they are renamed and resubmitted.

Database Exports from Exa

Format:	Matrix_filetype_version
Phase 3 IDC Example:	IDC-solid_Dbexport_V0.xlsx
Phase 4 Example:	GW_DBexport_V0.xlsx

Statistical Results from IDA

Format:	Matrix_filetype_version
Phase 3 IDC Example - report:	IDC-solid_report_V0.xlsx
Phase 3 IDC Example - supporting calculations:	IDC-solid_results_V0.xlsx
Phase 4 Example - report:	GW_report_V0.pdf
Phase 4 Example - supporting calculations:	GW_results_V0.xlsx

Matrix Codes:

Phase 3:

IDC-aqueous	Initial Demonstration of Capability - aqueous matrix
IDC-solid	Initial Demonstration of Capability - solid matrix
IDC-tissue	Initial Demonstration of Capability - tissue matrix

Phase 4:

GW	Groundwater
SW	Surface water
SD	Sediment
SS	Soil
TS	Tissue
WW	Wastewater
LC	Landfill Leachate
BS	Biosolids

Version codes:

v0	version 0 (original submission)
v1	version 1 (re-submission)
v2	version 2 (re-submission)
etc..	

Filetype codes:

DBexport	Database export including final results
report	Statistical analyses report
results	Files with supporting calculations

Appendix B3 - Quick Start Guide for IDA

SharePoint instructions	
Michael Tweiten will be in contact with you to provide credentials for accessing the MLV Study SharePoint site.	
Your folder can only be viewed by your team and select members of the MVS Team.	
Original and resubmitted files will be retained - do not delete any files from the SharePoint site.	
Please ensure you upload your files into the appropriate sub-folder within your main folder, as follows:	
Phase 3	
Aqueous IDC	
To IDA	Phase 3 IDC Database Exports will be provided in this folder (by Exa) for the aqueous matrix.
From IDA*	Upload the statistical results for the Phase 3 IDC water data into this folder.
Solids IDC	
To IDA	Phase 3 IDC Database Exports will be provided in this folder (by Exa) for the solids matrix.
From IDA	Upload the statistical results for the Phase 3 IDC solids data into this folder.
Tissue IDC	
To IDA	Phase 3 IDC Database Exports will be provided in this folder (by Exa) for the tissue matrix.
From IDA	Upload the statistical results for the Phase 3 IDC tissue data into this folder.
ICAL	
To IDA	Phase 3 ICAL results will be provided in this folder (by Exa/SEE).
From IDA	Upload the statistical results for the Phase 3 ICAL data into this folder.
Groundwater	
To IDA	Database Exports will be provided in this folder (by Exa) for the groundwater matrix.
From IDA	Upload the statistical results for the groundwater matrix.
Surface water	
To IDA	Database Exports will be provided in this folder (by Exa) for the surface water matrix.
From IDA	Upload the statistical results for the surface water matrix.
Sediment	
To IDA	Database Exports will be provided in this folder (by Exa) for the sediment matrix.
From IDA	Upload the statistical results for the sediment matrix.
Soil	
To IDA	Database Exports will be provided in this folder (by Exa) for the soil matrix.
From IDA	Upload the statistical results for the soil matrix.
Tissue	
To IDA	Database Exports will be provided in this folder (by Exa) for the tissue matrix.
From IDA	Upload the statistical results for the tissue matrix.
Wastewater	
To IDA	Database Exports will be provided in this folder (by Exa) for the wastewater matrix.
From IDA	Upload the statistical results for the wastewater matrix.
Landfill Leachate	
To IDA	Database Exports will be provided in this folder (by Exa) for the landfill leachate matrix.
From IDA	Upload the statistical results for the landfill leachate matrix.
Biosolids	
To IDA	Database Exports will be provided in this folder (by Exa) for the biosolids matrix.
From IDA	Upload the statistical results for the biosolids matrix.
* Results expected from IDA are: statistical report with appendices (pdf), along with supporting calculations (xlsx).	

Appendix C1 – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	TrackingID	text	Tracking ID from Tracking System; incorporated during ETL procedures	No	No	Yes		X	Yes	Yes
Lab_EDD_Results	LAB_ID	text	Laboratory Name. See Valid Value list.	Yes	Yes	Yes		X	Yes	No
Lab_EDD_Results	lab_num	number double	Lab_ID code, to keep laboratories anonymous. See Valid Value list.	No	Yes	Yes			No	Yes
Lab_EDD_Results	sdg_num	text	SDG number; incorporated during ETL procedures; extracted from Lab_Sample_ID	No	No	No			Yes	No
Lab_EDD_Results	SAMPLE_NO	text	For samples, these are the sample identification names (IDs) from the Chain of Custody. The Sample_No is the same, regardless of whether or not the sample is diluted or reanalyzed. For preparation batch QC, these are "MB" for the Method Blank, "OPR" for the OPR, and "LLOPR" for the LLOPR. For IDOC samples, "IPR" for the IPR samples, "MDLB" for the MDL_b samples, "MDLS" for the MDLs samples, and "LOQVER" for the LOQVER samples.	Yes	No	Yes		X	Yes	Yes
Lab_EDD_Results	LAB_SAMPLE_ID	text	The ID the laboratory assigns to the sample (which identifies the sample on the associated data files and reports). For samples that need to be re-analyzed for issues other than dilution, attach the following identifiers to the end of the lab sample identifier without a space between them (e.g., 02082022-01R): "R" for analytes, EISs & NISs reported from first re-analysis not due to dilution "R1" for analytes, EISs & NISs reported from second re-analysis not due to dilution "R2" for analytes, EISs & NISs reported from second re-analysis not due to dilution If more re-analyses not due to dilution are needed to be reported beyond three for a sample, continue on with the numbering (e.g., R3, R4, R5, etc.).	Yes	No	Yes		X	Yes	Yes
Lab_EDD_Results	ANALYSIS_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.	Yes	No	Yes			Yes	No
Lab_EDD_Results	ANALYSIS	text	Fill in "PFAS". See Valid Value list.	Yes	Yes	Yes	PFAS		Yes	No
Lab_EDD_Results	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	Yes	Yes	Yes		X	Yes	Yes
Lab_EDD_Results	DILUTION	number integer	Dilution made post extraction (e.g., extract diluted 1:10 is entered as "10"). If analyzed without dilution, enter "1".	Yes	No	Yes	1	X	Yes	Yes
Lab_EDD_Results	LAB_FLAG	text	Laboratory qualifiers. See Valid Value list.	Yes	Yes	No			Yes	No

Appendix C1 – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	CONC_FOUND	number double	Enter numeric quantitative result value only. Report to three significant figures. Do NOT enter any text string strings or symbols (e.g., "ND", "<"). For analytes that are not detected, the laboratory's sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account) is entered. Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. Report result units in "Unit" field, consistent for all sample fields.	Yes	No	Yes			Yes	No
Lab_EDD_Results	CONC_SPIKE	number double	For unspiked samples enter "0" for method analytes. For spiked samples, enter the spike concentration representing the estimated concentration in the final extract (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. For EIS and NIS, enter the spike concentration representing the concentration in the final extract in units consistent with sample result units. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	Yes			Yes	Yes
Lab_EDD_Results	PERCENT_REC	number double	For unspiked samples, leave blank. No text should be included in this field (e.g. N/A). For spiked samples (OPR, LLOPR, MDLs and LOQVER), enter the spike percentage recovery as a whole number (e.g., 95 versus 0.95). Do NOT include "%" symbol. For EIS and NIS recoveries, enter the spike % recovery as a whole number (e.g., 95 versus 0.95). Report to 3 significant figures. Do NOT include "%".	Yes	No	No			Yes	Yes
Lab_EDD_Results	MDL	number double	Method Detection Limit. Enter the sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	Yes	-9		Yes	Yes
Lab_EDD_Results	LOQ	number double	Limit of Quantitation. Enter the sample specific LOQ (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). Report to 3 significant figures. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	Yes	-9		Yes	Yes

Appendix C1 – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	UNIT	text	The reporting units must be consistent for the sample record including Conc_Found, MDL, LOQ etc. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Ensure that all values for the sample record are reported in the same units. See Valid Value list.	Yes	Yes	Yes			Yes	Yes
Lab_EDD_Results	SAMPLE_TRANSITION_RATIO	text	Enter the calculated Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte in the sample. Report to 3 significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g. N/A).	Yes	No	No			Yes	No
Lab_EDD_Results	EXPECTED_TRANSITION_RATIO	text	Enter the expected Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte per the method. Report to three significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g., N/A).	Yes	No	No			Yes	No
Lab_EDD_Results	RRT	text	Enter relative retention time	Yes	No	No			Yes	No
Lab_EDD_Results	SAMPLE_SIZE	number double	Enter volume (aqueous samples) or weight (solid samples) of sample extracted (in liters for aqueous samples, in kilograms for solids).	Yes	No	Yes			Yes	No
Lab_EDD_Results	SAMPLE_SIZE_UNIT	text	Will be liters (L) for aqueous samples or kilograms (Kg) for solid samples	Yes	No	Yes			Yes	No
Lab_EDD_Results	EXTRACTION_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.	Yes	No	Yes			Yes	No
Lab_EDD_Results	PERC_MOISTURE	number double	Percent moisture in solid samples only. Enter the percent moisture as a whole number (e.g., 73 versus 0.73). Do NOT include "%" symbol.	Yes	No	No			Yes	No
Lab_EDD_Results	MATRIX	text	Matrix analyzed. See Valid values list.	Yes	Yes	Yes			Yes	Yes
Lab_EDD_Results	METHOD	text	Laboratory SOP Name in format of "name(space)revision number"	Yes	No	Yes			Yes	No
Lab_EDD_Results	STUDY_PHASE	text	Multi-Lab Validation Study Phase. See Valid Value list.	Yes	Yes	Yes			Yes	No
Lab_EDD_Results	SAMPLE_TYPE	text	See Valid Value list.	Yes	Yes	Yes		X	Yes	Yes
Lab_EDD_Results	result_type	text	Code for Result Type. See Valid Value list.	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	spike_cat	text	Code for Spike Category. See Valid Value list.	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	validation_level	text	Stores information on the level of data validation that has been completed for the chemistry data.	No	No	Yes	Level 4		Yes	No
Lab_EDD_Results	validator	text	Code for Data Validator. See Valid Value list.	No	Yes	Yes			Yes	No
Lab_EDD_Results	val_qualifier	text	Code for Data Validator qualifiers. See Valid Value list.	No	Yes	No			Yes	No
Lab_EDD_Results	val_qualifier_reason	text	Data validation qualifier reason codes.	No	No	No			Yes	No

Appendix C1 – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	ValNotes_1	text	Include comments to distinguish the meaning of the val_qualifier assignment (e.g., distinguishing the -J qualifier)	No	No	No			Yes	No
Lab_EDD_Results	ValNotes_2	text	Additional information provided by validator deemed pertinent to their val_qualifier assignment.	No	No	No			Yes	No
Lab_EDD_Results	validator_result	number double	Validator recommended result for concentration. If this is provided, entries must be made in the valResultChange_yn field and valResultChange_desc fields.	No	No	No			Yes	No
Lab_EDD_Results	valResultChange_yn	Logical	Enter Y or N. Indicates whether the validator made a recommendation to change the result for concentration.	No	No	Yes			Yes	No
Lab_EDD_Results	valResultChange_desc	text	Description of the reasons for validator recommending a change to the result for concentration.	No	No	No			Yes	No
Lab_EDD_Results	valReviewer_qualifier	text	Code for qualifiers applied by NAVY/EPA reviewers of data validation results. See Valid Value list.	No	Yes	No			Yes	No
Lab_EDD_Results	valReviewer_notes	text	Notes from NAVY/EPA reviewers of data validation results.	No	No	No			Yes	No
Lab_EDD_Results	final_qualifier	text	Code for Final Qualifier. See Valid Value list.	No	Yes	No			No	Yes
Lab_EDD_Results	final_result	number double	Final result for concentration. Combines CONC_FOUND and validator_result fields.	No	No	Yes			No	Yes
dicValidValues	VVLField	text	Valid value field name.	No	No	Yes			No	No
dicValidValues	ValidValue	text	Acceptable valid value codes.	No	No	Yes			No	No
dicValidValues	VV_Description	text	Description of valid value codes.	No	No	Yes			No	No
dicValidValues	VVL_match_alt_field	text	Related valid value field name.	No	No	No			No	No
dicValidValues	VVL_match_alt_code	text	Matching valid value code.	No	No	No			No	No
LU_Compound	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Compound	COMPOUND	text	Use the names included in the example EDD. Method analytes, and EIS and NIS compounds must be reported for each sample. See Valid Value list.	Yes	Yes	Yes			Yes	Yes
LU_Compound	CAS_NO	text	Chemical Abstract Service Registration Number	Yes	Yes	Yes			Yes	Yes
LU_Compound	result_type	text	Code for Result Type. See Valid Value list.	No	Yes	Yes			No	No
LU_Spike_Levels	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Spike_Levels	Matrix	text	Name of the matrix. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Spike_Levels	PREP	text	Preparation code provided in the spiking level file.	No	No	Yes	REG		No	No
LU_Spike_Levels	Low_Spike_ngL	number double	Low spike concentration (ng/L)	No	No	Yes			No	Yes
LU_Spike_Levels	High_Spike_ngL	number double	High spike concentration (ng/L)	No	No	Yes			No	Yes
Convent	*As in EDDs for conventionals*									
TR1_GENERAL	--	--	*See Table describing the Tracking System.*	--	--	--			No	No

Appendix C1 – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
TR2_LABORATORY	--	--	*See Table describing the Tracking System.*	--	--	--			No	No
TR3_VALIDATOR	--	--	*See Table describing the Tracking System.*	--	--	--			No	No
TR4_DBASE	--	--	*See Table describing the Tracking System.*	--	--	--			No	No
TR5_STATS	--	--	*See Table describing the Tracking System.*	--	--	--			No	No

Note: The Required Fields may be revised during database development; maximum field lengths will be incorporated into the database structure during development.

Appendix C2 – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code
LAB_ID	ALPHA	Alpha Analytical	lab_num	3
LAB_ID	BATTELLE	Battelle	lab_num	6
LAB_ID	CALEPA	CalEPA DTSC	lab_num	2
LAB_ID	ELLET	Eurofins Lancaster Labs	lab_num	10
LAB_ID	ETA	ETA, Sacramento	lab_num	1
LAB_ID	GEL	GEL Laboratories	lab_num	8
LAB_ID	MDH	Maryland Department of Health	lab_num	5
LAB_ID	PACE	GCAL/Pace	lab_num	9
LAB_ID	SGSNA	SGS North America	lab_num	7
LAB_ID	VISTA	Vista Analytical	lab_num	4
MATRIX	LC	Landfill Leachate		
MATRIX	WW	Wastewater		
MATRIX	SW	Surface water		
MATRIX	GW	Groundwater		
MATRIX	QC	Quality Control Sample		
MATRIX	RT	Reference Tissue for tissue MBs		
MATRIX	OS	Ottawa sand for all solid MBs		
MATRIX	RW	Reagent water for all aqueous MBs		
MATRIX	BS	Biosolids		
MATRIX	TS	Tissue		
MATRIX	SS	Soil		
MATRIX	SD	Sediment		
MATRIX	IDC-water	Initial Demonstration of Capability - water matrix		
MATRIX	IDC-solid	Initial Demonstration of Capability - solid matrix		
MATRIX	IDC-tissue	Initial Demonstration of Capability - tissue matrix		
result_type	EIS	Extracted Internal Standard		
result_type	TRG	Target analyte		
result_type	NIS	Non-Extracted Internal Standard		
SAMPLE_TYPE	MDLB	Method analytes in MDL Blank IDC samples		
SAMPLE_TYPE	MDLS	Method analytes in MDL Spike IDC samples		
SAMPLE_TYPE	MDLLOQVER	Method analytes in MDL LOQVER IDC samples		
SAMPLE_TYPE	NORMAL	method analytes in field samples		
SAMPLE_TYPE	BLANK	method analytes in MBs		
SAMPLE_TYPE	OPR	method analytes in OPRs		
SAMPLE_TYPE	LLOPR	method analytes in LLOPRs		
SAMPLE_TYPE	EIS	EIS in all samples		
SAMPLE_TYPE	NIS	NIS in all samples		
spike_cat	HIGH	High		
spike_cat	LOW	Low		
STUDY_PHASE	Phase 3	Initial Demonstration of Capabilities (IDC), MDL Study, IPRs, and LOQ Verification		
STUDY_PHASE	Phase 4.4.1	GW, SW, LC, and WW matrices		
STUDY_PHASE	Phase 4.4.2	SS, SD, and BS matrices		
STUDY_PHASE	Phase 4.4.3	Tissue matrices		
validator	CHEMVAL	ChemVal		
validator	JACOBS	Jacobs Engineering		
validator	PYRON	Pyron Environmental		
Analysis	PFAS	NULL		
Lab_Flag	B	Detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than 1/10th the concentration in the sample, whichever is greatest. The MB must also be flagged with a "B" for all concentrations greater than ½ the LOQ.		
Lab_Flag	D	When the reported result is from a dilution, for analytes, EIS, NISs		

Appendix C2 – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code
Lab_Flag	I	Fail to meet ion ratio criteria		
Lab_Flag	J	At a concentration between the MDL and LOQ		
Lab_Flag	U	Not detected or were detected at a concentration less than the MDL		
UNIT	ug/kg	micrograms per kilogram	MATRIX	BS
UNIT	ng/L	nanograms per liter	MATRIX	GW
UNIT	ng/L	nanograms per liter	MATRIX	LC
UNIT	ug/kg	micrograms per kilogram	MATRIX	SD
UNIT	ug/kg	micrograms per kilogram	MATRIX	SS
UNIT	ng/L	nanograms per liter	MATRIX	SW
UNIT	ug/kg	micrograms per kilogram	MATRIX	TS
UNIT	ng/L	nanograms per liter	MATRIX	WW
dv_qualifier	I	Suspect		
dv_qualifier	X	Exclusion of data is recommended		
dv_qualifier	J	Estimated		
dv_qualifier	J-	If branched isomers were not included in the summed result reported, qualify associated detects as J- or Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J- when the %D is below acceptance criteria		
dv_qualifier	J+	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J+ when the %D is higher than acceptance criteria		
dv_qualifier	U	Values below the MDL are considered non-detects and are qualified as U at the stated MDL.		
dv_qualifier	UJ	Estimated non-detect or Verify that the %Ds are within the acceptance criteria. Non-detects are qualified as UJ in all associated samples for %D outside of acceptance criteria.		
valReviewer_qualifier	I	Suspect		
valReviewer_qualifier	X	Exclusion of data is recommended		
valReviewer_qualifier	J	Estimated		
valReviewer_qualifier	J-	If branched isomers were not included in the summed result reported, qualify associated detects as J- or Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J- when the %D is below acceptance criteria		
valReviewer_qualifier	J+	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J+ when the %D is higher than acceptance criteria		

Appendix C2 – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code
valReviewer_qualifier	U	Values below the MDL are considered non-detects and are qualified as U at the stated MDL.		
valReviewer_qualifier	UJ	Estimated non-detect or Verify that the %Ds are within the acceptance criteria. Non-detects are qualified as UJ in all associated samples for %D outside of acceptance criteria.		
valReviewer_qualifier	NQ	No qualifier. Use this code when the dv_qualifier should be over-ridden by no qualifier.		
dv_qualifier_reason	TBD	To be standardized in final database.		

Appendix C3 – Project Database – Valid Value Codes and Descriptions for Compounds

PFAS_ACRONYM	Compound	CAS_NO	Result_Type
13C2-4:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]hexanesulfonic acid	NA	EIS
13C2-6:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]octanesulfonic acid	NA	EIS
13C2-8:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]decanesulfonic acid	NA	EIS
13C2-PFDoA	Perfluoro-n-[1,2-13C2]dodecanoic acid	NA	EIS
13C2-PFTeDA	Perfluoro-n-[1,2-13C2]tetradecanoic acid	NA	EIS
13C3-HFPO-DA	Tetrafluoro-2-heptafluoropropoxy-13C3-propanoic acid	NA	EIS
13C3-PFBS	Perfluoro-1-[2,3,4-13C3]butanesulfonic acid	NA	EIS
13C3-PFHxS	Perfluoro-1-[1,2,3-13C3]hexanesulfonic acid	NA	EIS
13C4-PFBA	Perfluoro-n-[13C4]butanoic acid	NA	EIS
13C4-PFHpA	Perfluoro-n-[1,2,3,4-13C4]heptanoic acid	NA	EIS
13C5-PFHxA	Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid	NA	EIS
13C5-PFPeA	Perfluoro-n-[13C5]pentanoic acid	NA	EIS
13C6-PFDA	Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid	NA	EIS
13C7-PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid	NA	EIS
13C8-PFOA	Perfluoro-n-[13C8]octanoic acid	NA	EIS
13C8-PFOS	Perfluoro-1-[13C8]octanesulfonic acid	NA	EIS
13C8-PFOSA	Perfluoro-1-[13C8]octanesulfonamide	NA	EIS
13C9-PFNA	Perfluoro-n-[13C9]nonanoic acid	NA	EIS
D3-NMeFOSA	N-methyl-d3-perfluoro-1-octanesulfonamide	NA	EIS
D3-NMeFOSAA	N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	NA	EIS
D5-NEtFOSA	N-ethyl-d5-perfluoro-1-octanesulfonamide	NA	EIS
D5-NEtFOSAA	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid	NA	EIS
D7-NMeFOSE	N-methyl-d7-perfluorooctanesulfonamidoethanol	NA	EIS
D9-NEtFOSE	N-ethyl-d9-perfluorooctanesulfonamidoethanol	NA	EIS
13C2-PFDA	Perfluoro-n-[1,2-13C2]decanoic acid	NA	NIS
13C2-PFHxA	Perfluoro-n-[1,2-13C2]hexanoic acid	NA	NIS
13C3-PFBA	Perfluoro-n-[2,3,4-13C3]butanoic acid	NA	NIS
13C4-PFOA	Perfluoro-n-[1,2,3,4-13C4]octanoic acid	NA	NIS
13C4-PFOS	Perfluoro-n-[1,2,3,4-13C4]octanesulfonic acid	NA	NIS
13C5-PFNA	Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid	NA	NIS
18O2-PFHxS	Perfluoro-1-hexane[18O2]sulfonic acid	NA	NIS
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	TRG
3:3FTCA	3-Perfluoropropyl propanoic acid	356-02-5	TRG
4:2FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid	757124-72-4	TRG
5:3FTCA	2H,2H,3H,3H-Perfluorooctanoic acid	914637-49-3	TRG
6:2FTS	1H,1H,2H,2H-Perfluorooctane sulfonic acid	27619-97-2	TRG
7:3FTCA	3-Perfluoroheptyl propanoic acid	812-70-4	TRG
8:2FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid	39108-34-4	TRG
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	756426-58-1	TRG
ADONA	4,8-dioxa-3H-perfluorononanoic acid	919005-14-4	TRG
HFPO-DA	Hexafluoropropylene oxide dimer acid	13252-13-6	TRG
NEtFOSA	N-ethyl perfluorooctanesulfonamide	4151-50-2	TRG
NEtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid	2991-50-6	TRG
NEtFOSE	N-ethyl perfluorooctanesulfonamidoethanol	1691-99-2	TRG
NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	151772-58-6	TRG
NMeFOSA	N-methyl perfluorooctanesulfonamide	31506-32-8	TRG
NMeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid	2355-31-9	TRG
NMeFOSE	N-methyl perfluorooctanesulfonamidoethanol	24448-09-7	TRG
PFBA	Perfluorobutanoic acid	375-22-4	TRG

Appendix C3 – Project Database – Valid Value Codes and Descriptions for Compounds

PFAS_ACRONYM	Compound	CAS_NO	Result_Type
PFBS	Perfluorobutanesulfonic acid	375-73-5	TRG
PFDA	Perfluorodecanoic acid	335-76-2	TRG
PFDoA	Perfluorododecanoic acid	307-55-1	TRG
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	TRG
PFDS	Perfluorodecanesulfonic acid	335-77-3	TRG
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	113507-82-7	TRG
PFHpA	Perfluoroheptanoic acid	375-85-9	TRG
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	TRG
PFHxA	Perfluorohexanoic acid	307-24-4	TRG
PFHxS	Perfluorohexanesulfonic acid	355-46-4	TRG
PFMBA	Perfluoro-4-methoxybutanoic acid	863090-89-5	TRG
PFMPA	Perfluoro-3-methoxypropanoic acid	377-73-1	TRG
PFNA	Perfluorononanoic acid	375-95-1	TRG
PFNS	Perfluorononanesulfonic acid	68259-12-1	TRG
PFOA	Perfluorooctanoic acid	335-67-1	TRG
PFOS	Perfluorooctanesulfonic acid	1763-23-1	TRG
PFOSA	Perfluorooctanesulfonamide	754-91-6	TRG
PFPeA	Perfluoropentanoic acid	2706-90-3	TRG
PFPeS	Perfluoropentanesulfonic acid	2706-91-4	TRG
PFTeDA	Perfluorotetradecanoic acid	376-06-7	TRG
PFTrDA	Perfluorotridecanoic acid	72629-94-8	TRG
PFUnA	Perfluoroundecanoic acid	2058-94-8	TRG

Multi-Laboratory Validation Study Database Management

QA/QC and
Data Processing Procedures

WHITE PAPER

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1.0 Introduction

This white paper describes specific data quality checking processes and procedures for the Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. EPA Method 1633 is an interim draft method for analyzing per- and polyfluoroalkyl substances (PFAS), and now requires a Multiple-Laboratory Validation (MLV) Study. The study requires technically sound and legally admissible data; thus the QA/QC procedures documented in this white paper are a key element to project success. All of the data quality review steps are conducted in accordance with the MLV Study Plan (SERDP/ESCTP 2022).

Data management processes and procedures described herein are applicable to Phases 3-6 of the MLV Study Plan (SERDP/ESTCP 2022).

The Data Management Plan (DMP) for the MLV Study describes the objectives, framework, workflow, file management, and tracking procedures for the project (SERDP/ESCTP 2022; Attachment 4). This white paper provides additional details on the specific QA/QC procedures conducted on the electronic data deliverables (EDDs) from the study laboratories.

The checking routines as described here were developed under the auspices of the data management objectives for the project:
- To facilitate and coordinate with the MVS Team members to ensure that data management system meets overall project objectives;
- To ensure high quality data that provides an accurate representation of all data produced during the study;
- To standardize and store the data in a structured format to allow for accurate querying and statistical analyses;
- To ensure efficient and timely data processing;
- To store the data produced during the MLV Study in a secure location that restricts access to team members with appropriate credentials;
- To allow easy access to the data by project stakeholders; and,
- To implement documentation procedures that ensure the data is technically defensible and legally admissible.

The data management methodology is critical to ensure that laboratory analytical data, validation information, and final statistical calculations are of the highest quality to support and defend the publication of the final method.

1.1 Overview of the EDD Management Process

Electronic data from the laboratories are submitted to the MVS Team in a specific electronic data deliverable (EDD) format, as described in the Study Plan (SERDP/ESTCP 2022). The EDD records are imported into a ©Microsoft (MS) Access database using automated Visual Basic for Applications (VBA) code. In addition to the checking routines, there are additional functions to post-process the data which will be described in Section 6.0. The primary goal of this white paper is to provide a detailed description of the EDD and database QA/QC procedures conducted on data provided from the participating laboratories.

1.2 Overview of the EDD Checking Phases

There are four phases of EDD QA/QC in the workflow that have been developed:

1. Initial checks performed at receipt of data files from the laboratories;
2. Preliminary checks conducted upon import of the EDD;
3. Detailed checks conducted on individual EDDs, prior to submission of the data to the validators;
4. Database checks on the cumulative Master EDD Database conducted prior to submission to the data analysis (statistics) team.

Each of these phases of QA/QC procedures will be discussed in the following sections.

2.0 QA/QC Checks at Receipt

Upon receipt of data files submitted from the laboratories, the files are logged into the Tracking System and it is confirmed that the data files (data packages, EDDs, supplemental files) are appropriately filed on the MLV Study Library (SharePoint site). The Tracking System is described in the DMP (SERDP/ESCTP 2022; Attachment 4). Additionally, the file names are reviewed to ensure the file naming protocol was followed, and a high-level check is performed to ensure that the data package and EDD are associated with one another.

3.0 QA/QC Checks at Import

Import of the EDDs into the database is the first step of the MLV Study QA/QC tool (Figure 1). As each EDD is imported, a series of preliminary checks are conducted to ensure that the EDD imported properly. This includes checks to ensure all of the EDD template fields are present and named properly, and all of the EDD records were imported.
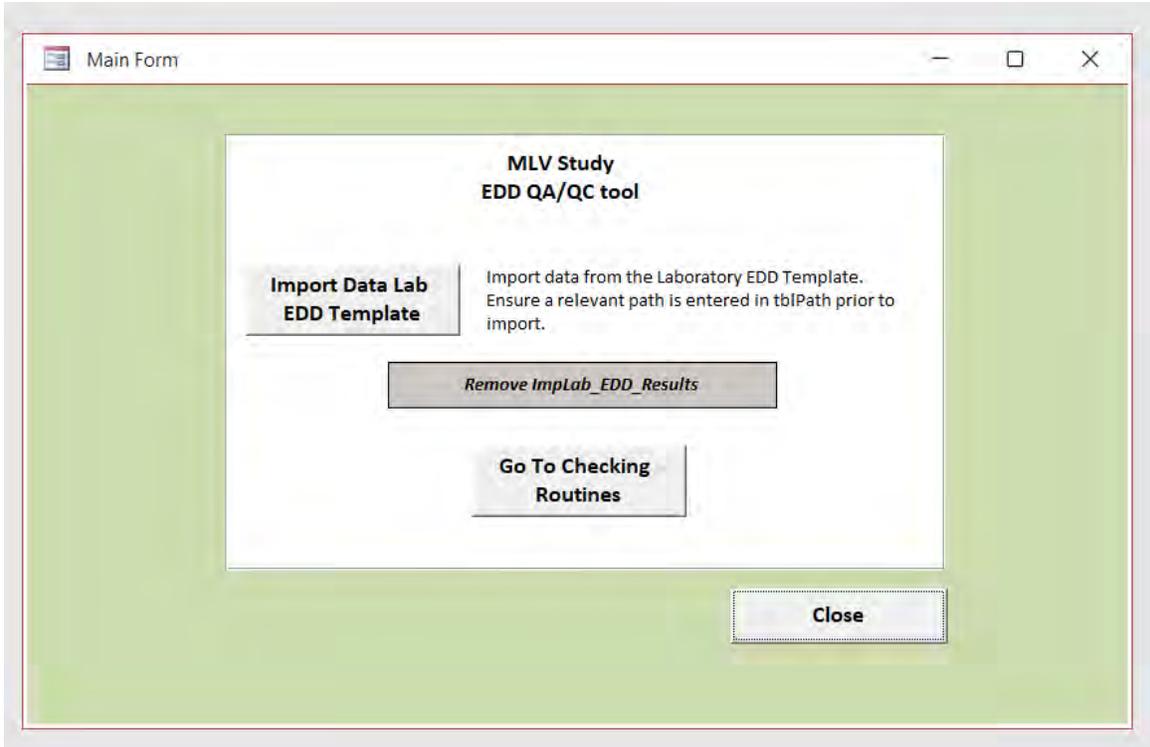
Figure 1. Opening Form of the MLV Study EDD QA/QC Tool

4.0 Detailed EDD QA/QC and Reporting

Once the EDD is imported as a stand-alone table into the database (e.g., not yet appended to the Master EDD Database), a series of automated QA/QC checks are conducted. These checks are under continual development as new issues arise, but the description provided below is comprehensive for the EDDs received for Phase 3 as of the publication of this white paper.

The EDD QA/QC checks are run in a sequential order (Routines 1-3, Figure 2). Results of the checks are written to the QA/QC Report for review and generating feedback to the data provider (Routine 4).

Figure 2. EDD Checking Routines and Reporting Form

The first routine checks that all required fields have entries. The list of required fields is drawn from the EDD Instructions and Format (SERDP/ESCTP 2022, Attachment 3, Table 1).

Table 1. List of Required Fields

Field Name		
ANALYSIS	LAB_SAMPLE_ID	SAMPLE_SIZE_UNIT
COMPOUND	MATRIX	SAMPLE_TYPE
CONC_FOUND	METHOD	STUDY_PHASE
CONC_SPIKE	PFAS_ACRONYM	UNIT
DILUTION	SAMPLE_NO	
LAB_ID	SAMPLE_SIZE	

In addition, the first routine also checks that relationships between tables are maintained – specifically the links between the EDD and the standardized SampleID (from the MatrixKey table) and the standardized compound codes in the compound table.

The second routine checks for unique records based on the following fields: LAB_ID, SAMPLE_NO, LAB_SAMPLE_ID, PFAS_ACRONYM, DILUTION, and SAMPLE_TYPE. If there is more than one record in the EDD with the same combination of these fields, this check will generate an error message.

The third routine ("Additional Checks" in Figure 2) includes a wide variety of automated QA/QC checks and summaries, some that require manual review. Range checks are conducted on numeric fields to ensure that the values are "reasonable" (e.g., percent moisture must be ≤ 100%, Table 2). All fields that are constrained to valid entries are checked for specific required content, including exact spelling for the following fields: ANALYSIS, LAB_FLAG, MATRIX, SAMPLE_TYPE, STUDY_PHASE, UNIT, PFAS_ACRONYM, COMPOUND, CAS_NO, Result_Type.

Table 2. Fields with Range Checks

Field Name	Min	Max	Default
CONC_FOUND	0.0001	1000	REQUIRED
PERC_MOISTURE	0	100	REQUIRED
CONC_SPIKE	1	1000	0
DILUTION	0	100	1
PERCENT_REC	0	170	NULL
SAMPLE_SIZE	0.004	1000	REQUIRED
MDL	0.0001	100	
LOQ	0.0001	100	

Following these checks, a series of additional QA/QC checks and review summaries are executed (Table 3). The Additional Checks can be easily modified, and new checks are added as needed.

Table 3. Detailed List of EDD QA/QC Checks

Type of Check	Description
Completeness	71 results for each sample
	All compounds have been reported for all samples
	Three sample types for each sample
	Review sample type counts
Units	Consistent units
	Correct units
Formatting	No suffixes added to SAMPLE_NO
	ANALYSIS_DATE is in the correct format
	PERC_MOISTURE must be a whole number
	PERCENT_REC must be a whole number
	Numeric entries in number fields
Null and placeholders	Null in CONC_FOUND field
	Null in CONC_SPIKE field
	Null in DILUTION field
	Null in LOQ field
	Null in MDL field
	Null in PERC_MOISTURE field
	Null in SAMPLE_SIZE field
	CONC_SPIKE = 0 ok
	CONC_SPIKE not equal to 0 ok
	PERCENT_REC = null ok
	PERCENT_REC not null ok
	CAS_NO can only be null for EIS or NIS
	PERC_MOISTURE should not be populated for aqueous samples
Sample Type/Matrix Coding	MATRIX is coded correctly for blank samples
	MATRIX is coded correctly for study samples
	MATRIX is coded correctly for QC samples
	SAMPLE_NO is coded correctly for blank samples
	SAMPLE_NO is coded correctly for QC samples
	SAMPLE_NO is coded correctly for study samples
	SAMPLE_TYPE is coded correctly for NIS and EIS result_type
	SAMPLE_TYPE is coded correctly for TRG result_type
	SAMPLE_TYPE is coded correctly for TRG result_type (not EIS or NIS)
Detection Limit / Qualifier Checks	MDL is not greater than the LOQ
	LAB_FLAG not set to J when CONC_FOUND > MDL and < LOQ
	LAB_FLAG not set to U when CONC_FOUND = MDL
	CONC_FOUND < MDL
	LAB_FLAG set to U when CONC_FOUND not = MDL
	MDL can only be null for EIS or NIS
	LOQ can only be null for EIS or NIS
	LOQ should not be populated for EIS/NIS
	MDL should not be populated for EIS/NIS
	Review if LAB_FLAG should be set to B
Calculations	PERCENT_REC calculations
	Review fields that must reported to 3 sig figs
	ANALYSIS_DATE is after the EXTRACTION_DATE

Many of the QA/QC checks listed in Table 3 are based on the Supplemental EDD Instructions (2022, unpublished), in particular the sample coding guidance (Table 4).

Table 4. Allowed Code Combinations for Sample_No, Matrix, and Sample_Type fields

Phase	SAMPLE_NO	Compound Type	Matrix	Sample_type		CONC_SPIKE	PERCENT_REC
Phase 4	GWA0	Method analytes	GW, SW, etc	NORMAL		0	NULL
Phase 4	GWA0	EIS	GW, SW, etc	EIS		Populated	Populated
Phase 4	GWA0	NIS	GW, SW, etc	NIS		Populated	Populated
Phase 4	MB	Method analytes	RW, OS, RT	BLANK		0	NULL
Phase 4	MB	EIS	RW, OS, RT	EIS		Populated	Populated
Phase 4	MB	NIS	RW, OS, RT	NIS		Populated	Populated
Phase 4	OPR*	Method analytes	QC	OPR		Populated	Populated
Phase 4	OPR*	EIS	QC	EIS		Populated	Populated
Phase 4	OPR*	NIS	QC	NIS		Populated	Populated
Phase 4	LLOPR*	Method analytes	QC	LLOPR		Populated	Populated
Phase 4	LLOPR*	EIS	QC	EIS		Populated	Populated
Phase 4	LLOPR*	NIS	QC	NIS		Populated	Populated
Phase 3 IDC	IPR*	Method analytes	QC	IPR		Populated	Populated
Phase 3 IDC	IPR*	EIS	QC	EIS		Populated	Populated
Phase 3 IDC	IPR*	NIS	QC	NIS		Populated	Populated
Phase 3 IDC	MDLB	Method analytes	RW, OS, RT	MDLB		0	NULL
Phase 3 IDC	MDLB	EIS	RW, OS, RT	EIS		Populated	Populated
Phase 3 IDC	MDLB	NIS	RW, OS, RT	NIS		Populated	Populated
Phase 3 IDC	MDLS*	Method analytes	QC	MDLS		Populated	Populated
Phase 3 IDC	MDLS*	EIS	QC	EIS		Populated	Populated
Phase 3 IDC	MDLS*	NIS	QC	NIS		Populated	Populated
Phase 3 IDC	LOQVER*	Method analytes	QC	LOQVER		Populated	Populated
Phase 3 IDC	LOQVER*	EIS	QC	EIS		Populated	Populated
Phase 3 IDC	LOQVER*	NIS	QC	NIS		Populated	Populated
Phase 3 IDC	MB	Method analytes	RW, OS, RT	BLANK		0	NULL
Phase 3 IDC	MB	EIS	RW, OS, RT	EIS		Populated	Populated
Phase 3 IDC	MB	NIS	RW, OS, RT	NIS		Populated	Populated

During the execution of the checking routines, an error report with standardized error messages is automatically generated for the checks that fail. This report can be viewed within the application by selecting "View Report" (Figure 3), and is also exported to an Excel file to generate the Error Summary Report to be provided to the laboratories.

Most of the QA/QC checks are associated with detailed queries that provide information on what the specific problem is (see the bottom part of the Figure 3, "Detailed QA/QC Queries"). For the errors that are applicable to a certain EDD, the query results are copied into separate worksheets of the Error Summary report and provided to the laboratories. The standardized error messages and the worksheets containing the detailed QA/QC query results are cross-referenced with the query name (e.g., *qry_Edd_review_sample_no*).

Finally, there is a manual step to modify the lab_rep field to account for re-analyses and dilutions.

Figure 3. QA/QC Report and Detailed QA/QC Queries Form

If the EDD passes the checking routines, the EDD is then ready to be appended into the Master EDD Database. Following that, an Amended EDD is generated to be submitted to the designated validator.

5.0 QA/QC Checks on Master EDD Database

As EDDs are compiled into the Master EDD Database, additional QA/QC checking routines have been developed to apply to the entire database. The purpose of this operation is to review the data across phases, laboratories and matrices to ensure that there are no internal inconsistencies or other issues that arise as the data are compiled. These checks will identify differences in how the data are reported from different laboratories and/or validators and ensure consistency in the data exports provided to the project statisticians. It is good practice to incorporate redundancy in the QA/QC procedures to ensure that issues are not overlooked.

Examples of these database-wide queries include:
- Min-max checks on number fields
- Dictionary checks
- Unique record check
- Date range checks
- Consistent unit checks
- Review of summary of lab_flag, lab_qual, dv_qualifier, reviewer_qual, final_qualifier
- Review of summary of conc_found, dv_result, final_result, dv_ResultChange_yn, dv_ResultChange_desc

6.0 Final Processing Steps

There are additional data processing steps that occur after the QA/QC routines have been executed on individual EDDs (Figure 4). Routine 5 populates the spike category (spike_cat) field, and Routine 6 appends the EDD to the Master EDD Database, with separate steps to populate the Tracking ID, LAB_ID and SDG fields. Finally, Routine 7 automatically exports an individual EDD in the Amended EDD format which will be provided to the data validators in Excel format. Table 5 lists the additional fields that are not in the EDD laboratory template but are included in the Amended EDD.

Figure 4. Append to Master EDD Database and Generate Amended EDD Form

Table 5. Additional Fields included in the Amended EDD

Field	Definition
TrackingID	Tracking ID from Tracking System; incorporated during ETL procedures
sdg_num	SDG number; incorporated during ETL procedures; extracted from Lab_Sample_ID
lab_rep	Lab rep number added to easily filter for re-analysis/dilutions.
Result_Type	Code for Result Type. See Valid Value list.
validation_level	Stores information on the level of data validation that has been completed for the chemistry data. Automatically populated with 'Level 4'.
validator	Code for Data Validator. See Valid Value list.
dv_qualifier	Code for Data Validator qualifiers. See Valid Value list.
dv_qualifier_reason	Data validation qualifier reason codes.
dv_notes1	Include comments to distinguish the meaning of the dv_qualifier assignment (e.g., distinguishing the -J qualifier)
dv_notes2	Additional information provided by validator deemed pertinent to their dv_qualifier assignment.
dv_result	Validator recommended result for concentration. If this is provided, entries must be made in the dv_ResultChange_yn field and dv_ResultChange_desc fields.
dv_ResultChange_yn	Enter Y or N. Indicates whether the validator made a recommendation to change the result for concentration.
dv_ResultChange_desc	Description of the reasons for validator recommending a change to the result for concentration.
Reviewer_qualifier	Code for qualifiers applied by NAVY/EPA reviewers of data validation results. See Valid Value list.
Reviewer_notes	Notes from NAVY/EPA reviewers of data validation results.

7.0 References

SERDP/ESTCP PFAS Method Validation Study Team. 2022. Study Plan for Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC- MS/MS. Prepared for Program Manager for Environmental Restoration, Strategic Environmental Research and Development Program (SERDP).

ADDENDUM
Data Management Plan for the

Multi-Laboratory Validation Draft EPA Method 1633 –
PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by
LC-MS/MS

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Addendum to Attachment 4 (Data Management Plan)

Key differences in the procedures outlined in the Data Management Plan and the actual procedures followed are described in this Addendum.

Section 2.0 Roles and Responsibilities
There have been changes to project personnel - Glenn Sutula (Exa) and Joe Vilain (HGL) were added to the data management team, and John Powell (HGL) took on a different role in the project.

Section 3.0 Data Sharing Plan and File Tracking

Section 3.1 MLV Study Library: Host and Software
The DMP's description of the MLV Study Library, commonly referred to as ExaBlue, has differences in the folder structure as originally envisioned (Figure 2 of DMP) and modifications to levels of access and permissions (Table 3 of DMP). There have been some updates to the actual content stored on Exa Blue (Table 2 of DMP); this continues to evolve as final documents are generated. For example, there is a variety of information that is stored in Documents/Laboratory Data/Other Lab Data, as follows: Corrective Action Reports, ERA Certificates of Spiking, ERA laboratory instructions, laboratory instructions, Wellington Certificates of Analysis.

Section 3.2 File Tracking System
Modifications and improvements were made in the procedures for tracking submissions from laboratories, validators and reviewers. The File Tracking System as originally designed was meant to have information entered by key team members (Exa, HGL, validators, NAVY/EPA reviewers). The actual process, however, has been that most tracking information is entered and tracked only by Exa personnel. The Tracking System (as described in Appendix A1 of the DMP) was streamlined, tracking information was not maintained in the Project Database, a live shared Excel status document was used for sharing status with the MLV Team (rather than weekly summary updates), and coordination with NAVSEA reviewers was conducted with a new tracking file 'EPA NAVSEA Review Tracker'.

Section 3.3 File-Naming Protocols
The DMP states: "Importantly, the laboratory must resubmit BOTH the EDD and the Data Package with a new version number, even if only one or the other was revised." However, as the project progressed, multiple submissions and version numbers of EDD/Data Packages were not updated together as required in the DMP due to the unforeseen magnitude of changes. Also, there were exceptions to the required file-naming conventions, as not all labs were in compliance.

Section 4.0 Data Management Processes and Procedures

Section 4.1 Workflow
This section of the DMP documented the plan for adhering to a specific, rigorous, and well-documented workflow for the data generated during the project. A diagram of the revised MLV Study workflow is provided in Figures 1a and 1b. The workflow outlines the sequence of

processes that are adhered to by all team members, including the Data Management Team (Exa/HGL), the MVS Team, laboratories, the validation team, and the statistical analysis team (IDA). This figure has been updated for this addendum to reflect the workflow that was ultimately established for the project.

Key changes to the workflow were:

- MLVS Team members were not informed of individual submissions of data packages and EDDs from the laboratories. Rather, the ExaBlue SharePoint was set up to automatically send email notifications to Team members that were interested in this level of detail.
- HGL completed a checklist when reviewing data packages submitted by the lab, and when the data package was approved, HGL posted the data package and the checklist to the Validator folders on ExaBlue.
- When validators found issues with the data packages/EDDs, in some cases they directly informed the laboratories of the action needed, instead of all communication flowing through HGL.
- Figure 3 in the DMP did not include the step for the data validators preparing and submitting the data validation report and the amended EDD (with validation qualifiers added) to ExaBlue
- A new review step was incorporated into the process, in which the Project Manager (Tim Thompson) reviewed the Data Validation Reports and resolved any inconsistencies prior to releasing it to NAVSEA/EPA for review.
- If the NAVSEA/EPA reviewers found issues in the Data Validation Report, they would request that the data validators resolve the issues and re-submit. NAVSEA/EPA reviewers added Reviewer Qualifiers directly in the amended EDD; in some cases, the data validator was asked to re-submit a revised amended EDD. After the EDD was finalized, it was uploaded and provided to Exa to incorporate the data validator and reviewer qualifiers into the database.
- A new step was added to the data processing procedures in that Exa calculated % recoveries considering the native concentration in the samples. The general calculation is:
  Final_result Spiked Sample/[spike_level + CONC_FOUND unspiked sample], and there were special data handling options if the Unspiked sample was a non-detect, or rejected
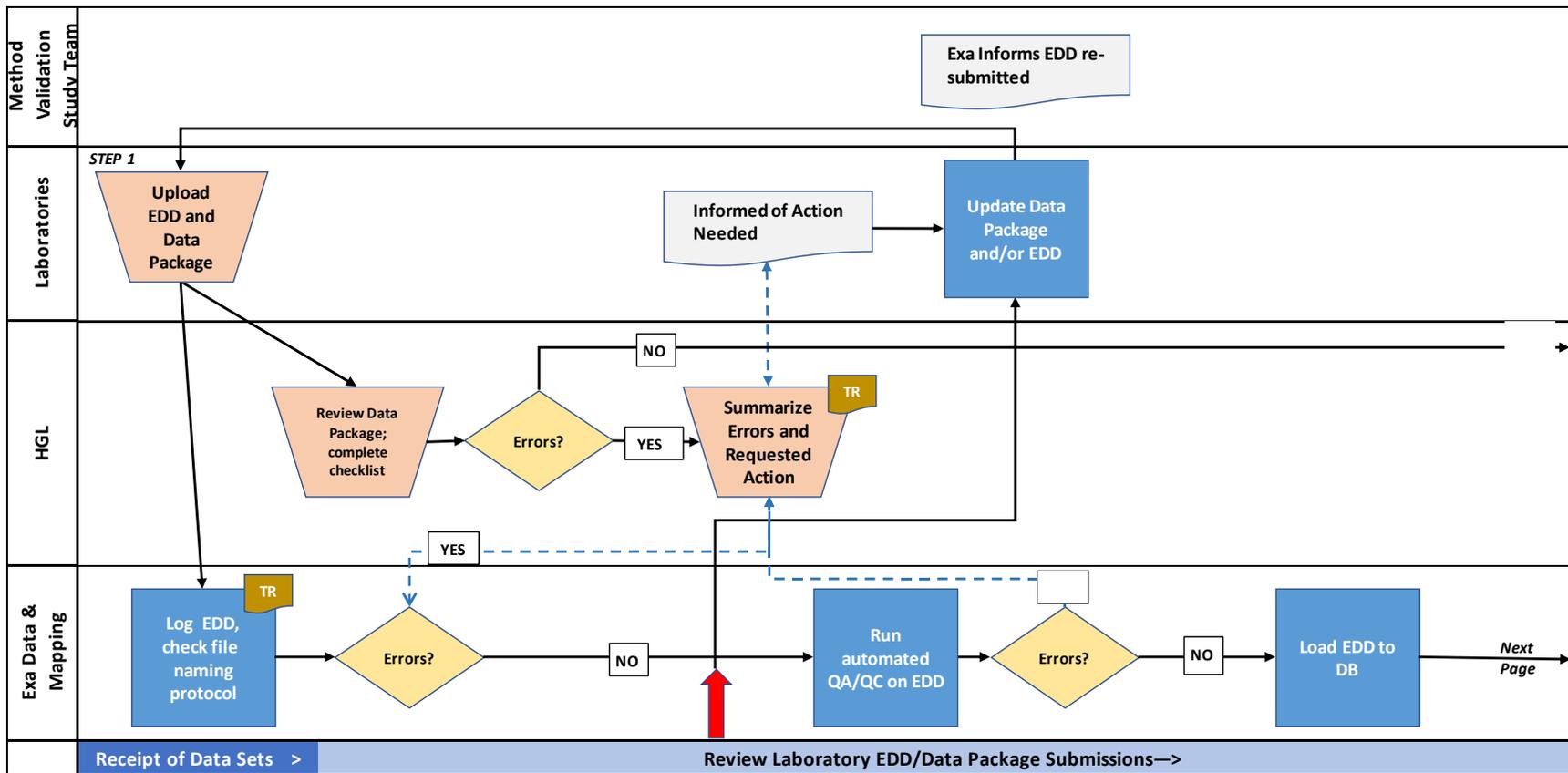
Figure 1a. Revised workflow for the PFAS Multi-Laboratory Validation Study
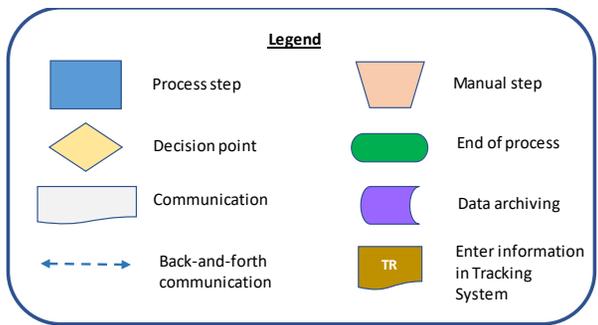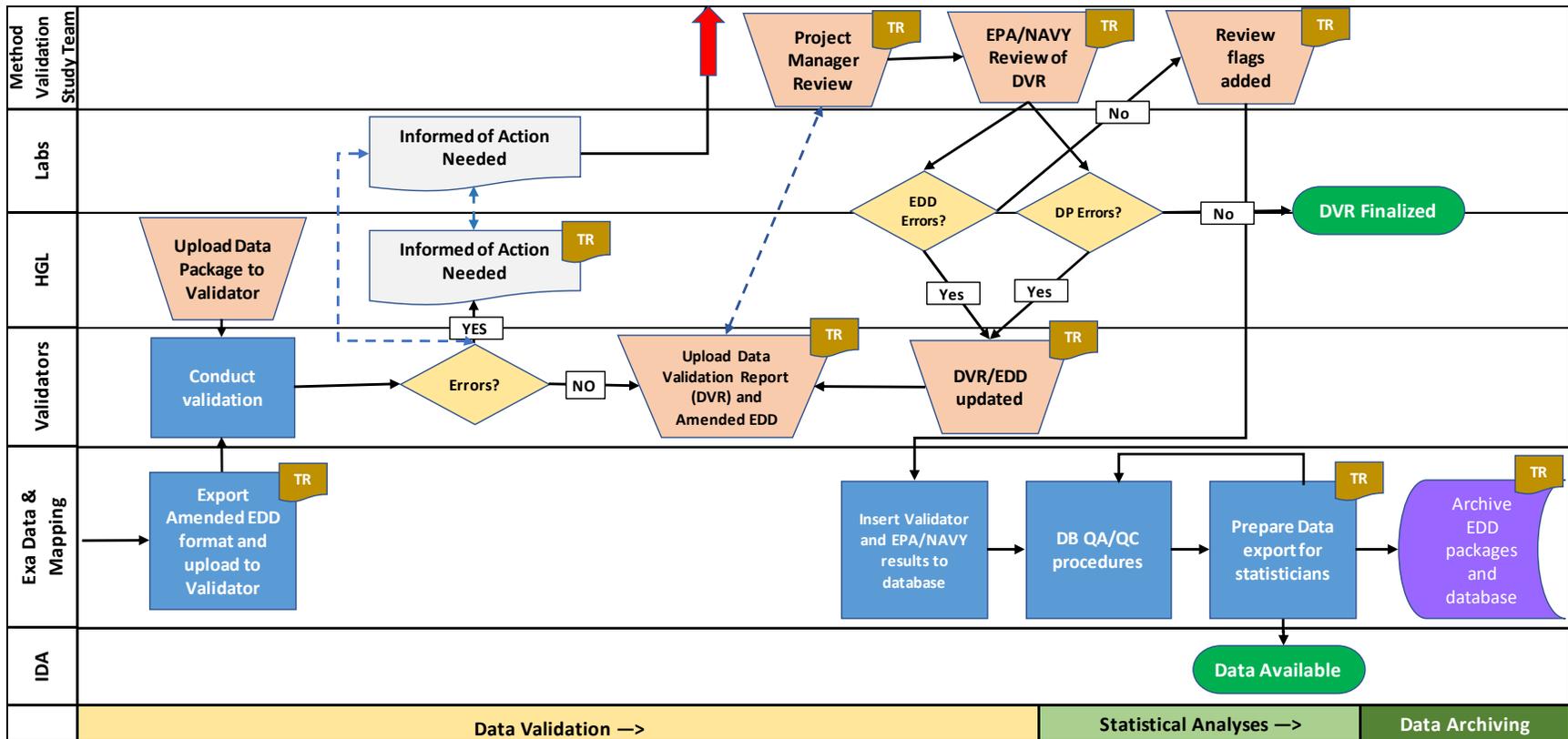
Figure 1b. Revised workflow for the PFAS Multi-Laboratory Validation Study (continued)

Section 4.1.6 Rejection and Resubmission Process

The Data Management Plan states that '…the Exa data managers will not conduct any editing or data cleaning procedures to amend the data provided by the laboratories.' In order to expedite the flow of EDDs, Exa directly edited minor inconsistencies in the EDDs, after receiving permission from the laboratories.

Section 4.2 Database and Tools

4.2.1   Database Structure

As the project progressed, there were modifications made to the structure of the Project Database, as follows:

- Tracking tables were removed
- The lu_matrixKey table was added, which provides a description of the test samples, the spike category of each sample, and whether it was selected for analysis
- Additional fields were added to the Lab_EDD_Results table: spike_level, conc_minus_native, spike_percent_rec

A revised version of the Entity-Relationship Diagram (ERD) for the Project Database is provided in Figure 2 and reflects the current database structure.
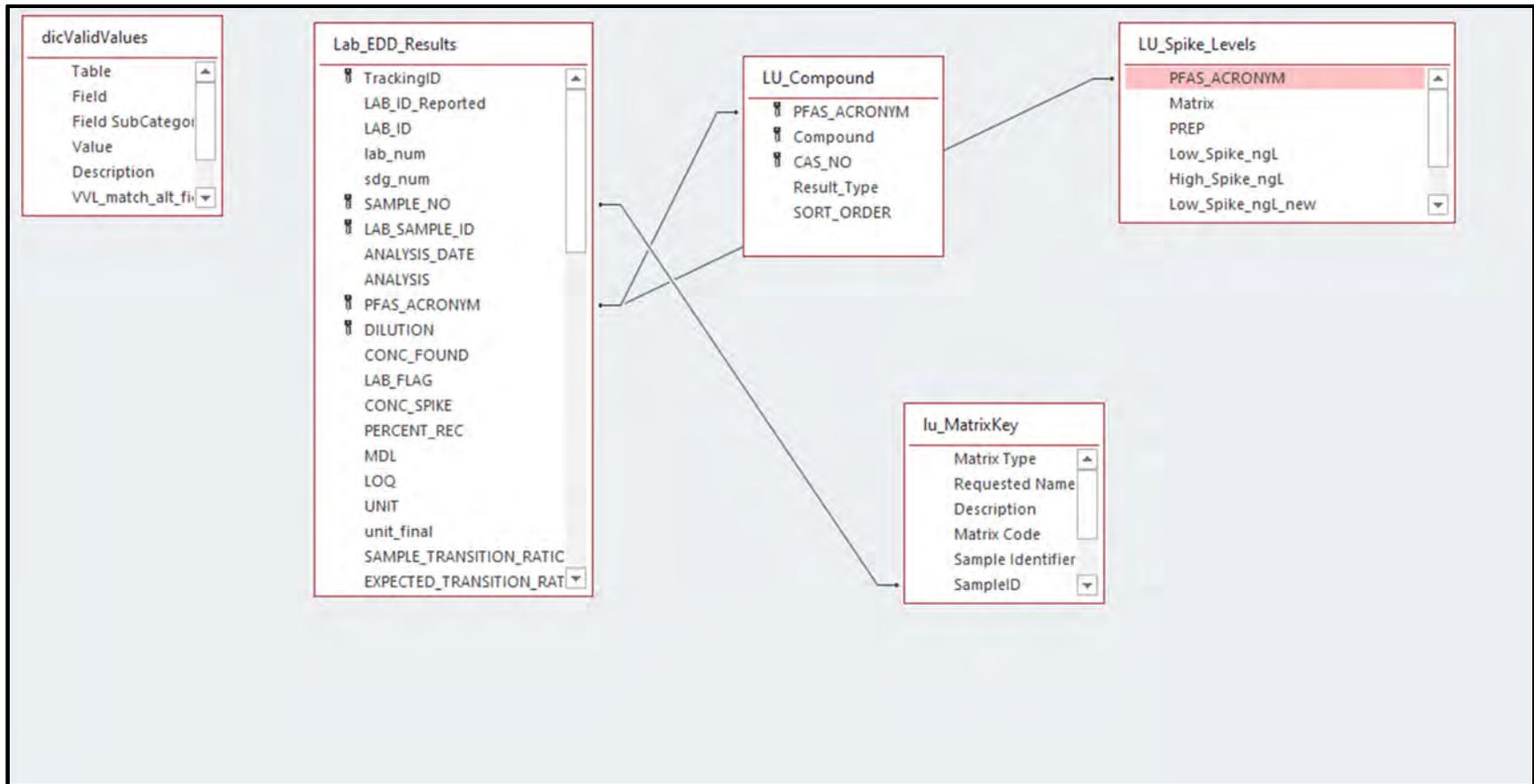
Figure 2. Revised Entity-Relationship Diagram (ERD) for the Project Database

4.2.3 Data Processing Tools

The procedures for data processing and QA/QC review of the EDDs was completed and is in use since the publication of the DMP. A 'QA/QC and Data Processing Procedures' white paper was prepared and submitted to the project team and is included in Appendix 1 of this Addendum.

In conjunction with developing the 'QA/QC and Data Processing Procedures' white paper, the EDD Template instructions were reviewed and 'MLV_Study_Supplemental_EDD_Instructions' were developed and distributed to the MLV Study team, to clarify some details of how the EDD Template is expected to be populated. The 'MLV_Study_Supplemental_EDD_Instructions' is included in Appendix 2 of this Addendum.

MLV Study - EDD Template Legend

Field	Data type	Definition	Valid Value Field	Required field*	Default Value	Primary Key
LAB_ID	text	Laboratory Name.		YES		
SAMPLE_NO	text	For samples, these are the sample identification names (IDs) from the Chain of Custody. The Sample_No is the same, regardless of whether or not the sample is diluted or reanalyzed. For preparation batch QC and Initial Demonstration of Capability samples: *Refer to the Sample Coding worksheet for information on populating this field.*		YES		X
LAB_SAMPLE_ID	text	The ID the laboratory assigns to the sample (which identifies the sample on the associated data files and reports). For samples that need to be re-analyzed for issues other than dilution, attach the following identifiers to the end of the lab sample identifier without a space between them (e.g., 02082022-01R): - "R" for analytes, EISs and NISs reported from first re-analysis not due to dilution; - "R1" for analytes, EISs and NISs reported from second re-analysis not due to dilution; and, - "R2" for analytes, EISs and NISs reported from second re-analysis not due to dilution If more re-analyses not due to dilution are needed to be reported beyond three for a sample, continue on with the numbering (e.g., R3, R4, R5, etc.).		YES		X
ANALYSIS_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.		YES		
ANALYSIS	text	Fill in "PFAS". See Valid Value list.	YES	YES	PFAS	
COMPOUND	text	PFAS compound name. Method analytes, and EIS and NIS compounds must be reported for each sample. See LU_Compound list.	YES	YES		
CAS_NO	text	Chemical Abstract Service Registration Number (CASRN). For compounds with no CASRN, leave blank. See LU_Compound list.	YES	YES		
PFAS_ACRONYM	text	Short form code for Compounds. See LU_Compound list.	YES	YES		X
DILUTION	number integer	Dilution made post extraction (e.g., extract diluted 1:10 is entered as "10"). If analyzed without dilution, enter "1".		YES	1	X
CONC_FOUND	number double	Measured concentration. Enter numeric quantitative result value only. Do NOT enter any text string strings or symbols (e.g., "ND", "<"). For analytes that are not detected, the laboratory's sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account) is entered. Report result units in "Unit" field.		YES		
LAB_FLAG	text	Laboratory qualifier codes. These flags apply to all samples (field and QC). See Valid Value list.	YES			
CONC_SPIKE	number double	Spiked concentration. For unspiked samples enter "0" for method analytes. For spiked samples, enter the spike concentration representing the estimated concentration in the final extract (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). For EIS and NIS, enter the spike concentration representing the concentration in the final extract in units consistent with sample result units.		YES		

MLV Study - EDD Template Legend

Field	Data type	Definition	Valid Value Field	Required field*	Default Value	Primary Key
PERCENT_REC	number double	Calculated percent recovery. For spiked samples (OPR, LLOPR, MDLs and LOQVER), enter the spike percentage recovery as a whole number (e.g., 95 versus 0.95). Do NOT include "%" symbol. For EIS and NIS recoveries, enter the spike % recovery as a whole number (e.g., 95 versus 0.95). For unspiked samples, leave blank. Do NOT include "%". ***Refer to the Sample Coding worksheet for information on populating this field.***			null	
MDL	number double	Method Detection Limit. Enter the sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account).			null	
LOQ	number double	Limit of Quantitation. Enter the sample specific LOQ (i.e. with extract dilution factor, sample volume/weight and final volume taken into account).			null	
UNIT	text	Reporting unit. See Valid Value list.	YES	YES		
SAMPLE_TRANSITION_F	number double	Calculated Transition Ratio (Quant Ion Area/Conf Ion Area). Enter for each analyte in the sample. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank.			null	
EXPECTED_TRANSITION	number double	Expected Transition Ratio (Quant Ion Area/Conf Ion Area). Enter for each analyte in the sample. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank.			null	
RRT	text	Relative retention time				
SAMPLE_SIZE	number double	Volume (aqueous samples) or weight (solid samples) of sample extracted (in liters for aqueous samples, in kilograms for solids).		YES		
SAMPLE_SIZE_UNIT	text	Will be liters (L) for aqueous samples or kilograms (Kg) for solid samples		YES		
EXTRACTION_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.		YES		
PERC_MOISTURE	number double	Percent moisture in solid samples only. Enter the percent moisture as a whole number (e.g., 73 versus 0.73). Do NOT include "%" symbol.				
MATRIX	text	Matrix of sample. See Valid values list. ***Refer to the Sample Coding worksheet for information on populating this field.***	YES	YES		
METHOD	text	Laboratory SOP Name in format of "name(space)revision number"		YES		
STUDY_PHASE	text	Multi-Lab Validation Study Phase. See Valid Value list.	YES	YES		
SAMPLE_TYPE	text	See Valid values list. ***Refer to the Sample Coding worksheet for information on populating this field.***	YES	YES		X

Combinations codes to use in the SAMPLE_NO, MATRIX and SAMPLE_TYPE fields.

Phase	SAMPLE_NO	Compound Type	Matrix	Sample_type	CONC_SPIKE	PERCENT_REC
Phase 4	GWA0	Method analytes	GW, SW, etc	NORMAL	0	NULL
Phase 4	GWA0	EIS	GW, SW, etc	EIS	Populated	Populated
Phase 4	GWA0	NIS	GW, SW, etc	NIS	Populated	Populated
Phase 4	MB	Method analytes	RW, OS, RT	BLANK	0	NULL
Phase 4	MB	EIS	RW, OS, RT	EIS	Populated	Populated
Phase 4	MB	NIS	RW, OS, RT	NIS	Populated	Populated
Phase 4	OPR*	Method analytes	QC	OPR	Populated	Populated
Phase 4	OPR*	EIS	QC	EIS	Populated	Populated
Phase 4	OPR*	NIS	QC	NIS	Populated	Populated
Phase 4	LLOPR*	Method analytes	QC	LLOPR	Populated	Populated
Phase 4	LLOPR*	EIS	QC	EIS	Populated	Populated
Phase 4	LLOPR*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	IPR*	Method analytes	QC	IPR	Populated	Populated
Phase 3 IDC	IPR*	EIS	QC	EIS	Populated	Populated
Phase 3 IDC	IPR*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	MDLB	Method analytes	RW, OS, RT	MDLB	0	NULL
Phase 3 IDC	MDLB	EIS	RW, OS, RT	EIS	Populated	Populated
Phase 3 IDC	MDLB	NIS	RW, OS, RT	NIS	Populated	Populated
Phase 3 IDC	MDLS*	Method analytes	QC	MDLS	Populated	Populated
Phase 3 IDC	MDLS*	EIS	QC	EIS	Populated	Populated
Phase 3 IDC	MDLS*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	LOQVER*	Method analytes	QC	LOQVER	Populated	Populated
Phase 3 IDC	LOQVER*	EIS	QC	EIS	Populated	Populated
Phase 3 IDC	LOQVER*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	MB	Method analytes	RW, OS, RT	BLANK	0	NULL
Phase 3 IDC	MB	EIS	RW, OS, RT	EIS	Populated	Populated
Phase 3 IDC	MB	NIS	RW, OS, RT	NIS	Populated	Populated

*spiked samples

Valid Value list for EDD Template fields with constrained entries:

Field	Field sub-category	Value	Description
ANALYSIS		PFAS	Default value.
LAB_FLAG		B	For analytes that were detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than 1/10th the concentration in the sample, whichever is greatest. The MB must also be flagged with a "B" for all concentrations greater than ½ the LOQ.
Lab_Flag		D	For analytes, EISs, and NISs reported from a dilution.
Lab_Flag		I	For analytes that fail to meet ion ratio criteria.
Lab_Flag		J	For analytes that were at a concentration between the MDL and LOQ.
Lab_Flag		U	For analytes that were not detected or were detected at a concentration less than the MDL.
MATRIX	Field samples	BS	Biosolid
MATRIX	Field samples	GW	Groundwater (method analytes, EIS, NIS)
MATRIX	Field samples	LC	Leachate
MATRIX	Field samples	SD	Sediment
MATRIX	Field samples	SS	Soil
MATRIX	Field samples	SW	Surface water
MATRIX	Field samples	TS	Tissue
MATRIX	Field samples	WW	Wastewater
MATRIX	Method blanks and MDLB	OS	Ottawa sand for all soil, sediment, and biosolid MBs
MATRIX	Method blanks and MDLB	RT	Reference Tissue for tissue MBs
MATRIX	Method blanks and MDLB	RW	Reagent water for all aqueous MBs
MATRIX	Quality Control sample	QC	Quality Control Sample
SAMPLE_TYPE		BLANK	method analytes in MBs
SAMPLE_TYPE		EIS	EIS in all samples
SAMPLE_TYPE		IPR	method analytes in IPR IDC samples
SAMPLE_TYPE		LLOPR	method analytes in LLOPRs
SAMPLE_TYPE		LOQVER	Method analytes in MDL LOQVER IDC samples
SAMPLE_TYPE		MDLB	Method analytes in MDL Blank IDC samples
SAMPLE_TYPE		MDLS	Method analytes in MDL Spike IDC samples
SAMPLE_TYPE		NIS	NIS in all samples
SAMPLE_TYPE		NORMAL	method analytes in field samples
SAMPLE_TYPE		OPR	method analytes in OPRs
STUDY_PHASE		Phase 3	Initial Demonstration of Capabilities (IDC)
STUDY_PHASE		Phase 4.4.1	GW, SW, LC, and WW matrices
STUDY_PHASE		Phase 4.4.2	SS, SD, and BS matrices
STUDY_PHASE		Phase 4.4.3	Tissue matrices
UNIT		ng/L	nanograms per liter
UNIT		ug/kg	micrograms per kilogram

Valid Value list for EDD Template fields PFAS_ACRONYM, COMPOUND and CAS_NO:

PFAS_ACRONYM	COMPOUND	CAS_NO	Result_Type
13C2-4:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]hexanesulfonic acid		EIS
13C2-6:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]octanesulfonic acid		EIS
13C2-8:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]decanesulfonic acid		EIS
13C2-PFDoA	Perfluoro-n-[1,2-13C2]dodecanoic acid		EIS
13C2-PFTeDA	Perfluoro-n-[1,2-13C2]tetradecanoic acid		EIS
13C3-HFPO-DA	Tetrafluoro-2-heptafluoropropoxy-13C3-propanoic acid		EIS
13C3-PFBS	Perfluoro-1-[2,3,4-13C3]butanesulfonic acid		EIS
13C3-PFHxS	Perfluoro-1-[1,2,3-13C3]hexanesulfonic acid		EIS
13C4-PFBA	Perfluoro-n-[13C4]butanoic acid		EIS
13C4-PFHpA	Perfluoro-n-[1,2,3,4-13C4]heptanoic acid		EIS
13C5-PFHxA	Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid		EIS
13C5-PFPeA	Perfluoro-n-[13C5]pentanoic acid		EIS
13C6-PFDA	Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid		EIS
13C7-PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid		EIS
13C8-PFOA	Perfluoro-n-[13C8]octanoic acid		EIS
13C8-PFOS	Perfluoro-1-[13C8]octanesulfonic acid		EIS
13C8-PFOSA	Perfluoro-1-[13C8]octanesulfonamide		EIS
13C9-PFNA	Perfluoro-n-[13C9]nonanoic acid		EIS
D3-NMeFOSA	N-methyl-d3-perfluoro-1-octanesulfonamide		EIS
D3-NMeFOSAA	N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid		EIS
D5-NEtFOSA	N-ethyl-d5-perfluoro-1-octanesulfonamide		EIS
D5-NEtFOSAA	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid		EIS
D7-NMeFOSE	N-methyl-d7-perfluorooctanesulfonamidoethanol		EIS
D9-NEtFOSE	N-ethyl-d9-perfluorooctanesulfonamidoethanol		EIS
13C2-PFDA	Perfluoro-n-[1,2-13C2]decanoic acid		NIS
13C2-PFHxA	Perfluoro-n-[1,2-13C2]hexanoic acid		NIS
13C3-PFBA	Perfluoro-n-[2,3,4-13C3]butanoic acid		NIS
13C4-PFOA	Perfluoro-n-[1,2,3,4-13C4]octanoic acid		NIS
13C4-PFOS	Perfluoro-n-[1,2,3,4-13C4]octanesulfonic acid		NIS
13C5-PFNA	Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid		NIS
18O2-PFHxS	Perfluoro-1-hexane[18O2]sulfonic acid		NIS
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	Method analytes

Valid Value list for EDD Template fields PFAS_ACRONYM, COMPOUND and CAS_NO:

PFAS_ACRONYM	COMPOUND	CAS_NO	Result_Type
3:3FTCA	3-Perfluoropropyl propanoic acid	356-02-5	Method analytes
4:2FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid	757124-72-4	Method analytes
5:3FTCA	2H,2H,3H,3H-Perfluorooctanoic acid	914637-49-3	Method analytes
6:2FTS	1H,1H,2H,2H-Perfluorooctane sulfonic acid	27619-97-2	Method analytes
7:3FTCA	3-Perfluoroheptyl propanoic acid	812-70-4	Method analytes
8:2FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid	39108-34-4	Method analytes
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	756426-58-1	Method analytes
ADONA	4,8-dioxa-3H-perfluorononanoic acid	919005-14-4	Method analytes
HFPO-DA	Hexafluoropropylene oxide dimer acid	13252-13-6	Method analytes
NEtFOSA	N-ethyl perfluorooctanesulfonamide	4151-50-2	Method analytes
NEtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid	2991-50-6	Method analytes
NEtFOSE	N-ethyl perfluorooctanesulfonamidoethanol	1691-99-2	Method analytes
NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	151772-58-6	Method analytes
NMeFOSA	N-methyl perfluorooctanesulfonamide	31506-32-8	Method analytes
NMeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid	2355-31-9	Method analytes
NMeFOSE	N-methyl perfluorooctanesulfonamidoethanol	24448-09-7	Method analytes
PFBA	Perfluorobutanoic acid	375-22-4	Method analytes
PFBS	Perfluorobutanesulfonic acid	375-73-5	Method analytes
PFDA	Perfluorodecanoic acid	335-76-2	Method analytes
PFDoA	Perfluorododecanoic acid	307-55-1	Method analytes
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	Method analytes
PFDS	Perfluorodecanesulfonic acid	335-77-3	Method analytes
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	113507-82-7	Method analytes
PFHpA	Perfluoroheptanoic acid	375-85-9	Method analytes
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	Method analytes
PFHxA	Perfluorohexanoic acid	307-24-4	Method analytes
PFHxS	Perfluorohexanesulfonic acid	355-46-4	Method analytes
PFMBA	Perfluoro-4-methoxybutanoic acid	863090-89-5	Method analytes
PFMPA	Perfluoro-3-methoxypropanoic acid	377-73-1	Method analytes
PFNA	Perfluorononanoic acid	375-95-1	Method analytes
PFNS	Perfluorononanesulfonic acid	68259-12-1	Method analytes
PFOA	Perfluorooctanoic acid	335-67-1	Method analytes

Valid Value list for EDD Template fields PFAS_ACRONYM, COMPOUND and CAS_NO:

PFAS_ACRONYM	COMPOUND	CAS_NO	Result_Type
PFOS	Perfluorooctanesulfonic acid	1763-23-1	Method analytes
PFOSA	Perfluorooctanesulfonamide	754-91-6	Method analytes
PFPeA	Perfluoropentanoic acid	2706-90-3	Method analytes
PFPeS	Perfluoropentanesulfonic acid	2706-91-4	Method analytes
PFTeDA	Perfluorotetradecanoic acid	376-06-7	Method analytes
PFTrDA	Perfluorotridecanoic acid	72629-94-8	Method analytes
PFUnA	Perfluoroundecanoic acid	2058-94-8	Method analytes

Supplemental guidance for correctly populating the EDD Template:

Item   **Guidance**

1   If possible, include all results in one worksheet.

2   We will be running QA/QC routines on the EDDs to ensure they are populated correctly. These will be delivered to you in an Excel file (e.g., LABNAME_RW_ver0_EDD_Error_Summary.xlsx).
   If you have questions/issues regarding the Error Summary report, please incorporate comments directly into the Excel file and send it back to us for review (via SharePoint).

3   Instructions for reporting CONC_FOUND for NIS compounds:
   1. Option 1: Report NIS Mass in CONC_FOUND, and in the accompanying report provide example calculation and point to where the data in the numerator (e.g., field sample) and the denominator (e.g. CCV) are found.
   2. Option2: Complete the EDDs with the NIS percent recoveries based on areas (i.e., no mass reported). CONC_FOUND does not need to be populated.

   a. If Option 2 is selected then
      i.   In the report provide the formula and example calculations for one sample per batch
      ii. Point to where those areas values are found in their data packages (i.e., ensure they have reported the NIS area for the field (target) sample and the comparative area from either the mid-point of the ICAL, CCV, or equivalent.

4   Instructions for populating EDD when a single sample is serving the purpose of the MDLB and MB samples:
   1. Copy the results and code the SAMPLE_NO field as 'MDLB' for one set of results, and 'MB' for the other set of results.
   2. Ensure that the case narrative clearly identifies that the blank sample was used for multiple purposes.

5   Ensure that B flags are applied as indicated by the instructions:
    "For analytes that were detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than 1/10th the concentration in the sample, whichever is greatest. The MB must also be flagged with a "B" for all concentrations greater than ½ the LOQ."
   If the CONC_FOUND is between the MDL and LOQ <u>AND</u> it is at a concentration greater than 1/2 LOQ, the flag should be BJ or JB.

6   Although the instructions for the CONC_FOUND field indicate "For analytes that are not detected, the laboratory's sample specific MDL", the exceptions are for the following sample types, where CONC_FOUND can be less than the MDL:
   MDLS
   LOQVER
   Blanks (MB and MDLB)

7   For instances where there are re-analysis not due to dilution, all results must be reported in the EDD (i.e., the original analysis and all subseqent re-analysis).

8   The Sample Coding spreadsheet lists the mandatory batch QC samples that are required for Phase 3 and Phase 4 submittals. All mandatory batch QC samples must be present in the EDD submittals.

Supplemental guidance for correctly populating the EDD Template:

Item	Guidance
9	Do not add suffixes to SAMPLE_NO (e.g., IPR1, MLDB 2) - LAB_SAMPLE_ID will differentiate samples.

10   Fields that must be reported to 3 significant figures:

         CONC_FOUND
         CONC_SPIKE
         PERCENT_REC
         MDL
         LOQ
         SAMPLE_TRANSITION_RATIO
         EXPECTED_TRANSITION_RATIO

         Note that the results in these fields must be rounded appropriately to 3 significant figures. Changing the display for the number of decimal places is not sufficient.

11   Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis.

12   UNIT field must be consistent across all samples.

13   The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples.

14   Do not include text in number fiels (e.g. N/A, %, ND, <).

15   Percent recovery must be reported in the EDD for IPR samples.

ATTACHMENT 5

STUDY DATA VALIDATION GUIDELINES

(Dated 01/10/22)

Data Validation Procedure for Per- and Polyfluoroalkyl Substances Results from the Multi-laboratory Validation Study

FINAL 01/10/2022

TABLE OF CONTENTS

Data Validation Procedure for Per- and Polyfluoroalkyl Substances Results from the Multi-laboratory Validation Study

1.0 Purpose

This document provides guidance on the validation of data generated by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis for per- and polyfluoroalkyl substances (PFAS) according to the Multi-Laboratory Validation (MLV) Study Method. The objective of this procedure is to provide the end user with a clear understanding of the quality and limitations of the data through documented validation procedures and to encourage consistency in the validation technique and reporting of data generated by the MLV Study of Draft EPA Method 1633.

This document assumes the user is familiar with data validation conventions and qualifiers used in the *DoD General Data Validation Guidelines (2019).* This document is not intended to obviate the need for professional judgment during the validation process.

2.0 Procedure

2.1 Introduction

This document was written with primary consideration to the MLV Study Plan and the MLV Study Method. This guidance is to be applied to PFAS data generated in support of the MLV Study of Draft EPA Method 1633. This guidance should be implemented by personnel familiar with the methodology contained herein.

Data validation personnel are responsible for implementing this procedure for validation of data and generation of data validation reports for the MLV Study of Draft EPA Method 1633.

2.2 Deliverables

Laboratory data deliverables consist of a combination of forms and raw data. The manner in which laboratories label their forms is not dictated nor specified. **The labeling convention below is used for simplicity.**

- Cover Sheet
- Table of Contents
- Case Narrative
- Sample Receipt and Conditions Summary
- Sample Results Summary
- Transition Ion Summary
- Sample Transition Ion Ratio Summary
- Extracted Internal Standard Recovery and Retention Time Summary
- Non-Extracted Internal Standard Recovery and Retention Time Summary
- Ongoing Precision and Recovery Sample/Low-Level Ongoing Precision and Recovery Sample Recovery Summary

- Method Blank Summary
- Sample Dilution and Reanalysis Summary
- Bile Salts Interference Check Summary
- Qualitative Identification Standards Summary
- Sequence and Preparation Logs (or equivalent to include Instrument Blanks)
- Mass Calibration and Mass Calibration Verification Check Summary
- Initial Calibration Summary (any equivalent to include the Initial Calibration Analyte Responses, Isomeric Profiles, Average Response Factors, and Regression)
- Calibration Verifications and Instrument Sensitivity Check Summary
- Manufacturer provided Certificate of Analysis for Standards
- Raw Data- including quantitative and confirmation transition ion chromatograms, peak areas, and ion ratios

2.3 Validation Stages

The types of laboratory data deliverables, staged data validation, and the relationship between the two are outlined in the *DoD General Data Validation Guidelines*.

Stage 1 data validation consists of a review of sample results forms, associated sample receipt summaries (chain of custody), and field QC data.

Stages 2A and **2B** data validation consist of review of summary forms only.

Stages 3 and **4** data validation require review of both summary forms and all associated raw data.

This MLV Study is using Stage 4 validation for all of the data review. Both the laboratory deliverable and the stage of validation required by the MLV Study are specified in MLV Study Plan. Specifically, each laboratory deliverable must include all of the elements required to conduct the level of data validation required for the study, which is Stage 4 data validation (see Section 7.0). Data review guidelines and how they apply to the different validation stages are indicated in the following sections.

3.0 Stage 1 Validation

The following documents should be reviewed for representativeness (compliance with required analytical protocols outlined in the MLV STUDY PLAN), completeness, and project sensitivity needs:

- Cover Sheet
- Table of Contents
- Case Narrative
- Sample Results form or equivalent Laboratory Report
- Transition Ion Summary
- Chain-of-Custody (CoC) forms, Laboratory Receipt Checklists, and other supporting records

Stage 1 is the validation of investigative and field QC samples**.**

3.1 Sample Results

Examine the Laboratory Report sample results and verify the following information, ensuring that:

- Holding times have been met, as applicable
- All project target analytes have been analyzed and are reported
- All ion transitions used for quantitation and confirmation are identified
- All project target analytes whose quantitation includes branched and linear isomers are identified
- All sample identification labels are unique, and match the chain of custody
- All laboratory reported Method Detection Limits (MDLs) and Limits of Quantitation (LOQs) have sample preparation factors and dilutions taken into account
- All analyte results are reported from the lowest dilution possible (i.e., the lowest dilution for which QC parameters are met)
- All reported units (e.g., ng/L) are accurate and reflect the requirements of the MLV Study Plan and Method and that units are consistent with the type of sample matrix
- Soil, sediment, and biosolid samples have been reported on a dry-weight basis
- Tissue samples have been reported on a wet-weight basis
- Each laboratory report has a case narrative that explains all non-conformities with the data

For sample results (assuming no other qualifications due to data quality issues):

Qualification of data is based upon the reporting requirements of the MLV Study Plan and Method.

Any detects between the MDL and LOQ are qualified as estimated **J.** Values below the MDL are considered non-detects and are qualified as **U** at the stated MDL.

Evaluation of the Laboratory Report

Any samples received for analysis that were not analyzed should be noted in the data validation report, along with the reason(s) for failure to analyze the samples, if the reason(s) can be determined; conversely, samples that were analyzed by LC/MS/MS but were not requested should also be noted.

Check that all 40 PFAS included in the scope of the MLV Study method have been reported by the laboratory.

Errors in reported units and case narrative non-conformities that call into question the quality of the data should be discussed in the data validation report.

Generally speaking, errors in quantitation limits or missing or misidentified samples may require a higher than Stage 1 validation, but this study is only using Stage 4. Data validators are encouraged to reach out to the MLV Study QA Manager (Janice.willey@navy.mil) and communicate issues when preparing the data validation report.

The transitions provided in the MLV Study Method, Table 2 must be used, unless interference renders the required product ion unusable. In these cases, MLV Study approval is required before using the alternative product ion. When evaluating the Sample Transition Ion Summary, if the ion transitions specified in the MLV Study Method, Table 2, are not used for quantitation, the technical justification provided in the case narrative should be reviewed. If a technical justification is not provided or the explanation provided does not provide a technical justification for the change, use professional judgment to qualify the data and all affected results must be noted in the data validation report. Use of any quantitative transitions other than those listed in the MLV Study Method, Table 2 must be identified in the data validation report.

3.2 Chain of Custody (CoC)

Examine the CoC form (some information may be included on Laboratory Receipt Checklists) for legibility and check that all of the samples have been analyzed and reported by the laboratory. Ensure that the CoC sample identification on the Laboratory Sample Results Form matches the sample identification on the CoC. Read the laboratory case narrative for additional information.

Evaluation of the CoC

Any discrepancies in sample naming between the CoC and sample results form should be noted in the data validation report with the correct sample name being identified in the report and on the appropriate summary form, if the correct sample name can be determined. These edit corrections should also be verified in any associated electronic data deliverables (EDDs). If any samples submitted to the laboratory were not analyzed, contact the MLV Study point of contact as soon as possible.

3.3 Sample Preservation, Handling, and Transport

Evaluate sample handling, transport, and laboratory receipt from the CoC and laboratory receipt checklists to ensure that the samples have been properly handled. The MLV Study Method specifies temperatures for sample shipment to the laboratory and sample storage at the laboratory. The following are general guidance if project specifications were not stipulated.

Evaluation of Preservation, Handling, and Transport

If the temperature of receipt is outside of the acceptable range required by the MLV Study Method, the discrepancy, identifying which samples were affected, should be documented in the data validation report.

In the event that both a cooler temperature and a temperature blank were measured, the temperature blank should be evaluated for temperature compliance as it best assimilates the condition of the samples; however, both temperatures shall be noted in the data validation report. If the temperature upon receipt at the laboratory was not recorded, note this in the data validation report.

3.4 HOLDING TIMES

Holding times for PFAS are measured from the date of collection (as shown on the CoC) to the time of sample extraction and analysis (as shown on the sample results form or extraction log). Based on input from the DoD Environmental Data Quality Workgroup (EDQW), holding time exceedances are calculated as follows:

Total holding time is based on the time frame (i.e., hours, days, or months) of the requirement. The following example gives guidance on how holding time exceedances are measured:

For a test with a recommended maximum holding time measured in **days**, the holding time is tracked by the **day**.

- An exceedance of holding time for a sample with a 14-day holding time will occur when the 15th day is reached. Therefore, a sample with a 14-day holding time collected at 8:30 AM on April 4th must be analyzed or extracted before 12:00 AM April 19th (midnight, the start of the 15th day), or an exceedance has occurred.

The MLV Study Method specifies the holding time requirements.

Evaluation of Holding Times

If the holding time is exceeded, document that holding time was exceeded in the data validation report.

4.0 Stage 2A Validation

Note: Stage 2A includes all of Stage 1

Stage 2A requires the review and qualification of the following summary documents:

- Sample Transition Ion Ratio Summary
- Extracted Internal Standard Recovery and Retention Time Summary
- Non-Extracted Internal Standard Recovery and Retention Time Summary
- Ongoing Precision and Recovery Sample/Low-Level Ongoing Precision and Recovery Sample Recovery Summary
- Method Blank Summary
- Extract Dilution and Reanalysis Summary
- Bile Salts Interference Check Summary
- Qualitative Identification Standards Summary

Stage 2A is the validation of preparation batch specific QC data in addition to any sample specific parameters included in Stage 1.

Generally, a "preparation batch" of samples consists of 20 field samples (maximum) along with blank, and control type QC samples. They must be analyzed together on a single instrument. If multiple instrumentation is used, it should be noted in the data validation report.

4.1 Ion Ratio

Ion ratios can be used to help determine if the matrix of the sample has resulted in a bias in the data. Ion ratios must be determined in accordance with the requirements of the MLV Study Method. To determine if a bias has potentially occurred, the ion ratio is evaluated against the ion ratio of the mid-point initial calibration standard, which do not contain matrix interferences. Ion ratios should not exceed 50-150% of the ion ratio observed in the mid-point initial calibration standard. In addition, if the concentration reported for an analyte falls between the MDL and LOQ, the ion ratios should also not exceed 50-150% of the ion ratio observed in the initial daily CV.

Evaluation of Ion Ratios

Verify the ion ratio(s) for each detect were reported and met the requirements of the MLV Study Method. For detects reported with ion ratios exceeding the 50-150% acceptance criteria, qualify the sample results as suspect **I** and note all affected results in the data validation report. Values reported with an **I** qualifier are estimated values. Ion ratio failures could be caused by matrix interference and/or be the result of the presence of isomers in the sample at different ratios than the ratio of isomers present in the calibration standards. A full evaluation (Stage 4 validation) of the raw data and quantitation report is necessary to fully evaluate the potential cause of the failure.

4.2 Extracted Internal Standard (EIS) Recovery

Extracted Internal Standard (EIS) recoveries are used to correct for bias associated with matrix interferences and sample preparation efficiencies, injection volume variances, chromatographic behavior, and mass spectrometry ionization efficiency. All samples, standards, QC samples (including blanks) are fortified with EIS compounds. EIS compounds are added to the solid sample prior to extraction and to an aqueous sample in the original sample container prior to extraction. EIS recoveries are quantitated with respect to Non-Extracted Internal Standard (NIS) responses using the response ratios or response factors from the most recent multi-level initial calibration.

Verify that EIS recoveries and acceptance limits were reported for all field samples, batch QC samples, standards, and instrument blanks.

Sample and batch QC sample EIS percent recoveries should be within target control limits of the MLV Study Method of 20 – 150%. Verify that no samples or batch QC have EIS percent recoveries outside the criteria.

The EIS retention times (RTs) for all field and QC samples should be within 0.40 minutes of the retention time of the midpoint standard in the ICAL, or on days when an ICAL is not performed, the initial CV is used.

Evaluation of Extracted Internal Standards

If EIS percent recoveries are out of specification with no evidence of reanalysis, justification should be noted in the laboratory case narrative (e.g., limited extract volume prevented reanalysis). If justification is not noted, the point of contact identified for the MLV Study Plan should be reached for further guidance.

If the EIS percent recovery control criteria displayed in the deliverable are not the same ranges stipulated in the MLV Study Method, reference the required control ranges for evaluation instead of the summarized ranges in the deliverable. The point of contact identified for the MLV Study should be informed to implement changes to the current deliverables or those to be created in the future.

If extremely low area counts are reported (< 10%), detects and non-detects should be qualified **X**.

If an EIS retention time varies by more than 0.40 minutes, use professional judgment to qualify the sample results and note all affected results in the data validation report.

EIS results may not be reported as "diluted out" since they are used as the internal standard for calculation of the native analyte. A full evaluation (Stage 4 validation) of the sample, chromatogram, mass spectral ions and quantitation report may be necessary to determine that diluted analytes are quantified correctly.

Some extracts may require dilution to bring analytes within the calibration range or resolve interferences with EIS compounds. This can result in EIS dilution to the point that EIS recoveries may not be sufficiently measurable and would require EIS fortification to the diluted extract. In these instances, detects for analytes quantified from this type of diluted extract should be identified in the data validation report.

4.3 Non-Extracted Internal Standard (NIS) Recovery

Non-Extracted Internal Standard (NIS) peak areas are used to quantify EIS recoveries. NIS analytes are labeled PFAS compounds spiked into the concentrated extract immediately prior to injection of an aliquot of the extract into the LC-MS/MS.

Verify that NIS recoveries and acceptance limits were reported for all field samples, batch QC samples, standards, and instrument blanks.

Sample and batch QC NIS peak areas must be greater than 30% of the average area of the corresponding NIS in the calibration standards. Verify that no samples or batch QC have NIS peak areas outside the criteria.

If any NIS peak area is out of specification, then a reanalysis should be performed and reported. The laboratory should have reported the first run if the second was still unsuccessful. If the second run did not confirm the failure, it should have been reported.

The NIS retention times (RTs) for all field and QC samples should be within 0.40 minutes of the retention time of the midpoint standard in the ICAL, or on days when an ICAL is not performed, the initial CV is used.

Evaluation of Non-Extracted Internal Standards

If NIS peak areas are out of specification, justification should be noted in the laboratory case narrative (e.g., limited sample extract volume prevented reanalysis). If justification is not noted, the point of contact identified in the MLV Study Plan should be reached for further guidance.

If the control range documented in the deliverable does not correlate to 30% of the average area of the corresponding NIS in the calibration standards, reference the required control ranges for evaluation instead of the summarized ranges in the deliverable. The point of contact identified in the MLV Study Plan should be informed to implement changes to the current deliverables or those to be created in the future.

If low area counts are reported (< 30%), detects and non-detects should be qualified **X**.

If an NIS retention time varies by more than 0.40 minutes, use professional judgment to qualify the sample results and note all affected results in the data validation report.

NIS results may not be reported as "diluted out" since they are used as the internal standard for calculation of the EIS recoveries. A full evaluation (Stage 4 validation) of the sample, chromatogram, mass spectral ions and quantitation report may be necessary to determine that diluted analytes are quantified correctly.

4.4 Ongoing Precision and Recovery (OPR) Sample and Low-Level Ongoing Precision and Recovery (LLOPR) Sample

An OPR is an analyte-free reference matrix spiked with known amounts of the analytes of interest and taken through all sample preparation, cleanup and analytical steps. OPRs establish the method precision and bias for a specific batch of samples. LLOPRs verify the LOQ. An LLOPR is an OPR spiked at a low concentration (2x the LOQ), while the OPR is spiked at mid-level concentration relative to the calibration range.

OPR (sometimes called a "Blank Spike") and LLOPR recoveries should be within 40-150% recovery.

Evaluation of OPR/LLOPR

Verify that results (from appropriate summary form), spiking levels, percent recoveries, and acceptance limits were reported for all target analytes.

If the spike percent recovery control criteria displayed in the deliverable are not the same range (i.e., outside or wider than) as those stipulated in the MLV Study Method, reference the required control ranges for evaluation instead of the summarized ranges in the deliverable. The point of contact identified in the MLV Study Plan should be informed to implement changes to the current deliverables or those to be created in the future.

If the analyte recoveries in the OPR or LLOPR are outside of the MLV Study Method target recovery criteria of 40 – 150% or are not spiked at the required level, qualify the affected data with a J and identify the non-conformance in the data validation report.

4.5 Method Blanks

A method blank is used to identify systemic contamination originating in the laboratory that may have a detrimental effect on project sample results. The validator should identify samples associated with each method blank using a method blank summary form (or equivalent). Verify that the method blank has been reported per batch. Compare the results of each method blank with the associated sample results. The reviewer should note that the blank analyses may not involve the same weights, volumes, percent moistures, or dilution

factors as the associated samples. Care should be taken to factor in the percent moisture or dilution factor when doing comparisons between detects in the sample and the method blank.

In the method blank, no analytes should be detected > ½ LOQ or > 1/10th the amount measured in any sample, whichever is greater.

Evaluation of Method Blanks

If no method blank was prepared and analyzed with a batch of samples, identify the non-conformance in the data validation report. The point of contact identified in the MLV Study Plan should be informed of this nonconformance as soon as possible.

Compare the results of each method blank with the associated sample results. The reviewer should note that the blank analyses.

- If an analyte is detected in the method blank, but not in the associated samples, no action is taken.
- If an analyte is detected in the method blank (at any concentration) and in the associated samples, the action taken depends on both the blank and sample concentrations (Table I).

Table I: Blank Qualifications

Sample		
Result	**Validated Result**	**Validation Qualifier**
≤ MDL	Report at MDL	U
> MDL and ≤ 5x MB result	Report at Sample Result	J+
> 5x MB result	Report at Sample Result	None

MDL = Method Detection Limit

Note: The laboratory B qualifier is maintained, and the validation qualifier is added in addition to the laboratory qualifier.

4.6 Extract Dilution and Reanalysis

The MLV Study Plan and Method require aqueous samples to be prepared using the entire sample volume received; sample dilutions are not permitted. If the entire sample received by the laboratory was not prepared, document the nonconformance in the data validation report. Dilutions of sample extracts are required by the MLV Study Method when concentrations of target analytes exceed the quantification range or EIS failures are associated with a sample and matrix interference is suspected.   Reanalysis of samples is required by the MLV Study Method when NIS or EIS compounds fail to meet the MLV Study Method acceptance criteria.

Evaluation of Extract Dilution and Reanalysis

When sample results are reported for a sample at more than one dilution due to analyte concentrations exceeding the calibration curve, the dilution that results in the lowest MDL/LOQ should be used each target analyte unless a QC criterion has been exceeded.

The data validation report should indicate the reason for all reported dilutions resulting in elevated sensitivity limits for non-detected results.

When reanalysis has occurred due to quality control non-conformities, the validator should ensure that the non-conformity was corrected during the reanalysis. If that is not the case, then the appropriate qualifier should be placed on the reported results.

In some cases, using professional judgment, the validator may determine that an alternate result was more appropriate than the one reported. In those cases, explain the rationale for accepting the alternate result in the data validation report.

4.7 Bile Salts Interference Check

A bile salts interference check standard consisting of taurodeoxycholic acid (TDCA) when the mobile phase used for analysis is acetonitrile, or taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDA), and tauroursodeoxycholic acid (TUDCA) when an alternate mobile phase is used, must be analyzed daily, prior to analysis of all matrix types (aqueous, solid, and tissue). During the retention time calibration process, conditions are adjusted to ensure that bile salt peaks do not coelute with any of the target analytes, EIS, or NIS standards. Analytical conditions must be set to allow a separation of at least 1 minute between retention time of the bile salts and the retention time window of PFOS.

All MLV Study Method requirements for evaluation of the relationship of the retention time of the TDCA peak to the retention time of PFOS must be met. The retention time of PFOS applies to the retention time of all isomers of PFOS.

Evaluation of the Bile Salts Interference Check

If no bile salts interference check standard was analyzed or the required separation was not achieved, discuss the nonconformance in the data validation report.

4.8 Qualitative Identification Standard

A qualitative identification standard(s) containing a mixture of the branched and linear isomers of PFOA, PFNA, PFOSA, NMeFOSA, NEtFOSA, NEtFOSE, and NMeFOSE must be analyzed daily, prior to analysis of all samples. This qualitative standard should be used to determine the retention time of branched isomers of these target analytes in samples. The only target analytes that should include branched isomers in their quantitation are those whose retention times match those determined by a qualitative standard(s) or quantitative standard that contained an isomeric mixture of the target analyte that was used to create the calibration standards (PFOS, PFHxS, NMeFOSAA, and NEtFOSAA).

Evaluation of the qualitative standard

The qualitative identification standard summary should include the retention times and retention time windows for each target analyte determined by qualitative identification standard. If the required qualitative standards were not analyzed with the samples, the target analyte quantitation included branched isomers not identified in the qualitative standard, or the target analyte quantitation did not include branched isomers identified in

the qualitative standard and present in the sample, discuss the nonconformance in the data validation report.

5.0 Stage 2B Validation

Note: Stage 2B includes all of Stage 1, and Stage 2A

Stage 2B requires the review and qualification of the following summary documents for each instrument.

- Sequence and Preparation Logs (or equivalent to include Instrument Blanks)
- Mass Calibration and Mass Calibration Verification Summary
- Initial Calibration Summary (any equivalent to include the Initial Calibration Analyte Responses, Spike Concentrations, Isomeric Profiles, Response Ratios (RRs) or Response Factors (RFs), RR or RF Relative Standard Deviation or Relative standard Error)
- Instrument Blank Summary
- Calibration Verification and Instrument Sensitivity Check Summaries

Stage 2B adds for review, the validation of instrument specific QC data.

5.1 Sequence and Preparation Logs

Sequence logs are reviewed by the data validator to ensure all QC samples (both batch- and instrument-specific) had been analyzed within a specific batch, in the correct order (Section 13.0 of MLV Study Method). Preparation logs are reviewed by the data validator to ensure that samples had the proper extraction performed (Section 12 of MLV Study Method), within specified holding times. The logs themselves do not require validation. However, non-conformities uncovered in the review of the logs may point the validator to specific samples that require further review. Non-conformities uncovered in preparation or sequence logs should be noted in the data validation report.

Sequence logs are helpful in identifying when multiple instruments are used to analyze a batch of samples. For example, it is not uncommon to analyze a single batch of 20 samples at the same time on two or more instruments. At a minimum, mass calibration and mass calibration verification documentation should be included for each instrument used. Batch QC should be reviewed on each instrument, as appropriate. Non-conformities involving the use of multiple instruments should be noted in the data validation report.

5.2 Mass Calibration and Mass Calibration Verifications

A mass calibration of the LC/MS/MS instrument is required prior to analysis of an initial calibration curve. The mass calibration must meet all of the requirements included in the MLV Study Method. A mass calibration verification is performed after the mass calibration to ensure mass resolution, identification, and to some degree, sensitivity are all within criteria. Conformance is determined using reference standards; therefore, acceptance criteria should be met in all circumstances. Check that all samples and associated QC analyses are associated with an acceptable mass calibration verification.

Make certain that a mass calibration verification has been performed prior to the initial calibration used. The mass calibration verification should verify a mass range which includes the ion masses of all quantitative and qualitative ion masses of the target analytes of this method. Unit resolution should be such that the value of the peak width at half-height is within 0.5 ± 0.1 amu or Da. The peak apex should not shift more than 0.1 Da from the expected masses for each target analyte.

Evaluation of Mass Calibration and Mass Calibration Verifications

If the mass calibration and/or mass calibration verifications do not meet the requirements of the SLV Study Method, those non-conformities should be noted in the data validation report.

5.3 Initial Calibration

The objective of initial calibration is to ensure that the instrument is capable of producing acceptable qualitative and quantitative data. Initial calibration demonstrates that the instrument is capable of acceptable performance prior to sample analysis and of producing an acceptable calibration curve.

The instrument should be calibrated for all target analytes and isotopically labeled analogs of target analytes (EIS compounds and NIS compounds) with least six solutions, with at least five of the six calibration standards being within the quantification range (LOQ to highest calibration standard that meet criteria). (If a second-order calibration model is used, then one additional concentration is required.) The EIS and NIS compounds listed in the MLV Study Method should be used; no other NIS or EIS compounds should be included. The target analyte-EIS compound and EIS compound-NIS compound associations stated in the MLV Study Method should be used.

The instrument calibration summary should identify which analytes were calibrated using standards that contained branched and linear isomers of the analyte. Branched and linear isomers that should be used for calibration standards are listed in Table II. The target analyte response for analytes containing branched and linear isomer should be result of the summation of peaks from all isomers. A certified linear standard should be used to build the calibration curve for all other target analytes.

Table II: Currently Available Certified PFAS Standards Containing Branched and Linear Isomers
Perfluorohexanesulfonic acid (PFHxS)
Perfluorooctanesulfonic acid (PFOS)
2-(N-methylperfluorooctanesulfonamido) acetic acid (NMeFOSAA)
2-(N-ethylperfluorooctanesulfonamido) acetic acid (NEtFOSAA)

Evaluation of Initial Calibration

If target analytes were not calibrated, qualify associated non-detects and detects as **X**, exclusion of data is recommended.

If less than the required minimum number of calibration standards were used, make note in the data validation report and notify the MLV Study point of contact as soon as possible.

If the laboratory has analyzed more than the required number of calibration standards and picked out the "best" set (e.g., analyzed seven calibration standards and picked the five "best" to pass calibration criteria), make note of this in the data validation report.

Any other manipulation of calibration points (such as 'dropping' calibration levels at the ends of the calibration curve) should have a technical justification documented in the laboratory report. This study is providing laboratories with commercially prepared calibration mixtures, and it is expected that some laboratories may not be able to calibrate the analytes through the full calibration range in the mixtures. It is not acceptable to 'drop' a calibration point in between two points that are used. Use professional judgment to evaluate the data. If no technical justification is provided, then make note of this in the data validation report.

The lowest calibration standard should be at or below the LOQ. If the LOQ is below the lowest calibration standard, then the LOQ is inconsistent with MLV Study requirements. If the concentration of the lowest calibration standard was greater than the LOQ and the concentration of the associated Instrument Sensitivity Check (ISC) is at the LOQ and meets its acceptance criteria, no qualification is needed. If the concentration of the lowest calibration standard was greater than the LOQ and the associated ISC concentration is greater than the LOQ or it fails to meet acceptance criteria, qualify all associated data that are at a concentration below the concentration of the lowest calibration standard that meets acceptance criteria as **X** and make note of this in the data validation report.

Verify isotope dilution quantitation was used for all target analytes where isotopically labeled analogs are commercially available and EIS quantitation was used for all other target analytes. Verify the target analyte-EIS compound and EIS compound-NIS compound associations stated in the MLV Study Method were used. If either of these criteria were not met, make note of this in the data validation report and inform the point of contact for the MLV Study as soon as possible.

In order to produce acceptable sample results, the response of the instrument must be within the quantification range established by the initial calibration. Any sample detections above the working range of the calibration curve should be accompanied by a dilution that is within the quantification range. If dilutions were not performed, qualify all detections above the initial calibration working range as estimated **J,** and make note of the lack of dilution(s) in the data validation report.

If dilution(s) were performed that were within the working range of the initial calibration, then qualification of the data is not necessary. Make note in the data validation report that dilution(s) were performed. If reported concentration exceeded the calibration range, qualify detects as estimated **J**.

If branched isomers were not included in the summed result reported, qualify associated detects as **J-.**

5.3.1 Response Ratios (RRs), Response Factors (RFs), Relative Standard Deviation (%RSD), and Relative Standard Error (RSE)

Evaluate the average response ratio (RR) for each target analyte calibrated by isotope dilution and each response factor (RF) for each target analyte calibrated by extracted internal standard. The response factor of each EIS compound is quantified by non-

extracted internal standard (NIS). RRs/RFs are an indicator of the sensitivity of the analyte to detection and quantitation by Mass Spectrometry (the higher the RR/RF the more sensitive the analyte).

All target analytes should have either an associated %RSD or %RSE of ≤ 20% for an average calibration fit. Second order fits should use a %RSE of ≤ 20% criteria.

Evaluation of RRs/RFs, %RSD, and %RSE

Evaluate the %RSD or %RSE for all target analytes. If any target analyte has a %RSD or %RSE > 20% and ≤ 30%, flag detects for the affected analytes as **J** and non-detects as **UJ** in the associated samples.

If the %RSD or %RSE for any target analyte is excessively high (defined as > 30%), qualify associated sample results as **X**, exclusion of data is recommended.

5.4 Calibration Verification, and Instrument Sensitivity Check

The LOQ should be verified following the initial calibration and daily at the beginning of the analytical sequence, with a standard that is prepared at the concentration of the LOQ. This standard is called the ISC. The ISC should contain all of the target analytes. Note that multiple ISCs may be analyzed to encompass all of the target analytes. A CV containing all target compounds at the concentration of the mid-level calibration standard should be analyzed at the beginning of every analytical sequence prior to sample analysis, after every ten field samples, and at the end of the analytical sequence. These ISCs and CVs verify satisfactory performance of the instrument on a day-to-day basis.

Verify the CVs have been run prior to sample analysis, every ten field samples, and at the end of the analytical sequence.

Verify the ISC was analyzed following the initial calibration and contained all target analytes. Verify the ISCs have been run daily prior to sample analysis.

The ISC, and CV percent difference (%D) or percent drift for each target analyte and EIS analytes should be within ± 30%.

Evaluating the CV and ISC

Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated **J+** when the %D is higher than acceptance criteria and **J-** when below acceptance criteria. Non-detects are qualified as **UJ** in all associated samples for %D outside of acceptance criteria.

For gross exceedances of %D (defined as > 50% for ISC/CV) qualify all associated data as **X**.

If the ISC have not been performed after an initial calibration and daily, prior to sample analysis, qualify all associated data as **X**, exclusion of the data is recommended. No samples should have been analyzed without a valid ISC.

If the CV and/or ISC have not been analyzed (either continuing or end-of-run), qualify all associated data as **X**. No samples should have been analyzed without a valid CV and ISC.

If CVs have been analyzed at a frequency less than every ten field samples, qualify the associated sample detects as **J** and the non-detects as **UJ**.

5.5 Instrument Blanks

Instrument blanks (IBs) are used to ensure that the LC/MS/MS system does not contribute unacceptable concentrations of a target analyte into a sample result. The IB should be analyzed immediately following the highest calibration standard, prior to the ISC, after the qualitative identification standards, and after every CV. In order to quantify contamination, the IBs should contain EIS and NIS compounds. Each analyte in the IB should meet the acceptance criteria defined in the MLV Study Method (target analytes concentrations should be < ½ LOQ).

Evaluation of Instrument Blanks

Careful consideration should be given to any reported results that accompany an instrument blank that does not meet criteria. If the MLV Study method criteria is not met, note affected samples in the data validation report.

6.0 Stage 3 Validation

Note: Stage 3 validation includes all of Stage 1, Stage 2A and Stage 2B

The following documents are used for a Stage 3 validation:

- Raw data (including any laboratory forms, instrument outputs, spreadsheets, or handwritten calculations necessary for recalculation and re-quantification)
- Standards traceability forms and worksheets
- Method Detection Limit Studies Summaries
- Limit of Quantitation Verification Studies Summaries
- Initial Precision and Recovery Determinations Summaries

Stage 3 validation includes the recalculation and re-quantification of selected samples, and method and instrument QC. The types of results that should be recalculated and re-quantified include target analytes, analytes with detects above the LOQ, and field QC samples (blanks and duplicates). For method QC results, spiked recoveries and method blanks should be considered. For instrument QC, calibrations (including response factors and regressions), and calibration verifications, EIS recoveries should be recalculated and re-quantified. Some calculations may include the need to review standards preparation and serial dilutions.

6.1 Samples and QC Samples

When choosing samples, QC samples, and analytes for re-quantification and recalculation, consideration should be given to the laboratory's batching scheme to ensure a representative subsample of recalculations is performed. Recalculations should include some target analytes that have both salt and acid/anion concentrations provided on the

manufacturer's certificate of analysis to ensure the appropriate concentration was used in calculations (the acid/anion concentration). Other circumstances that should be prioritized for re-quantification and recalculation are diluted samples, manual integrations, or re-runs of samples.

As a minimum, 10% of the sample results should be re-quantified and recalculated. Sample recalculations should include the raw instrument result, re-quantified from the instrument response against the calibration function, and the final reported sample result, including any dilution, preparation factor, or percent moisture (if applicable). The equations in Appendix A can be used to calculate a sample result from the corresponding reported calibration or regression function, as appropriate.

Verify that one or more of the laboratory's sample-specific MDLs and LOQs are calculated correctly for the non-detects and reported accordingly. Verify that the MDLs are less than the LOQs for each target analyte.

Re-quantitate all detected target analytes in the 10% sample data chosen. For some samples, all results may be non-detects, therefore recalculation would typically not be necessary. In the case of method blanks, non-detects should be verified to ensure peaks are identified when all of the qualitative requirements of the method are met. Verify that sample-specific results have been adjusted correctly to reflect percent solids, original sample mass/volume, and any applicable dilutions.

When recalculations require rounding of data, the rounding should be completed only once at the end of all calculations to minimize rounding errors. Calculations should be rounded to the significant figures of the underlying criteria. For example, an OPR criteria of 80 - 117% would still be considered acceptable if the recalculation was 117.4%.

Evaluation of Sample and QC Samples recalculations

If the laboratory's LOQs are calculated incorrectly, then continue to recalculate limits until it is determined that the problem is systemic (such as incorrect equations used) or isolated (such as a transcription or rounding errors).

In all cases of nonconformance, the MLV Study point of contact should be notified as soon as possible, and all affected results noted in the data validation report, including listing the calculation errors. It may be necessary to engage the point of contact as identified in the MLV Study Plan to contact the laboratory so they can provide revised (corrected) results.

6.2 Method QC

Re-quantification of batch QC sample results should use raw instrument response in tandem with the reported calibration factor, response factor, or slope; the preparation information; and percent moisture for solid samples to recreate the reported result.

6.2.1 EIS Compound Spike

Verify the concentrations of EIS compounds from the raw data. Verify that the EIS compound result and percent recovery were calculated and reported correctly by re-calculating all EIS compounds in the 10% of the sample results and method QC that were originally selected.

6.2.2 NIS Compound Spike

Verify the peak areas of NIS compounds from the raw data. Verify that the NIS compound result and percent recovery were calculated and reported correctly by re-calculating all NIS compounds in the 10% of chosen sample data and method QC that were originally selected.

6.2.3 OPR/LLOPR

To check that the spike percent recovery was calculated and reported correctly, using the equation in Appendix A, re-quantitate and then recalculate a random 10% of the analytes in the OPR/LLOPR.

6.2.6 Method Blanks

Method blank analytical results are assessed to determine the existence and magnitude of contamination problems associated with sample extraction (if applicable) and analysis. If problems with any method blank exist, all associated data should be carefully evaluated to determine whether there is any bias associated with the data, or if the problem is an isolated occurrence not affecting other data. Results may not be corrected by subtracting any blank values.

Re-quantitate one or more detects found in the method blank (if applicable) from the reported average RF (or higher order regression, if used) per each batch of samples.

Evaluation of all EIS Compound Spike, NIS Compound Spike, OPR, LLOPR, and Method Blank Recalculations

If transcription errors (or other minor issues such as rounding errors) are found in method QC results, use professional judgment to qualify the data. It may be necessary to engage the point of contact as identified in the MLV Study Plan to contact the laboratory so they can provide revised (corrected) results. In all cases, if method QC calculation errors affect project target analytes, including peaks that should have been identified in method blanks but were not, the MLV Study point of contact should be notified, and all affected results noted in the data validation report, including listing the calculation errors.

6.3 Instrument QC

6.3.1 Response Ratios, Response Factors, Instrument Sensitivity Checks, and Calibration Verifications

Initial calibration recalculations should use the raw instrument response for the target analytes and associated EIS and NIS compounds, to recreate the calibration curve from the individual calibration standards. If multiple types (e.g., first order or second order curve fit) of calibration curves are employed a data package, at least one analyte per curve type should be recalculated.

Commercial PFAS standards available as salts are acceptable, providing the measured mass is corrected to the neutral acid concentration. Results shall be reported as the neutral acid with CAS numbers provided in the MLV Study Method. If sample results were not corrected to the neutral acid but reported from the salt, the MLV Study point of contact should be notified, and all affected results noted in the data validation report.

Re-quantitate and recalculate the individual and average RRs/RFs for at least 10% of the target analytes.

Re-quantitate and recalculate the CV, ISC, %D, %RSD or %RSE for at least 10% of the target analytes, proportionally selecting analytes based on each calibration curve type.

The laboratory may employ a linear or weighted linear least squares regression. The low standard should be recalculated using the calibration curve and evaluated. If the ICAL included refitting of the data back to the model (relative standard error), then recalculate 10% of the target analytes for the relative standard error in each ICAL.

Evaluation of Instrument Performance Checks, ICAL, Calibration Factors, Regressions, CV/ISC, and EIS Recalculations

If the files provided do not match the quantitation report, the RFs) reported are likely to be from another initial calibration and the laboratory report should be revised. The MLV Study point of contact should be reached to get a revised (corrected) report from the laboratory.

In all cases where instrument QC are calculated incorrectly, the MLV Study point of contact should be notified and noted in the data validation report.

6.4 Standards Traceability

Evaluate the calibration standards used for the analytes of concern. From the Certificate of Analysis (however named), verify that the "true values" of each analyte of concern were correctly applied to create the calibration curve, that all analytes of concern were in the calibration mix, and contained both branched and linear isomers, if commercially available. Some standards are made by manufacturers using the salt of a PFAS. In these cases, the concentration of those PFAS should be corrected to the neutral acid concentration. Results should be reported as the neutral acid with appropriate CAS number.

Check that the stock standards were diluted properly into working standards by recalculating the dilutions of one or more calibration standards. Recalculate one or more method QC sample dilutions (such as OPR or LLOPR) from the stock to the working standard.

Note: It is not the role of the data validator to evaluate the Certificate of Analysis for compliance with the *ISO-17034 Standard*, but to verify that stock and working standards were correctly applied in the creation of calibration curves.

Evaluation of Standards

If calculation errors have been identified, the MLV Study point of contact should be contacted to get a revised (corrected) report from the laboratory and the nonconformances should be noted in the data validation report.

For expired standards, the nonconformances should be noted in the data validation report.

6.5 Method Detection Limit Studies

A Method Detection Limit (MDL) study for each media type (aqueous, solid, and tissue) will be included in the Phase 3 laboratory submittals, as required by the MLV Study Plan. The MLV Study Plan requires the MDL to be determined using the MDL procedure at 40 CFR Part 136, Appendix B. The MLV Study Plan requires the laboratory's submittal include a summary that tabulates the individual MDL sample results, the computed MDL values based on the method blanks (MDL_b), the MDL values based on spiked samples (MDL_s), and the final MDLs.

Evaluation of Detection Limit Studies

The criteria for evaluating a MDL study is provided in the MDL procedure at 40 CFR Part 136, Appendix B. A minimum of seven method blanks and seven spiked samples should be prepared over the course of three days (i.e., three separate batches) and analyzed over three analytical sequences. The Student's t-values and the standard deviations should be checked for error.

If transcription errors (or other minor issues such as rounding errors) are found in detection/quantitation limit studies, note the errors in the data validation report. It may be necessary to engage the MLV Study point of contact to communicate with the laboratory, so they can provide revised (corrected) results. In all cases, if calculation errors affect MDLs, the point of contact should be notified, and all affected results noted in the data validation report, including listing the calculation errors.

6.6 Limit of Quantitation Verification Studies

A Limit of Quantitation Verification (LOQVER) study for each media type (aqueous, solid, and tissue) will be included in the Phase 3 laboratory submittals, as required by the MLV Study Plan. The MLV Study Plan requires the limit of quantitation in each media type to be verified by a method blank and a reference matrix sample spiked with method analytes, EIS compounds, and NIS compounds and will be carried through the entire analytical process (sample preparation and analysis) in accordance with the MLV Study Method. Exact spike concentrations will be determined by each participating laboratory based on the results of the MDL study and acceptable initial calibration range. EIS compounds will be spiked at the same quantity in every sample, QC sample, and calibration injection; typically the EIS is spiked close to the midpoint of the calibration curve. Target analytes are spiked between 1-2 times the LOQ. The MLV Study plan requires the target analytes in the LOQVER to recover within the 40-150% of their true value, EIS compounds to recover within 20-150%, and NIS compounds to recover greater than 30%.

Evaluation of Limit of Quantitation Verification Studies

If recoveries fail to meet the criteria stated in the MLV Study Plan, qualify the affected data with a J and note the nonconformance in the data validation report. It may be necessary to engage the MLV Study point of contact to communicate with the laboratory, so they can provide revised (corrected) results. In all cases, if recoveries fail to meet the criteria stated in the MLV Study Plan, the point of contact should be notified, and all affected results noted in the data validation report.

6.7 Initial Precision and Recovery Studies

An Initial Precision and Recovery (IPR) study for each media type (aqueous, solid, and tissue) will be included in the Phase 3 laboratory submittals, as required by the MLV Study Plan. The MLV Study Plan requires the limit of quantitation in each media type to be verified by a method blank and four reference matrix samples spiked with method analytes, EIS compounds, and NIS compounds and will be carried through the entire analytical process (sample preparation and analysis) in accordance with the MLV Study Method. Exact spike concentrations will be determined by each participating laboratory based on the results of the MDL study and acceptable initial calibration range. Target analytes and EIS compounds will be spiked around the midpoint of the calibration curve. The MLV Study plan requires the target analytes in each IPR to recover within the 40-150% of their true value, EIS compounds to recover within 20-150%, and NIS compounds to recover greater than 30%.

Evaluation of Initial Precision and Recovery Studies

If mean recoveries of the target analytes fail to meet the criteria stated in the MLV Study Plan, note the nonconformance in the data validation report. If recoveries of the EIS compounds fall below the 20% criteria and/or the recoveries of NIS compounds fall below 30% for NIS compounds, qualify the affected data with a J. It may be necessary to engage the MLV Study point of contact to communicate with the laboratory, so they can provide revised (corrected) results. In all cases, if recoveries fail to meet the criteria stated in the MLV Study Plan, the point of contact should be notified, and all affected results noted in the data validation report.

7.0 Stage 4 Validation

Note: Stage 4 validation includes all of Stage 1, Stage 2A, Stage 2B and Stage 3

Raw Data (including any instrument outputs, mass spectra, chromatograms, instrument parameters such as mobile phases and mobile phase gradients)

Stage 4 is a qualitative review of non-detected and detected results from instrument outputs. Chromatograms are checked for peak integration (10% of automated integration and 100% of manual integrations), baseline, and interferences; mass spectra are checked for minimum quantitative ion and qualitative ion signal-to-noise ratio, transition ion ratios, retention times or relative retention times are within method requirements for analyte identification. Raw data quantitation reports and ion transition chromatograms are required to perform review of the instrument outputs.

7.1 Target Compound Identification

The objective of the criteria for LC/MS/MS qualitative analysis is to minimize the number of erroneous identifications of target compounds. An erroneous identification can either be false positive (reporting a compound present when it is not) or a false negative (not reporting a compound that is present).

The identification criteria can be applied more easily in detecting false positives than false negatives. More information is available for false positives because of the requirement for

submittal of data supporting positive identifications. Negatives or non-detects, on the other hand, represent an absence of data and are therefore more difficult to assess.

The peak area of the branched isomers, if present, should be summed with the peak area integration of the linear isomer. Branched isomers elute prior to the linear isomer of a target analyte.

Target analyte detections should display a signal-to-noise of ≥ 3:1 for both the quantitative ion and confirmation ion (where one exists), have proper peak integration, and display all ions at the correct retention times with passing ion ratios (50 - 150%).

The retention time of each target analyte and EIS compound should be within ± 0.40 minutes of the predicted retention and updated with the latest daily CV. Check a minimum of 10% of the reported target analyte detects for retention time. RT performance in samples with only non-detects can be evaluated by reviewing the EIS times.

Evaluation of Target Compound Identification

The application of qualitative criteria for LC/MS/MS analysis of target analytes requires professional judgment. It is up to the reviewer's discretion to obtain additional information from their MLV Study point of contact if qualitative identification problems are uncovered. The point of contact should arrange with the laboratory to obtain a revised (corrected) laboratory report. All qualitative identification problems should be discussed in the data validation report. If it is determined that incorrect identifications were made, or if a confirmed positive detect was made, but the confirmation ion was not detected (when available), then all affected data should be qualified as **X**, exclusion of data recommended.

If evaluation of the ion ratios, retention times, or signal-to-noise for a detected target analyte is considered invalid, document the nonconformances in the data validation report.

While retention time windows are usually less critical to mass spectrometry systems, retention times have an acute effect on LC/MS/MS using Multiple Reaction Monitoring (MRM) mode. For example, retention time window drift on an MRM system can have a direct impact on the reported results. Professional judgment should be used to qualify the data.

7.2 Manual Integrations

For Stage 4, the reviewer should examine and verify the validity of all manual integrations.

Performing improper manual integrations, including peak shaving, peak enhancing, or baseline manipulation to meet QC criteria or to avoid corrective actions is unwarranted manipulation and misrepresents the data. All manual integrations should be reviewed by the data validator. When manual integrations are performed, raw data records should include a complete audit trail for those manipulations (i.e., the chromatograms obtained before and after the manual integration should be retained to permit reconstruction of the results). This requirement applies to all analytical runs including calibration standards and QC samples. The person performing the manual integration should sign and date each manually integrated chromatogram and record the rationale for performing manual

integration (electronic signature is acceptable). Any manual integration should be fully discussed in the case narrative, including the cause and justification.

Evaluation of Manual Integrations

Some level of manual integration is considered necessary for the normal operation of chromatographic systems. Instances of properly integrated peaks do not require qualification, but should be noted in the data validation report. However, excessive manual integrations may show a lack of routine maintenance by the laboratory, a rush to complete samples, or the results of analyzing excessively 'dirty' samples. Excessive manual integrations may also be the result of faulty software peak/baseline integration.

The data validator should use professional judgment in the review of manual integrations. All instances of manual integrations should be noted in the data validation report. Instances of incomplete information for manual integrations (such as failure to provide justification) should be reported to the MLV Study point of contact to obtain a revised (corrected) laboratory report.

If, in the professional judgment of the validator, there are instances of unwarranted manipulation of data (such as multiple manual integrations used to 'pass' QC criteria), then those cases should be reported to the MLV Study point of contact as soon as practical.

Appendix A: Formulas used in Stages 3 and 4 Data Validation

Calibration:

Response Ratio (RR):

$$RR = \frac{Area_n \, M_l}{Area_l \, M_n}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the corresponding isotopically labeled PFAS added to the sample before extraction

M_l = The mass of the isotopically labeled compound in the calibration standard

M_n = The mass of the native compound in the calibration standard

Response Factor (RF) of Target Analytes:

$$RF = \frac{Area_s \, M_{EIS}}{Area_{EIS} \, M_s}$$

where:

$Area_s$ = The measured area of the Q1 m/z for the target (unlabeled) PFAS

$Area_{EIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the extracted internal standard (EIS)

M_{EIS} = The mass of the isotopically labeled PFAS used as the extracted internal standard (EIS) in the calibration standard

M_s = The mass of the target (unlabeled) PFAS in the calibration standard

Response Factor (RF) of EIS Compounds:

$$RF = \frac{Area_l \, M_{NIS}}{Area_{NIS} \, M_l}$$

where:

$Area_l$ = The measured area of the Q1 m/z for the isotopically labeled PFAS standard added to the sample before extraction

$Area_{NIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the non-extracted internal standard (NIS)

M_{NIS} = The mass of the isotopically labeled compound used as the non-extracted internal standard (NIS) in the calibration standard

M_l =   The mass of the isotopically labeled PFAS standard added to the sample before extraction

Relative Retention time:

$$RRT = \frac{Retention\ time\ of\ the\ analyte}{Retention\ time\ of\ the\ extracted\ internal\ standard}$$

Percent Difference:

$$\%D = \frac{C_s - C_k}{C_k} \times 100$$

where:

C_s =   Concentration, reported

C_k =   Concentration, known

Sample Concentration:

Target Analyte Reported Values:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_n\ M_l}{Area_l(\overline{RR}\ or\ \overline{RF})} \times \frac{1}{W_S}$$

where:

$Area_n$ =   The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ =   The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS). *See note below.*

M_l =   The mass of the isotopically labeled compound added (ng)

$\overline{RR}$ =   Average response ratio used to quantify target compounds by the isotope dilution method

$\overline{RF}$ =   Average response factor used to quantify target compounds by the extracted internal standard method

W_S =   Sample volume (L) or weight (g)

EIS Compound Reported Values:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_l\ M_{nis}}{Area_{nis}\overline{RF_s}} \times \frac{1}{W_S}$$

where:

Area$_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS)

Area$_{nis}$ = The measured area of the Q1 m/z for the non-extracted internal standard (NIS)

M$_{nis}$ = The mass of the added non-extracted internal standard (NIS) compound (ng)

W$_S$ = Sample volume (L) or weight (g) (wet weight for tissue, dry weight for solids)

$\overline{RF_s}$ = Average response factor used to quantify the isotopically labeled compound by the non-extracted internal standard method

EIS, OPR, or LLOPR Percent Recovery:

$$Percent\ Recovery = \frac{C_s}{C_K}\ x\ 100$$

Where:

C$_s$ = Concentration, Reported

C$_K$ = Concentration, Known

Transition Ion Ratio:

$$IR = \frac{Q_q}{Q_c}$$

Where:

IR = Ion Ratio

Qq = quantitative ion abundance

Qc = confirmation ion abundance

Ion Ratio Percent Recovery:

$$Percent\ Recovery = \frac{R_S}{R_K}\ x\ 100$$

where:

Rs= Ion Ratio, Reported in Sample

R_K= Ion Ratio, Reported in mid-point initial calibration standard and/or initial daily CV.

ATTACHMENT 6

WELLINGTON LABORATORIES, INC. CERTIFICATE OF ANALYSIS DOCUMENTATION FOR PFAS REFERENCE STANDARD MIXTURES

WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

MPFAC-HIF-ES
Mass-Labelled Per- and Poly-fluoroalkyl Substance
Extraction Standard Solution

PRODUCT CODE: MPFAC-HIF-ES
LOT NUMBER: MPFACHIFES0821
SOLVENT(S):   Methanol/Isopropanol (1%)/Water (<1%)
DATE PREPARED: (mm/dd/yyyy)   08/05/2021
LAST TESTED: (mm/dd/yyyy)   08/16/2021
EXPIRY DATE: (mm/dd/yyyy)   08/16/2024
RECOMMENDED STORAGE: Refrigerate ampoule

DESCRIPTION:

MPFAC-HIF-ES is a solution/mixture of ten mass-labelled (^{13}C) perfluoroalkylcarboxylic acids (C_4-C_{12}, C_{14}), three mass-labelled (^{13}C) perfluoroalkanesulfonates (C_4, C_6, and C_8), three mass-labelled (one ^{13}C and two ^{2}H) perfluoro-1-octanesulfonamides, three mass-labelled (^{13}C) fluorotelomer sulfonates (4:2, 6:2, and 8:2), two mass-labelled (^{2}H) perfluorooctanesulfonamidoacetic acids, two mass-labelled (^{2}H) perfluorooctane-sulfonamidoethanols, and mass-labelled (^{13}C) hexafluoropropylene oxide dimer acid. The components and their concentrations are given in Table A.

The individual mass-labelled perfluoroalkylcarboxylic acids, mass-labelled perfluoroalkanesulfonates, mass-labelled fluorotelomer sulfonates, perfluoro-1-($^{13}C_8$)octanesulfonamide, and mass-labelled hexafluoropropylene oxide dimer acid all have chemical purities of >98% and isotopic purities of $\geq$99%. The individual mass-labelled perfluorooctanesulfonamidoacetic acids, mass-labelled perfluorooctanesulfon-amidoethanols, and two mass-labelled (^{2}H) perfluoro-1-octanesulfonamides all have chemical purities of >98% and isotopic purities of $\geq$98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acids to their respective methyl esters.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters $x_1, x_2,...x_n$ on which it depends is:

$$u_c(y(x_1, x_2,...x_n)) = \sqrt{\sum_{i=1}^{n} u(y, x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class A tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

QUALITY MANAGEMENT:

This product was produced using a Quality Management System registered to the latest versions of ISO 9001 by SAI Global, ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation Inc. (CALA; A1226), and ISO 17034 by ANSI National Accreditation Board (ANAB; AR-1523).

For additional information or assistance concerning this or any other products from Wellington Laboratories Inc., please visit our website at www.well-labs.com or contact us directly at info@well-labs.com

Table A: MPFAC-HIF-ES; Components and Concentrations (ng/mL, ± 5% in Methanol/Isopropanol (1%)/Water (<1%))

Compound	Acronym	Concentration (ng/mL)	Peak Assignment in Figure 1
Perfluoro-n-($^{13}C_4$)butanoic acid	MPFBA	2000	1
Perfluoro-n-($^{13}C_5$)pentanoic acid	M5PFPeA	1000	2
Perfluoro-n-(1,2,3,4,6-$^{13}C_5$)hexanoic acid	M5PFHxA	500	5
Perfluoro-n-(1,2,3,4-$^{13}C_4$)heptanoic acid	M4PFHpA	500	7
Perfluoro-n-($^{13}C_8$)octanoic acid	M8PFOA	500	10
Perfluoro-n-($^{13}C_9$)nonanoic acid	M9PFNA	250	11
Perfluoro-n-(1,2,3,4,5,6-$^{13}C_6$)decanoic acid	M6PFDA	250	14
Perfluoro-n-(1,2,3,4,5,6,7-$^{13}C_7$)undecanoic acid	M7PFUdA	250	17
Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	MPFDoA	250	19
Perfluoro-n-(1,2-$^{13}C_2$)tetradecanoic acid	M2PFTeDA	250	22
Perfluoro-1-($^{13}C_8$)octanesulfonamide	M8FOSA	500	18
N-methyl-d_3-perfluoro-1-octanesulfonamide	d-N-MeFOSA	500	21
N-ethyl-d_5-perfluoro-1-octanesulfonamide	d-N-EtFOSA	500	24
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	d3-N-MeFOSAA	1000	15
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	d5-N-EtFOSAA	1000	16
2-(N-methyl-d_3-perfluoro-1-octanesulfonamido)ethan-d_4-ol	d7-N-MeFOSE	5000	20
2-(N-ethyl-d_5-perfluoro-1-octanesulfonamido)ethan-d_4-ol	d9-N-EtFOSE	5000	23
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)($^{13}C_3$)propanoic acid	M3HFPO-DA	2000	6

Compound	Acronym	Concentration* (ng/mL)		Peak Assignment in Figure 1
		as the salt	as the acid	
Sodium perfluoro-1-(2,3,4-$^{13}C_3$)butanesulfonate	M3PFBS	500	466	3
Sodium perfluoro-1-(1,2,3-$^{13}C_3$)hexanesulfonate	M3PFHxS	500	474	8
Sodium perfluoro-1-($^{13}C_8$)octanesulfonate	M8PFOS	500	479	12
Sodium 1H,1H,2H,2H-perfluoro-(1,2-$^{13}C_2$)hexanesulfonate	M2-4:2FTS	1000	938	4
Sodium 1H,1H,2H,2H-perfluoro-(1,2-$^{13}C_2$)octanesulfonate	M2-6:2FTS	1000	951	9
Sodium 1H,1H,2H,2H-perfluoro-(1,2-$^{13}C_2$)decanesulfonate	M2-8:2FTS	1000	960	13

* Concentrations have been rounded to three significant figures.

Certified By: _____ Date: 08/27/2021
B.G. Chittim, General Manager   (mm/dd/yyyy)

Figure 1: MPFAC-HIF-ES; LC/MS Data (SIR)

16AUG2021_MPFACHIFES_007   16-Aug-2021   13:21:01

MPFACHIFES0821 1/10 dil

1: SIR of 10 Channels ES-TIC
1.80e6

2: SIR of 6 Channels ES-TIC
2.20e6

4: SIR of 7 Channels ES-TIC
3.08e6

3: SIR of 1 Channel ES-TIC
1.29e5

Conditions for Figure 1:

Waters Acquity Ultra Performance LC
Waters Xevo TQ-S micro MS

Chromatographic Conditions:

Column:	Acquity UPLC BEH Shield RP$_{18}$
	1.7 µm, 2.1 x 100 mm

Mobile phase: Gradient
Start: 50% H_2O / 50% (80:20 MeOH:ACN)
(both with 10 mM NH_4OAc buffer)
Ramp to 90% organic over 9 min and hold for
2 min before returning to initial conditions in 1 min.
Time: 15 min

Flow: 300 µL/min

MS Parameters:

Experiment: SIR

Source: Electrospray (negative)
Capillary Voltage (kV) = 2.00
Cone Voltage (V) = variable (2-44)
Desolvation Temperature (°C) = 350
Desolvation Gas Flow (L/hr) = 1000

Figure 2: MPFAC-HIF-ES; LC/MS/MS Data (Selected MRM Transitions)

16aug2021_MPFACHIFES_008   16-Aug-2021   13:36:55
MPFACHIFES0821 1/10 dil

MPFBA $[^{13}C_4F_7O_2]^- \longrightarrow [^{13}C_3F_7]^-$ — **217 > 172** 6.83e5

M5PFPeA $[^{13}C_5F_9O_2]^- \longrightarrow [^{13}C_4F_9]^-$ — **268 > 223** 3.31e5

M3PFBS $[^{13}C_3{}^{12}C_1F_9SO_3]^- \longrightarrow [SO_3]^-$ — **302 > 80** 7.94e4

M2-4:2FTS $[^{13}C_2{}^{12}C_4H_4F_9SO_3]^- \longrightarrow [^{13}C_2{}^{12}C_4H_3F_8SO_3]^-$ — **329 > 309** 8.15e4

M5PFHxA $[^{13}C_5{}^{12}C_1F_{11}O_2]^- \longrightarrow [^{13}C_4{}^{12}C_1F_{11}]^-$ — **318 > 273** 2.36e5

M3HFPO-DA $[^{13}C_2{}^{12}C_3F_{11}O]^- \longrightarrow [^{12}C_3F_7]^-$ — **287 > 169** 1.30e5

M4PFHpA $[^{13}C_4{}^{12}C_3F_{13}O_2]^- \longrightarrow [^{13}C_3{}^{12}C_3F_{13}]^-$ — **367 > 322** 3.05e5

M3PFHxS $[^{13}C_3{}^{12}C_3F_{13}SO_3]^- \longrightarrow [SO_3]^-$ — **402 > 80** 9.02e4

M2-6:2FTS $[^{13}C_2{}^{12}C_6H_4F_{13}SO_3]^- \longrightarrow [^{13}C_2{}^{12}C_6H_3F_{12}SO_3]^-$ — **429 > 409** 1.07e5

Figure 2: MPFAC-HIF-ES; LC/MS/MS Data (Selected MRM Transitions)

MPFACHIFES0821 1/10 dil

M8PFOA $[^{13}C_8F_{15}O_2]^- \longrightarrow [^{13}C_7F_{15}]^-$ 421 > 376   3.67e5

M9PFNA $[^{13}C_9F_{17}O_2]^- \longrightarrow [^{13}C_8F_{17}]^-$ 472 > 427   1.86e5

M8PFOS $[^{13}C_8F_{17}SO_3]^- \longrightarrow [SO_3]^-$ 507 > 80   8.98e4

M2-8:2FTS $[^{13}C_2{}^{12}C_8H_4F_{17}SO_3]^- \longrightarrow [^{13}C_2{}^{12}C_8H_3F_{16}SO_3]^-$ 529 > 509   1.15e5

M6PFDA $[^{13}C_6{}^{12}C_4F_{19}O_2]^- \longrightarrow [^{13}C_5{}^{12}C_4F_{19}]^-$ 519 > 474   2.04e5

d3-N-MeFOSAA $[^{12}C_{11}{}^2H_3{}^1H_2F_{17}NO_4S]^- \longrightarrow [^{12}C_8F_{17}]^-$ 573 > 419   1.34e5

d5-N-EtFOSAA $[^{12}C_{12}{}^2H_5{}^1H_2F_{17}NO_4S]^- \longrightarrow [^{12}C_8F_{17}]^-$ 589 > 419   1.23e5

M7PFUdA $[^{13}C_7{}^{12}C_4F_{21}O_2]^- \longrightarrow [^{13}C_6{}^{12}C_4F_{21}]^-$ 570 > 525   1.82e5

M8FOSA $[^{13}C_8HF_{17}NO_2S]^- \longrightarrow [NO_2S]^-$ 506 > 78   3.53e5

Figure 2: MPFAC-HIF-ES; LC/MS/MS Data (Selected MRM Transitions)

16aug2021_MPFACHIFES_008   16-Aug-2021   13:36:55
MPFACHIFES0821 1/10 dil

615 > 570
1.85e5

MPFDoA $[^{13}C_2{}^{12}C_{10}F_{23}O_2]^-$ ⟶ $[^{13}C_1{}^{12}C_{10}F_{23}]^-$

623 > 59
4.12e5

d7-N-MeFOSE $[C_{11}{}^{2}H_7{}^{1}H_1F_{17}NO_3S]\cdot[C^1H_3COO]^-$ ⟶ $[C^1H_3COO]^-$
adduct

515 > 169
7.92e4

d-N-MeFOSA $[C_9{}^{2}H_3F_{17}NO_2S]^-$ ⟶ $[C_3F_7]^-$

715 > 670
1.28e5

M2PFTeDA $[^{13}C_2{}^{12}C_{12}F_{27}O_2]^-$ ⟶ $[^{13}C_1{}^{12}C_{12}F_{27}]^-$

639 > 59
8.20e5

d9-N-EtFOSE $[C_{12}{}^{2}H_9{}^{1}H_1F_{17}NO_3S]\cdot[C^1H_3COO]^-$ ⟶ $[C^1H_3COO]^-$
adduct

531 > 169
5.08e4

d-N-EtFOSA $[C_{10}{}^{2}H_5F_{17}NO_2S]^-$ ⟶ $[C_3F_7]^-$

Time

Conditions for Figure 2:

Injection: On-column (MPFAC-HIF-ES)

Mobile phase: Same as Figure 1

Flow: 300 µL/min

MS Parameters:

Collision Gas (mbar) = 3.41e-3
Collision Energy (eV) = 4-64 (variable)

MPFAC-HIF-IS
Mass-Labelled Perfluoroalkyl Substance
Injection Standard Solution

PRODUCT CODE: MPFAC-HIF-IS

LOT NUMBER: MPFACHIFIS0921

SOLVENT(S):   Methanol/Water (<1%)

DATE PREPARED: (mm/dd/yyyy)   09/07/2021

LAST TESTED: (mm/dd/yyyy)   09/07/2021

EXPIRY DATE: (mm/dd/yyyy)   09/07/2026

RECOMMENDED STORAGE: Store ampoule in a cool, dark place

DESCRIPTION:

MPFAC-HIF-IS is a solution/mixture of five mass-labelled (^{13}C) perfluoroalkylcarboxylic acids (C_4, C_6, C_8-C_{10}) and two mass-labelled (^{18}O and ^{13}C) perfluoroalkanesulfonates (C_6 and C_8).The components and their concentrations are given in Table A.

The individual mass-labelled perfluoroalkylcarboxylic acids and mass-labelled perfluoroalkanesulfonates all have chemical purities of >98% and isotopic purities of ≥99% per ^{13}C or >94% per ^{18}O.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acids to their respective methyl esters.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters x_1, x_2,...x_n on which it depends is:

$$u_c(y(x_1, x_2, ...x_n)) = \sqrt{\sum_{i=1}^{n} u(y, x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class A tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

QUALITY MANAGEMENT:

This product was produced using a Quality Management System registered to the latest versions of ISO 9001 by SAI Global, ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation Inc. (CALA; A1226), and ISO 17034 by ANSI National Accreditation Board (ANAB; AR-1523).

CALA
Testing
Accreditation No. A1226

ANAB
ANSI National Accreditation Board
ACCREDITED
ISO 17034
REFERENCE MATERIAL PRODUCER

For additional information or assistance concerning this or any other products from Wellington Laboratories Inc., please visit our website at www.well-labs.com or contact us directly at info@well-labs.com

Table A: MPFAC-HIF-IS; Components and Concentrations (ng/mL, ± 5% in methanol/water (<1%))

Compound	Acronym	Concentration (ng/mL)	Peak Assignment in Figure 1
Perfluoro-n-(2,3,4-$^{13}C_3$)butanoic acid	M3PFBA	1000	1
Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	MPFHxA	500	2
Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	MPFOA	500	4
Perfluoro-n-(1,2,3,4,5-$^{13}C_5$)nonanoic acid	MPFNA	250	5
Perfluoro-n-(1,2-$^{13}C_2$)decanoic acid	MPFDA	250	7

Compound	Acronym	Concentration* (ng/mL)		Peak Assignment in Figure 1
		as the salt	as the acid	
Sodium perfluoro-1-hexane($^{18}O_2$)sulfonate	MPFHxS	500	474	3
Sodium perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	MPFOS	500	479	6

* Concentrations have been rounded to three significant figures.

Certified By: _____   **Date:** 09/23/2021
B.G. Chittim, General Manager   (mm/dd/yyyy)

Figure 1: MPFAC-HIF-IS; LC/MS Data (SIR)

07sept2021_MPFACHIFIS_003   07-Sep-2021   19:29:01

MPFACHIFIS921 1/10 dil

1: SIR of 5 Channels ES-TIC
8.88e5

2: SIR of 2 Channels ES-TIC
1.22e6

Conditions for Figure 1:

Waters Acquity Ultra Performance LC
Waters Xevo TQ-S micro MS

Chromatographic Conditions:

Column: Acquity UPLC BEH Shield RP$_{18}$
            1.7 μm, 2.1 x 100 mm

Mobile phase: Gradient
            Start: 50% H$_2$O / 50% (80:20 MeOH:ACN)
            (both with 10 mM NH$_4$OAc buffer)
            Ramp to 90% organic over 9 min and hold for
            2 min before returning to initial conditions in 1 min.
            Time: 15 min

Flow: 300 μL/min

MS Parameters:

Experiment: SIR

Source: Electrospray (negative)
Capillary Voltage (kV) = 2.00
Cone Voltage (V) = variable (2-6)
Desolvation Temperature (°C) = 350
Desolvation Gas Flow (L/hr) = 1000

Figure 2:   MPFAC-HIF-IS; LC/MS/MS Data (Selected MRM Transitions)

07sept2021_MPFACHIFIS_002   **07-Sep-2021   19:13:08**
MPFACHIFIS921 1/10 dil

216 > 172
3.43e5

M3PFBA $[^{13}C_3{}^{12}CF_7O_2]^- \rightarrow [^{13}C_3F_7]^-$

315 > 270
2.75e5

MPFHxA $[^{13}C_2{}^{12}C_4F_{11}O_2]^- \rightarrow [^{13}C_1{}^{12}C_4F_{11}]^-$

403 > 103
4.33e4

MPFHxS $[C_6F_{13}S^{18}O_2{}^{16}O]^- \rightarrow [FS^{18}O_2{}^{16}O]^-$

417 > 372
4.16e5

MPFOA $[^{13}C_4{}^{12}C_4F_{15}O_2]^- \rightarrow [^{13}C_3{}^{12}C_4F_{15}]^-$

468 > 423
1.16e5

MPFNA $[^{13}C_5{}^{12}C_4F_{17}O_2]^- \rightarrow [^{13}C_4{}^{12}C_4F_{17}]^-$

503 > 99
5.25e4

MPFOS $[^{13}C_4{}^{12}C_4F_{17}SO_3]^- \rightarrow [FSO_3]^-$

515 > 470
1.33e5

MPFDA $[^{13}C_2{}^{12}C_8F_{19}O_2]^- \rightarrow [^{13}C_1{}^{12}C_8F_{19}]^-$

Time

Conditions for Figure 2:

Injection: On-column (MPFAC-HIF-IS)

Mobile phase: Same as Figure 1

Flow: 300 µL/min

MS Parameters:

Collision Gas (mbar) = 3.18e-3
Collision Energy (eV) = 4-64 (variable)

PFAC-MXF

**Native Replacement PFAS
Solution/Mixture**

PRODUCT CODE:	PFAC-MXF
LOT NUMBER:	PFACMXF1219
SOLVENT(S):	Methanol / Water (<1%)
DATE PREPARED: (mm/dd/yyyy)	12/03/2019
LAST TESTED: (mm/dd/yyyy)	05/04/2020
EXPIRY DATE: (mm/dd/yyyy)	05/04/2023
RECOMMENDED STORAGE:	Refrigerate ampoule

DESCRIPTION:

PFAC-MXF is a solution/mixture of sodium dodecafluoro-3H-4,8-dioxanonanoate (NaDONA), the major and minor components of F-53B (9Cl-PF3ONS and 11Cl-PF3OUdS), and GenX (HFPO-DA). The components and their concentrations are given in Table A.

The individual native components of this mixture all have chemical purities of >98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acid to the methyl ester.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters x_1, x_2,...x_n on which it depends is:

$$u_c(y(x_1, x_2,...x_n)) = \sqrt{\sum_{i=1}^{n} u(y,x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class A tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

QUALITY MANAGEMENT:

This product was produced using a Quality Management System registered to the latest versions of ISO 9001 by SAI Global, ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation Inc. (CALA; A1226), and ISO 17034 by ANSI-ASQ National Accreditation Board (ANAB; AR-1523).

CALA
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Accreditation No. A1226

ANAB
ACCREDITED
ISO 17034
REFERENCE MATERIAL
PRODUCER

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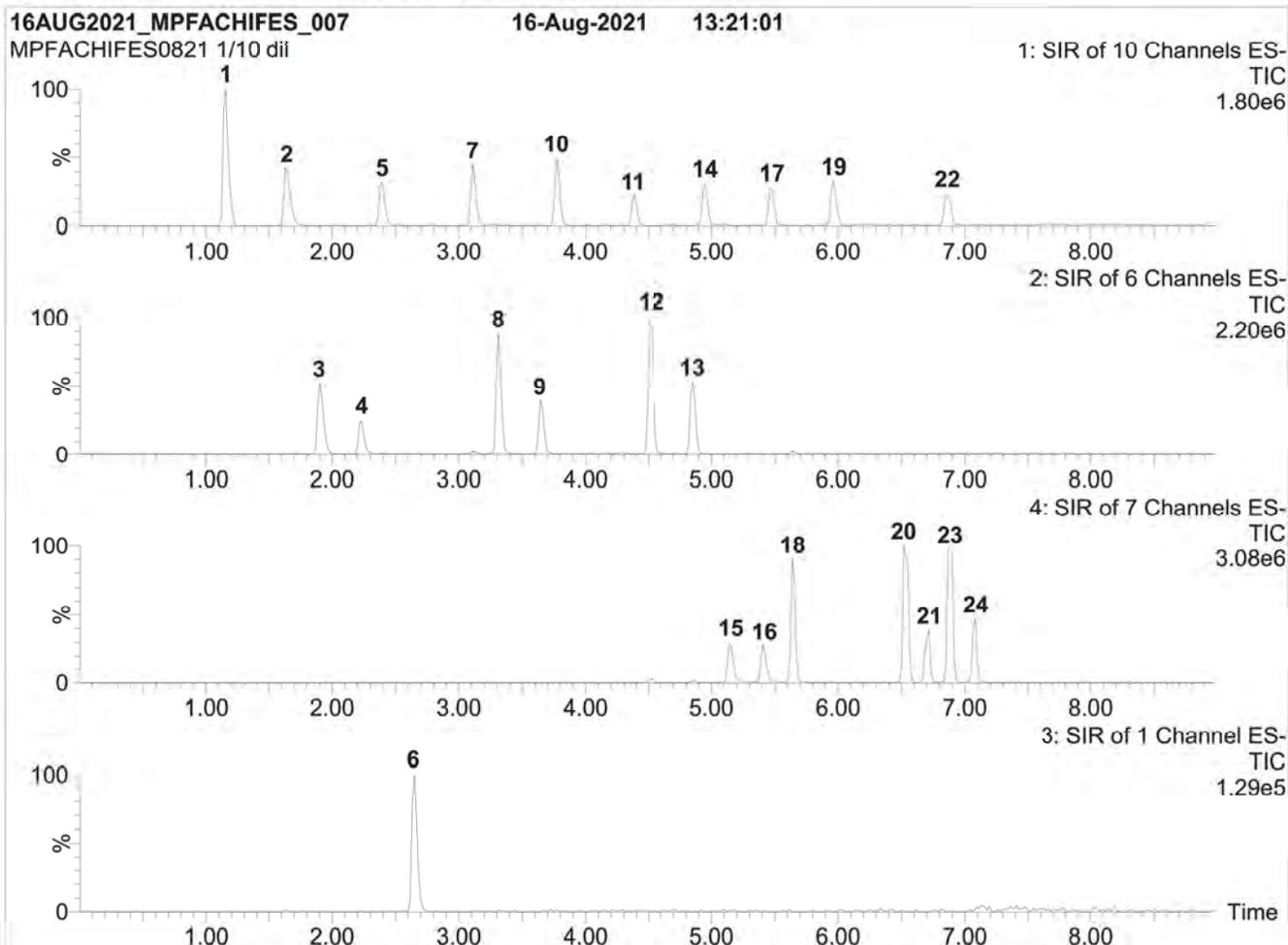## Table A: PFAC-MXF; Components and Concentrations (ng/ml; ± 5% in Methanol / Water (<1%))

Compound	Abbreviation	Concentration* (ng/ml)		Peak Assignment in Figure 1
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA	2000		A

Compound	Abbreviation	Concentration* (ng/ml)		Peak Assignment in Figure 1
		as the salt	as the acid	
Sodium dodecafluoro-3H-4,8-dioxanonanoate	NaDONA	2000	1890	B
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS	2000	1870	C
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	2000	1890	D

*   Concentrations have been rounded to three significant figures.

Certified By: _____   Date: __05/27/2020__
                                                                          (mm/dd/yyyy)
B.G. Chittim, General Manager

Figure 1: PFAC-MXF; LC/MS Data (SIR)

12dec2019_PFACMXF_003   12-Dec-2019   13:28:01

PFACMXF1219 50 ng/ml ea

SIR of 5 Channels ES-TIC
1.70e6

Conditions for Figure 1:

LC: **Waters Acquity Ultra Performance LC**
MS: **Waters Xevo TQ-S micro MS**

Chromatographic Conditions

Column: Acquity UPLC BEH Shield RP$_{18}$
           1.7 µm, 2.1 x 100 mm

Mobile phase: Gradient
           Start: 50% (80:20 MeOH:ACN) / 50% H$_2$O
           (both with 10 mM NH$_4$OAc buffer)
           Ramp to 90% organic over 8 min and
           hold for 2 min before returning
           to initial conditions in 0.75 min.
           Time: 12 min

Flow: 300 µl/min

MS Parameters

Experiment: SIR

Source: Electrospray (negative)
Capillary Voltage (kV) = 2.00
Cone Voltage (V) = variable (15-74)
Desolvation Temperature (°C) = 350
Desolvation Gas Flow (l/hr) = 1000

Figure 2: PFAC-MXF; LC/MS/MS Data (Selected MRM Transitions)

12dec2019_PFACMXF_002   **12-Dec-2019**   **13:15:06**
PFACMXF1219 50 ng/ml ea

285 > 169
2.00e5

HFPO-DA $[C_5F_{11}O]^-$ ⟶ $[C_3F_7]^-$

377 > 251
8.86e5

NaDONA $[C_7HF_{12}O_4]^-$ ⟶ $[C_4F_9O_2]^-$

531 > 351
4.70e5

9Cl-PF3ONS $[C_8F_{16}ClSO_4]^-$ ⟶ $[C_6F_{12}ClO]^-$

631 > 451
2.77e5

11Cl-PF3OUdS $[C_{10}F_{20}ClSO_4]^-$ ⟶ $[C_8F_{16}ClO]^-$

Time
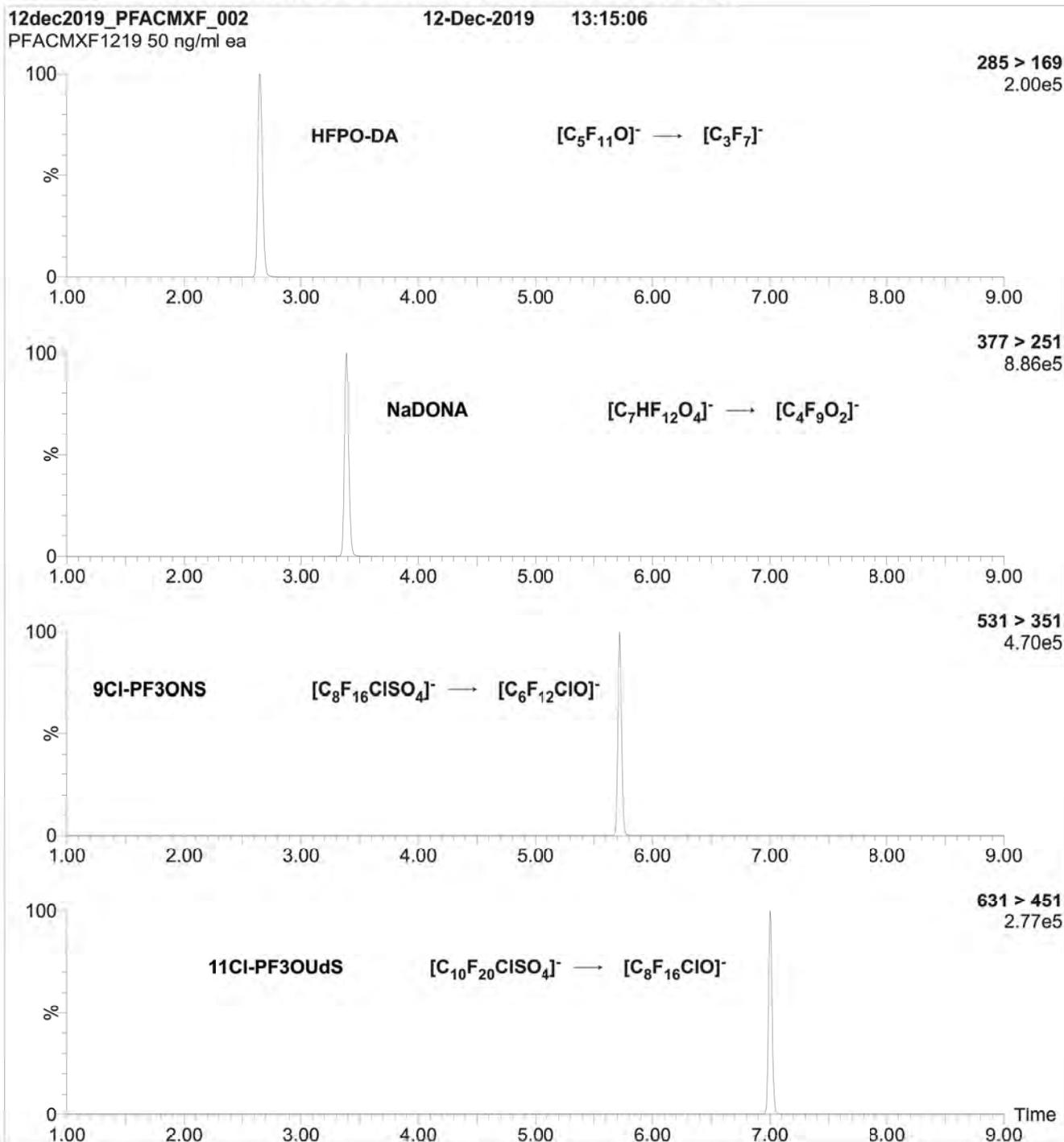
Conditions for Figure 2:

Injection: On-column (PFAC-MXF)

Mobile phase: Same as Figure 1

Flow: 300 µl/min

MS Parameters
Collision Gas (mbar) = 3.59e-3
Collision Energy (eV) = 8-24 (variable)

WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

PFAC-MXG

Native Perfluoroalkyl Ether Carboxylic Acids and Sulfonate Solution/Mixture

PRODUCT CODE: PFAC-MXG

LOT NUMBER: PFACMXG1219

SOLVENT(S):   Methanol/Water (<1%)

DATE PREPARED: (mm/dd/yyyy)   12/03/2019

LAST TESTED: (mm/dd/yyyy)   05/04/2020

EXPIRY DATE: (mm/dd/yyyy)   05/04/2025

RECOMMENDED STORAGE: Store ampoule in a cool, dark place

DESCRIPTION:

PFAC-MXG is a solution/mixture of three native perfluoroalkyl ether carboxylic acids and a native perfluoroalkyl ether sulfonate. The components and their concentrations are given in Table A.

The individual components all have chemical purities of >98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acids to their respective methyl esters.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters $x_1, x_2,...x_n$ on which it depends is:

$$u_c(y(x_1,x_2,...x_n)) = \sqrt{\sum_{i=1}^{n} u(y,x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class A tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

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QUALITY MANAGEMENT:

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CALA
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For additional information or assistance concerning this or any other products from Wellington Laboratories Inc., please visit our website at www.well-labs.com or contact us directly at info@well-labs.com

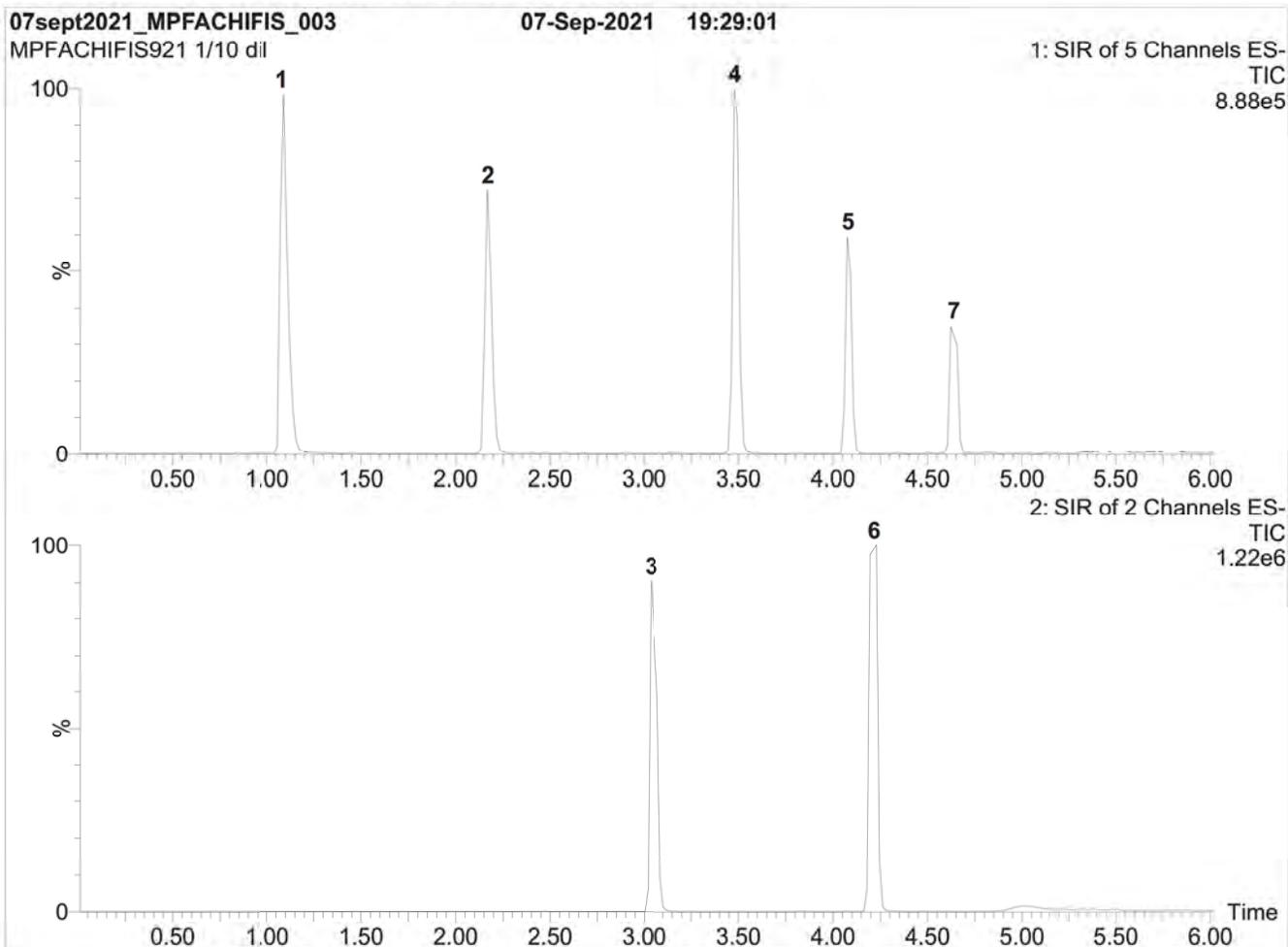## Table A: PFAC-MXG; Components and Concentrations (ng/mL; ± 5% in methanol/water (<1%))

Compound	Acronym	Concentration (ng/mL)		Peak Assignment in Figure 1
Perfluoro-4-oxapentanoic acid	PF4OPeA	2000		A
Perfluoro-5-oxahexanoic acid	PF5OHxA	2000		B
Perfluoro-3,6-dioxaheptanoic acid	3,6-OPFHpA	2000		D
Compound	**Acronym**	**Concentration* (ng/mL)**		**Peak Assignment in Figure 1**
		as the salt	as the acid	
Potassium perfluoro(2-ethoxyethane)sulfonate	PFEESA	2000	1780	C

* Concentrations have been rounded to three significant figures.
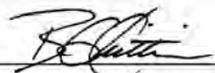
Certified By: _____ Date: __07/30/2021__
B.G. Chittim, General Manager   (mm/dd/yyyy)

Figure 1: PFAC-MXG; LC/MS Data (SIR)
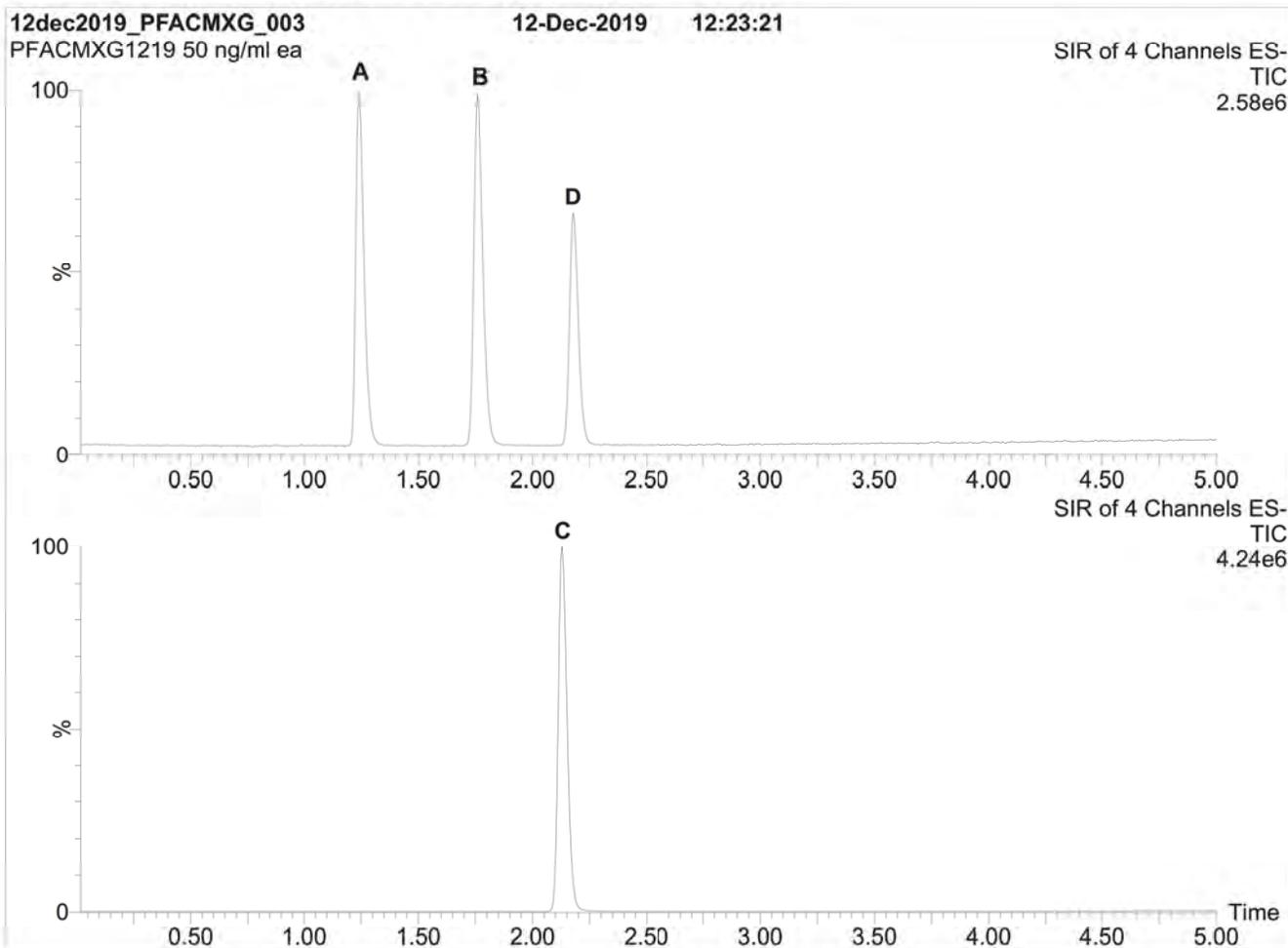
12dec2019_PFACMXG_003　　　　12-Dec-2019　12:23:21

PFACMXG1219 50 ng/ml ea

SIR of 4 Channels ES-
TIC
2.58e6

SIR of 4 Channels ES-
TIC
4.24e6

Conditions for Figure 1:

Waters Acquity Ultra Performance LC
Waters Xevo TQ-S micro MS

Chromatographic Conditions:

| Column: | Acquity UPLC BEH Shield RP$_{18}$ |
| | 1.7 µm, 2.1 x 100 mm |

Mobile phase: Gradient
　　　　Start: 50% H$_2$O / 50% (80:20 MeOH:ACN)
　　　　(both with 10 mM NH$_4$OAc buffer)
　　　　Ramp to 90% organic over 8 min and hold for 2 min
　　　　before returning to initial conditions in 0.75 min.
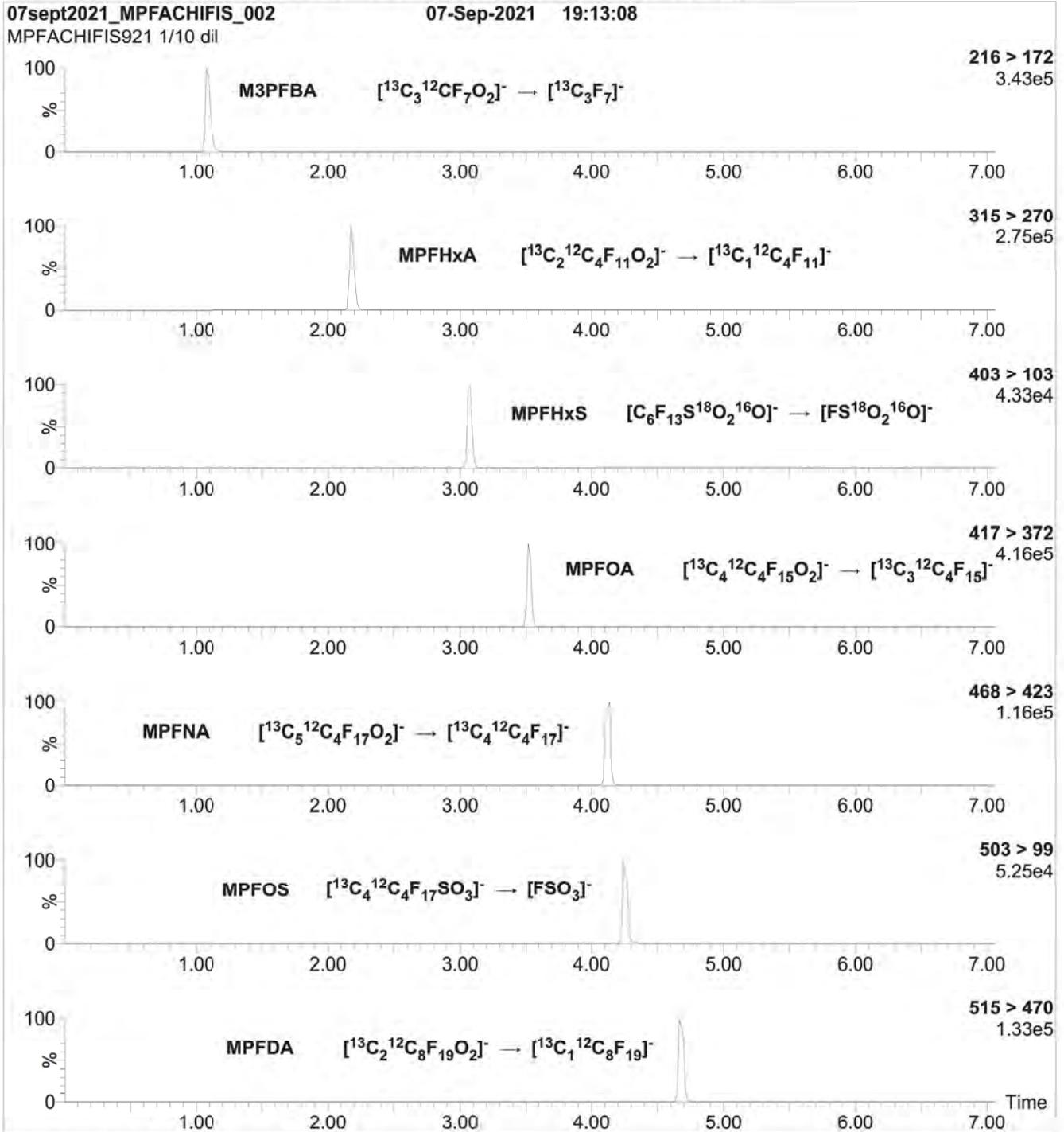　　　　Time: 12 min

Flow:　　　300 µL/min

MS Parameters:

Experiment: SIR

Source: Electrospray (negative)
Capillary Voltage (kV) = 1.00
Cone Voltage (V) = variable (15-35)
Desolvation Temperature (°C) = 500
Desolvation Gas Flow (L/hr) = 1000

Figure 2: PFAC-MXG; LC/MS/MS Data (Selected MRM Transitions)

PFACMXG1219 50 ng/ml ea   12-Dec-2019   12:10:28
12dec2019_PFACMXG_002

PF4OPeA $[C_4F_7O_3]^-$ $\longrightarrow$ $[CF_3O]^-$ **229 > 85**
1.10e6

PF5OHxA $[C_5F_9O_3]^-$ $\longrightarrow$ $[CF_3O]^-$ **279 > 85**
1.24e6

PFEESA $[C_4F_9SO_4]^-$ $\longrightarrow$ $[C_2F_5O]^-$ **315 > 135**
1.51e6

3,6-OPFHpA $[C_5F_9O_4]^-$ $\longrightarrow$ $[C_3F_7O_2]^-$ **295 > 201**
1.29e4

Time

Conditions for Figure 2:

Injection: On-column (PFAC-MXG)

Mobile phase: Same as Figure 1

Flow: 300 µL/min

MS Parameters:
Collision Gas (mbar) = 3.59e-3
Collision Energy (eV) = 8-48 (variable)

WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

PFAC-MXH

Native Per- and Poly-fluoroalkyl Substance Solution/Mixture

PRODUCT CODE: PFAC-MXH
LOT NUMBER: PFACMXH0921
SOLVENT(S): Methanol / Isopropanol (2%) / Water (<1%)
DATE PREPARED: (mm/dd/yyyy) 09/09/2021
LAST TESTED: (mm/dd/yyyy) 09/14/2021
EXPIRY DATE: (mm/dd/yyyy) 09/14/2026
RECOMMENDED STORAGE: Refrigerate ampoule

DESCRIPTION:

PFAC-MXH is a solution/mixture of eleven native linear perfluoroalkylcarboxylic acids (C_4-C_{14}), eight native perfluoroalkanesulfonates (C_4, C_5, C_7, C_9, C_{10} and C_{12} linear; C_6 and C_8 linear and branched), three native fluorotelomer sulfonates (4:2, 6:2, and 8:2), two native linear and branched perfluorooctanesulfonamidoacetic acids, and perfluoro-1-octanesulfonamide (FOSA). The components and their concentrations are given in Table A.

The individual components of this mixture all have chemical purities of >98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Table B: Isomeric Components and Percent Composition of br-NMeFOSAA
Table C: Isomeric Components and Percent Composition of br-NEtFOSAA
Table D: Isomeric Components and Percent Composition of PFHxSK
Table E: Isomeric Components and Percent Composition of PFOSK
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acids to their respective methyl esters.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

Wellington Laboratories Inc., 345 Southgate Dr. Guelph ON N1G 3M5 CANADA
519-822-2436 • Fax: 519-822-2849 • info@well-labs.com

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters x_1, x_2,...x_n on which it depends is:

$$u_c(y(x_1, x_2,...x_n)) = \sqrt{\sum_{i=1}^{n} u(y, x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class A tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

QUALITY MANAGEMENT:

This product was produced using a Quality Management System registered to the latest versions of ISO 9001 by SAI Global, ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation Inc. (CALA; A1226), and ISO 17034 by ANSI National Accreditation Board (ANAB; AR-1523).

CALA
Testing
Accreditation No. A1226

ANAB
ANSI National Accreditation Board
ACCREDITED
ISO 17034
REFERENCE MATERIAL PRODUCER

For additional information or assistance concerning this or any other products from Wellington Laboratories Inc., please visit our website at www.well-labs.com or contact us directly at info@well-labs.com

Table A: PFAC-MXH; Components and Concentrations
(µg/mL, ± 5% in methanol / isopropanol (2%) / water (<1%))
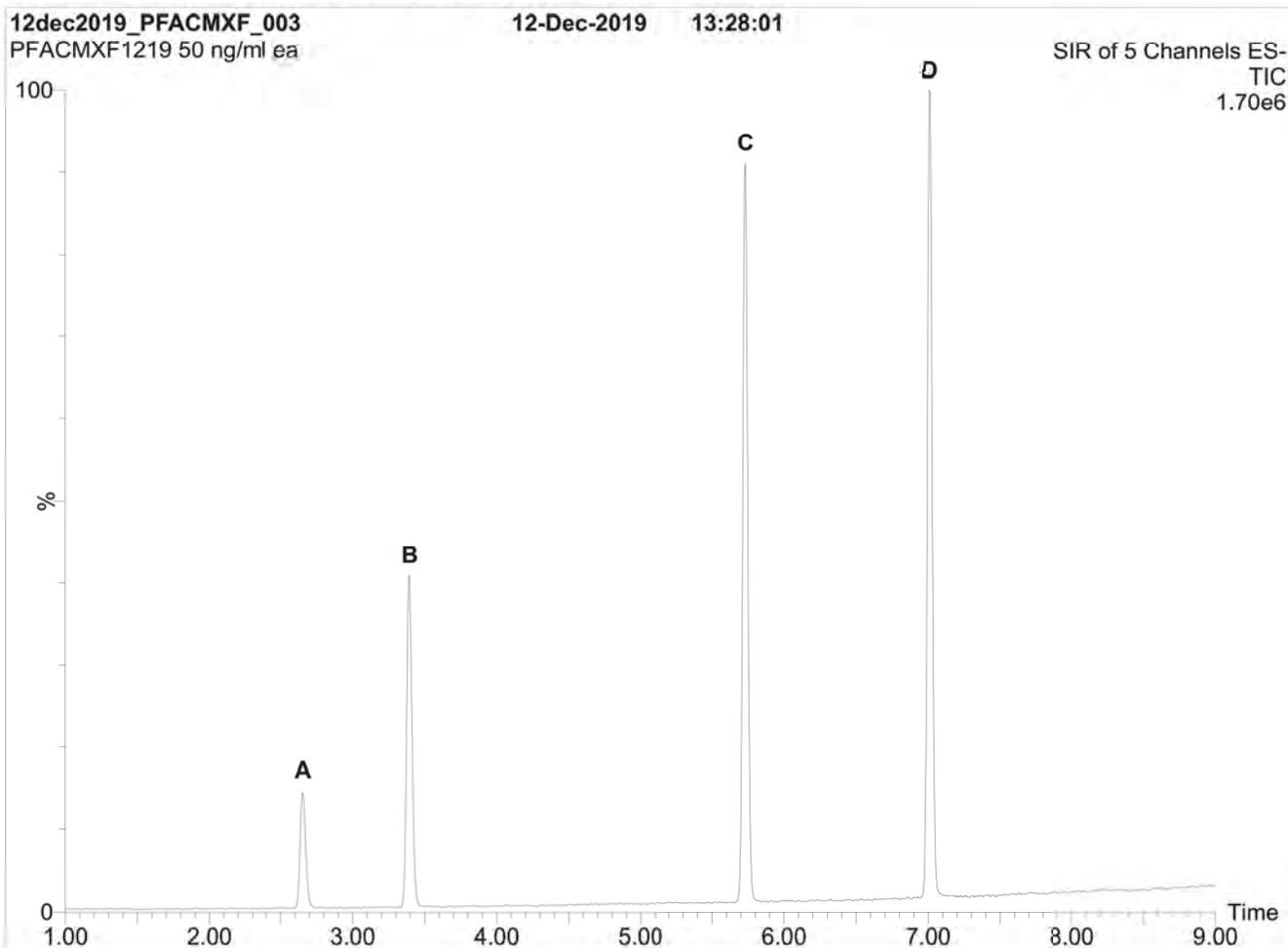
Compound	Acronym	Concentration* (µg/mL)	Peak Assignment in Figure 1
Perfluoro-n-butanoic acid	PFBA	4.00	1
Perfluoro-n-pentanoic acid	PFPeA	2.00	2
Perfluoro-n-hexanoic acid	PFHxA	1.00	5
Perfluoro-n-heptanoic acid	PFHpA	1.00	7
Perfluoro-n-octanoic acid	PFOA	1.00	11
Perfluoro-n-nonanoic acid	PFNA	1.00	14
Perfluoro-n-decanoic acid	PFDA	1.00	18
Perfluoro-n-undecanoic acid	PFUdA	1.00	23
Perfluoro-n-dodecanoic acid	PFDoA	1.00	26
Perfluoro-n-tridecanoic acid	PFTrDA	1.00	27
Perfluoro-n-tetradecanoic acid	PFTeDA	1.00	29
Perfluoro-1-octanesulfonamide	FOSA	1.00	25
N-methylperfluorooctanesulfonamidoacetic acid [a]	N-MeFOSAA: linear isomer	0.760	20
	N-MeFOSAA: ∑ branched isomers	0.240	17
N-ethylperfluorooctanesulfonamidoacetic acid [b]	N-EtFOSAA: linear isomer	0.775	22
	N-EtFOSAA: ∑ branched isomers	0.225	21

Compound	Acronym	Concentration* (µg/mL)		Peak Assignment in Figure 1
		as the salt	as the acid	
Potassium perfluoro-1-butanesulfonate	L-PFBS	1.00	0.887	3
Sodium perfluoro-1-pentanesulfonate	L-PFPeS	1.00	0.941	6
Potassium perfluorohexanesulfonate [c]	PFHxSK: linear isomer	0.811	0.741	9
	PFHxSK: ∑ branched isomers	0.189	0.173	8
Sodium perfluoro-1-heptanesulfonate	L-PFHpS	1.00	0.953	12
Potassium perfluorooctanesulfonate [d]	PFOSK linear isomer	0.788	0.732	15
	PFOSK ∑ branched isomers	0.211	0.196	13
Sodium perfluoro-1-nonanesulfonate	L-PFNS	1.00	0.962	19
Sodium perfluoro-1-decanesulfonate	L-PFDS	1.00	0.965	24
Sodium perfluoro-1-dodecanesulfonate	L-PFDoS	1.00	0.970	28
Sodium 1H,1H,2H,2H-perfluorohexanesulfonate	4:2FTS	4.00	3.75	4
Sodium 1H,1H,2H,2H-perfluorooctanesulfonate	6:2FTS	4.00	3.80	10
Sodium 1H,1H,2H,2H-perfluorodecanesulfonate	8:2FTS	4.00	3.84	16

[a] See Table B for percent composition of linear and branched N-MeFOSAA isomers.
[b] See Table C for percent composition of linear and branched N-EtFOSAA isomers.
[c] See Table D for percent composition of linear and branched PFHxSK isomers.
[d] See Table E for percent composition of linear and branched PFOSK isomers.

* Concentrations have been rounded to three significant figures.

Certified By: _____ Date: __09/23/2021__
B.G. Chittim, General Manager   (mm/dd/yyyy)

Table B: br-NMeFOSAA; Isomeric Components and Percent Composition (by ^{19}F-NMR)*

Isomer	Compound	Structure	Percent Composition by ^{19}F-NMR	
1	N-methylperfluoro-1-octanesulfonamidoacetic acid	$CF_3(CF_2)_7SO_2NCH_2CO_2H$ CH_3	76.0	76.0
2	N-methylperfluoro-3-methylheptanesulfonamidoacetic acid	$CF_3(CF_2)_3CF(CF_2)_2SO_2NCH_2CO_2H$ CF_3 CH_3	0.7	
3	N-methylperfluoro-4-methylheptanesulfonamidoacetic acid	$CF_3(CF_2)_2CF(CF_2)_3SO_2NCH_2CO_2H$ CF_3 CH_3	2.0	
4	N-methylperfluoro-5-methylheptanesulfonamidoacetic acid	$CF_3CF_2CF(CF_2)_4SO_2NCH_2CO_2H$ CF_3 CH_3	6.0	24.0
5	N-methylperfluoro-6-methylheptanesulfonamidoacetic acid	$CF_3CF(CF_2)_5SO_2NCH_2CO_2H$ CF_3 CH_3	14.0	
6	N-methylperfluoro-5,5-dimethylhexanesulfonamidoacetic acid	CF_3 $CF_3C(CF_2)_4SO_2NCH_2CO_2H$ CF_3 CH_3	0.2	
7	Other Unidentified Isomers		1.1	

* Percent of total N-methylperfluorooctanesulfonamidoacetic acid isomers only.

Table C: **br-NEtFOSAA; Isomeric Components and Percent Composition (by ^{19}F-NMR)***

Isomer	Compound	Structure	Percent Composition by ^{19}F-NMR	
1	N-ethylperfluoro-1-octanesulfonamidoacetic acid	$CF_3(CF_2)_7SO_2NCH_2CO_2H$ C_2H_5	77.5	77.5
2	N-ethylperfluoro-3-methylheptanesulfonamidoacetic acid	$CF_3(CF_2)_3CF(CF_2)_2SO_2NCH_2CO_2H$ CF_3 C_2H_5	2.3	
3	N-ethylperfluoro-4-methylheptanesulfonamidoacetic acid	$CF_3(CF_2)_2CF(CF_2)_3SO_2NCH_2CO_2H$ CF_3 C_2H_5	2.2	
4	N-ethylperfluoro-5-methylheptanesulfonamidoacetic acid	$CF_3CF_2CF(CF_2)_4SO_2NCH_2CO_2H$ CF_3 C_2H_5	5.4	
5	N-ethylperfluoro-6-methylheptanesulfonamidoacetic acid	$CF_3CF(CF_2)_5SO_2NCH_2CO_2H$ CF_3 C_2H_5	10.4	22.5
6	N-ethylperfluoro-5,5-dimethylhexanesulfonamidoacetic acid	CF_3 $CF_3C(CF_2)_4SO_2NCH_2CO_2H$ CF_3 C_2H_5	0.3	
7	N-ethylperfluoro-4,5-dimethylhexanesulfonamidoacetic acid	CF_3 $CF_3CFCF(CF_2)_3SO_2NCH_2CO_2H$ CF_3 C_2H_5	0.3	
8	N-ethylperfluoro-3,5-dimethylhexanesulfonamidoacetic acid	CF_3 $CF_3CFCF_2CF(CF_2)_2SO_2NCH_2CO_2H$ CF_3 C_2H_5	0.3	
9	Other Unidentified Isomers		1.3	

*   Percent of total N-ethylperfluorooctanesulfonamidoacetic acid isomers only.

Table D: PFHxSK; Isomeric Components and Percent Composition (by ^{19}F-NMR)*

Isomer	Compound	Structure	Percent Composition by ^{19}F-NMR	
1	Potassium perfluoro-1-hexanesulfonate	$CF_3CF_2CF_2CF_2CF_2CF_2SO_3^-K^+$	81.1	81.1
2	Potassium 1-trifluoromethylperfluoropentanesulfonate**	$CF_3CF_2CF_2CF_2CFSO_3^-K^+$ $\qquad\qquad\quad\; CF_3$	2.9	
3	Potassium 2-trifluoromethylperfluoropentanesulfonate	$CF_3CF_2CF_2CFCF_2SO_3^-K^+$ $\qquad\qquad\quad CF_3$	1.4	
4	Potassium 3-trifluoromethylperfluoropentanesulfonate	$CF_3CF_2CFCF_2CF_2SO_3^-K^+$ $\qquad\qquad CF_3$	5.0	
5	Potassium 4-trifluoromethylperfluoropentanesulfonate	$CF_3CFCF_2CF_2CF_2SO_3^-K^+$ $\qquad\; CF_3$	8.9	18.9
6	Potassium 3,3-di(trifluoromethyl)perfluorobutanesulfonate	$\qquad\; CF_3$ $CF_3CCF_2CF_2SO_3^-K^+$ $\qquad\; CF_3$	0.2	
7	Other Unidentified Isomers		0.5	

* Percent of total perfluorohexanesulfonate isomers only.
** Systematic Name: Potassium perfluorohexane-2-sulfonate.

Table E: PFOSK; Isomeric Components and Percent Composition (by ^{19}F-NMR)*

Isomer	Compound	Structure	Percent Composition by ^{19}F-NMR	
1	Potassium perfluoro-1-octanesulfonate	$CF_3CF_2CF_2CF_2CF_2CF_2CF_2CF_2SO_3^-K^+$	78.8	78.8
2	Potassium 1-trifluoromethylperfluoroheptanesulfonate**	$CF_3CF_2CF_2CF_2CF_2CF_2CFSO_3^-K^+$ $\mid$ CF_3	1.2	
3	Potassium 2-trifluoromethylperfluoroheptanesulfonate	$CF_3CF_2CF_2CF_2CF_2CFCF_2SO_3^-K^+$ $\mid$ CF_3	0.6	
4	Potassium 3-trifluoromethylperfluoroheptanesulfonate	$CF_3CF_2CF_2CF_2CFCF_2CF_2SO_3^-K^+$ $\mid$ CF_3	1.9	
5	Potassium 4-trifluoromethylperfluoroheptanesulfonate	$CF_3CF_2CF_2CFCF_2CF_2CF_2SO_3^-K^+$ $\mid$ CF_3	2.2	
6	Potassium 5-trifluoromethylperfluoroheptanesulfonate	$CF_3CF_2CFCF_2CF_2CF_2CF_2SO_3^-K^+$ $\mid$ CF_3	4.5	
7	Potassium 6-trifluoromethylperfluoroheptanesulfonate	$CF_3CFCF_2CF_2CF_2CF_2CF_2SO_3^-K^+$ $\mid$ CF_3	10.0	21.1
8	Potassium 5,5-di(trifluoromethyl)perfluorohexanesulfonate	CF_3 $\mid$ $CF_3CCF_2CF_2CF_2CF_2SO_3^-K^+$ $\mid$ CF_3	0.2	
9	Potassium 4,4-di(trifluoromethyl)perfluorohexanesulfonate	CF_3 $\mid$ $CF_3CF_2CCF_2CF_2CF_2SO_3^-K^+$ $\mid$ CF_3	0.03	
10	Potassium 4,5-di(trifluoromethyl)perfluorohexanesulfonate	CF_3 $\mid$ $CF_3CFCFCF_2CF_2CF_2SO_3^-K^+$ $\mid$ CF_3	0.4	
11	Potassium 3,5-di(trifluoromethyl)perfluorohexanesulfonate	CF_3 $\mid$ $CF_3CFCF_2CFCF_2CF_2SO_3^-K^+$ $\mid$ CF_3	0.07	

*   Percent of total perfluorooctanesulfonate isomers only.
**   Systematic Name: Potassium perfluorooctane-2-sulfonate.

Figure 1: PFAC-MXH; LC/MS Data (SIR)

14sep2021_PFACMXH_003 **14-Sep-2021** **14:48:50**

PFACMXH0921 1/20 dil

1: SIR of 11 Channels ES-TIC 2.27e6

4: SIR of 9 Channels ES-TIC 4.50e6

3: SIR of 8 Channels ES-TIC 3.55e6

5: SIR of 6 Channels ES-TIC 7.43e5

5: SIR of 6 Channels ES-TIC 6.04e5

Conditions for Figure 1:

Waters Acquity Ultra Performance LC
Waters Xevo TQ-S micro MS

Chromatographic Conditions:

Column: Acquity UPLC BEH Shield RP$_{18}$
1.7 µm, 2.1 x 100 mm

Mobile phase: Gradient
Start: 50% H$_2$O / 50% (80:20 MeOH:ACN)
(both with 10 mM NH$_4$OAc buffer)
Ramp to 90% organic over 9 min and hold for 2 min
before returning to initial conditions in 1 min.
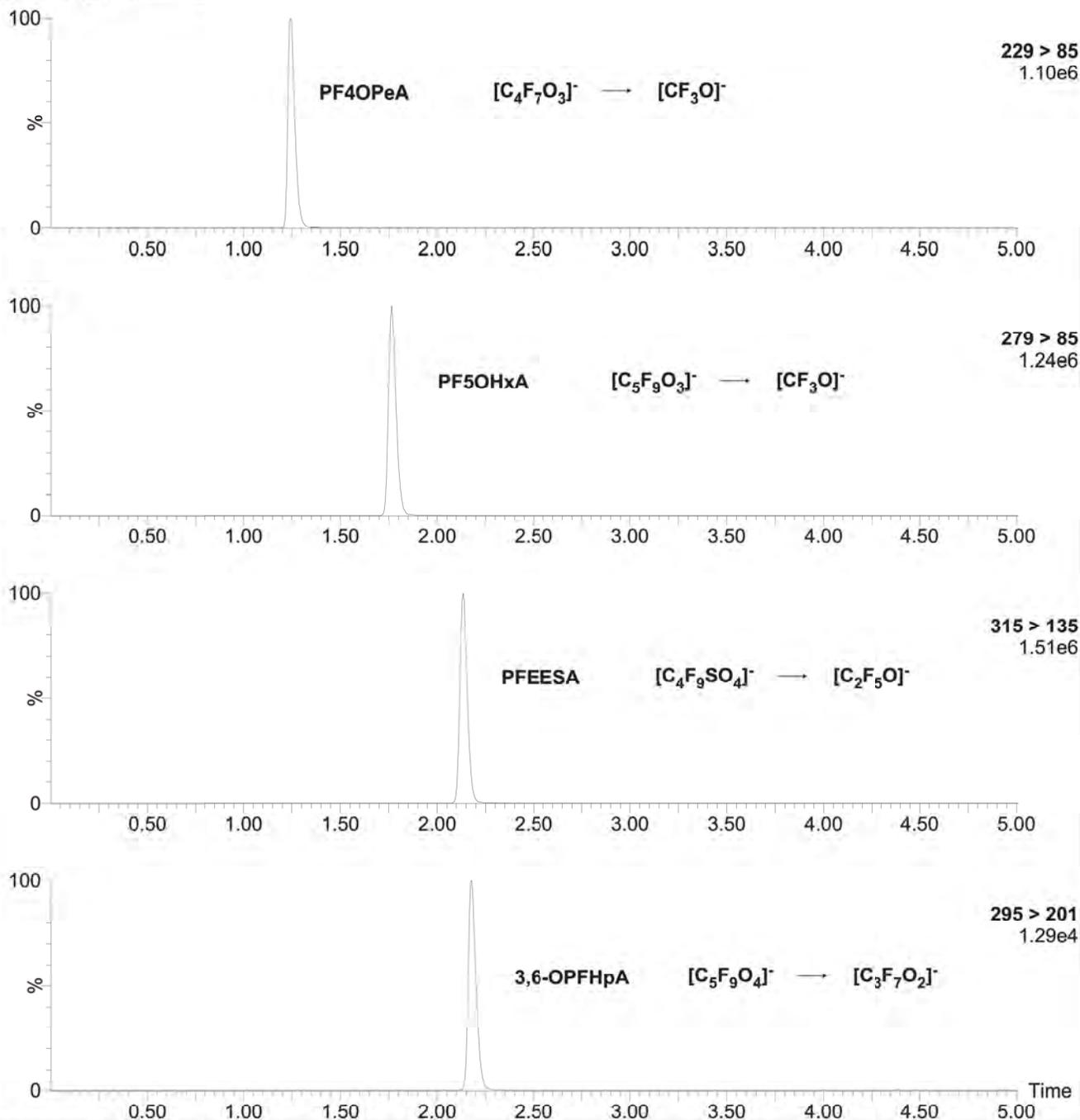Time: 15 min

Flow: 300 µL/min

MS Parameters:

Experiment: SIR

Source: Electrospray (negative)
Capillary Voltage (kV) = 2.50
Cone Voltage (V) = variable (2-74)
Desolvation Temperature (°C) = 350
Desolvation Gas Flow (L/hr) = 1000

Figure 2: PFAC-MXH; LC/MS/MS Data (Selected MRM Transitions)

14sep2021_PFACMXH_002   14-Sep-2021   14:32:55
PFACMXH0921 1/20 dil

213 > 169
9.74e5
PFBA $[C_4F_7O_2]^- \longrightarrow [C_3F_7]^-$

263 > 219
5.52e5
PFPeA $[C_5F_9O_2]^- \longrightarrow [C_4F_9]^-$

299 > 80
1.29e5
L-PFBS $[C_4F_9SO_3]^- \longrightarrow [SO_3]^-$

327 > 307
2.55e5
4:2FTS $[C_6H_4F_9SO_3]^- \longrightarrow [C_6H_3F_8SO_3]^-$

313 > 269
3.90e5
PFHxA $[C_6F_{11}O_2]^- \longrightarrow [C_5F_{11}]^-$

349 > 80
1.46e5
L-PFPeS $[C_5F_{11}SO_3]^- \longrightarrow [SO_3]^-$

363 > 319
5.27e5
PFHpA $[C_7F_{13}O_2]^- \longrightarrow [C_6F_{13}]^-$

399 > 99
1.01e5
PFHxSK $[C_6F_{13}SO_3]^- \longrightarrow [FSO_3]^-$

427 > 407
3.27e5
6:2FTS $[C_8H_4F_{13}SO_3]^- \longrightarrow [C_8H_3F_{12}SO_3]^-$

Time

14sep2021_PFACMXH_002   14-Sep-2021   14:32:55
PFACMXH0921 1/20 dil

Figure 2: PFAC-MXH; LC/MS/MS Data (Selected MRM Transitions)

14sep2021_PFACMXH_002   14-Sep-2021   14:32:55
PFACMXH0921 1/20 dil

PFUdA $[C_{11}F_{21}O_2]^- \longrightarrow [C_{10}F_{21}]^-$ **563 > 519** 7.23e5

L-PFDS $[C_{10}F_{21}SO_3]^- \longrightarrow [SO_3]^-$ **599 > 80** 1.39e5

FOSA $[C_8HF_{17}NO_2S]^- \longrightarrow [NO_2S]^-$ **498 > 78** 3.97e5

PFDoA $[C_{12}F_{23}O_2]^- \longrightarrow [C_{11}F_{23}]^-$ **613 > 569** 4.55e5

PFTrDA $[C_{13}F_{25}O_2]^- \longrightarrow [C_{12}F_{25}]^-$ **663 > 619** 7.12e5

L-PFDoS $[C_{12}F_{25}SO_3]^- \longrightarrow [SO_3]^-$ **699 > 80** 1.94e5

PFTeDA $[C_{14}F_{27}O_2]^- \longrightarrow [C_{13}F_{27}]^-$ **713 > 669** 6.18e5

Conditions for Figure 2:

Injection: On-column (PFAC-MXH)

Mobile phase: Same as Figure 1

Flow: 300 µL/min

MS Parameters:
Collision Gas (mbar) = 3.31e-3
Collision Energy (eV) = 6-60 (variable)

WELLINGTON LABORATORIES

PFAC-MXI

Native Perfluorooctanesulfonamide and Perfluorooctanesulfonamidoethanol Solution/Mixture

PRODUCT CODE: PFAC-MXI

LOT NUMBER: PFACMXI0921

SOLVENT(S): Methanol

DATE PREPARED: (mm/dd/yyyy) 09/08/2021

LAST TESTED: (mm/dd/yyyy) 09/14/2021

EXPIRY DATE: (mm/dd/yyyy) 09/14/2026

RECOMMENDED STORAGE: Store ampoule in a cool, dark place

DESCRIPTION:

PFAC-MXI is a solution/mixture of two native perfluorooctanesulfonamides (FOSAs) and two native perfluorooctanesulfonamidoethanols (FOSEs). The components and their concentrations are given in Table A.

The individual components have a chemical purity of >98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters x_1, x_2,...x_n on which it depends is:

$$u_c(y(x_1, x_2,...x_n)) = \sqrt{\sum_{i=1}^{n} u(y, x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class A tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

QUALITY MANAGEMENT:

This product was produced using a Quality Management System registered to the latest versions of ISO 9001 by SAI Global, ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation Inc. (CALA; A1226), and ISO 17034 by ANSI National Accreditation Board (ANAB; AR-1523).

For additional information or assistance concerning this or any other products from Wellington Laboratories Inc., please visit our website at www.well-labs.com or contact us directly at info@well-labs.com

Table A: PFAC-MXI; Components and Concentrations (µg/mL; ± 5% in methanol)

Compound	Acronym	Concentration (µg/mL)	Peak Assignment in Figure 1
N-methylperfluoro-1-octanesulfonamide	N-MeFOSA	1.00	B
N-ethylperfluoro-1-octanesulfonamide	N-EtFOSA	1.00	D
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	N-MeFOSE	10.0	A
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	N-EtFOSE	10.0	C

Certified By: _____   Date: __09/23/2021__
B.G. Chittim, General Manager   (mm/dd/yyyy)

Figure 1:   PFAC-MXI; LC/MS Data (SIR)

14sep2021_PFACMXI_004   14-Sep-2021   15:52:39

PFACMXI0921 1/40 dil   5: SIR of 6 Channels ES-
                                                        TIC
                                                        6.05e6

                                                        5: SIR of 6 Channels ES-
                                                        TIC
                                                        1.54e6

Conditions for Figure 1:

Waters Acquity Ultra Performance LC
Waters Xevo TQ-S micro MS

Chromatographic Conditions:

Column:	Acquity UPLC BEH Shield RP$_{18}$
	1.7 µm, 2.1 x 100 mm

Mobile phase: Gradient
Start: 50% H_2O / 50% (80:20 MeOH:ACN)
(both with 10 mM NH_4OAc buffer)
Ramp to 90% organic over 9 min and hold for
2 min before returning to initial conditions in 1 min.
Time: 15 min

Flow: 300 µL/min

MS Parameters:

Experiment: SIR

Source: Electrospray (negative)
Capillary Voltage (kV) = 2.50
Cone Voltage (V) = variable (2-74)
Desolvation Temperature (°C) = 350
Desolvation Gas Flow (L/hr) = 1000

Figure 2:   PFAC-MXI; LC/MS/MS Data (Selected MRM Transitions)

14sep2021_PFACMXI_002   14-Sep-2021   15:20:46
PFACMXI0921 1/40 dil

616 > 59
7.31e5

N-MeFOSE adduct

$[C_{11}H_8F_{17}NO_3S] \cdot [CH_3COO]^- \longrightarrow [CH_3COO]^-$

512 > 219
3.51e4

N-MeFOSA

$[C_9H_3F_{17}NO_2S]^- \longrightarrow [C_4F_9]^-$

630 > 59
7.56e5

N-EtFOSE adduct

$[C_{12}H_{10}F_{17}NO_3S] \cdot [CH_3COO]^- \longrightarrow [CH_3COO]^-$

526 > 219
4.48e4

N-EtFOSA

$[C_{10}H_5F_{17}NO_2S]^- \longrightarrow [C_4F_9]^-$

Time
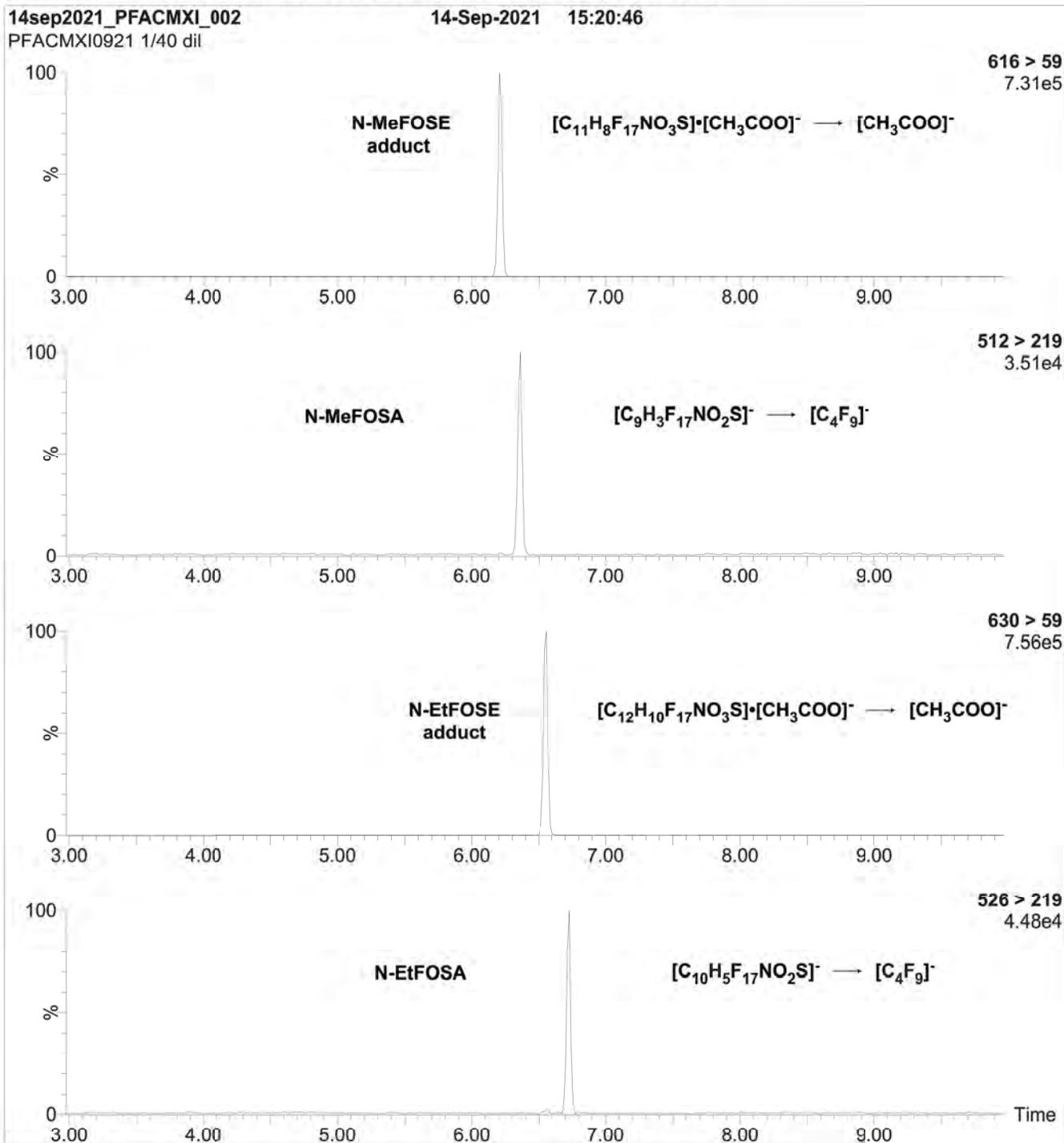
Conditions for Figure 2:

Injection: On-column (PFAC-MXI)

Mobile phase: Same as Figure 1

Flow: 300 µL/min

MS Parameters:
Collision Gas (mbar) = 3.31e-3
Collision Energy (eV) = 6-60 (variable)

PFAC-MXJ

Native Fluorotelomer Propanoic Acid
Solution/Mixture

PRODUCT CODE: PFAC-MXJ

LOT NUMBER: PFACMXJ0921

SOLVENT(S): Methanol

DATE PREPARED: (mm/dd/yyyy) 09/08/2021

LAST TESTED: (mm/dd/yyyy) 09/14/2021

EXPIRY DATE: (mm/dd/yyyy) 09/14/2026

RECOMMENDED STORAGE: Store ampoule in a cool, dark place

DESCRIPTION:

PFAC-MXJ is a solution/mixture of three native fluorotelomer propanoic acids. The components and their concentrations are given in Table A.

The individual components have a chemical purity of >98%.

DOCUMENTATION/ DATA ATTACHED:

Table A: Components and Concentrations of the Solution/Mixture
Figure 1: LC/MS Data (SIR)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

INTENDED USE:

The products prepared by Wellington Laboratories Inc. are for laboratory use only. This certified reference material (CRM) was designed to be used as a standard for the identification and/or quantification of the specific chemical compounds it contains.

HANDLING:

This product should only be used by qualified personnel familiar with its potential hazards and trained in the handling of hazardous chemicals. Due care should be exercised to prevent unnecessary human contact or ingestion. All procedures should be carried out in a well-functioning fume hood and suitable gloves, eye protection, and clothing should be worn at all times. Waste should be disposed of according to national and regional regulations. Safety Data Sheets (SDSs) are available upon request.

SYNTHESIS / CHARACTERIZATION:

Our products are synthesized using single-product unambiguous routes whenever possible. They are then characterized, and their structures and purities confirmed, using a combination of the most relevant techniques, such as NMR, GC/MS, LC/MS/MS, SFC/UV/MS/MS, x-ray crystallography, and melting point. Isotopic purities of mass-labelled compounds are also confirmed using HRGC/HRMS and/or LC/MS/MS.

HOMOGENEITY:

Prior to solution preparation, crystalline material is tested for homogeneity using a variety of techniques (as stated above) and its solubility in a given diluent is taken into consideration. Duplicate solutions of a new product are prepared from the same crystalline lot and, after the addition of an appropriate internal standard, they are compared by GC/MS, LC/MS/MS, and/or SFC/UV/MS/MS. The relative response factors of the analyte of interest in each solution are required to be <5% RSD. New solution lots of existing products, as well as mixtures and calibration solutions, are compared to older lots in a similar manner. This further confirms the homogeneity of the crystalline material as well as the stability and homogeneity of the solutions in the storage containers. In order to maintain the integrity of the assigned value(s), and associated uncertainty, the dilution or injection of a subsample of this product should be performed using calibrated measuring equipment.

UNCERTAINTY:

The maximum combined relative standard uncertainty of our reference standard solutions is calculated using the following equation:

The combined relative standard uncertainty, $u_c(y)$, of a value y and the uncertainty of the independent parameters $x_1, x_2, ... x_n$ on which it depends is:

$$u_c(y(x_1, x_2, ... x_n)) = \sqrt{\sum_{i=1}^{n} u(y, x_i)^2}$$

where x is expressed as a relative standard uncertainty of the individual parameter.

The individual uncertainties taken into account include those associated with weights (calibration of the balance) and volumes (calibration of the volumetric glassware). An expanded maximum combined percent relative uncertainty of ±5% (calculated with a coverage factor of 2 and a level of confidence of 95%) is stated on the Certificate of Analysis for all of our products.

TRACEABILITY:

All reference standard solutions are traceable to specific crystalline lots. The microbalances used for solution preparation are regularly calibrated by an external ISO/IEC 17025 accredited laboratory. In addition, their calibration is verified prior to each weighing using calibrated external weights traceable to an ISO/IEC 17025 accredited laboratory. All volumetric glassware used is calibrated, of Class A tolerance, and traceable to an ISO/IEC 17025 accredited laboratory. For certain products, traceability to international interlaboratory studies has also been established.

EXPIRY DATE / PERIOD OF VALIDITY:

Ongoing stability studies of this product have demonstrated stability in its composition and concentration, until the specified expiry date, in the unopened ampoule. Monitoring for any degradation or change in concentration of the listed analyte(s) is performed on a routine basis.

LIMITED WARRANTY:

At the time of shipment, all products are warranted to be free of defects in material and workmanship and to conform to the stated technical and purity specifications.

QUALITY MANAGEMENT:

This product was produced using a Quality Management System registered to the latest versions of ISO 9001 by SAI Global, ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation Inc. (CALA; A1226), and ISO 17034 by ANSI National Accreditation Board (ANAB; AR-1523).

CALA
Testing
Accreditation No. A1226

ANAB
ANSI National Accreditation Board
ACCREDITED
ISO 17034
REFERENCE MATERIAL PRODUCER

For additional information or assistance concerning this or any other products from Wellington Laboratories Inc., please visit our website at www.well-labs.com or contact us directly at info@well-labs.com

Table A: PFAC-MXJ; Components and Concentrations (µg/mL; ± 5% in methanol)

Compound	Acronym	Concentration (µg/mL)	Peak Assignment in Figure 1
3-Perfluoropropyl propanoic acid	FPrPA	4.00	A
3-Perfluoropentyl propanoic acid	FPePA	20.0	B
3-Perfluoroheptyl propanoic acid	FHpPA	20.0	C
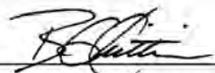
Certified By: _____   Date: ___09/23/2021___
B.G. Chittim, General Manager   (mm/dd/yyyy)

Figure 1: PFAC-MXJ; LC/MS Data (SIR)

14sept2021_PFACMXJ_008   14-Sep-2021   12:22:41

PFACMXJ0921 1/50 dil   2: SIR of 7 Channels ES-
                                                                                 TIC
                                                                                4.13e5

                                                                       2: SIR of 7 Channels ES-
                                                                                 TIC
                                                                                4.19e6

                                                                       2: SIR of 7 Channels ES-
                                                                                 TIC
                                                                                4.89e6

Conditions for Figure 1:

Waters Acquity Ultra Performance LC
Waters Xevo TQ-S micro MS

Chromatographic Conditions:

Column:	Acquity UPLC BEH Shield RP$_{18}$
	1.7 µm, 2.1 x 100 mm

Mobile phase: Gradient
Start: 50% H_2O / 50% (80:20 MeOH:ACN)
(both with 10 mM NH_4OAc buffer)
Ramp to 90% organic over 9 min and hold for
2 min before returning to initial conditions in 1 min.
Time: 15 min

Flow: 300 µL/min

MS Parameters:

Experiment: SIR

Source: Electrospray (negative)
Capillary Voltage (kV) = 2.50
Cone Voltage (V) = variable (2-74)
Desolvation Temperature (°C) = 350
Desolvation Gas Flow (L/hr) = 1000

Figure 2: PFAC-MXJ; LC/MS/MS Data (Selected MRM Transitions)

14sept2021_PFACMXJ_001   14-Sep-2021   09:45:06

PFACMXJ0921 1/50 dil

241 > 177
2.56e4

FPrPA $[C_6H_4F_7O_2]^- \longrightarrow [C_5H_3F_6]^-$

341 > 237
4.24e5

FPePA $[C_8H_4F_{11}O_2]^- \longrightarrow [C_7HF_8]^-$

441 > 317
1.52e5

FHpPA $[C_{10}H_4F_{15}O_2]^- \longrightarrow [C_9F_{11}]^-$

Time
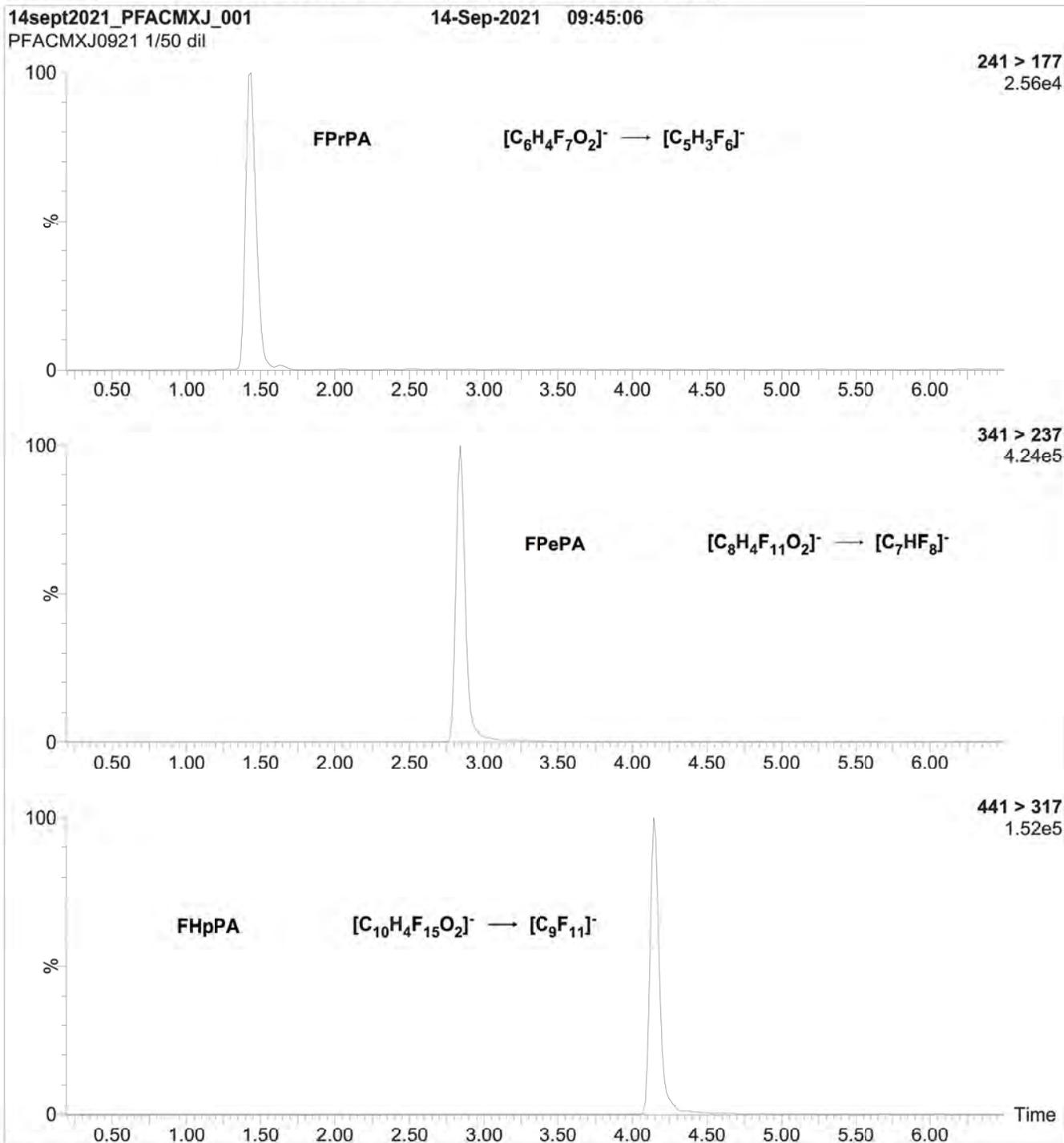
Conditions for Figure 2:

Injection: On-column (PFAC-MXJ)

Mobile phase: Same as Figure 1

Flow: 300 µL/min

MS Parameters:
Collision Gas (mbar) = 3.31e-3
Collision Energy (eV) = 6-60 (variable)

ATTACHMENT 7

ERA COOLER STUDY REPORT IN SUPPORT OF THE HGL/DOD PFAS MULTI-LABORATORY METHOD VALIDATION STUDY – JANUARY 2022

(Dated January 2022)

ERA Cooler Study Report in Support of the HGL/DoD PFAS Multi-Laboratory Method Validation Study- January 2022

Table of Contents

1. **Purpose:**
   - To assess the ability of the aqueous matrix samples to retain a temperature of < 6°C during the scheduled 24 hr. shipping process and to measure/document sample temperatures out to 120 hours under ambient external temperature conditions.

2. **Objective:**
   - To assess if pre-frozen aqueous samples will be able to maintain a < 6°C temperature under ambient shipping conditions when packed per normal ERA protocol and shipped in ERA coolers to participating laboratories.

3. **Procedure:**
   1. (7) large shipping coolers (25" x 15.5" x 17" Styrofoam cooler in a cardboard shipping box) were assembled for the study.
   2. (108) 1L HDPE bottles were filled with approximately 500 mL of tap water to represent study samples. Bottles were stored at ≤ 0 deg C for a minimum of 16 hrs. or until frozen.
   3. Each cooler was packed according to ERA Work Instruction 730002425 *Whole Volume Cooler Shipping*.
      - A minimum of 26 freezer packs (blue ice type) were used per cooler.
      - A layer of freezer packs was placed in the bottom and top of the cooler.
      - Each cooler was filled with 13 pre-frozen 1L Amber HDPE bottles containing tap water and packed per this protocol for shipping.
      - Any remaining space around and on top of the bottles was filled with additional insulating material (foam sheets, bubble wrap or packing paper) or ice packs.
   4. Each cooler was labeled 1-7 corresponding to the different time interval of 12, 24, 36, 48, 72, 96 & 120 hours.
   5. Initial sample temperatures for each of the seven coolers was taken with an IR temperature gun prior to cooler sealing. Sample #1 was checked for this temperature.
   6. The (7) coolers were placed at ambient temperature in a central location of ERA's lab under four large work benches for this project.
      - This location is environmentally controlled and is suggested to best represent temperature conditions during shipment (according to the planned schedule).
      - A thermometer was placed adjacent to but outside of the coolers to monitor ambient room temperature.

7. One cooler was opened, and the temperature was checked at each time intervals: 12, 24, 36, 48, 72, 96 & 120 hours (± 30 minutes). At each interval, the time was recorded, and the following performed:
    - The temperature for the 3 samples from across the cooler was checked with an IR temperature gun and the results were recorded.
    - The ambient room temperature was checked and recorded.
8. Results were compiled in an Excel spreadsheet which is included at the end of this document.

4. **Study Evaluation/Results:**

The 1L Amber bottles exhibited either a very slight or no apparent bulge in the side of the bottle after the water became frozen. If the bulge was noticed it was located at the top of the frozen water level from the expansion of the ice (see photo below). None of the 108 frozen bottles broke and all bulges that were observed were insignificant and would have no impact on the structural integrity of the bottle.

The first five (of seven) coolers were packed on 1/10/2022 and three samples in each cooler were randomly labeled 1-3. The Ambient temperature and the temperature of a single bottle from each cooler was recorded at the time of the initial cooler packing. See photos of packing configurations within the coolers below.

At each time interval the temperature of the three bottles, the ambient temperature and the thermometer IDs were recorded. All data for the cooler study were recorded and entered in a spread sheet (which is included at the end of this document). Samples for the 12 and 24 hr. check remained completely frozen. A small amount of thawed water (~ 10 mL) was noticeable in the bottles at the 36 hr. check. At the 72 hr. check, bottles were about ¾ frozen and ¼ thawed. It was decided to extend the cooler study to 96 hr. and 120 hr. checks to anticipate any delays that may occur with the delivery carrier for the planned overnight deliveries. At the 96 hr. check, bottles 1 and 3 (which were located along the outside of the cooler) were ¾ frozen and ¼ thawed. Bottle 1 (located in the middle of the cooler)

had only ~ 20 ml of thawed water. At 120 hrs., all sample temperatures were below 0°C and the volumes of thawed water can be seen in the following two pictures below.

Pictures Representing Volume(s) of Thawed Water After 120 hrs.:
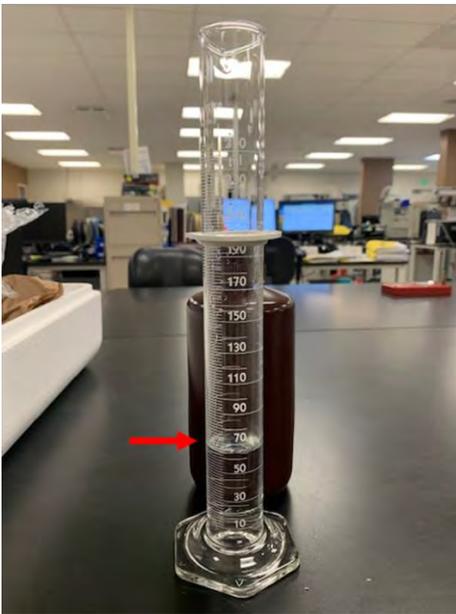
Bottle #1 – 60 mL Bottle #2 – 180 mL Bottle #3 – ~300 mL

5. **Conclusion:**

The current cooler packing design will keep all samples in the coolers frozen for the planned shipping duration of 24 hrs. and if the samples are shipped within the originally scheduled proposed timeline. The coolers will keep the samples at least half frozen and below 0°C for a duration of 120 hrs. (under the conditions of test). If it is decided

that the samples need to be received completely frozen and/or additional shipping conditions or if the distribution schedule is adjusted to a time when the ambient temperature may be significantly higher, further testing may be needed.

Appendix:

ERA
A Waters Company

HGL/DOD PFAS Cooler Temperature Study Log

Cooler ID	Temp. Check @	Start Date/Time of Cooler Study	Initial Temp. Check*	Scheduled Date/Time to be Checked	Bottle #1 Temp.	Bottle #2 Temp.	Bottle #3 Temp.	IR Thermometer ID	Ambient Temp.	Ambient Thermometer ID	Initials	Date/Time Checked	Comment
Cooler #1	12 hr.	1/10/2022 16:40	-19°C	1/11/2022 5:30	-1.9°C	-3.1°C	-2.6°C	TH-118	21.1°C	TH-65	ES	1/11/2020 5:30	Samples completely frozen.
Cooler #2	24 hr.	1/10/2022 16:40	-14°C	1/11/2022 17:30	-1.6°C	-1.8°C	-1.6°C	TH-118	21.1°C	TH-65	AJC	1/11/2022 16:40	Samples completely frozen.
Cooler #3	36 hr.	1/10/2022 16:50	-18°C	1/12/2022 5:30	-1.4°C	-2.3°C	-2.4°C	TH-118	20.8°C	TH-65	ES	1/12/2022 5:30	Little thawed water noticeable ~10 mL.
Cooler #4	48 hr.	1/10/2022 17:00	-19°C	1/12/2022 17:30	-1.4°C	-1.8°C	-1.9°C	TH-118	21.3°C	TH-65	MG	1/12/2022 16:50	N/A
Cooler #5	72 hr.	1/10/2022 17:00	-19°C	1/13/2022 17:30	-1.0°C	-1.6°C	-1.1°C	TH-118	21.1°C	TH-65	AJC	1/13/2022 16:35	Samples 3/4 frozen, 1/4 thawed.
Cooler #6	96 hr.	1/14/2022 10:30	-16°C	1/18/2022 10:30	-0.9°C	-1.6°C	-0.5°C	TH-118	21.1°C	TH-65	BPM	1/18/2022 10:32	Bottle #2 middle cooler location ~20mL thawed, outside bottle 1 & 3 ~3/4 frozen, ~1/4 thawed.
Cooler #7	120 hr.	1/14/2022 10:30	-17°C	1/19/2022 10:30	-0.9°C	-0.6°C	-0.4°C	TH-118	20.0°C	TH-65	BPM	1/19/2022 10:35	Bottle #1 middle cooler location 60mL thawed, thawed. Bottle #2 outside location 180 mL thawed and bottle #3 outside location ~300mL thawed.

Mark bottles, randomize marked bottles in coolers.

All coolers stored under micro benches in the lab.

* Initial Temperature Check performed using IR Thermometer ID : TH-118. All on Bottle #1

Initial ambient Temp = 20.9°C

Appendix B

Preparation of PFAS-Spiked Samples

Content Table

1   PURPOSE

1.1   The purpose of this procedure/work instruction is to supply samples for a characterization determination and a Multi-Laboratory Validation Study for per-and polyfluoroalkyl substances (PFAS).

2   SCOPE

2.1   The scope of this procedure is to prepare the defined sample matrices for characterization testing and then spiking the prepared matrices with known amounts of PFAS analytes for a Multi-Laboratory Validation Study for analytical Method 1633 – October 2021.

2.1.1   Matrix Characterization for PFAS and Chemical Characteristics:

Sample Chart 1 - PFAS Characterization Samples

Matrix	Matrix Type	Number Matrix Types	Number of Unspiked Samples	Total Number of Samples Shipping/ Matrix Type	Volume/Mass to be provided for each Sample
Water	Wastewater	9 [1]	3	27	550 mL
	Groundwater	3	3	9	550 mL
	Surface Water	4 [2]	3	12	500 mL
	Landfill Leachate	3	3	9	125 mL
Soil/Sediment	Soil	8 [3]	3	24	10.0 g
	Sediment	3	3	9	10.0 g
	Biosolids	3	3	9	1.00 g
Tissue	Fish/Clams [4]	3	3	9	10.0 g
Total Number				108	

Footnotes:
[1] Eight wastewaters were received by ERA and ERA will manufacture one substitute wastewater following ASTM method

D5905-98 (Reapproved 2018).

[2] Four different surface water lots were received by ERA.

[3] Eight different soil lots were received by ERA. One received soil lot does not contain enough sample to supply all samples for the study.

[4] Two fish filets (salmon and walleye) with skin were received and un-shucked clams.

Sample Chart 2 - Chemical Characteristics Testing

Analyte	Analytical Method	Container	Preservation	Minimum Sample Volume/Mass	Number of Lots [1]	Number of Unspiked Samples per Lot	Holding Time
Aqueous Samples							
Alkalinity (total, carbonate and bicarbonate)	SM 2320B	250 mL HDPE	≤ 6°C	100 ml	19	1	14 days
Ammonia	EPA 350.1	250 mL Amber Glass	≤ 6°C, H_2SO_4	250 ml	19	1	28 days
Calcium, sodium	SW 6010C	250 mL HDPE	≤ 6°C, HNO_3	50 ml	19	1	6 months
Chloride, sulfate	SW 9056A	250 mL HDPE	≤ 6°C	100 ml	19	1	28 days
Conductivity	SW 9050A	250 mL HDPE	≤ 6°C	50 ml	19	1	28 days
Oil and grease	EPA 1664B	1000 mL Amber Glass	≤ 6°C, H_2SO_4	1 L	19	1	28 days
pH	SW 9040C	250 mL HDPE	≤ 6°C	100 ml	19	1	Immediately
Total dissolved solids	SM 2540C	250 mL HDPE	≤ 6°C	100 ml	19	1	7 days
Total suspended solids	SM 2540D	250 mL HDPE	≤ 6°C	100 ml	19	1	7 days
Soil and Sediment Samples							
Grain Size	ASTM D422	16 oz Plastic or Glass Jar	None	500 g	11	1	Not defined
Moisture	ASTM D2216	4 oz Glass Jars	≤ 6°C	20 g	11	1	1 year
pH	SW 9045D	4 oz Glass Jars	≤ 6°C	50 g	11	1	Immediately
Salinity *(sediment only)*	SM 2520B	4 oz Glass Jars	≤ 6°C	50 g	3	1	6 months
Total Organic Carbon	SW 9060A	4 oz Glass Jars	≤ 6°C	10 g	11	1	28 days
Tissue							
Lipids	SM 2540B	4 oz Glass Jar	≤ 6°C	20 g	3	1	1 year
Biosolids							
pH	SW 9045D	4 oz Glass Jar	≤ 6°C	50 g	3	1	Immediately
Total Number of Samples Shipping: 188							

Notes:

EPA Methods - USEPA Methods for Chemical Analysis of Water and Wastes (MCAWW) USEPA/600/4-79-020, Revised March 1983.

SW Methods - USEPA Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, Third Edition, 1998.

SM Methods - Standards Methods for the Examination of Water and Wastewater, Revised 2018

ASTM Methods - ASTM International, Revised 2019

Footnotes:

[1] Refer to Sample Chart 1 for further information regarding Matrix Type.

2.1.1.1   Samples listed in Sample Chart 1 will be sent to SGS AXYS for PFAS characterization testing and samples listed in Sample Chart 2 will be sent to Eurofins TestAmerica Denver for sample chemical characteristics testing.

2.1.2   Multiple Laboratory Validation Study Samples:

2.1.2.1   The chart below lists the matrix types, spiking levels and sample quantities required for the Multiple Laboratory Validation testing.

Multiple Laboratory Validation Study Sample Chart

Matrix	Matrix Type	Number Matrix Types	Number of Unspiked Samples/ Matrix Type	Number of Replicates Spiked at Low Level[1]/ Matrix Type	Number of Replicates Spiked at High Level[1,2]/ Matrix Type	Total Number of Samples Shipping/ Matrix Type	Spiked Volume/Mass to be provided for each Sample
Water	Wastewater	7	1	3	3	49	500 mL[3]
	Groundwater	3	1	3	3	21	500 mL[3]
	Surface Water	3	1	3	3	21	500 mL[3]
	Landfill Leachate	3	1	3	3	21	100 mL[3]
					3	21	5.00 g[3]
	Sediment	3	1	3	3	21	5.00 g[3]
	Biosolids	3	1	3	3	21	0.500 g[3]
Tissue	Fish/Clams	3	1	3	3	21	2.00 g[3]
Total Number						**205**	

Footnotes:

[1] Low and High levels for spiking defined in analytical method "Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS", October 2021.

[2] Except for FTSs spiked at 1.5 times low level.

[3] Labs will be instructed to use the entire sample contents for analysis and assume the volume or mass listed.

2.1.2.2   Samples listed in the Multiple Laboratory Validation Study Sample Chart will be sent to the labs that were selected for the Multiple Laboratory Validation Study.

2.1.2.3   ERA will prepare 5 extra sets of prepped samples for use as: replacement samples, future testing, and/or other use by the client.

2.1.3   Analyte lists as determined from the analytical Method 1633 – October 2021 (as referenced above) will be confirmed by the SERDP/ESTCP team. New analyte addition(s) to be reviewed and approved by ERA Technical department.

2.1.4   Final product concentrations as determined from the analytical Method 1633 – October 2021 (as referenced above) will be confirmed by the SERDP/ESTCP team.

3   DEFINITIONS AND ABBREVIATIONS

3.1   PFAS - Per-and Polyfluoroalkyl Substances

3.2   GHS - Globally Harmonized System

3.3   HDPE - High Density Polyethylene

3.4   PWS - Performance Work Statement

3.5   NIST - National Institute of Standards & Technology

3.6   CofA - Certificate of Analysis

3.7   DWBSS - Dry Weight Basis Sample Size is the state of the soil without the presence of water.

4   ROLES AND RESPONSIBILITIES

4.1   Technical Manager

4.1.1   Responsible for the project evaluation and project scope feasibility.

4.1.2   Documentation review.

4.1.3   Providing technical assistants for the steps described in this Work Procedure.

4.2   Product Line Manager

4.2.1   Responsible for the overall project management and task scheduling of the project.

4.2.2   Documentation review.

4.2.3   Overseeing project progress for the steps described in this Work Procedure.

4.3   Chemist/Chemistry Technician

4.3.1   Responsible for performing the sample homogenizing and packaging.

4.3.2   Manufacturing PFAS concentrates and verification analysis of concentrates.

4.3.3   Perform sample spiking.

4.3.4   Preparing samples and coolers for shipping.

4.4   Production Technician

4.4.1   Responsible for performing the sample homogenizing and packaging.

4.4.2   Preparing samples and coolers for shipping.

5   REFERENCES / RELATED DOCUMENTS

5.1   The listed ERA ISO 17025 Accredited Work Procedures in the table below are associated with this procedure.

Doc No:	Document Title
730002414	Environmental Product Packaging and Labeling
730002350	Organic Liquid Standards Preparation
730002570	Semi-volatile Analytical Verification by High Performance Liquid Chromatography
730002497	Analytical Verification of Grease and Oil in Water Samples
730002520	Analytical Verification by ICP-OES
730002523	Analytical Verification of Anions, Perchlorate and DBPs by Dionex Ion Chromatography
730002515	Analytical Verification of Total Alkalinity in Water Samples
730002510	Analytical Verification of Ammonia in Water and Soil Samples
730002501	Analytical Verification of pH in Water and Soil Samples
730002508	Analytical Verification of Specific Conductance in Water and Soil Samples
730002492	Analytical Verification of Total Dissolved Solids in Water Samples
730002493	Analytical Verification of Total Suspended Solids (TSS) in Water Samples
730002434	Reagents Manufacturing
730002412	Environmental Product Manual Certificate of Analysis Generation

730002425	Whole Volume Cooler Shipping
730002254	Control of Quality Records

6   FORMS

6.1   Records

6.1.1   All records associated with this procedure will be retained by Quality according to OP 730002254 Control of Quality Records.

7   HEALTH & SAFETY

7.1   GHS Evaluation

7.1.1   ERA will perform GHS (Globally Harmonized System) evaluation as needed for product shipments.

8   EQUIPMENT / MATERIALS

8.1   Mixing Equipment:

8.1.1   30-gal Blue HDPE Open Top Drum

8.1.2   Stainless Steel Mixer

8.1.3   Rotational Mixer

8.1.4   Food Processor

8.1.5   Mixing Bins – for solids/tissues

8.2   ERA will procure other required (non-chemical) materials. Quantities will be based upon number of laboratories and samples. Items will include but are not limited to:

8.2.1   1000 mL amber HDPE Narrow Mouth Bottles - Thermo Scientific Part# 03-313-8F

8.2.2   1000 mL Amber Glass Bottles – ESS Part# 1000-0150-PC

8.2.3   250 mL HDPE Narrow Mouth Bottles - Thermo Scientific Part# 312002-0016

8.2.4   250 mL Amber Glass Bottles – ESS Part# 0250-0150-PC

8.2.5   125 mL HDPE Narrow Mouth Bottles - Thermo Scientific Part# 312089-0004

8.2.6   4 oz Glass Jars – Berlin Part# - GLA-00869

8.2.7   24 mL clear glass screw top vial – Berlin Packaging Part# 293339, Cap Part# CAP-00145

8.2.8   12 oz Blue Gel Ice Packs - Katzke Paper Part# MM-PP12

8.2.9   Large Styrofoam Coolers - Katzke Paper Part# CP211312C

8.2.10   Medium Coolers - Katzke Paper Part# CP121213

8.2.11   Reusable Matrix Coolers

8.2.12   Packaging Material

8.3   ERA will work with Client to source high level PFAS starting materials from an ERA approved vendor. ERA will consult Client with any availability or lead time concerns for vendor supplied starting materials.

8.4   ERA will procure PFAS starting materials. Lead time will be determined by vendor.

8.5   Client Supplied Matrices for preparation of PFAS spiking:

    8.5.1   8 - Client supplied Wastewater Matrix Lots, 1 ASTM manufactured by ERA, 7 lots will be chosen for the study

    8.5.2   3 - Client supplied Leachate matrix lots all 3 will used for the study.

    8.5.3   4 - Client supplied Surface Water matrix lots, 3 will be chosen for the study.

    8.5.4   3 - Client supplied Ground Water matrix lots all 3 will used for the study.

    8.5.5   8 - Client supplied Soil lots, 3 will be chosen for the study.

    8.5.6   3 - Client supplied Sediment lots all 3 will used for the study.

    8.5.7   3 - Client supplied Biosolids lots all 3 will used for the study.

    8.5.8   3 - Client supplied Fish/Clam Tissue lots all 3 will used for the study.

9   PROCEDURE / INSTRUCTION

9.1   Matrix Check-in & Preparation Process:

    9.1.1   Check-In - When each matrix is received containers will be:

        9.1.1.1   Labelled:

            9.1.1.1.1   With matrix type

            9.1.1.1.2   Source ID from clients Chain of Custody (When Provided)

            9.1.1.1.3   Container #

        9.1.1.2   Login all matrices into an electronic matrix log for record keeping & organization. File is available to client. File Location - K:Customs-WholeVolumes\Hydrogeologic PFAS Round Robin 2020\Matrix Log.

        9.1.1.3   Store labelled and logged in matrices in a walk-in refrigerator. Fish/clam tissues are stored in a freezer until needed for preparation.

        9.1.1.4   Client will be notified when each matrix has been received.

    9.1.2   Matrix Preparation & Homogenizing:

        9.1.2.1   Prior to the homogenization and final packaging of the matrices, composite samples will be prepared for each matrix lot by taking subsamples from each homogenized container and combining.

            9.1.2.1.1   The unspiked composite samples of each matrix lot will be sent to SGS Axys (PFAS) and Eurofins TestAmerica Denver (characteristic parameters) for background analysis.

                9.1.2.1.1.1   HGL will advise ERA of any dilutions or adjustments required to any of the matrices.

                9.1.2.1.1.2   Any required dilutions will be made using approximate volumes.

9.1.2.1.2   Any matrix adjustment (to inorganic background parameters) will be verified in ERA's laboratory.

9.1.2.2   Aqueous Matrices:

9.1.2.2.1   All samples for each individual aqueous matrix will be combined and homogenized in a pre-cleaned and sealable 30-gal blue HDPE open top drum with a powered stainless-steel mixer.

9.1.2.2.2   Each aqueous matrix will mix for 1hr and be stored in a labeled 30-gal blue HDPE sealable container until the specific aqueous matrices for the study have been selected by the SERDP/ESTCP team.

9.1.2.2.3   Once the specific aqueous matrices are selected, they will be packaged in 1000 mL amber HDPE bottles for the Waste, Surface & Ground waters, 125 mL HDPE bottles for the Leachate, while mixing.

9.1.2.2.4   Wastewater, Surface Water & Ground Water bottles will be filled to a nominal fill of 500 mL in a 1000 mL amber HDPE bottle and Leachate bottles will be filled to a nominal fill of 100 mL in a 125 mL HDPE bottle.

9.1.2.2.5   The labs will be directed in the sample instructions to use the entire contents of the sample and assume 500 mL for Waste, Surface & Ground waters and 100 mL for Leachate Waters.

9.1.2.2.6   The packaging sequence for each lot will be recorded for reference.

9.1.2.2.7   Label each bottle with an adhesive label listing matrix type and lot number, that is listed in the Appendices section under the Sample Labeling Scheme.

9.1.2.2.8   Filled bottles will then be stored in a walk-in refrigerator until the spiking event.

9.1.2.2.9   The steps above will be repeated for each aqueous matrix.

9.1.2.3   Soil/Sediment/Biosolids:

9.1.2.3.1   All sample containers for each Soil, Sediment and Biosolid matrix will be combined and homogenized in pre-cleaned sealable containers. Sediment samples containing standing liquid will be decanted prior to homogenizing.

9.1.2.3.2   Each solid matrix in the bulk container will be mixed until thoroughly homogenized for at least 1hr in a rotational mixer.

9.1.2.3.3   All soil matrices will be tested for the percent moisture content and recorded.

9.1.2.3.4   Each soil matrix will be stored in a bulk labeled sealable container until the specific matrices for the study have been selected by the SERDP/ESTCP team.

9.1.2.3.5   The labs will be directed in the sample instructions for the soil, sediment and biosolid matrices to use the entire contents of the sample and rinse the vial out to remove any PFAS material that may adhere to the vial walls. The labs will also be directed in the instructions to assume 5.0 g dry weight basis for soil/sediment samples and 0.5 g dry weight basis for the biosolids is packaging in the vial. Instructions will also include that moisture content and moisture correction will not be necessary by the labs.

9.1.2.3.6   When the specific matrices are chosen, 5 g dry weight basis ± 0.5 g soil and sediment sample sizes will be packaged in 24 mL clear glass screw top vials. The dry weight basis sample size is the state of the soil without the presence of water.

9.1.2.3.7   0.5 g dry weight basis ± 0.05 g sample size for each biosolid matrix will be packaged in 24 mL clear glass screw top vials.

9.1.2.3.8   Percent Moisture Content (MC) and Dry Weight Basis Sample Size (DWBSS) will be calculated by ERA as follows.

% Moisture Content:

$$MC = \frac{M - D}{D} \times 100$$

MC = % Moisture Content
M = Weight of Moist Soil
D = Weight of Dry Soil

Dry Weight Basis Sample Size:

$$DWBSS = \left(D \times \left(\frac{MC}{100} \right) \right) + D$$

DWBSS = Dry Weight Sample Size
MC = % Moisture Content
D = Weight of Dry Soil

9.1.2.3.9   The packaging sequence for each lot will be recorded for reference.

9.1.2.3.10   Label each vial with an adhesive label listing matrix type and lot number, that is listed in the Appendices section under the Sample Labeling Scheme.

9.1.2.3.11   Filled vials will then be stored in a walk-in refrigerator until spiking and shipping.

9.1.2.3.12   Steps above will be repeated for each solid matrix.

9.1.2.4   Fish/Clam Tissue:

9.1.2.4.1   Each tissue will be homogenized separately in a pre-cleaned bin.

9.1.2.4.2   Fish tissue including skin and shucked clams will be processed with a food processer until a smooth paste consistency is achieved. Due to the amount of the tissue and capacity of the food processor, the tissue may need to be processed in separate batches and combined in the pre-cleaned bin.

9.1.2.4.3   After the paste consistency is achieved each tissue will be mixed until thoroughly homogenized for at least 1hr in a pre-cleaned bin. 2 g ± 0.5 g fish/clam tissue sample size will be packaged in 24 mL clear glass screw top vials.

9.1.2.4.4   The packaging sequence for each lot will be recorded for reference.

9.1.2.4.5   Label each vial with an adhesive label listing matrix type and lot number, that is listed in the Appendices section under the Sample Labeling Scheme.

9.1.2.4.6   Filled vials will then be stored in a freezer until spiking and shipping.

9.1.2.4.7   Labs will be directed in the tissue sample instructions to use the entire contents of the sample and rinse the vial out to remove any PFAS material that may adhere to the vial walls. The labs will also be directed in the sample instructions to assume 2.0 g for each tissue sample is packaging in the vial.

9.1.2.4.8   Steps above will be repeated for each fish/clam tissue matrix.

9.2   **Matrix Characteristics Testing:**

9.2.1   All aqueous, soil, sediments, and tissue matrices will be sent to Eurofins TestAmerica Denver for sample chemical characteristics testing.

9.2.2   Reference Sample Chart 2 in section 2.1.1 for a summary of sample requirements for the chemical characteristic analysis for each matrix. Detailed are the testing parameters, testing method, packaging container, sample preservative, volume/mass and holding times.

9.2.3   After external characteristic testing is completed, data will be reviewed by the Method Validation Team and they will determine if any adjustments are needed to any of the sample matrices.

9.2.4   If any adjusting is required, ERA will adjust the determined lot to meet any outstanding criteria. Adjustments will be analytically verified by ERA using the ERA work procedures listed in section 5.1 of this work procedure.

9.3   **PFAS Spiking Concentrate Manufacturing & Verification Process:**

9.3.1   ERA will manufacture an estimate of 4 unique mixed PFAS spiking concentrates, depending on analyte concentrations using gravimetric balances and volumetric glassware traced to NIST weights following ERA ISO 17025 Accredited Work Instruction 730002350 (Organic Liquid Standards Preparation).

9.3.2   The 4 mixed spiking concentrates will consist of 2 mixed spiking concentrate solutions for the aqueous matrices and 2 mixed spiking concentrate solutions for the solid matrices will be used to manufacture all final products. See "MARTIX SPIKING LEVELS" in Appendices section of this procedure for spiking levels.

9.3.3   New analyte addition(s) may affect the total number of spiking concentrates and/or the originally planned concentrations. PFAS analytes, concentrations, and levels will be approved by the client prior to manufacturing commencement. Depending on background PFAS levels more spiking concentrates maybe be required.

9.3.4   ERA will analytically verify the 4 PFAS spiking concentrates by LC/MS/MS following ERA ISO 17025 Accredited Work Procedure 730002570 (Semi-volatile Analytical Verification by High Performance Liquid Chromatography). A minimum of n=5 for each concentration will be verified.

9.3.5   Method development will be performed as needed for any new analyte addition(s).

9.4   **Sample PFAS Spiking Process:**

9.4.1   Sample Spiking Organization:

9.4.1.1   Sample spiking, freezing and packing timeline will be determined by the SERDP/ESTCP team and communicated to ERA.

9.4.1.2   Whole volume spiking bench sheets will be created and reviewed prior to shipment.

9.4.1.2.1   Two spiking concentrates will be used to spike all aqueous final whole volume products at varying levels.

9.4.1.2.2   Two spiking concentrates will be used to spike all solid/tissue final whole volume products at varying levels.

9.4.1.3   Sample Organization:

9.4.1.3.1   The designated samples for each week will be pulled from refrigeration/freezer on the day the of the spiking.

9.4.1.3.2   Samples will be organized according to the spiking levels.

9.4.1.3.3   Spiking concentrations will be pulled from refrigeration storage and allowed to reach ambient temperature prior to spiking samples.

9.4.1.4   Sample Spiking:

9.4.1.4.1   Once samples and spiking concentrates reach room temperature they will be mixed/vortexed prior to being opened and aliquots withdrawn. They will be organized on physically separated work benches according to spiking levels.

9.4.1.4.2   A validated repeating pipettor delivery system will be used to deliver the aliquots on to the samples.

9.4.1.4.3   Bench sheets will list the amount of each spiking concentrate to deliver to the specific sample. Spiking aliquots will be determined upon determination of final concentrations.

9.4.1.4.4   Detailed records of the spiking events will be recorded and a peer witness review of samples, spiking concentrates and pipettor will be performed prior to and during actual spiking event.

9.4.1.4.5   Once the designated aliquot of spiking concentrate has been delivered, each sample will be sealed and placed in a designated completed area to avoid double spiking.

9.4.1.4.6   A peer witness review of samples, spiking concentrates and pipettor will be performed during the actual spiking event and documented.

9.4.1.4.7   Aqueous sample bottles will be inverted to homogenize and stored in the walk-in freezer until they are scheduled to ship. Soil/Sediments/Biosolids and fish tissue will be stored in a walk-in refrigerator until they are scheduled to ship.

9.4.1.4.8   Samples will be spiked in advanced by the Friday prior to the scheduled shipping week and then ship overnight by Wednesday to give ample time for samples to freeze.

9.5   **Sample Shipping Process:**

9.5.1   Order confirmations for shipments will be setup by ERA in consultation with client.

9.5.2   Sample shipping will follow the determined schedule by the SERDP/ESTCP team and will be communicated to ERA.

9.5.3   Frozen Blue Ice Gel packs and packing material will be used with each shipment to chill and protect samples.

9.5.4   Shipment Preparation:

9.5.4.1   Cooler shipments will be prepared following ERA work instruction 730002425 (Whole Volume Cooler Shipping). Each order will include the following listed below.

9.5.4.1.1   A set number of coolers will be determined by matrix type.

9.5.4.1.2   The samples that are prescheduled for each shipping event.

9.5.4.1.3   Temperature Blank

9.5.4.1.4   Completed chain of custody form.

9.5.4.1.5   Sample Preparation Instructions

9.5.4.1.6   Blue Gel Ice Packs

9.5.4.1.7   A 4x4 label applied to outside of cooler box stating, "Upon arrival, contact (LAB POC) immediately."

9.5.4.2   Laboratory contacts and client personnel will be notified by email of shipment and supplied with shipment tracking numbers.

9.6   **Certificate of Analysis and Sample Instruction Generation:**

9.6.1   CofA's for each spiked level matrix will be created using ERA ISO 17025 Accredited Work Instruction 730002412 (Environmental Product Manual Certificate of Analysis Generation).

9.6.2   Sample instructions for each matrix type will generated.

9.6.3   CofA's & Sample instructions content will be discussed and mutually agreed upon with client.

9.6.4   CofA's & Sample instructions will be distributed to personnel designated by the client.

10   APPENDICES / ATTACHMENTS

SAMPLE LABELING SCHEME

Matrix Type	Requested Name	Description	Matrix Code	Sample Identifier	Characterization Sample Lot#	Spiked Study Sample Lot Numbers						
						Unspiked	Low Replicate 1	Low Replicate 2	Low Replicate 3	High Replicate 1	High Replicate 2	High Replicate 3
Ground Water	USACE	GW #1, Midwest	GW	A	GWA0	GWA1	GWA2	GWA3	GWA4	GWA5	GWA6	GWA7
Ground Water	LRPCD	GW #2, Southwest	GW	B	GWB0	GWB1	GWB2	GWB3	GWB4	GWB5	GWB6	GWB7
Ground Water	USACE	GW #13	GW	C	GWC0	GWC1	GWC2	GWC3	GWC4	GWC5	GWC6	GWC7
Surface Water	Lake Harsha, OH	SW OH 9/10	SW	D	SWD0	SWD1	SWD2	SWD3	SWD4	SWD5	SWD6	SWD7
Surface Water	Norwell, MA	SW MA 9/24	SW	E	SWE0	SWE1	SWE2	SWE3	SWE4	SWE5	SWE6	SWE7
Surface Water	Burley Creek, WA	Burley Creek	SW	F	SWF0	SWF1	SWF2	SWF3	SWF4	SWF5	SWF6	SWF7
Surface Water	Sequim Bay, WA	Sequim Seawater	SW	G	SWG0	SWG1	SWG2	SWG3	SWG4	SWG5	SWG6	SWG7
Waste Water	Metal Finisher	Metal Finisher	WW	H	WWH0	WWH1	WWH2	WWH3	WWH4	WWH5	WWH6	WWH7
Waste Water	Hospital	Hospital	WW	I	WWI0	WWI1	WWI2	WWI3	WWI4	WWI5	WWI6	WWI7
Waste Water	POTW Influent	POTW Influent	WW	J	WWJ0	WWJ1	WWJ2	WWJ3	WWJ4	WWJ5	WWJ6	WWJ7
Waste Water	ASTM Substitute	ASTM Substitute	WW	K	WWK0	WWK1	WWK2	WWK3	WWK4	WWK5	WWK6	WWK7
Waste Water	WW Bus Washing Station	WW Bus Wash	WW	L	WWL0	WWL1	WWL2	WWL3	WWL4	WWL5	WWL6	WWL7
Waste Water	Playa Del Ray, CA	Plant Effluent	WW	M	WWM0	WWM1	WWM2	WWM3	WWM4	WWM5	WWM6	WWM7
Waste Water	P&P WW	#1- 28	WW	N	WWN0	WWN1	WWN2	WWN3	WWN4	WWN5	WWN6	WWN7
Waste Water	POTW Effluent	POTW Effluent	WW	O	WWO0	WWO1	WWO2	WWO3	WWO4	WWO5	WWO6	WWO7
Waste Water[1]	Dairy Wastewater[1]	Dairy Wastewater	WW	P	WWP0[1]	WWP1	WWP2	WWP3	WWP4	WWP5	WWP6	WWP7
Soil	Musselshell, Clark Co. MT	AA (2016-106), L32547-2	SS	R	SSR0	SSR1	SSR2	SSR3	SSR4	SSR5	SSR6	SSR7
Soil	Ivy, Cashe Co. UT	BB (2017-111), L32547-3	SS	S	SSS0	SSS1	SSS2	SSS3	SSS4	SSS5	SSS6	SSS7
Soil	Fruitland, San Juan Co. NM	CC (2018-105), L32547-4	SS	T	SST0	SST1	SST2	SST3	SST4	SST5	SST6	SST7
Soil	Armijo, Dona Ana Co. NM	DD (2018-116), L32547-5	SS	U	SSU0	SSU1	SSU2	SSU3	SSU4	SSU5	SSU6	SSU7
Soil	Drummer, Dekalb Co. IL	EE (2019-107), L32547-6	SS	V	SSV0	SSV1	SSV2	SSV3	SSV4	SSV5	SSV6	SSV7
Soil	Brock, Wheatley Co. TN	FF (2019-110), L32547-7	SS	W	SSW0	SSW1	SSW2	SSW3	SSW4	SSW5	SSW6	SSW7
Soil	Delhi, Fresno County. CA	2014-107	SS	X	SSX0	SSX1	SSX2	SSX3	SSX4	SSX5	SSX6	SSX7
Sediment	Burley 1 Sed. Burley Creek, WA	Burley 1 Sed.	SD	Y	SDY0	SDY1	SDY2	SDY3	SDY4	SDY5	SDY6	SDY7
Sediment	Burley 2 Sed. Burley Creek, WA	Burley 2 Sed.	SD	Z	SDZ0	SDZ1	SDZ2	SDZ3	SDZ4	SDZ5	SDZ6	SDZ7
Sediment	Sequim Bay Sediment	Sequim Bay Sediment	SD	AA	SDAA0	SDAA1	SDAA2	SDAA3	SDAA4	SDAA5	SDAA6	SDAA7

Fish Tissue	Walleye (low lipid fish)	Walleye	TS	AB	TSAB0	TSAB1	TSAB2	TSAB3	TSAB4	TSAB5	TSAB6	TSAB7
Fish Tissue	Salmon (high lipid fish)	Salmon	TS	AC	TSAC0	TSAC1	TSAC2	TSAC3	TSAC4	TSAC5	TSAC6	TSAC7
Fish Tissue	Clams	Clams	TS	AD	TSAD0	TSAD1	TSAD2	TSAD3	TSAD4	TSAD5	TSAD6	TSAD7
Leachate	MSW LF Leachate Sample	MSW LF Leachate Sample	LC	AE	LCAE0	LCAE1	LCAE2	LCAE3	LCAE4	LCAE5	LCAE6	LCAE7
Leachate	CDD Landfill	CDD	LC	AF	LCAF0	LCAF1	LCAF2	LCAF3	LCAF4	LCAF5	LCAF6	LCAF7
Leachate	Ash leachate	Ash leachate	LC	AG	LCAG0	LCAG1	LCAG2	LCAG3	LCAG4	LCAG5	LCAG6	LCAG7
Biosolids	Playa Del Ray, CA	Wetcake	BS	AH	BSAH0	BSAH1	BSAH2	BSAH3	BSAH4	BSAH5	BSAH6	BSAH7
Biosolids	Biosolids #1 East	Biosolids #1 East	BS	AI	BSAI0	BSAI1	BSAI2	BSAI3	BSAI4	BSAI5	BSAI6	BSAI7
Biosolids	South Plant Biosolids	South Plant Biosolids	BS	AJ	BSAJ0	BSAJ1	BSAJ2	BSAJ3	BSAJ4	BSAJ5	BSAJ6	BSAJ7

[1] Dairy Wastewater determined to not be fit for the study and will not be used.

Matrices highlighted in yellow above are to be excluded from MLV study.

Template 730000483 V08

It is the responsibility of the user to verify they are using the current released version.
HARD COPY UNCONTROLLED UNLESS STAMPED, SIGNED AND DATED BY AUTHORISED PERSONNEL

MATRIX SPIKING LEVELS

PFAS Compound	Wastewater, Ground Water and Surface Water Low Spike[1] (ng/L)	High Spike[1] (ng/L)	Leachates Low Spike[1] (ng/L)	High Spike[1] (ng/L)	Soils, Sediments, Biosolids and Tissues Low Spike[1] (ng/Kg)	High Spike[1] (ng/Kg)
PFBA	80	400				
PFPEA	40	200				
PFHXA	20	100				
PFHPA	20	100				
PFOA	20	100				
PFNA	20	100				
PFDA	20	100				
PFUNA	20	100				
PFDOA	20	100				
PFTRDA	20	100				
PFTEDA	20	100				
PFBS	20	100				
PFPES	20	100				
PFHXS	20	100				
PFHPS	20	100				
PFOS	20	100				
PFNS	20	100				
PFDS	20	100				
PFDOS	20	100				
4:2FTS	80	240				
6:2FTS	80	240				
8:2FTS	80	240				
PFOSA	20	100				
NMeFOSA	20	100				
NEtFOSA	20	100				
NMeFOSAA	20	100				
NEtFOSAA	20	100				
NMeFOSE	160	400				
NEtFOSE	160	400				
HFPO-DA	80	240				
ADONA	80	240				
9CL-PF3ONS	80	240				
11CL-PF3OUDS	80	240				
3:3FTCA	80	400				
5:3FTCA	120	2000				
7:3FTCA	120	2000				
PFEESA	40	200				
PFMPA	40	200				
PFMBA	40	200				
NFDHA	40	200				
All spike concentrations are presented as acid concentrations; not salts						

PFAS Method Validation Study:

Wastewater Sample Preparation Guidelines

Shipment Contents

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

Sample Description

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

Before You Begin

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

Sample Instructions

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

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

PFAS Method Validation Study:

Surface & Ground Water Sample Preparation Guidelines

Shipment Contents

- (4) 25"x15.5"x17" Styrofoam box coolers
- (3) Surface Waters Lots
- (3) Ground Water Lots
- (42) 1L amber HDPE bottles
- Temperature blank
- Sample Preparation Guidelines
- Sample Chain of Custody (COC)

Sample Description

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

Before You Begin

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

Sample Instructions

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

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

Appendix C

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

Data Management Summary Report
for the
Multi-Laboratory Validation Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids,
and Tissue Samples by LC-MS/MS

DRAFT

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July 19, 2023

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LIST OF ACRONYMS AND ABBREVIATIONS

AFFF   Aqueous Film-Forming Foams
ASTM   American Society for Testing and Materials
DoD   US Department of Defense
DFARS   Defense Federal Acquisition Regulations Supplement
DMP   Data Management Plan
DP   Data Package
DVR   Data Validation Report
GDIT   General Dynamics Information Technology
EDD   electronic data deliverable
EIS   extracted internal standard
EPA   US Environmental Protection Agency
ESTCP   Environmental Security Technology Certification Program
GCC   Government Community Cloud
HGL   HydroGeoLogic, Inc.
IDA   Institute for Defense Analysis
IDC   initial demonstrations of capability
IPR   initial precision and recovery
ITAR   International Traffic in Arms Regulations
LC-MS/MS   Liquid Chromatography/Tandem Mass Spectrometry
MLV   Multi-Laboratory Validation
MVS Team   Method Validation Study Team
NAVSEA   Naval Sea Systems Command
NIS   Non-extracted internal standard
PFAS   per- and polyfluoroalkyl substances
QA   quality assurance
QC   quality control
SDG   sample delivery group
SEE   Science and Engineering for the Environment
SERDP   Strategic Environmental Research and Development Program
SLV   Single-Laboratory Validation
SOP   Standard Operating Procedures
USACE   US Army Corps of Engineers
VBA   Visual Basic for Applications

1.0 INTRODUCTION

This document describes data management processes and procedures for the Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. EPA Method 1633 is an interim draft method for analyzing per- and polyfluoroalkyl substances (PFAS), and now requires a Multiple-Laboratory Validation (MLV) Study. A Data Management Plan (DMP; SERDP/ESCTP 2023, Attachment 4) was generated at the outset of the project to describe the design of the data management system which outlined the processes and procedures intended to be used for the transmission, tracking, verification, review, storage, and delivery of laboratory data and associated validation and analyses data collected in support of the MLV Study. During the course of the project, the intended design of certain elements of the data management system were adjusted; the processes and procedures actually used during the project are documented in this report.

To meet study requirements for the acquisition of technically sound and legally admissible data, a traceable audit trail was established from the shipment of sample matrices to each participating laboratory through the archiving of information and data. Each step was conducted in accordance with the MLV Study Work Plan (SERDP/ESTCP 2023). All potential variations in the analytical and reporting process were documented and retained with other laboratory data and digital information generated during the MLV Study.

1.1 Background

The MLV Study was conducted by the US Department of Defense's (DoD) Strategic Environmental Research and Development Program (SERDP) in cooperation with the US Environmental Protection Agency (EPA), the US Navy (Navy), the US Air Force (Air Force), and the US Army Corps of Engineers (USACE). Members from each of these agencies comprise the advisory Method Validation Study (MVS) Team. The study is being conducted as SERDP Project ER19-1409. The end goal of the MLV Study is to use the findings to revise, as necessary, draft Method 1633, and to submit the supporting data packages to the EPA Office of Water for consideration as a final method under the Clean Water Act.

As part of the method validation, the MVS Team worked with Federal, municipal, state, and regional contacts to obtain sufficient volumes/masses of samples from eight different environmental matrices, including wastewater, landfill leachate, groundwater, surface water, fish tissue/clams, biosolids, sediment, and soil. Sample matrices were collected and transferred under chain of custody between September and December 2020. A replacement biosolids sample was collected in October 2021 and an ASTM substitute wastewater sample was developed in December 2021.

Specific steps of the MLV Study are to (a) develop the analytical method, (b) conduct single and multi-laboratory validation studies, and (c) perform statistical analyses of the resultant analytical data to develop appropriate Quality Assurance (QA) and Quality Control (QC) criteria for the method. The draft EPA Office of Water Method 1633 for PFAS has been demonstrated in the Single-Laboratory Validation (SLV) Study conducted under ER19-1409 (Willey *et al.* 2021). The Method was evaluated and determined to be sufficiently robust to proceed to the Multi-Laboratory Validation Study. A Final EPA Office of Water Method 1633 for PFAS is critical to DoD Remedial Project Managers working at aqueous film-forming foams (AFFF)-impacted sites. The method is also of critical importance nationally to wastewater permit writers, ecological and human health risk assessments.

1.2 Phases of Data Management

The data management processes and procedures described herein are applicable to Phases 3-6 of the MLV Study Work Plan (SERDP/ESTCP 2023). The six phases of the plan include:

- Phase 1 – Soliciting Laboratories: This phase involved soliciting proposals and awarding subcontracts to laboratories and suppliers to participate in the Study.

- Phase 2 – Procuring Standards and Study Samples: This phase involved procuring the standards, acquiring and characterizing sample media, and creating the Study Samples.

- Phase 3 – Calibration and Demonstration of Capability: involves using the Study Method (SERDP/ESTCP 2023, Attachment 1), which includes MLV Study-specific requirements and guidance to (1) perform the initial steps (calibration, initial demonstrations of capability [IDCs], initial precision and recovery [IPR], MDLs, and verify limits of quantitation [LOQs]), (2) demonstrate laboratory capability with standards and clean matrices, and (3) generate an applicable standard operating procedure (SOP). Data/information for this phase includes laboratory-specific SOPs, calibration data, and results from the IDC as well as records related to document reviews, corrections, and approvals.

- Phase 4 – Analyses of Study Samples: This phase involves all participant laboratories using the Study Method to analyze the Study Samples. Data/information for this phase includes laboratory-specific data for each Study Sample (electronic data deliverables [EDDs] and Data Packages).

- Phase 5.1 – Data Verification: This phase involves data verification of all study results by the HydroGeoLogic (HGL) Project Chemist and automated checks of the EDDs by Exa. HGL's Project Chemist performed an initial evaluation of the data from each phase of the study with the MVS Team before authorization is given to proceed with the next phase of the study. Data/information for this phase includes the data package completeness review checklist and EDD Error Summary reports.

- Phase 5.2 – Data Validation: An independent third-party conducted data validation for all study results. Data/information for this phase includes data reviews, updated/corrected EDDs and Data Packages, correspondence related to corrections, and approvals.

- Phase 6 – Development of QC Acceptance Criteria: Data/information for this phase includes results from the statistical analysis of data from the MLV Study, quality control (QC) acceptance criteria, recommendations for revisions to draft Method 1633, and a MLV Study Report submitted to the EPA.

1.3 Data Management System Objectives

The primary objective of the data management system (DMS) is to provide an efficient and organized method of data management to streamline data flow and ensure the highest quality data are compiled. Specific objectives are:

- To facilitate and coordinate with the MVS Team members to ensure that the data management system meets overall project objectives;
- To ensure high quality data that provides an accurate representation of all data produced during the study;
- To standardize and store the data in a structured format to allow for accurate querying and statistical analyses;
- To ensure efficient and timely data processing;
- To store the data produced during the MLV Study in a secure location that restricts access to team members with appropriate credentials;
- To allow easy access to the data by project stakeholders; and,
- To implement documentation procedures that ensure the data is technically defensible and legally admissible.

The data management methodology is critical to ensure that laboratory analytical data, validation information, and final statistical calculations are of the highest quality to support and defend the publication of the final method.

2.0   ROLES AND RESPONSIBILITIES

Under the leadership and guidance of the MVS Team, the Data Management Team developed the data management system and ensured that the project objectives and scope were achieved. The Data Management Team consists of Exa Data & Management, Inc. (Exa) and HydroGeoLogic, Inc. (HGL; Figure 1). The Data Management Team coordinated with the MVS Team and data providers, including the analytical laboratories, the validator team, and the statistics and analysis team (Institute for Defense Analysis [IDA]). HGL was responsible for managing the laboratories and reviewing the data packages submitted by the laboratories to ensure contractual compliance and coordinating communication between data validators and laboratories. Exa was responsible for maintaining the project database, reviewing EDDs submitted by the laboratories, and assisting with coordinating the multi-level review process (described in Section 4.0).
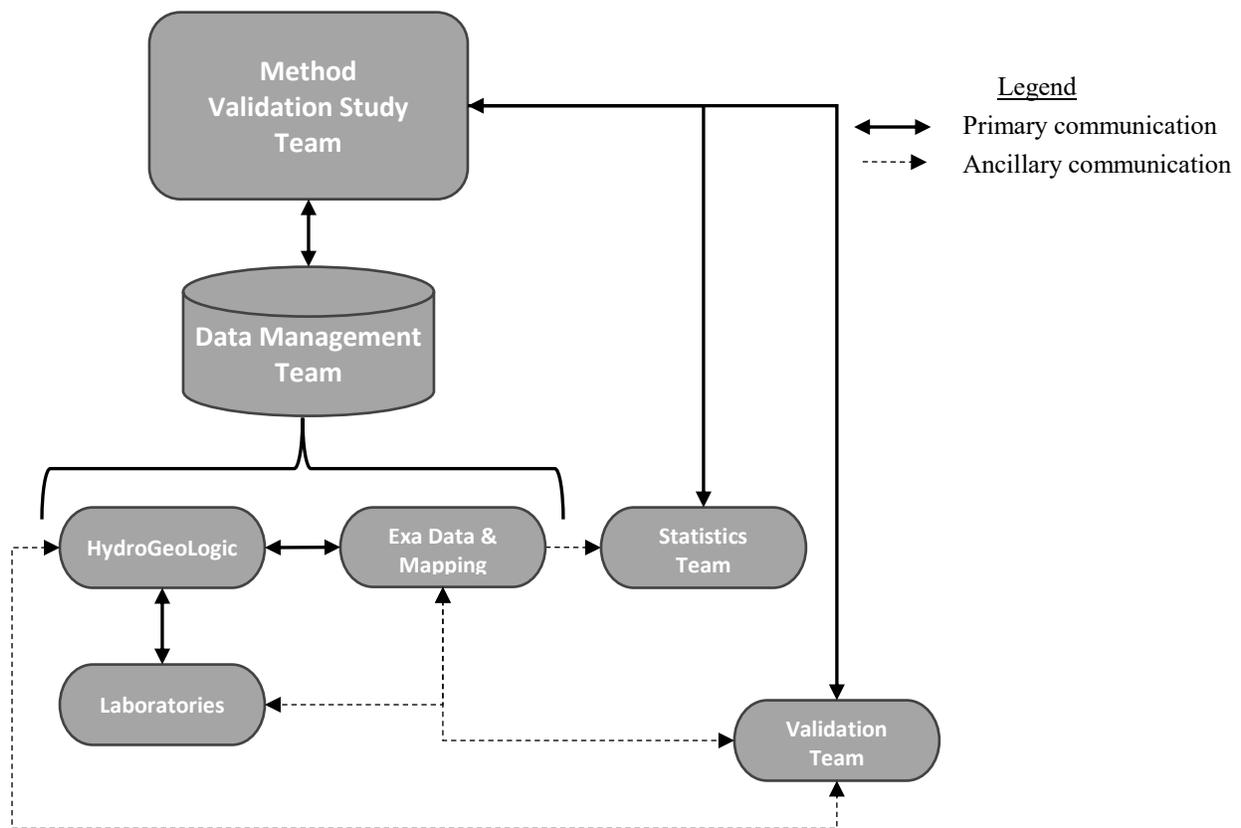
Figure 1. Organization of the Multi-Laboratory Validation Study Teams Relative to their Roles in Managing Data

Specific roles of individual team members are provided in Table 1. Ms. Dawn Smorong, Exa's Project/Database Manager, has overall responsibility for ensuring the data are managed in accordance with the approved MLV Study Work Plan (SERDP/ESTCP 2023) and other related documents. Other Exa team roles include that of Ms. Peggy Myre, who served an oversight role to

ensure that project data management goals and target schedule milestones were met. Dr. Michael Tweiten of Exa had primary responsibility for management of the centralized file sharing system discussed in Section 3.0. Mr. Glenn Sutula assisted with data management processes.

The HGL data management role will be to coordinate incoming data from the laboratories, and to perform initial checks of data acceptability as described in Section 4.1.2.

Table 1. MLV Study Data Management Team Member Roles and Responsibilities

Organization	Team Member	Role	Data Management Responsibility
Exa Data & Mapping	Dawn Smorong	Exa PM; Data Manager	Exa project completion and database management
	Peggy Myre	Exa Data Quality Officer	Ensure compliance with project goals and the DMP
	Michael Tweiten	Exa Data Library Manager	Setup and manage MLV Study Library storage and users
	Glenn Sutula	Data Manager	Database management
HydroGeoLogic	Joe Skibinski	HGL Project Manager	HGL project completion, lab coordination
	John Powell	HGL Program Chemist	Laboratory coordination, chemistry review
	Denise Rivers	HGL Project Chemist	Laboratory coordination, laboratory data compliance, chemistry review
	Ken Rapuano	HGL Project Chemist	Laboratory data compliance, chemistry review
	Joe Vilain	HGL Project Chemist	Laboratory data compliance, chemistry review
	Andrea Fletcher	HGL Data Manager	Laboratory EDD and data package tracking and coordination

3.0   DATA SHARING PLAN AND FILE TRACKING

A critical element to ensure proper organization of the data collected for the MLV Study is managing the files generated to support the project. A file storage server was deployed to serve as a repository for all documents and data for the project, termed the ExaBlue SharePoint (Section 3.1). As part of the file organization strategy, a File Tracking System was developed (Section 3.2), including strict rules for file-naming (Section 3.3).

3.1   ExaBlue SharePoint: Host and Software

All project data and information were stored on a centralized, secure server managed by the Exa team. Table 2 provides a listing of the general data types stored on the server, as well as the MVS Team member responsible for upload and maintenance of the associated files.

Access is strictly controlled to ensure the protection of all proprietary data. The selected platform was Microsoft$^©$ (MS) Office 365 Enterprise software; the SharePoint application was used for the central storage and accessing of documents, data, and other information related to the MLV Study. This section details server specifications, the folder structure, as well as the list of users and their access level (permissions).

The ExaBlue SharePoint is hosted on the Microsoft Azure Government Community Cloud (GCC) High and DoD environments to ensure cloud-service compliance, including Federal Risk and Authorization Management Program (FedRAMP) High, Department of Defense Security Requirements Guidelines, Defense Federal Acquisition Regulations Supplement (DFARS), and International Traffic in Arms Regulations (ITAR). The ExaBlue SharePoint utilizes a Microsoft Office 365 E1 Enterprise environment, including Microsoft Office 365 software tools to enable file sharing, editing, team communications and identity/access management.

The ExaBlue SharePoint employs access restrictions with requirements for authentication and user credentials to gain access. Exa's Michael Tweiten was responsible for setting up the system, assigning users and user privileges based on assigned project roles and responsibilities, and making required adjustments as the project progressed. The laboratory and data validator participants are only allowed to upload/view their own data. Additional details regarding users and defined privileges are provided in Section 3.1.2.

The ExaBlue SharePoint was set up to automatically send email notifications to Team members when files were posted in certain folders.

Table 2. MLV Study General Data Types

Data Type	Example Data	Team Member Responsible for Upload/Maintenance
Project Documents	Background Documents - UFP-QAPP and PMP, Study Work Plan, Background Analytical Data (SGS AXYS), conventional analyses	HGL/Exa
	Correspondence	All
	Final DVR and Data - final versions of the laboratory data package, HGL data package review checklist, data validation reports, validated EDDs, EPA/NAVSEA review documents.	Exa
	Meetings and Schedules - Schedule, Contact list, Meeting Minutes	HGL, SEE
Project Reports	Working and final versions of reports generated for the MLV Study: Aqueous, Biosolids/Landfill Leachate, Soil/Sediment, Tissue	MVS Team
Laboratory Data	EDDs (csv)	Individual labs
	Data packages (pdf)	Individual labs
	Sample receipts - Sample acknowledgment forms, chain-of-custody records	HGL
	Standard Operating Procedures (SOP)	Individual labs
	Corrective action reports	HGL
	ERA laboratory instructions	HGL
	Spike Levels and Wellington Certificates of Analysis	HGL
	Data package completenes review checklists	HGL
Validator Data	*To validator:*	
	Amended EDDs (xlsx), Lab Data Packages (pdf)	Exa/HGL
	From validator:	
	DV Report (pdf)	Individual validators
	Amended EDDs with validator fields populated (xlsx)	Individual validators
	Evidence of 10% verification (xlsx)	Individual validators
	Data validation checklist (doc)	Individual validators
Statistics Data	*To IDA:*	
	Database exports (xlsx)	Exa
	From IDA:	
	Report with appendices (pdf) and supporting calculations (xlsx)	IDA
Database	Database (accdb), documentation (pdf)	Exa
Tracking	MLVS Review Status (xlsx); Exa internal tracking (xlsx); EPA/NAVSEA Review Tracker (xlsx)	Exa/SEE/NAVSEA

3.1.1 Folder Structure

The ExaBlue SharePoint employs a strict, hierarchical folder structure, and displays a list of files and key information about the files, such as who was the last person to modify the file. The folder and sub-folder structure supported access permissions as described in Section 3.1.2. The top-level folders indicate the type of data and other content available in the folder (Figure 2).

Appendix A - Description of the File Tracking System

TRACKING WORKSHEET	TRACKING FIELD	VV Field	TRACKING FIELD DESCRIPTION
GENERAL	Tracking ID		Root file name
	Project Phase		Project Phase. See 'ValidValues'
	EDD File Name		EDD File name
	Data Package File Name		Data Package File Name
	Laboratory Name	Yes	Laboratory Name. See 'ValidValues'
	Matrix	Yes	Matrix. See 'ValidValues'
	Notes		Notes regarding submitted files
	Log Date		Date the reciept of files was logged into the Tracking System
LABORATORY	EDD/DP Due Date		Due date for the Lab EDD/Data Package (mm/dd/yyyy)
	EDD/DP Date Received		Date Lab EDD/Data Package received (mm/dd/yyyy; uploaded to Sharepoint)
	HGL Reviewer		Initials of HGL staff conducting the Data Package review
	Date HGL Review Complete		Date HGL review complete (mm/dd/yyyy)
	EDD Rejected or Approved - HGL	Yes	Indicate whether EDD/Data Package was rejected by HGL. See 'ValidValues'
	Summary of Errors - HGL		Brief summary of issues found during HGL Data Package review
	Exa Reviewer		Initials of Exa staff conducting the automated EDD review
	Date Exa Review Complete		Date Exa review complete (mm/dd/yyyy)
	EDD Rejected or Approved - Exa	Yes	Indicate whether EDD/Data Package was rejected by Exa. See 'ValidValues'
	Summary of Errors - Exa		Brief summary of issues found during Exa EDD review
	Date Data Package to DV		Date the Data Package was posted to the Validator folder (mm/dd/yyyy).
	Date Amended EDD to DV		Date the Amended EDD was posted to the Validator folder (mm/dd/yyyy).
VALIDATOR	Data Validator	Yes	Data Validator. See 'ValidValues'.
	DP version reviewed		Current version number of the Data Package provided to the data validator for review.
	Amended EDD version reviewed		Current version number of the Amended EDD provided to the data validator for review.
	Date DV Report/Files Received		Date of receipt for the current data validator report/files (mm/dd/yyyy).
	DV Amended EDD version		Current version number of the Amended EDD with validator fields populated (posted by the data validator).
	DV Report version		Current version number of the data validator report (posted by the data validator).
	DV Verification version		Current version number of the Verification file (posted by the data validator).
	DV Checklist version		Current version number of the Checklist (posted by the data validator).
	EDD Rejected or Approved - DV	Yes	Indicate whether EDD/Data Package was rejected by the Data Validator. See 'ValidValues'
	Notes		Notes regarding submitted files
	Date EPA/NAVY Files Received		Date of receipt for the current review files posted by EPA/NAVY reviewers (mm/dd/yyyy).

Acronyms:
DB - Project Database
DP - Data Package
DV - Data Validator
EDD - Electronic Data Deliverable

Appendix B - File Tracking System Valid Values List

Worksheet	Field	Valid Value Code	Valid Value Code Description
GENERAL	Laboratory Name	ALPHA	Alpha Analytical
GENERAL	Laboratory Name	BATTELLE	Battelle
GENERAL	Laboratory Name	CALEPA	CalEPA DTSC
GENERAL	Laboratory Name	ELLET	Eurofins Lancaster Labs
GENERAL	Laboratory Name	ETA	ETA, Sacramento
GENERAL	Laboratory Name	GEL	GEL Laboratories
GENERAL	Laboratory Name	MDH	Maryland Department of Health
GENERAL	Laboratory Name	PACE	GCAL/Pace
GENERAL	Laboratory Name	SGSNA	SGS North America
GENERAL	Laboratory Name	VISTA	Vista Analytical
GENERAL	Matrix	GW	Groundwater
GENERAL	Matrix	SW	Surface water
GENERAL	Matrix	SD	Sediment
GENERAL	Matrix	SS	Soil
GENERAL	Matrix	TS	Tissue
GENERAL	Matrix	WW	Wastewater
GENERAL	Matrix	LC	Landfill Leachate
GENERAL	Matrix	BS	Biosolids
GENERAL	Project Phase	Phase 3 - ICAL	Initial Calibration
GENERAL	Project Phase	Phase 3 - IDC	Initial Demonstration of Capabilities (IDC)
GENERAL	Project Phase	Phase 4.4.1	GW, SW, and WW matrices
GENERAL	Project Phase	Phase 4.4.2	SS and SD matrices
GENERAL	Project Phase	Phase 4.4.3	Tissue matrices
GENERAL	Project Phase	Phase 4.4.4	LC and BS matrices
LABORATORY	EDD Rejected or Approved - HGL	Approved	Passed review
LABORATORY	EDD Rejected or Approved - HGL	Rejected	Did not pass review
LABORATORY	EDD Rejected or Approved - HGL	NA	Not applicable (not reviewed)
LABORATORY	EDD Rejected or Approved - HGL	To Validator	Bypassed review and went straight to validator.
LABORATORY	EDD Rejected or Approved - HGL	Re-submitting	Re-submission from the lab expected
LABORATORY	EDD Rejected or Approved - HGL	Pending	Lab has submitted a data package and it's pending review
LABORATORY	EDD Rejected or Approved - HGL	Not Submitted	Lab hasn't submitted
LABORATORY	EDD Rejected or Approved - Exa	Approved	Passed review
LABORATORY	EDD Rejected or Approved - Exa	Rejected	Did not pass review
LABORATORY	EDD Rejected or Approved - Exa	NA	Not applicable (not reviewed)
LABORATORY	EDD Rejected or Approved - Exa	Re-submitting	Re-submission from the lab expected
LABORATORY	EDD Rejected or Approved - Exa	Pending	Lab has submitted an EDD and it's pending review
LABORATORY	EDD Rejected or Approved - Exa	Not submitted	Lab hasn't submitted
VALIDATOR	Data Validator	CHEMVAL	ChemVal
VALIDATOR	Data Validator	PYRON	Pyron Environmental
VALIDATOR	Data Validator	JACOBS	Jacobs Engineering
VALIDATOR	EDD Rejected or Approved - DV	Approved	Approved as is (no DV input)
VALIDATOR	EDD Rejected or Approved - DV	Revised	Approved with DV input added
VALIDATOR	EDD Rejected or Approved - DV	Rejected	Did not pass review
VALIDATOR	EDD Rejected or Approved - DV	NA	Not applicable (not reviewed)

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	TrackingID	text	Tracking ID from Tracking System; incorporated during ETL procedures	No	No	Yes		X	Yes	No
Lab_EDD_Results	Lab_ID_Reported	text	LAB_ID reported by the laboratories in the EDD	Yes	No	Yes			No	No
Lab_EDD_Results	LAB_ID	text	Laboratory Name. See Valid Value list.	No	Yes	Yes		X	Yes	No
Lab_EDD_Results	lab_num	number	Lab_ID code, to keep laboratories anonymous. See Valid Value list.	No	Yes	Yes			No	Yes
Lab_EDD_Results	sdg_num	text	SDG number; incorporated during ETL procedures; extracted from Lab_Sample_ID	No	No	No			Yes	Yes
Lab_EDD_Results	SAMPLE_NO	text	For samples, these are the sample identification names (IDs) from the Chain of Custody. The Sample_No is the same, regardless of whether or not the sample is diluted or reanalyzed. For preparation batch QC, these are "MB" for the Method Blank, "OPR" for the OPR, and "LLOPR" for the LLOPR. For IDOC samples, "IPR" for the IPR samples, "MDLB" for the MDL$_b$ samples,"MDLS" for the MDLs samples, and "LOQVER" for the LOQVER samples.	Yes	No	Yes		X	Yes	Yes
Lab_EDD_Results	LAB_SAMPLE_ID	text	The ID the laboratory assigns to the sample (which identifies the sample on the associated data files and reports). For samples that need to be re-analyzed for issues other than dilution, attach the following identifiers to the end of the lab sample identifier without a space between them (e.g., 02082022-01R): "R" for analytes, EISs & NISs reported from first re-analysis not due to dilution "R1" for analytes, EISs & NISs reported from second re-analysis not due to dilution "R2" for analytes, EISs & NISs reported from second re-analysis not due to dilution If more re-analyses not due to dilution are needed to be reported beyond three for a sample, continue on with the numbering (e.g., R3, R4, R5, etc.).	Yes	No	Yes		X	Yes	Yes
Lab_EDD_Results	ANALYSIS_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.	Yes	No	Yes			Yes	No
Lab_EDD_Results	ANALYSIS	text	Fill in "PFAS". See Valid Value list.	Yes	Yes	Yes	PFAS		Yes	No
Lab_EDD_Results	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	Yes	Yes	Yes		X	Yes	Yes
Lab_EDD_Results	lab_rep	text	Lab replicate identifier	No	No	Yes	1		Yes	Yes

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	DILUTION	number	Dilution made post extraction (e.g., extract diluted 1:10 is entered as "10"). If analyzed without dilution, enter "1".	Yes	No	Yes	1	X	Yes	Yes
Lab_EDD_Results	LAB_FLAG	text	Laboratory qualifiers. See Valid Value list.	Yes	Yes	No			Yes	No
Lab_EDD_Results	CONC_FOUND	number	Enter numeric quantitative result value only. Report to three significant figures. Do NOT enter any text string strings or symbols (e.g., "ND", "<"). For analytes that are not detected, the laboratory's sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account) is entered. Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. Report result units in "Unit" field, consistent for all sample fields.	Yes	No	Yes			Yes	No
Lab_EDD_Results	CONC_SPIKE	number	For unspiked samples enter "0" for method analytes. For spiked samples, enter the spike concentration representing the estimated concentration in the final extract (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. For EIS and NIS, enter the spike concentration representing the concentration in the final extract in units consistent with sample result units. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	Yes			Yes	Yes
Lab_EDD_Results	PERCENT_REC	number	For unspiked samples, leave blank. No text should be included in this field (e.g. N/A). For spiked samples (OPR, LLOPR, MDLs and LOQVER), enter the spike percentage recovery as a whole number (e.g., 95 versus 0.95). Do NOT include "%" symbol. For EIS and NIS recoveries, enter the spike % recovery as a whole number (e.g., 95 versus 0.95). Report to 3 significant figures. Do NOT include "%".	Yes	No	No			Yes	Yes
Lab_EDD_Results	MDL	number	Method Detection Limit. Enter the sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	No	-9		Yes	Yes

Appendix C – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	LOQ	number	Limit of Quantitation. Enter the sample specific LOQ (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). Report to 3 significant figures. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	No	-9		Yes	Yes
Lab_EDD_Results	UNIT	text	The reporting units must be consistent for the sample record including Conc_Found, MDL, LOQ etc. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Ensure that all values for the sample record are reported in the same units. See Valid Value list.	Yes	Yes	Yes			No	No
Lab_EDD_Results	unit_final	text	The reporting unit, standardized	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	SAMPLE_ TRANSITION_RATIO	text	Enter the calculated Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte in the sample. Report to 3 significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g. N/A).	Yes	No	No			Yes	No
Lab_EDD_Results	EXPECTED_ TRANSITION_RATIO	text	Enter the expected Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte per the method. Report to three significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g., N/A).	Yes	No	No			Yes	No
Lab_EDD_Results	RRT	text	Enter relative retention time	Yes	No	No			Yes	No
Lab_EDD_Results	SAMPLE_SIZE	number	Enter volume (aqueous samples) or weight (solid samples) of sample extracted (in liters for aqueous samples, in kilograms for solids).	Yes	No	Yes			Yes	No
Lab_EDD_Results	SAMPLE_SIZE_UNIT	text	Will be liters (L) for aqueous samples or kilograms (Kg) for solid samples	Yes	No	Yes			Yes	No
Lab_EDD_Results	EXTRACTION_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.	Yes	No	Yes			Yes	No
Lab_EDD_Results	PERC_MOISTURE	number	Percent moisture in solid samples only. Enter the percent moisture as a whole number (e.g., 73 versus 0.73). Do NOT include "%" symbol.	Yes	No	No			Yes	No
Lab_EDD_Results	MATRIX	text	Matrix analyzed. See Valid values list.	Yes	Yes	Yes			Yes	Yes

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	METHOD	text	Laboratory SOP Name in format of "name(space)revision number"	Yes	No	Yes			Yes	No
Lab_EDD_Results	STUDY_PHASE	text	Multi-Lab Validation Study Phase. See Valid Value list.	Yes	Yes	Yes			Yes	No
Lab_EDD_Results	SAMPLE_TYPE	text	See Valid Value list.	Yes	Yes	Yes		X	Yes	Yes
Lab_EDD_Results	result_type	text	Code for Result Type. See Valid Value list.	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	spike_cat	text	Code for Spike Category. See Valid Value list.	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	validation_level	text	Stores information on the level of data validation that has been completed for the chemistry data.	No	No	Yes	Level 4		Yes	No
Lab_EDD_Results	validator	text	Code for Data Validator. See Valid Value list.	No	Yes	Yes			Yes	No
Lab_EDD_Results	dv_qualifier	text	Code for Data Validator qualifiers. See Valid Value list.	No	Yes	No			Yes	No
Lab_EDD_Results	dv_qualifier_reason	text	Data validation qualifier reason codes.	No	No	No			Yes	No
Lab_EDD_Results	dv_notes1	text	Include comments to distinguish the meaning of the dv_qualifier assignment (e.g., distinguishing the -J qualifier)	No	No	No			Yes	No
Lab_EDD_Results	dv_notes2	text	Additional information provided by validator deemed pertinent to their dv_qualifier assignment.	No	No	No			Yes	No
Lab_EDD_Results	dv_result	number	Validator recommended result for concentration. If this is provided, entries must be made in the dv_ResultChange_yn field and dv_ResultChange_desc fields.	No	No	No			Yes	No
Lab_EDD_Results	dv_ResultChange_yn	Logical	Enter Y or N. Indicates whether the validator made a recommendation to change the result for concentration.	No	No	Yes			Yes	No
Lab_EDD_Results	dv_ResultChange_desc	text	Description of the reasons for validator recommending a change to the result for concentration.	No	No	No			Yes	No
Lab_EDD_Results	Reviewer_qualifier	text	Code for qualifiers applied by NAVY/EPA reviewers of data validation results. See Valid Value list.	No	Yes	No			Yes	No
Lab_EDD_Results	Reviewer_notes	text	Notes from NAVY/EPA reviewers of data validation results.	No	No	No			Yes	No
Lab_EDD_Results	final_qualifier	text	Code for Final Qualifier. See Valid Value list.	No	Yes	No			No	Yes
Lab_EDD_Results	final_result	number	Final result for concentration. Combines CONC_FOUND and validator_result fields.	No	No	Yes			No	Yes
Lab_EDD_Results	lab_rep	text	Data manager assigned. Laboratory replicate number; to assist with completeness and duplicate checks.	No	No	Yes			Yes	No
Lab_EDD_Results	sample_rep	text	Data manager assigned. From lu_MatrixType.Rep field; to assist with spike_percent_rec calculation	No	No	No			No	No
Lab_EDD_Results	sample_root	text	Data manager assigned. Sample_NO without the Reg suffix; to assist with spike_percent_rec calculation	No	No	No			No	No
Lab_EDD_Results	spike_level	number	Data manager assigned. From lu_SpikeLevel table; to assist with spike_percent_rec calculation	No	No	No			No	Yes

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	conc_minus_native	number	Data manager calculated. Interim value in spike_percent_rec calculation	No	No	No			No	Yes
Lab_EDD_Results	spike_percent_rec	number	Data manager calculated. Matrix spike percent recovery.	No	No	No			No	Yes
Lab_EDD_Results	spk_pct_rec_DNC	text	Data manager assigned. Determination of Calculate/DNC (do not calculate)for spike_percent_rec calculation	No	No	No			No	Yes
Lab_EDD_Results	CONC_FOUND_val	text	Data manager assigned. CONC_FOUND as a value; to assist with database-wide QAQC checks.	No	No	No			No	No
Lab_EDD_Results	LOQ_val	text	Data manager assigned. LOQ as a value; to assist with database-wide QAQC checks.	No	No	No			No	No
Lab_EDD_Results	MDL_val	text	Data manager assigned. MDL as a value; to assist with database-wide QAQC checks.	No	No	No			No	No
Lab_EDD_Results	LAB_SAMPLE_IDclean	text	Data manager assigned. Standardized LAB_SAMPLE_ID to remove re-analysis suffixes; to assist with completeness and duplicate checks.	No	No	Yes			No	Yes
Lab_EDD_Results	qaqc_dup	text	Data manager assigned. Identifies LLOPR, OPR and MB double-duty samples and exclude one of the results for the 'all in' database exports.	No	No	No			No	No
Lab_EDD_Results	DM_notes	text	Data manager notes.	No	No	No			No	No
Lab_EDD_Results	DM_notes2	text	Data manager notes - 2.	No	No	No			No	No
Lab_EDD_Results	EditDate	text	Date of append to Lab_EDD_Results table.	No	No	Yes			No	No
dicValidValues	Table	text	Valid value table name.	No	No	Yes			No	No
dicValidValues	Field	text	Valid value field name.	No	No	Yes			No	No
dicValidValues	Value	text	Acceptable valid value codes.	No	No	Yes			No	No
dicValidValues	Description	text	Description of valid value codes, if necessary	No	No	Yes			No	No
dicValidValues	VVL_match_alt_field	text	Related valid value field name.	No	No	No			No	No
dicValidValues	VVL_match_alt_code	text	Matching valid value code.	No	No	No			No	No
dicValidValues	Validator	text	Data validator assoicated with each LAB_ID_CODE	No	No	No			No	No
LU_Compound	SORT_ORDER	number	Sort order to apply to data summary tables.	No	No	Yes			No	No
LU_Compound	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Compound	COMPOUND	text	Use the names included in the example EDD. Method analytes, and EIS and NIS compounds must be reported for each sample. See Valid Value list.	Yes	Yes	Yes			Yes	Yes
LU_Compound	CAS_NO	text	Chemical Abstract Service Registration Number	Yes	Yes	Yes			Yes	Yes
LU_Compound	result_type	text	Code for Result Type. See Valid Value list.	No	Yes	Yes			No	No
LU_Spike_Levels	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Spike_Levels	Matrix	text	Name of the matrix. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Spike_Levels	Low_Spike	number	Low spike concentration	No	No	Yes			No	No
LU_Spike_Levels	High_Spike	number	High spike concentration	No	No	Yes			No	No

Appendix C – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
LU_Spike_Levels	Unit	text	Unit of spike concentration	No	No	Yes			No	No
lu_MatrixKey	Matrix Type	text	Matrix (full name)	No	No	Yes			No	No
lu_MatrixKey	Requested Name	text	Descriptive name of sample	No	No	Yes			No	No
lu_MatrixKey	Description	text	Description of sample	No	No	Yes			No	No
lu_MatrixKey	Matrix Code	text	Matrix (code)	No	No	Yes			No	No
lu_MatrixKey	Sample Identifier	text	Sample identifier (middle component of SampleID)	No	No	Yes			No	No
lu_MatrixKey	SampleID	text	SampleID (EDD SAMPLE_NO must match to this code)	No	No	Yes			No	No
lu_MatrixKey	spike_cat	text	Spike category (low, high, etc)	No	No	Yes			No	No
lu_MatrixKey	Rep	text	Sample replicate number (last component of SampleID)	No	No	Yes			No	No
lu_MatrixKey	Selected	text	Indicates if sample was selected for use in the study	No	No	Yes			No	No

Note: The Required Fields may be revised during database development; maximum field lengths will be incorporated into the database structure during development.

Appendix D – Supplemental guidance for correctly populating the EDD Template	
Item	**Guidance**
1	If possible, include all results in one worksheet.
2	We will be running QA/QC routines on the EDDs to ensure they are populated correctly. These will be delivered to you in an Excel file (e.g., LABNAME_RW_ver0_EDD_Error_Summary.xlsx).
	If you have questions/issues regarding the Error Summary report, please incorporate comments directly into the Excel file and send it back to us for review (via SharePoint).
3	Instructions for reporting CONC_FOUND for NIS compounds:
	1. Option 1: Report NIS Mass in CONC_FOUND, and in the accompanying report provide example calculation and point to where the data in the numerator (e.g., field sample) and the denominator (e.g. CCV) are found.
	2. Option2: Complete the EDDs with the NIS percent recoveries based on areas (i.e., no mass reported). CONC_FOUND does not need to be populated.
	a. If Option 2 is selected then
	i. In the report provide the formula and example calculations for one sample per batch
	ii. Point to where those areas values are found in their data packages (i.e., ensure they have reported the NIS area for the field (target) sample and the comparative area from either the mid-point of the ICAL, CCV, or equivalent.
4	Instructions for populating EDD when a single sample is serving the purpose of the MDLB and MB samples:
	1. Copy the results and code the SAMPLE_NO field as 'MDLB' for one set of results, and 'MB' for the other set of results.
	2. Ensure that the case narrative clearly identifies that the blank sample was used for multiple purposes.
5	Ensure that B flags are applied as indicated by the instructions:
	"For analytes that were detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than 1/10th the concentration in the sample, whichever is greatest. The MB must also be flagged with a "B" for all concentrations greater than ½ the LOQ."
	If the CONC_FOUND is between the MDL and LOQ <u>AND</u> it is at a concentration greater than 1/2 LOQ, the flag should be BJ or JB.
6	Although the instructions for the CONC_FOUND field indicate "For analytes that are not detected, the laboratory's sample specific MDL", the exceptions are for the following sample types, where CONC_FOUND can be less than the MDL:
	MDLS
	LOQVER
	Blanks (MB and MDLB)
7	For instances where there are re-analysis not due to dilution, all results must be reported in the EDD (i.e., the original analysis and all subseqent re-analysis).

	Appendix D – Supplemental guidance for correctly populating the EDD Template
8	The Sample Coding spreadsheet lists the mandatory batch QC samples that are required for Phase 3 and Phase 4 submittals. All mandatory batch QC samples must be present in the EDD submittals.
9	Do not add suffixes to SAMPLE_NO (e.g., IPR1, MLDB 2) - LAB_SAMPLE_ID will differentiate samples.
10	Fields that must be reported to 3 significant figures:
	CONC_FOUND
	CONC_SPIKE
	PERCENT_REC (DOES need to be reported to 3 sig figs; report as a whole number (95.1), not a fraction (0.951)
	MDL
	LOQ
	SAMPLE_TRANSITION_RATIO
	EXPECTED_TRANSITION_RATIO
	Note that the results in these fields must be rounded appropriately to 3 significant figures. Changing the display for the number of decimal places is not sufficient.
11	Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis.
12	UNIT field must be consistent across all samples.
13	The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples.
14	Do not include text in number fiels (e.g. N/A, %, ND, <).
15	Percent recovery must be reported in the EDD for IPR samples.

Appendix E – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code	Validator
LAB_ID_CODE	ALPHA	Alpha Analytical	lab_num	3	JACOBS
LAB_ID_CODE	BATTELLE	Battelle	lab_num	6	JACOBS
LAB_ID_CODE	CALEPA	CalEPA DTSC	lab_num	2	PYRON
LAB_ID_CODE	ELLET	Eurofins Lancaster Labs	lab_num	10	JACOBS
LAB_ID_CODE	ETA	ETA, Sacramento	lab_num	1	PYRON
LAB_ID_CODE	GEL	GEL Laboratories	lab_num	8	JACOBS
LAB_ID_CODE	MDH	Maryland Department of Health	lab_num	5	JACOBS
LAB_ID_CODE	PACE	GCAL/Pace	lab_num	9	JACOBS
LAB_ID_CODE	SGSNA	SGS North America	lab_num	7	PYRON
LAB_ID_CODE	VISTA	Vista Analytical	lab_num	4	PYRON
MATRIX	BS	Biosolids			
MATRIX	GW	Groundwater			
MATRIX	LC	Landfill Leachate			
MATRIX	OS	Ottawa sand for all soil, sediment, and biosolid MBs			
MATRIX	QC	Quality Control Sample			
MATRIX	RT	Reference Tissue for tissue MBs			
MATRIX	RW	Reagent water for all aqueous MBs			
MATRIX	SD	Sediment			
MATRIX	SS	Soil			
MATRIX	SW	Surface water			
MATRIX	TS	Tissue			
MATRIX	WW	Wastewater			
result_type	EIS	Extracted Internal Standard			
result_type	NIS	Non-Extracted Internal Standard			
result_type	TRG	Target analyte			
SAMPLE_TYPE	BLANK	method analytes in MBs			
SAMPLE_TYPE	EIS	EIS in all samples			
SAMPLE_TYPE	IPR	method analytes in IPR IDC samples			
SAMPLE_TYPE	LLOPR	method analytes in LLOPRs			
SAMPLE_TYPE	LOQVER	Method analytes in MDL LOQVER IDC samples			
SAMPLE_TYPE	MDLB	Method analytes in MDL Blank IDC samples			
SAMPLE_TYPE	MDLS	Method analytes in MDL Spike IDC samples			
SAMPLE_TYPE	NIS	NIS in all samples			
SAMPLE_TYPE	NORMAL	method analytes in field samples			
SAMPLE_TYPE	OPR	method analytes in OPRs			
spike_cat	HIGH	High			
spike_cat	LOW	Low			
STUDY_PHASE	Phase 3 - ICAL	Initial Calibration			
STUDY_PHASE	Phase 3 - IDC	Initial Demonstration of Capabilities (IDC)			
STUDY_PHASE	Phase 4.4.1	GW, SW, and WW matrices			
STUDY_PHASE	Phase 4.4.2	SS and SD matrices			
STUDY_PHASE	Phase 4.4.3	Tissue matrices			
STUDY_PHASE	Phase 4.4.4	LC and BS matrices			
validator	CHEMVAL	ChemVal			
validator	JACOBS	Jacobs Engineering			
validator	PYRON	Pyron Environmental			
Analysis	PFAS	NULL			
Lab_Flag	B	Detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than 1/10th the concentration in the sample, whichever is greatest.			

Appendix E – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code	Validator
Lab_Flag	D	When the reported result is from a dilution			
Lab_Flag	I	Fail to meet ion ratio criteria			
Lab_Flag	J	At a concentration between the MDL and LOQ			
Lab_Flag	U	Not detected or were detected at a concentration less than the MDL			
UNIT	ng/L	nanograms per liter	MATRIX	GW	
UNIT	ng/L	nanograms per liter	MATRIX	LC	
UNIT	ng/L	nanograms per liter	MATRIX	SW	
UNIT	ng/L	nanograms per liter	MATRIX	WW	
UNIT	ug/kg	micrograms per kilogram	MATRIX	BS	
UNIT	ug/kg	micrograms per kilogram	MATRIX	SD	
UNIT	ug/kg	micrograms per kilogram	MATRIX	SS	
UNIT	ug/kg	micrograms per kilogram	MATRIX	TS	
dv_qualifier	I	Suspect			
dv_qualifier	J	Estimated			
dv_qualifier	J-	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J- when the %D is below acceptance criteria			
dv_qualifier	J-	If branched isomers were not included in the summed result reported, qualify associated detects as J-			
dv_qualifier	J+	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J+ when the %D is higher than acceptance criteria			
dv_qualifier	U	Values below the MDL are considered non-detects and are qualified as U at the stated MDL.			
dv_qualifier	UJ	Verify that the %Ds are within the acceptance criteria. Non-detects are qualified as UJ in all associated samples for %D outside of acceptance criteria.			
dv_qualifier	UJ	Estimated non-detect			
dv_qualifier	X	Exclusion of data is recommended			
Reviewer_qualifier	I	Suspect			
Reviewer_qualifier	J	Estimated			
Reviewer_qualifier	J-	If branched isomers were not included in the summed result reported, qualify associated detects as J-			
Reviewer_qualifier	J-	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J- when the %D is below acceptance criteria			
Reviewer_qualifier	J+	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J+ when the %D is higher than acceptance criteria			

Appendix E – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code	Validator
Reviewer_qualifier	U	Values below the MDL are considered non-detects and are qualified as U at the stated MDL.			
Reviewer_qualifier	UJ	Verify that the %Ds are within the acceptance criteria. Non-detects are qualified as UJ in all associated samples for %D outside of acceptance criteria.			
Reviewer_qualifier	UJ	Estimated non-detect			
Reviewer_qualifier	X	Exclusion of data is recommended			

Appendix F – Project Database – Valid Value Codes and Descriptions for Compounds

PFAS_ACRONYM	Compound	CAS_NO	Result_Type
13C2-4:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]hexanesulfonic acid	NA	EIS
13C2-6:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]octanesulfonic acid	NA	EIS
13C2-8:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]decanesulfonic acid	NA	EIS
13C2-PFDoA	Perfluoro-n-[1,2-13C2]dodecanoic acid	NA	EIS
13C2-PFTeDA	Perfluoro-n-[1,2-13C2]tetradecanoic acid	NA	EIS
13C3-HFPO-DA	Tetrafluoro-2-heptafluoropropoxy-13C3-propanoic acid	NA	EIS
13C3-PFBS	Perfluoro-1-[2,3,4-13C3]butanesulfonic acid	NA	EIS
13C3-PFHxS	Perfluoro-1-[1,2,3-13C3]hexanesulfonic acid	NA	EIS
13C4-PFBA	Perfluoro-n-[13C4]butanoic acid	NA	EIS
13C4-PFHpA	Perfluoro-n-[1,2,3,4-13C4]heptanoic acid	NA	EIS
13C5-PFHxA	Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid	NA	EIS
13C5-PFPeA	Perfluoro-n-[13C5]pentanoic acid	NA	EIS
13C6-PFDA	Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid	NA	EIS
13C7-PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid	NA	EIS
13C8-PFOA	Perfluoro-n-[13C8]octanoic acid	NA	EIS
13C8-PFOS	Perfluoro-1-[13C8]octanesulfonic acid	NA	EIS
13C8-PFOSA	Perfluoro-1-[13C8]octanesulfonamide	NA	EIS
13C9-PFNA	Perfluoro-n-[13C9]nonanoic acid	NA	EIS
D3-NMeFOSA	N-methyl-d3-perfluoro-1-octanesulfonamide	NA	EIS
D3-NMeFOSAA	N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	NA	EIS
D5-NEtFOSA	N-ethyl-d5-perfluoro-1-octanesulfonamide	NA	EIS
D5-NEtFOSAA	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid	NA	EIS
D7-NMeFOSE	N-methyl-d7-perfluorooctanesulfonamidoethanol	NA	EIS
D9-NEtFOSE	N-ethyl-d9-perfluorooctanesulfonamidoethanol	NA	EIS
13C2-PFDA	Perfluoro-n-[1,2-13C2]decanoic acid	NA	NIS
13C2-PFHxA	Perfluoro-n-[1,2-13C2]hexanoic acid	NA	NIS
13C3-PFBA	Perfluoro-n-[2,3,4-13C3]butanoic acid	NA	NIS
13C4-PFOA	Perfluoro-n-[1,2,3,4-13C4]octanoic acid	NA	NIS
13C4-PFOS	Perfluoro-n-[1,2,3,4-13C4]octanesulfonic acid	NA	NIS
13C5-PFNA	Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid	NA	NIS
18O2-PFHxS	Perfluoro-1-hexane[18O2]sulfonic acid	NA	NIS
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	TRG
3:3FTCA	3-Perfluoropropyl propanoic acid	356-02-5	TRG
4:2FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid	757124-72-4	TRG
5:3FTCA	2H,2H,3H,3H-Perfluorooctanoic acid	914637-49-3	TRG
6:2FTS	1H,1H,2H,2H-Perfluorooctane sulfonic acid	27619-97-2	TRG
7:3FTCA	3-Perfluoroheptyl propanoic acid	812-70-4	TRG
8:2FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid	39108-34-4	TRG
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	756426-58-1	TRG
ADONA	4,8-dioxa-3H-perfluorononanoic acid	919005-14-4	TRG
HFPO-DA	Hexafluoropropylene oxide dimer acid	13252-13-6	TRG
NEtFOSA	N-ethyl perfluorooctanesulfonamide	4151-50-2	TRG
NEtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid	2991-50-6	TRG
NEtFOSE	N-ethyl perfluorooctanesulfonamidoethanol	1691-99-2	TRG
NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	151772-58-6	TRG
NMeFOSA	N-methyl perfluorooctanesulfonamide	31506-32-8	TRG
NMeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid	2355-31-9	TRG
NMeFOSE	N-methyl perfluorooctanesulfonamidoethanol	24448-09-7	TRG
PFBA	Perfluorobutanoic acid	375-22-4	TRG
PFBS	Perfluorobutanesulfonic acid	375-73-5	TRG

Appendix F – Project Database – Valid Value Codes and Descriptions for Compounds

PFAS_ACRONYM	Compound	CAS_NO	Result_Type
PFDA	Perfluorodecanoic acid	335-76-2	TRG
PFDoA	Perfluorododecanoic acid	307-55-1	TRG
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	TRG
PFDS	Perfluorodecanesulfonic acid	335-77-3	TRG
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	113507-82-7	TRG
PFHpA	Perfluoroheptanoic acid	375-85-9	TRG
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	TRG
PFHxA	Perfluorohexanoic acid	307-24-4	TRG
PFHxS	Perfluorohexanesulfonic acid	355-46-4	TRG
PFMBA	Perfluoro-4-methoxybutanoic acid	863090-89-5	TRG
PFMPA	Perfluoro-3-methoxypropanoic acid	377-73-1	TRG
PFNA	Perfluorononanoic acid	375-95-1	TRG
PFNS	Perfluorononanesulfonic acid	68259-12-1	TRG
PFOA	Perfluorooctanoic acid	335-67-1	TRG
PFOS	Perfluorooctanesulfonic acid	1763-23-1	TRG
PFOSA	Perfluorooctanesulfonamide	754-91-6	TRG
PFPeA	Perfluoropentanoic acid	2706-90-3	TRG
PFPeS	Perfluoropentanesulfonic acid	2706-91-4	TRG
PFTeDA	Perfluorotetradecanoic acid	376-06-7	TRG
PFTrDA	Perfluorotridecanoic acid	72629-94-8	TRG
PFUnA	Perfluoroundecanoic acid	2058-94-8	TRG

Figure 2. Folder Structure for the ExaBlue SharePoint

Each top-level folder contains sub-folders for different types of data, from different sources:

Project Documents
- **Background Documents** – This folder includes reports and documentation that guide the MLV Study (e.g., Study Work Plan, UFP-QAPP, Background Analytical Data from SGS AXYS, conventional analyses).
- **Correspondence** – MVS Team members ensured that project communication (including email) is backed up. Correspondence included on the ExaBlue SharePoint are: any written communication (including emails) that document major decisions and information regarding study status and/or problems; a log documenting verbal communication with team participants regarding study status or issues.
- **Final DVR and Data** – When the review processes for a matrix were completed, the final versions of key documents were moved into this folder to provide efficient access for MVS Team members tasked with preparing the MLV Study reports. This included: final versions of the laboratory data package, HGL data package review checklist, data validation reports (DVR), validated EDDs, EPA and Naval Sea Systems Command (NAVSEA) review documents.
- **Meetings and Schedules** – This folder is a repository for meeting notes and status reports.

Project Reports – MVS Team members with appropriate permissions (Section 3.1.2) maintain working and final versions of the following reports generated during the MLV Study:
- Aqueous
- Biosolids/Landfill Leachate
- Soil/Sediment
- Tissue

Laboratory Data
- **Lab Name** – Each laboratory has their own folder including the same structure of sub-folders.
    - **Phase 3 IDC** – This folder includes the Phase 3 Initial Demonstration of Capability (IDC) EDDs and Data Packages for aqueous, solid and tissue matrices.
    - **Phase 3 ICAL** – This folder includes the Phase 3 Initial Calibration (ICAL) Data Package.
    - **Matrix** – There is one sub-folder for each of eight matrices (e.g, wastewater, surface water, groundwater, soil, sediment, biosolids, landfill leachate, tissue), and includes the current version of the EDDs and Data Packages. There are additional directories for:

- **Communications** – This folder is used to store documents provided to the laboratories (e.g., EDDs with comments incorporated, resubmission requests).
- **Archive –** This folder includes versions of the EDDs and Data Packages that have been replaced with re-submissions.
  o **SOP** – This folder stores each laboratory's Standard Operating Procedures.
- **Other Lab Data -** This folder includes sub-directories to store files for:
  o **Corrective Actions**
  o **ERA Laboratory Instructions**
  o **Spike Levels**
  o **Wellington Certificates of Analysis**
- **PFAS Compound Names** – The folder includes the file listing the Compound Name, CAS_NO, and PFAS_Acronyms to be used by the laboratory.
- **Shared** – This folder includes miscellaneous files providing instruction to the laboratories.

Each sub-folder in the Laboratory sub-directory includes an Archive folder to store versions of database exports and files with statistical results that have been replaced with revised file versions.

Validator Data
- **Validator Name** – Each validator has their own folder including the same structure of sub-folders.
  o **Phase 3 IDC**– This folder includes sub-directories for each of the three IDC matrices (aqueous, solid and tissue).
    - **To Validator** - Phase 3 IDC data packages and Amended EDDs are uploaded to this folder (by HGL and Exa, respectively) for each laboratory (not shown on Figure 2).
    - **From Validator** – Phase 3 IDC results provided by the data validator are posted to this folder (data validation report, Amended EDD with validator fields populated, evidence of 10% verification, checklist) for each laboratory (not shown on Figure 2).
  o **Matrix** – There is one sub-folder for each of eight matrices.
    - **To Validator** – data packages and Amended EDDs are uploaded to this folder (by HGL and Exa, respectively), for each laboratory.
    - **From Validator** – results provided by the data validator are posted to this folder (data validation report, Amended EDD with validator fields populated, evidence of 10% verification and checklist), for each laboratory.
- **Shared** – This folder includes miscellaneous files providing directions to the validators.

Each sub-folder in the Validator Data sub-directory includes an Archive folder to store versions of Amended EDDs, Data Packages and data validator submissions that have been replaced with re-submissions.

Statistics

- **Phase 3** – There is one folder for each of the three IDC matrices and the ICAL data.
  - ○ **Phase 3 IDC** – There is one folder for each of the three IDC matrices (aqueous, solid and tissue).
    - ▪ **To IDA** – Database Exports are posted to this folder (from Exa; not shown on Figure 2).
    - ▪ **From IDA** – IDA posts the statistical report with appendices (pdf), along with supporting calculations (csv; not shown on Figure 2) to this folder.
  - ○ **ICAL**
    - ▪ **To IDA** – This folder stores the tabular version of the ICAL results (from Science and Engineering for the Environment [SEE] and HGL; not shown on Figure 2).
    - ▪ **From IDA** – IDA posts the statistical report with appendices (pdf), along with supporting calculations (csv; not shown on Figure 2).
- **Matrix** – There is one sub-folder for each of eight matrices, as well as folders with combined matrices (e.g., "Aqueous_Combined; not shown on Figure 2).
  - ○ **To IDA** – Database Exports are posted to this folder (from Exa).
  - ○ **From IDA** – IDA posts the statistical report with appendices (pdf), along with supporting calculations (csv) to this folder.
  - ○ **Report Statistics** – When final versions of Database Exports and IDA reports are prepared, they are posted to this folder to provide access to EPA and General Dynamics Information Technology (GDIT) contractors.

Each sub-folder in the Statistics sub-directory includes an Archive folder to store versions of database exports and files with statistical results that have been replaced with revised file versions.

Database

- **Database** – A copy of the Project Database (MS Access) is posted to this folder when the MLV Study reports are completed. The version will be indicated by the matrix included (AQ for aqueous media; SOL for soils/sediment; TS for tissue; BLL for biosolids/landfill leachate) and the posted date in the filename (e.g., MLVS_AQ_Database_20230630).
- **Documentation** – When MLV Study reports are completed, current database documentation files (e.g., database dictionary, valid value lists, QA/QC application, scripts, archived database copies) are posted in this folder.

Tracking – this folder contains the shared files used for logging submissions from laboratories, data validators and reviewers, and tracking project status. This main directory includes the following folders:

- **Exa Internal Tracking** – stores detailed information on file submissions and the status of the review process.
- **Archive** – stores backup versions of the tracking files.

Laboratories have access to their specific folders ONLY as described in Section 3.1.2. The Project Documents folder also allowed limited access as described below. Exa and HGL are responsible for reviewing the files uploaded to the site by the laboratory, validator, and statistician team members, ensuring their documents are properly filed and the file-naming protocols are adhered to (described in Section 3.3).

3.1.2 Access and Permissions

There is a strict permission structure limiting access to certain folders to specific team members. Exa is the only organization with Administrator permission, with full control to audit all site content and receive administrative messages.

SharePoint information is permissions-trimmed, meaning that individuals only have access to designated folders within the ExaBlue SharePoint folder structure. In other words, members from each individual laboratory can only access their own EDDs and Data Packages in order to maintain control of proprietary data. Similarly, individuals from the validation or statistics groups are only able to access the folders designated to them.

Specific members of the MVS Team have access to different folders. For example, under the top-level "Statistics" folder, some MVS Team members have access to the "To IDA" and "From IDA" folders which store the draft versions of database exports and statistical results. A smaller group will have permissions to access the final versions included in the "Report Statistics" folder. Table 3 includes the list of project participants that have access to the ExaBlue SharePoint.

Table 3. Project Participants with Access to the ExaBlue SharePoint

Team / Organization	Team Member
Method Validation Study Team	
NAVSEA	Janice Willey
SEE LLC	Tim Thompson
AFCEC	Hunter Anderson
SERDP/ESTCP	Andrea Leeson
EPA (OW)	Adrian Hanley
SERDP/ESTCP	Anastasia Nickerson
SERDP/ESTCP	Stephen Levitas
GDIT	Chip McCarty
GDIT	Mirna Alpizar
Data Management Team	
Exa	Dawn Smorong, Michael Tweiten, Peggy Myre, Glenn Sutula
HGL	Joe Skibinski, Denise Rivers, Ken Rapuano, Andrea Fletcher, Joe Vilain, John Powell
Laboratories	
California DTSC	Katie Hamblin
Pace	Stephen Somerville
SGS	Andrea Colby
Battelle	Jon Thorn
GEL	Vonda Fields
Vista Analytical	Anne Wilhoit; Jamie Fox
Maryland DOH	Sin Urban
Alpha Analytical	Alycia Mogayzel
Eurofins Lancaster	Bradley Ayars
ETA - Sacramento	Jill Kellman
Validators	
Pyron	Mingta Lin
Jacobs	Maggie Radford, Jeremy Bishop
Chem Val	Kathi Gumpper; John Gumpper
Statisticians (IDA)	Allyson Buytendyk

3.2 File Tracking System

A File Tracking System ("Tracking System") was developed to ensure that files and information provided by project participants are logged at each stage of the project. The workflow for handling data from the laboratories, through the validators and the statisticians, is discussed in Section 4.1. In this section, the format of the Tracking System is defined and described.

The Tracking System includes three shared documents, posted in the ExaBlue SharePoint:
1.   **MLVS_FileTracking.xlsx** - Exa internal tracking, which is a detailed log of files received.
2.   **MLVS_Review_Status.xlsx –** summary status information for the overall project.
3.   **EPA NAVSEA Review Tracker.**xlsx – status of the review of data validation reports conducted by EPA and NAVSEA personnel.

3.2.1   Exa Internal Tracking

The purpose of Exa's internal tracking file (MLVS_FileTracking.xlsx) is to log the receipt of submissions from laboratories, validators and reviewers, record the status of the review process, and ensure that file versioning is recorded and monitored. Exa is responsible for updating the internal tracking tables as files are submitted.

Exa Internal Tracking includes three main stages of logging files, partitioned into separate worksheets in the file:
- General – Defines the basis for a unique set of files from the laboratory, including the EDD and the Data Package, linked to the laboratory of origin and the matrix analyzed. If an EDD is rejected and resubmitted, then the resubmitted EDD/Data Package receives a new version number and are tracked separately from the original.
- Laboratory – This element of the Tracking System defines the status of laboratory data receipt and review by the Data Management Team.
- Validator – This element of the Tracking System defines the status of receipt, processing, and return of the laboratory data to and from the data validators.

The MLVS_FileTracking.xlsx file also includes the following ancillary worksheets:
- EDD for DB – identifies the final Amended EDD versions, with data validator and review input added, which were incorporated in the Project Database
- NIS updates - tracks the revisions of percent recovery results for non-extracted internal standards (NIS) in the Project Database, which were received separately from the main EDD for three laboratories.
- ICAL_files – a listing of Phase 3 ICAL files received from the laboratories.
- ValidValues – a listing of acceptable entries for fields in the Tracking System constrained by valid values.
- Field Descriptions – a listing of fields, with field descriptions in the three main tracking worksheets.

The status of data provided to the statistics team (IDA) is not included in the Tracking System, since they receive Database Exports for each matrix (i.e., not on an EDD-specific basis).

A summary of the tracking fields is provided in Appendix A. Several Tracking System fields are limited to specific content ("valid values"); the list of acceptable entries for the valid value fields is provided in Appendix B.

3.2.2   MLV Review Status

The purpose of the shared 'MLVS_Review_Status.xlsx' document is to communicate overall status information to the MVS Team. Each project phase is included in separate worksheets and each matrix has a separate table to record the status of review progress. Table 4 shows an example of a Review Status table. Exa and SEE are responsible for updating the MLV Review Status tables as files are submitted and review steps are completed.

Table 4. Example Review Status Table

Phase 3 IDOC - Reagent Water (RW)

Laboratory Name	Current Version (DP/EDD)	DP Approval Status (HGL)	EDD Approval Status (Exa)	Data Package to DV?	Amended EDD to DV?	DV Reports Received?	SEE DVR Review complete?	DVR Ready for Navy/EPA Review	EDD Ready for Navy/EPA Review	Navy/EPA DV Review complete?	DV qualifiers added to EDD?	Navy/EPA qualifiers added to EDD?	Data Ready for IDA?
ALPHA	8	Approved	Approved	10-Oct-22	06-Mar-23	Tech Memo V0 - yes	Documents confirmed 3/30/23	Tech Memo V0 - yes	Yes V2 3/30/2023	Yes	No	No	√
BATTELLE	2	Approved	Approved	27-Apr-22	29-Apr-22	V2-yes	All documents confirmed 9/16	Yes V2 9/16/2022	Yes V0 9/16/2022	Yes	No	No	√
ELLET	3	Approved	Approved	29-Mar-23	18-Aug-22	V3 - yes	All documents confirmed 9/27	Yes V3 9/27/2022	Yes V2 9/20/2022	Yes	Yes	Yes	√
GEL	--	Rejected	Rejected	--	--	--	--	NA	NA	--	--	--	NA
PACE	1	Approved	Approved	27-Apr-22	29-Apr-22	V1 - yes	All documents confirmed 9/16	Yes V1 9/16/2022	Yes V0 9/16/2022	Yes	No	No	√
CALEPA	5	Approved	Approved	12-Aug-22	12-Aug-22	V2 - Yes	All documents confirmed 9/15	Yes V2 9/15/2022	Yes V2 9/15/2022	Yes	No	No	√
ETA	5	Approved	Approved	10-Mar-23	21-Mar-23	V2 - yes	All documents confirmed 9/27	Yes V2 9/27/2022	Yes V2 9/20/2022	Yes	Yes	No	√
MDH	2/6	Approved	Approved	27-Apr-22	20-Sep-22	V2 - yes	All documents confirmed 10/4	Yes V2 10/4/2022	Yes V3 10/7/2022	Yes	Yes	Yes	√
SGSNA	4/6	Approved	Approved	18-Aug-22	18-Aug-22	V3 - yes	All documents confirmed 9/16	Yes V3 9/16/2022	Yes V1 9/16/2022	V3 DVR - Yes V1 EDD - Yes	Yes	No	√
VISTA	4/3	Approved	Approved	21-Jul-22	30-Aug-22	V4 - yes	All documents confirmed 9/16	Yes V4 9/16/2022	Yes V2 9/16/2022	V4 DVR - Yes V2 EDD - Yes	No	No	√

Acronyms: DP - data package; DV - data validator; DVR - data validation report; EDD - electronic data deliverable; HGL - HydroGeoLogic: IDA - Institute for Defence Analyses; SEE - Science, Engineering and the Environment LLC

3.2.3 EPA NAVSEA Review Tracker

The purpose of the shared 'EPA NAVSEA Review Tracker.xlsx' document is to communicate the status and summary results from the review of data validation reports conducted by EPA and NAVSEA personnel. NAVSEA and EPA are responsible for updating the 'EPA NAVSEA Review Tracker.xlsx' tables as reviews are completed.

3.3   File-Naming Protocols

As part of the File Tracking System, a strict file-naming protocol was devised and guidance produced for the laboratories, validators, and statisticians. Each laboratory EDD and accompanying Data Package (DP) was to be named according to the laboratory, the matrix, and the version of the data. If the delivered data is a resubmission (Section 4.1.6), then the file name reflects that the data are of a new version (Table 5). Similar file-naming protocols were developed for the validators and statisticians.

The DMP states: "Importantly, the laboratory must resubmit BOTH the EDD and the Data Package with a new version number, even if only one or the other was revised." However, as the project progressed, multiple submissions and version numbers of EDD/Data Packages were not updated together as indicated in the DMP due to the unforeseen magnitude of changes. Also, there were exceptions to the required file-naming conventions, as not all labs were in compliance.

The Quick Start Guides provided to the participating laboratories, validators and statisticians to give instructions on file-naming protocols and using the ExaBlue SharePoint site are available in Appendices B1 – B3 of the Data Management Plan (SERDP/ESTCP 2023, Attachment 4).

Table 5. Laboratory Data File-Naming Protocol Examples

Tracking ID	EDD File Name	Data Package File Name	Laboratory Name Code	Matrix Code	Description
ALPHA_GW_ver0	ALPHA_GW_ver0.csv	ALPHA_GW_ver0.pdf	ALPHA	GW	First EDD/DP submitted by Alpha for groundwater
ALPHA_GW_ver1	ALPHA_GW_ver1.csv	ALPHA_GW_ver1.pdf	ALPHA	GW	First revision of Alpha EDD/DP for groundwater
ALPHA_GW_ver2	ALPHA_GW_ver2.csv	ALPHA_GW_ver1.pdf	ALPHA	GW	Second revision of Alpha EDD/DP for groundwater
ETA_SD_ver0	ETA_SD_ver0.csv	ETA_SD_ver0.pdf	ETA	SD	First EDD/DP submitted by ETA for sediment
ETA_SD_ver1	ETA_SD_ver1.csv	ETA_SD_ver1.pdf	ETA	SD	First revision of ETA EDD/DP for sediment

4.0 DATA MANAGEMENT PROCESSES AND PROCEDURES

In addition to the ExaBlue SharePoint, a data management system (DMS) was developed to compile the laboratory EDD data generated for the project, as well as the data validation and EPA/NAVSEA reviewer results. The overall DMS consists of several elements. This section provides an overview of the DMS, including the main components of the workflow (Section 4.1.1 – 4.1.5), a description of the rejection criteria and resubmission process (Section 4.1.6), an overview of the QA/QC procedures applied to laboratory EDD submissions (Section 4.2), and a description of the database and related tools for processing data (Section 4.3).

4.1   Workflow

An important element for meeting project goals and objectives is the use of a specific, rigorous, and well-documented workflow for the data generated during the project. This section provides detailed descriptions of every step of that workflow. At each step, dates of actions and descriptions of decisions are logged in the Tracking System.

The workflow designed and described in the DMP was modified as the project progressed to accommodate requests and requirements from the MVS Team. The workflow that reflects the actual process used during the MLV Study is provided as a flowchart diagram in Figures 3a and 3b. The workflow outlines the sequence of processes that were followed by all team members, including the Data Management Team (Exa/HGL), the MVS Team, laboratories, the validation team, and the statistical analysis team (IDA).

One of the key elements of workflow is the multiple stages of data QA/QC by the Data Management Team, the validators, and the MVS Team. At each stage, the Exa Data Manager ensured that the review information was captured in the Tracking System so that the MVS Team could always understand the status of the review procedures.

4.1.1   Receipt of Data Sets

As shown in Figure 3a, the first component of the workflow is the receipt of data sets, where the laboratories upload EDDs and Data Packages to the ExaBlue SharePoint site. The Exa team is responsible for logging the receipt of the submission in the Tracking System.
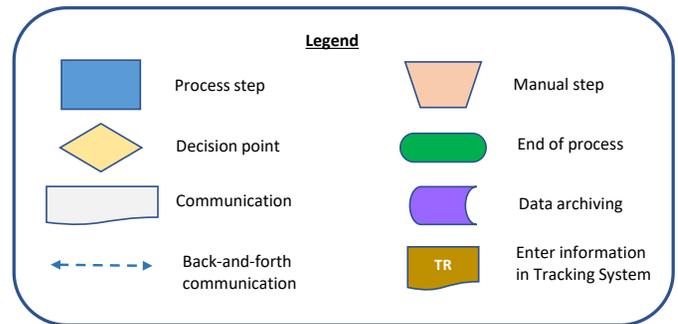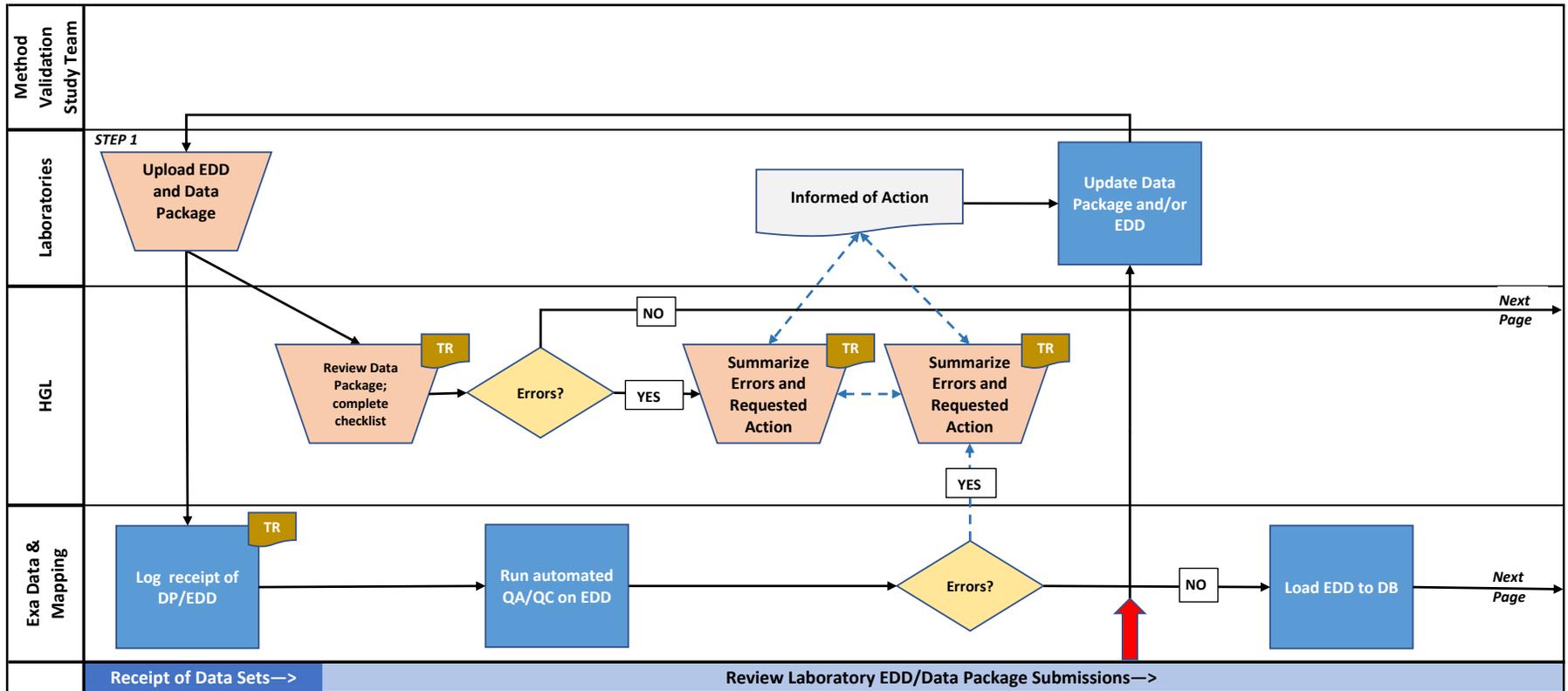
Figure 3a. Workflow for the PFAS Multi-Laboratory Validation Study
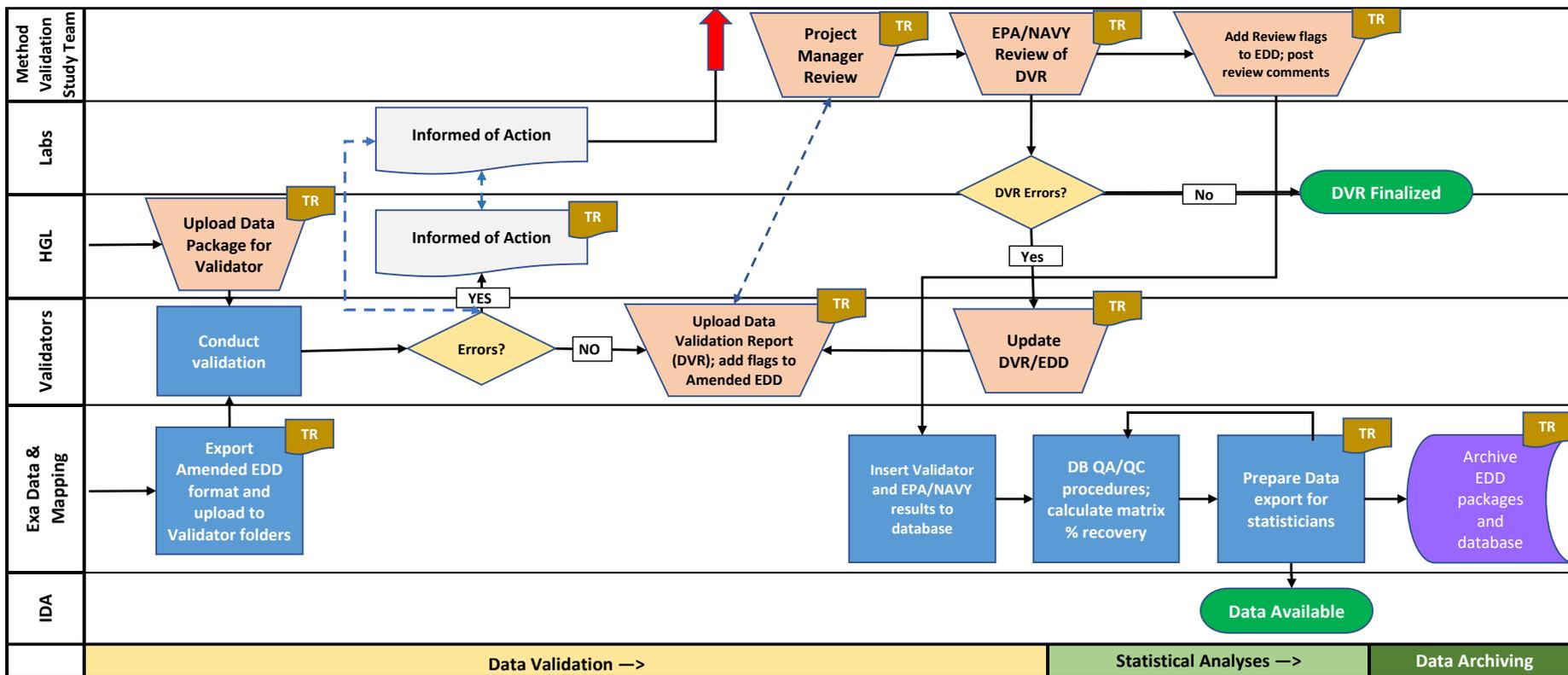
Figure 3b. Workflow for the PFAS Multi-Laboratory Validation Study (continued)

4.1.2   Review Laboratory Data Package/EDD Submissions

The next step in the workflow (Figure 3a) is to conduct a detailed review of the data submitted from the laboratory. This involves two major steps. First, the HGL Project Chemists review the Data Package for completeness and record the findings in a checklist. If errors or omissions were found, HGL rejected the Data Package and informed the laboratories that they must address the issue(s) and resubmit the Data Package. HGL managed the timetable for submissions and this was logged separately from the Tracking System. If there were no issues with the Data Package, HGL posted the Data Package and the completed checklist to the Validator folders on the ExaBlue SharePoint.

The second step of this part of the workflow involves Exa conducting automated QA/QC checks on the EDD using a customized application. If errors were found, the Exa team completed an Error Report with the reasons for rejection and sent this to the laboratory, and noted the EDD as rejected in the Tracking System.

The automated QA/QC checks ensured that the EDDs contained all information required by the template guidance (SERDP/ESTCP 2023, Attachment 3), and each data field in the EDD was completed in accordance with those instructions. Section 4.2 provides the specific details on the EDD checking procedures.

The final step of this component of the workflow was to load the EDD data into the Project Database.

Details on the rejection criteria and resubmission process are described in Section 4.1.6.

4.1.3   Data Validation

The next component of the workflow is Data Validation and is shown in Figure 3b. Three independent third-party validators were responsible for the validation of Data Packages and EDDs in accordance with the study data validation guidelines (SERDP/ESTCP 2023, Attachment 5). Following review and approval of the EDD and Data Packages by the Data Management Team, the Exa team generated an Amended EDD file from the database, which included the laboratory EDD fields, as well as several additional fields incorporated for the validator to populate. The format of the validator Amended EDD is described in Section 4.3.5 and Appendix C. The Amended EDD was then posted for the validator in the appropriate folder on the ExaBlue SharePoint. Amended EDDs were posted for the validators review only after HGL approved the Data Package.

The validator then conducted the data validation procedures. If the validator found errors in the laboratory data that require the laboratory to revise the information and data submitted, the validator informed the MVS Team to determine the course of action to be followed. In some cases the data validators communicated directly with the laboratories regarding the action(s) needed. If a laboratory resubmission was warranted, HGL managed the timetable for submissions and this was logged separately from the Tracking System.

If no errors were found in the laboratory data, the validator completed the data validation procedures and provided: a data validation report; the associated Amended EDD with the validator fields populated; a file with evidence of 10% verification; and, the data validation checklist. The Exa team is responsible for logging the receipt of the submitted files into the Tracking System.

The next step in the workflow is for the SEE Co-Principal Investigator to review the files submitted by the data validators to ensure it is complete and ready for EPA/NAVSEA review. If issues were found, SEE communicated this to the data validators and they revised the data validation files and posted updated files with a revised version number. When this review was complete, SEE was responsible for updating the Tracking System.

The next step in the workflow is for NAVYSEA and EPA members of the MVS Team to review the validator results. If the EPA/NAVSEA reviewers disagree with the validator qualifiers, they enter qualifiers and comments into the Reviewer_qualifier and Reviewer_notes fields of the Amended EDD; these changes are communicated to the data validator. If the EPA/NAVSEA reviewers found issues with the data validation report and associated files, they posted a narrative of their review findings to the validator folders on the ExaBlue SharePoint. The data validators then revised the data validation report and posted updated files with a revised version number.

If a resubmission of the data validation report was not warranted, the EPA/NAVSEA reviewers will upload the revised Amended EDD, with the Reviewer fields populated and a suffix on the file name indicating the review is complete, as well as a narrative of their review findings (.doc), to the appropriate folder in the ExaBlue SharePoint. The Exa Database Manager then ran a routine to link the Project Database to the appropriate file to incorporate validator and validator reviewer qualifiers and comments.

Exa was responsible for logging the receipt of the files submitted by the data validators and EPA/NAVSEA reviewers into the Tracking System.

4.1.4   *Statistical Analyses*

The next step of the workflow is Statistical Analyses and is shown in Figure 3b. Once the database is complete for a matrix, the Exa Data Manager executed automated database-level checks to

ensure results are consistent for the given matrix to ensure the dataset is ready for statistical analysis. In addition, the Exa team calculated matrix percent recovery considering the native concentration in the samples (see Section 4.3.4).

The Exa team then exported the complete dataset for that matrix and posted it to the statistics team member (IDA) in the Statistics folder in the ExaBlue SharePoint. The format of this Database Export is described in Section 4.3.5 and Appendix C. Upon completion of the statistical analysis, IDA uploaded all statistical files to the appropriate ExaBlue SharePoint folder [for each matrix, this included a report with appendices (pdf), supporting calculations (csv), figures (png), and their log file (txt) documenting the statistical output].

The Phase 3 ICAL results had a slightly different workflow – these data were compiled from the laboratory Data Packages by HGL and then provided to Exa for QA/QC review. Subsequently, Exa uploaded the file to the appropriate folder in the ExaBlue SharePoint (Phase 3 ICAL/To IDA). After statistical analyses were complete, IDA posted the files with statistical results to the ExaBlue SharePoint. Note that Phase 3 ICAL data is not stored in the Project Database.

If the statisticians encountered issues with the Database Export provided to them by Exa, the issues were resolved and a new Database Export was provided with a revised version number.

4.1.5 Data Archiving

The final step of the workflow (Figure 3b) is to archive the data, both during the project and at project completion. When the review procedures are completed for a given matrix, all of the final files are moved from the various folders on the ExaBlue SharePoint and compiled in the 'Final DVR and Data' folder (in the Project Documents top-level folder). This includes the final versions for: laboratory data package, HGL data package review checklist, data validation reports, validated EDDs, and EPA/NAVSEA review documents.

The master version of the Project Database will be backed up regularly on Exa's servers. When a Study report is drafted, a copy of the Project Database is posted on the ExaBlue SharePoint for the matrices addressed in the Study report, with the date of posting.

At project completion, the Exa team will provide a final report documenting data processing procedures and a summary of the contents of the final database. This report will take the form of a Data Management Final Report. This report will summarize components of data management for the project, including workflow, database structure, data sharing, Tracking System, and the processes and procedures established for managing the data and conducting QA/QC procedures.

Prior to the completion of the MLV project, Exa will coordinate with team members to assess options for archiving the data from the project. Some of the long-term data needs that will be addressed include:

- The level of long-term access required by the MVS team, other government entities, or the public;
- Whether to utilize existing government data archive resources and platforms;
- Data security level required;
- The options for accessible data formats;
- The need to retain preliminary versions of files from the laboratories, data validators and statisticians;
- Requirements for metadata, if any; and
- Consistency with other SERDP programs and/or databases.

A Data Archive Plan will be produced and delivered as part of this project. Upon acceptance, the data will be archived to these specifications at the completion of the project and documented in the Data Management Report.

As a part of project completion, Exa will work with team partners to ensure smooth technology transition of all work products to the SERDP and the EPA. This delivery will include documentation that provides information on the data structure, all developed processes, automated tools and scripts, and related export products. Sufficient documentation will accompany the archived data to fully describe the source, contents, and structure of the data to ensure future usability.

The ExaBlue SharePoint has several features as a part of the Microsoft Enterprise environment, to ensure the information stored on the SharePoint site is always recoverable (Microsoft 2022). For example, the Microsoft datacenters are geo-distributed to mitigate the impact of a natural disaster or local power outage; backups are retained for 14 days and can be restored to any point in time.

4.1.6   Rejection and Resubmission Process

There are several steps along the workflow where laboratory EDDs/Data Packages could be rejected, as shown in Figure 3a and 3b and described in Sections 4.1.1 through 4.1.4. If the laboratory EDD and/or Data Package includes any inconsistencies with the instructions provided in their contract, or they did not follow the instructions for populating the EDD template, the submission was rejected. In addition, the laboratory EDD/Data Package could be rejected if the data validators found issues with the data that required re-analysis. If the EDD/Data Package was rejected, the laboratory was informed that they must address the errors and resubmit. HGL managed the timetable for submissions, and this was logged separately from the Tracking System. The resubmittal was given a revised version number as described above and shown in Table 5.

The Data Management Plan states that "…the Exa data managers will not conduct any editing or data cleaning procedures to amend the data provided by the laboratories." However, in order to expedite the flow of EDDs, Exa directly edited minor inconsistencies in the EDDs after receiving

permission from the laboratories. There were also instances where updates were made to the original database entries at the request of the MVS Team. When edits were made the Exa data manager added comments in the DM_Notes field of the Project Database, to document the revision.

4.2 EDD QA/QC Procedures

The purpose of this section of the report is to describe specific data quality checking processes and procedures conducted on the electronic data deliverables (EDDs) from the study laboratories. The study requires technically sound and legally admissible data; thus the QA/QC procedures documented in this section are a key element to project success. The data management methodology is critical to ensure that laboratory analytical data, validation information, and final statistical calculations are of the highest quality to support and defend the publication of the final method.

Electronic data from the laboratories are submitted to the MVS Team in a specific electronic data deliverable (EDD) format, as described in the Study Plan (SERDP/ESTCP 2023; Attachment 3). The EDD records are imported into a Microsoft$^©$ (MS) Access database using automated Visual Basic for Applications (VBA) code. In addition to the checking routines, there are additional functions to post-process the data which will be described in Section 4.2.3.

There are three phases of EDD QA/QC in the workflow:

1. Preliminary checks conducted upon import of the EDD;
2. Detailed checks conducted on individual EDDs, prior to submission of the data to the validators;
3. Database checks on the cumulative Project Database conducted prior to submission to the data analysis (statistics) team.

The custom application for processing data for the MLV Study was designed and developed by the Exa team and tested extensively. Testing involved multiple Exa team members running the procedures on multiple test data sets to identify bugs and inconsistencies. Fixes were then incorporated into the automated routines.

Each of these phases of QA/QC procedures will be discussed in the following sections.

4.2.1 QA/QC Checks at Import

Upon receipt of data files submitted from the laboratories, the files are logged into the Tracking System and HGL confirms that the data files (data packages, EDDs, supplemental files) are appropriately filed on the ExaBlue SharePoint.

Import of the EDDs into the database is the first step of the MLV Study QA/QC tool (Figure 4). As each EDD is imported, a series of preliminary checks are conducted to ensure that the EDD is imported properly. This includes checks to ensure all EDD template fields are present and named properly, and all of the EDD records were imported.
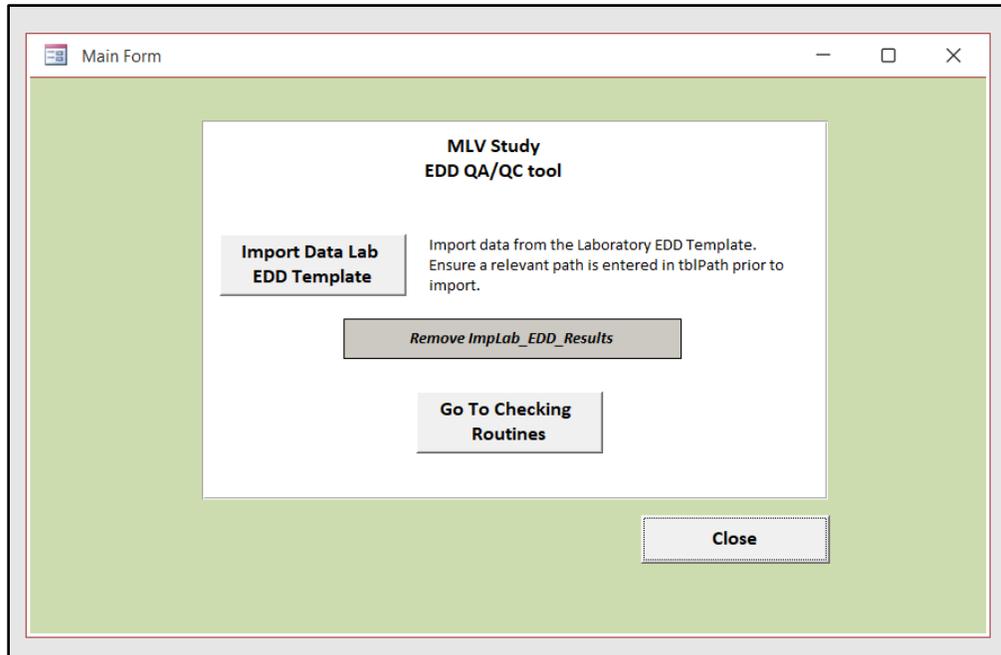
Figure 4. Opening Form of the MLV Study EDD QA/QC Tool

4.2.2 Detailed EDD QA/QC and Reporting

Once the EDD is imported as a stand-alone table into the database (i.e., not yet appended to the main database tables), a series of automated QA/QC checks are conducted. These checks underwent modification as the project progressed, but the description provided below is comprehensive for the EDDs received for Phase 4 as of the publication of this report.

The EDD QA/QC checks are executed in a sequential order (Routines 1-3, Figure 5). Results of the checks are written to the QA/QC Report for review and generating feedback to the data provider (Routine 4).

Figure 5. EDD Checking Routines and Reporting Form

The first routine checks that all required fields have been fully populated. The list of required fields (Table 6) is drawn from the EDD Instructions and Format (SERDP/ESTCP 2023, Attachment 3, Table 1).

Table 6. List of Required Fields

Field Name		
ANALYSIS	LAB_SAMPLE_ID	SAMPLE_SIZE_UNIT
COMPOUND	MATRIX	SAMPLE_TYPE
CONC_FOUND	METHOD	STUDY_PHASE
CONC_SPIKE	PFAS_ACRONYM	UNIT
DILUTION	SAMPLE_NO	
LAB_ID	SAMPLE_SIZE	

In addition, the first routine also checks that relationships between tables are maintained – specifically the links between the EDD and the standardized SampleID (from the lu_MatrixKey table) and the standardized compound codes in the lu_compound table.

The second routine checks for unique records based on the following fields: LAB_ID, SAMPLE_NO, LAB_SAMPLE_ID, PFAS_ACRONYM, DILUTION, and SAMPLE_TYPE. If there is more than one record in the EDD with the same combination of these fields, this check will generate an error message.

The third routine ("Additional Checks" in Figure 5) includes a wide variety of automated QA/QC checks and summaries, some that require manual review (Table 7). Range checks are conducted on numeric fields to ensure that the values are "reasonable" (e.g., dilution is checked if is less than 0 or greater than 100, Table 8). Fields that are constrained to valid entries are checked for specific required content, including exact spelling. These fields are: ANALYSIS, LAB_FLAG, MATRIX, SAMPLE_TYPE, STUDY_PHASE, UNIT, PFAS_ACRONYM, COMPOUND, CAS_NO, Result_Type.

Table 7. Detailed List of EDD QA/QC Checks

Type of Check	Description
Completeness	71 results for each sample All compounds have been reported for all samples Three sample types for each sample Review sample type counts Re-analysis results without the original analysis reported Missing SAMPLE_NO Missing compounds (from all samples)
Units	Consistent units Correct units Consistent sample_size_units Correct sample_size_units SAMPLE_SIZE is consistent within EDD and across the matrix
Formatting	No suffixes added to SAMPLE_NO ANALYSIS_DATE is in the correct format PERCENT_REC must be a whole number Numeric entries in number fields Re-analysis has incremented lab_rep
Null and placeholders	Null in CONC_FOUND field Null in CONC_SPIKE field Null in DILUTION field Null in LOQ field Null in MDL field Null in SAMPLE_SIZE field CONC_SPIKE = 0 ok CONC_SPIKE not equal to 0 ok PERCENT_REC = null ok PERCENT_REC not null ok CAS_NO can only be null for EIS or NIS
Sample Type/Matrix Coding	MATRIX is coded correctly for blank samples MATRIX is coded correctly for study samples MATRIX is coded correctly for QC samples SAMPLE_NO is coded correctly for blank samples SAMPLE_NO is coded correctly for QC samples SAMPLE_NO is coded correctly for study samples SAMPLE_TYPE is coded correctly for NIS and EIS result_type SAMPLE_TYPE is coded correctly for TRG result_type SAMPLE_TYPE is not EIS or NIS for TRG result_type (not EIS or NIS) Mis-coded Normal samples Mis-coded compounds
Detection Limit / Qualifier Checks	MDL is not greater than the LOQ LAB_FLAG not set to J when CONC_FOUND >MDL and <LOQ LAB_FLAG not set to U when CONC_FOUND = MDL CONC_FOUND < MDL LAB_FLAG set to U when CONC_FOUND not = MDL CONC_FOUND > MDL and U flagged MDL can only be null for EIS or NIS LOQ can only be null for EIS or NIS LOQ should not be populated for EIS/NIS MDL should not be populated for EIS/NIS
Calculations	PERCENT_REC calculations ($\geq$100) PERCENT_REC calculations ($\geq$10 and <100) PERCENT_REC calculations (<10) Review fields that must reported to 3 sig figs ANALYSIS_DATE is after the EXTRACTION_DATE NIS PERCENT_REC are not all 100

Table 8. Fields with Range Checks

Field Name	Min	Max	Default
CONC_FOUND	0.0001	1000	REQUIRED
CONC_SPIKE	1	1000	0
DILUTION	0	100	1
PERCENT_REC	0	170	NULL
SAMPLE_SIZE	0.004	1000	REQUIRED
MDL	0.0001	100	
LOQ	0.0001	100	

In conjunction with developing the data management system, the EDD Template instructions were reviewed and 'MLV_Study_Supplemental_EDD_Instructions' were developed and distributed to the MLV Study team, to clarify some details of how the EDD Template is expected to be populated. Many of the QA/QC checks listed in Table 7 are based on the Supplemental EDD Instructions (2022, unpublished), in particular, the sample coding guidance (Table 9). Narrative guidance included in the Supplemental EDD Instructions are found in Appendix D.

Table 9. Allowed Code Combinations for Sample_No, Matrix, and Sample_Type fields

Phase	SAMPLE_NO	Compound Type	Matrix	Sample_type	CONC_SPIKE	PERCENT_REC
Phase 4	GWA0	Method analytes	GW, SW, etc	NORMAL	0	NULL
Phase 4	GWA0	EIS	GW, SW, etc	EIS	Populated	Populated
Phase 4	GWA0	NIS	GW, SW, etc	NIS	Populated	Populated
Phase 4	MB	Method analytes	RW, OS, RT	BLANK	0	NULL
Phase 4	MB	EIS	RW, OS, RT	EIS	Populated	Populated
Phase 4	MB	NIS	RW, OS, RT	NIS	Populated	Populated
Phase 4	OPR*	Method analytes	QC	OPR	Populated	Populated
Phase 4	OPR*	EIS	QC	EIS	Populated	Populated
Phase 4	OPR*	NIS	QC	NIS	Populated	Populated
Phase 4	LLOPR*	Method analytes	QC	LLOPR	Populated	Populated
Phase 4	LLOPR*	EIS	QC	EIS	Populated	Populated
Phase 4	LLOPR*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	IPR*	Method analytes	QC	IPR	Populated	Populated
Phase 3 IDC	IPR*	EIS	QC	EIS	Populated	Populated
Phase 3 IDC	IPR*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	MDLB	Method analytes	RW, OS, RT	MDLB	0	NULL
Phase 3 IDC	MDLB	EIS	RW, OS, RT	EIS	Populated	Populated
Phase 3 IDC	MDLB	NIS	RW, OS, RT	NIS	Populated	Populated
Phase 3 IDC	MDLS*	Method analytes	QC	MDLS	Populated	Populated
Phase 3 IDC	MDLS*	EIS	QC	EIS	Populated	Populated
Phase 3 IDC	MDLS*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	LOQVER*	Method analytes	QC	LOQVER	Populated	Populated
Phase 3 IDC	LOQVER*	EIS	QC	EIS	Populated	Populated
Phase 3 IDC	LOQVER*	NIS	QC	NIS	Populated	Populated
Phase 3 IDC	MB	Method analytes	RW, OS, RT	BLANK	0	NULL
Phase 3 IDC	MB	EIS	RW, OS, RT	EIS	Populated	Populated
Phase 3 IDC	MB	NIS	RW, OS, RT	NIS	Populated	Populated

*spiked samples

During the execution of the checking routines, an error report with standardized error messages is automatically generated for the checks that failed. This report can be viewed within the application by selecting "View Report" (Figure 6) and is also exported to an Excel file to generate the Error Summary Report that was provided to the laboratories. This auto-generated report was carefully reviewed by the Exa team and apparent errors were examined prior to sending to laboratories.

Most of the QA/QC checks are associated with detailed queries that provide information on what the specific problem is (see the bottom part of Figure 6, "Detailed QA/QC Queries"). For the errors that are applicable to a certain check, the query results are copied into separate worksheets of the Error Summary report and provided to the laboratories, to assist them in identifying the issue(s) in the submitted EDD. The standardized error messages and the worksheets containing the detailed QA/QC query results are cross-referenced with the query name (e.g., *qry_Edd_review_sample_no*).

Figure 6. QA/QC Report and Detailed QA/QC Queries Form

4.2.3 Final Processing Steps

There are additional data processing steps that occur after the QA/QC routines have been executed on individual EDDs (Figure 7). Routine 5 populates the spike category (spike_cat) field, and Routine 6 appends the EDD to the main database table (Lab_EDD_Results), with separate steps to populate the Tracking ID, LAB_ID and SDG fields. Finally, Routine 7 automatically exports an individual EDD in the Amended EDD format which will be provided to the data validators in Excel format. Table 10 lists the additional fields that are not in the EDD laboratory template but are included in the Amended EDD (also see Appendix C).

Figure 7. Append to Master EDD Database and Generate Amended EDD Form

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Table 10. Additional Fields Included in the Amended EDD

Field	Definition
TrackingID	Tracking ID from Tracking System; incorporated during ETL procedures
sdg_num	SDG number; incorporated during ETL procedures; extracted from Lab_Sample_ID
lab_rep	Lab rep number added to easily filter for re-analysis/dilutions.
Result_Type	Code for Result Type. See Valid Value list.
validation_level	Stores information on the level of data validation that has been completed for the chemistry data. Automatically populated with 'Level 4'.
validator	Code for Data Validator. See Valid Value list.
dv_qualifier	Code for Data Validator qualifiers. See Valid Value list.
dv_qualifier_reason	Data validation qualifier reason codes.
dv_notes1	Include comments to distinguish the meaning of the dv_qualifier assignment (e.g., distinguishing the -J qualifier)
dv_notes2	Additional information provided by validator deemed pertinent to their dv_qualifier assignment.
dv_result	Validator recommended result for concentration. If this is provided, entries must be made in the dv_ResultChange_yn field and dv_ResultChange_desc fields.
dv_ResultChange_yn	Enter Y or N. Indicates whether the validator made a recommendation to change the result for concentration.
dv_ResultChange_desc	Description of the reasons for validator recommending a change to the result for concentration.
Reviewer_qualifier	Code for qualifiers applied by NAVY/EPA reviewers of data validation results. See Valid Value list.
Reviewer_notes	Notes from NAVY/EPA reviewers of data validation results.

4.3 Database and Tools

The purpose of this section is to describe the structure and associated tools for compiling the EDDs into the Project Database. The Project Database is a relational database using MS Access as the selected database software. MS Access was chosen due to its common usage and ease of transforming the data to other formats, as necessary. The master version of the Project Database will be stored on Exa's local server, ensuring that access to the 'working' database is limited to the Database Managers. If project participants requested access to the Project Database, they were given permissions to access a copy of the database posted on the ExaBlue SharePoint. Alternatively, Exa generated customized data exports for specific purposes, when requested.

The goals of the Project Database and associated toolsets are as follows:

- Maximize the reliability of the database by designing and implementing automated QA/QC and verification checks;
- Store the data in a structured database with rules that restrict entries for certain fields to specific valid values, and that follow relational database rules such as primary keys and inter-table relationships;
- Promote accurate and rapid transfer of data to a variety of export and imports formats for use by team members (validators, IDA) and reporting to the MVS Team.

The custom application for processing data for the MLV Study was designed and developed by the Exa team and tested extensively. Testing involved multiple Exa team members running the procedures on multiple test data sets to identify bugs and inconsistencies. Fixes were then incorporated into the automated routines.

4.3.1 Database Structure

The structure of the database is provided in Figure 8 as an entity-relationship diagram (ERD), which describes the tables and fields in the database and how they are related. The field definitions are compiled in the database dictionary as seen in Appendix C.

The main EDD data table (Lab_EDD_Results) parallels the format of the laboratory EDD (SERDP/ESTCP 2023, Attachment 3), with EDD field names shown in all capital letters in Figure 8. In addition to the EDD fields, several additional fields were added to the main EDD table, shown in lower case, including the TrackingID, result_type, spike_level, and fields to capture results from the data validators and EPA/NAVSEA (Appendix C).

The valid value tables (dicValidValues, lu_Compound) were maintained separately but linked to the main EDD table to enforce entries in fields constrained by valid values, and are shown in

Appendices E and F, respectively. Retaining strict valid values enable both the validators and IDA to accurately filter and analyze the output data.

The database structure includes the lu_SpikeLevels table which stores the spike concentrations reported in the ERA Certificates of Spiking. This facilitates the matrix spike percent recovery calculations (see Section 4.3.4).

The lu_MatrixType table stores the required sample nomenclature, matrix types and spike levels from the Study Plan (SERDP/ESTCP 2023; Attachment 2). This facilitates the matrix spike percent recovery calculations (see Section 4.3.4) and QA/QC EDD checks that ensure the Sample Identifiers used by the laboratories are compliant.

The dicEDD table stores information on whether individual EDDs are Approved or Rejected; it was decided by the MVS Team to include rejected EDDs in the database, when possible.

As the project progressed, modifications were made to the structure of the Project Database, as needed.
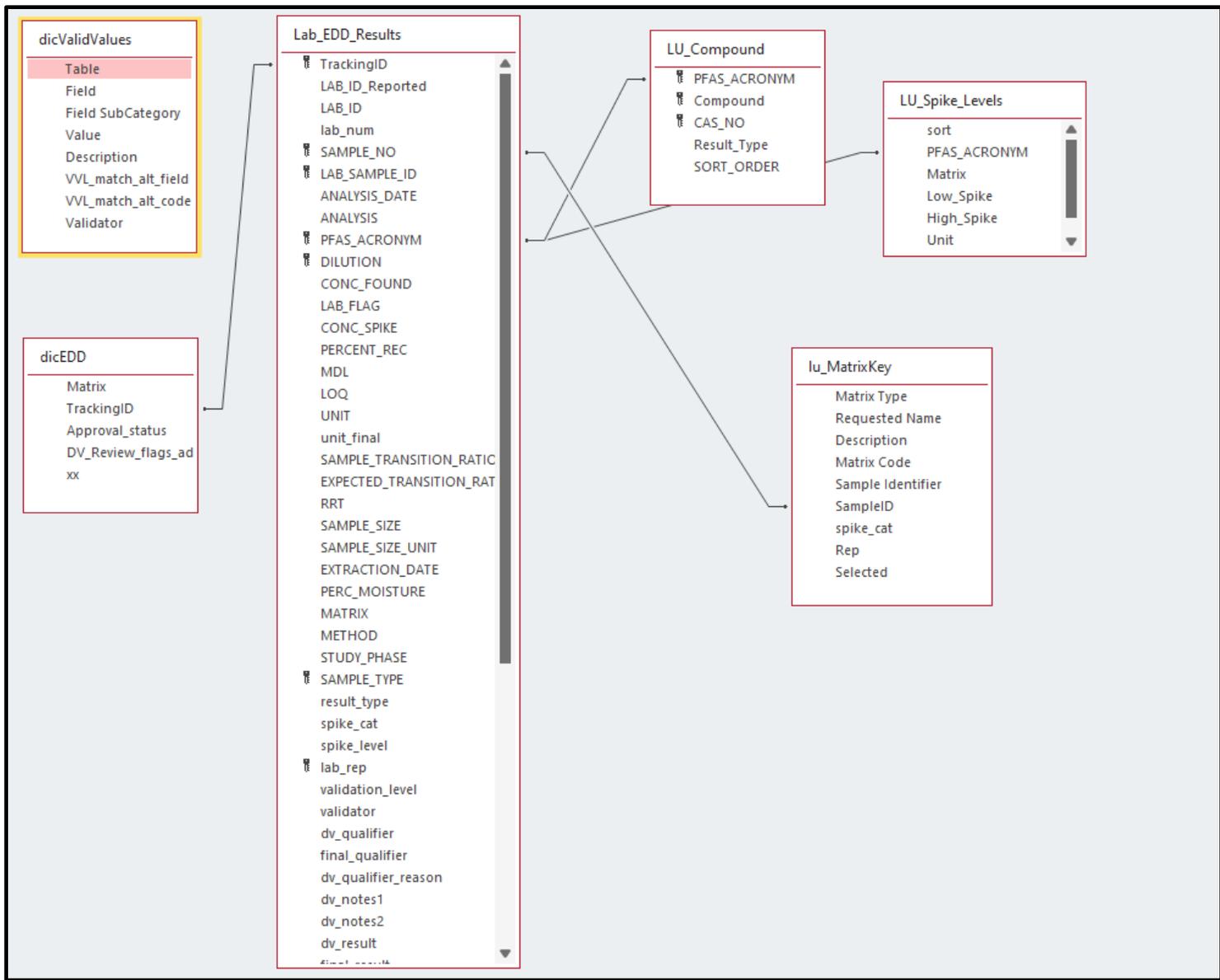
Figure 8. Entity-Relationship Diagram (ERD) for the Project Database

4.3.2 Populating Final_Result and Final_Qualifier

After data are compiled and finalized for a matrix, the Exa team populates the Final_Qualifier field based on these rules:

- Data validators only populate the dv_qualifier field if they do not agree with the laboratory qualifier. If a qualifier should be changed, they enter the new qualifier; if they want to remove a qualifier, they enter "[null]".
- EPA/NAVSEA only populate the reviewer_qualifier field if they do not agree with the dv_qualifier or LAB_FLAG qualifier. If a qualifier should be changed, they enter the new qualifier; if they want to remove a qualifier, they enter "[null]".
- The following logic is used for updating final_qualifier: 1. update with LAB_FLAG entry; 2. Overide with dv_qualifier entry; 3. Override with reviewer_qualifier entry; 4. Update [null] entries to null.

Similar logic would be employed to populate the Final_Result field (CONC_FOUND would be overridden with the dv_result entry, but at the date of publishing this report the data validators have not included entries in the dv_result field. Therefore, final_result has been updated with the CONC_FOUND entry.

4.3.3 QA/QC Checks on Master EDD Database

In addition to the checks applied to individual EDDs, there are additional QA/QC checking routines that were developed to apply to the entire database. The purpose of this operation is to review the data across Study Phases, laboratories and matrices to ensure that there are no internal inconsistencies or other issues that arise as the data are compiled. These checks identify differences in how the data are reported from different laboratories and/or validators and ensure consistency in the data exports provided to the project statisticians. It is good practice to incorporate redundancy in the QA/QC procedures to ensure that issues are not overlooked.

Examples of these database-wide queries include:
- Min-max checks on number fields
- Dictionary checks
- Unique record check
- Date range checks
- Consistent unit checks
- Review of summary of lab_flag, lab_qual, dv_qualifier, reviewer_qual, final_qualifier
- Review of summary of conc_found, dv_result, final_result, dv_ResultChange_yn, dv_ResultChange_desc

4.3.4 Matrix Spike Percent Recovery Calculation Procedures

After data are compiled and finalized for a matrix, the Exa team calculates matrix percent recovery considering the native concentration in the samples. This calculation applies to target compounds in matrix samples (i.e., it did not apply to QA/QC samples). The general calculation is:

Final_result Spiked Sample/[spike_level + Final_result Unspiked sample]

There were specific data handling options developed by the MVS Team for certain scenarios and the specific procedures used for calculating matrix percent recovery are provided in Table 11.

Table 11. Data Rules for Calculating Percent Matrix Spike Recoveries

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

Notes: [ ] - reported analyte concentration; X-flagged data are excluded from calculations and excluded from statistical analyses.

4.3.5 Import and Export File Structures

The primary import structure for the Project Database is the laboratory EDD, provided as Attachment 3 in the Study Plan (SERDP/ESTCP 2023).

There are several other import and export routines that were used in the overall workflow of the MLV Study using queries in the database:

- Export of the Amended EDD for the validators – Includes the laboratory EDD results, and additional fields to be populated by the validator when reviewing the results provided by one laboratory for one matrix.
- Import of the Amended EDD, with validation fields populated – Used to update the Project Database with the results from the validator and the EPA/NAVSEA reviewers.
- Database Export for the statistics team – Used to create a dataset for a single matrix, or a combination of matrices, for IDA in generating statistics and analysis for the project. This

database export includes final results and qualifiers, considering laboratory, data validator and data validator reviewer results.

- EDD archiving – Procedures were developed to extract and archive EDDs loaded into the Project Database and subsequently rejected (i.e., EDDs that passed initial QA/QC checks but were then rejected by the data validators; these EDDs were replaced by re-submissions).

The output formats provided to the validators and IDA are available in Appendix C (see columns named 'Include in Amended EDD for DV' and 'Include in Exports for IDA', respectively).

5.0 REFERENCES

Microsoft. 2022. How SharePoint and OneDrive safeguard your data in the cloud.
https://docs.microsoft.com/en-us/sharepoint/safeguarding-your-data

SERDP/ESTCP PFAS Method Validation Study Team. 2023. Study Plan for Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC- MS/MS. Prepared for Program Manager for Environmental Restoration, Strategic Environmental Research and Development Program (SERDP).

Willey, J., R. Anderson, A. Hanley, M. Mills, C. Hamilton, T. Thompson, and A. Leeson. 2021. Report on the Single-Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS. Strategic Environmental Research and Development Program (SERDP) Project ER19-1409. https://serdp-estcp.org/content/download/54966/539631/file/Single-Laboratory%20Validation%20Study%20Report.pdf

Appendix A - Description of the File Tracking System

TRACKING WORKSHEET	TRACKING FIELD	VV Field	TRACKING FIELD DESCRIPTION
GENERAL	Tracking ID		Root file name
	Project Phase		Project Phase. See 'ValidValues'
	EDD File Name		EDD File name
	Data Package File Name		Data Package File Name
	Laboratory Name	Yes	Laboratory Name. See 'ValidValues'
	Matrix	Yes	Matrix. See 'ValidValues'
	Notes		Notes regarding submitted files
	Log Date		Date the reciept of files was logged into the Tracking System
LABORATORY	EDD/DP Due Date		Due date for the Lab EDD/Data Package (mm/dd/yyyy)
	EDD/DP Date Received		Date Lab EDD/Data Package received (mm/dd/yyyy; uploaded to Sharepoint)
	HGL Reviewer		Initials of HGL staff conducting the Data Package review
	Date HGL Review Complete		Date HGL review complete (mm/dd/yyyy)
	EDD Rejected or Approved - HGL	Yes	Indicate whether EDD/Data Package was rejected by HGL. See 'ValidValues'
	Summary of Errors - HGL		Brief summary of issues found during HGL Data Package review
	Exa Reviewer		Initials of Exa staff conducting the automated EDD review
	Date Exa Review Complete		Date Exa review complete (mm/dd/yyyy)
	EDD Rejected or Approved - Exa	Yes	Indicate whether EDD/Data Package was rejected by Exa. See 'ValidValues'
	Summary of Errors - Exa		Brief summary of issues found during Exa EDD review
	Date Data Package to DV		Date the Data Package was posted to the Validator folder (mm/dd/yyyy).
	Date Amended EDD to DV		Date the Amended EDD was posted to the Validator folder (mm/dd/yyyy).
VALIDATOR	Data Validator	Yes	Data Validator. See 'ValidValues'.
	DP version reviewed		Current version number of the Data Package provided to the data validator for review.
	Amended EDD version reviewed		Current version number of the Amended EDD provided to the data validator for review.
	Date DV Report/Files Received		Date of receipt for the current data validator report/files (mm/dd/yyyy).
	DV Amended EDD version		Current version number of the Amended EDD with validator fields populated (posted by the data validator).
	DV Report version		Current version number of the data validator report (posted by the data validator).
	DV Verification version		Current version number of the Verification file (posted by the data validator).
	DV Checklist version		Current version number of the Checklist (posted by the data validator).
	EDD Rejected or Approved - DV	Yes	Indicate whether EDD/Data Package was rejected by the Data Validator. See 'ValidValues'
	Notes		Notes regarding submitted files
	Date EPA/NAVY Files Received		Date of receipt for the current review files posted by EPA/NAVY reviewers (mm/dd/yyyy).

Acronyms: DB - Project Database
             DP - Data Package
             DV - Data Validator
             EDD - Electronic Data Deliverable

Appendix B - File Tracking System Valid Values List

Worksheet	Field	Valid Value Code	Valid Value Code Description
GENERAL	Laboratory Name	ALPHA	Alpha Analytical
GENERAL	Laboratory Name	BATTELLE	Battelle
GENERAL	Laboratory Name	CALEPA	CalEPA DTSC
GENERAL	Laboratory Name	ELLET	Eurofins Lancaster Labs
GENERAL	Laboratory Name	ETA	ETA, Sacramento
GENERAL	Laboratory Name	GEL	GEL Laboratories
GENERAL	Laboratory Name	MDH	Maryland Department of Health
GENERAL	Laboratory Name	PACE	GCAL/Pace
GENERAL	Laboratory Name	SGSNA	SGS North America
GENERAL	Laboratory Name	VISTA	Vista Analytical
GENERAL	Matrix	GW	Groundwater
GENERAL	Matrix	SW	Surface water
GENERAL	Matrix	SD	Sediment
GENERAL	Matrix	SS	Soil
GENERAL	Matrix	TS	Tissue
GENERAL	Matrix	WW	Wastewater
GENERAL	Matrix	LC	Landfill Leachate
GENERAL	Matrix	BS	Biosolids
GENERAL	Project Phase	Phase 3 - ICAL	Initial Calibration
GENERAL	Project Phase	Phase 3 - IDC	Initial Demonstration of Capabilities (IDC)
GENERAL	Project Phase	Phase 4.4.1	GW, SW, and WW matrices
GENERAL	Project Phase	Phase 4.4.2	SS and SD matrices
GENERAL	Project Phase	Phase 4.4.3	Tissue matrices
GENERAL	Project Phase	Phase 4.4.4	LC and BS matrices
LABORATORY	EDD Rejected or Approved - HGL	Approved	Passed review
LABORATORY	EDD Rejected or Approved - HGL	Rejected	Did not pass review
LABORATORY	EDD Rejected or Approved - HGL	NA	Not applicable (not reviewed)
LABORATORY	EDD Rejected or Approved - HGL	To Validator	Bypassed review and went straight to validator.
LABORATORY	EDD Rejected or Approved - HGL	Re-submitting	Re-submission from the lab expected
LABORATORY	EDD Rejected or Approved - HGL	Pending	Lab has submitted a data package and it's pending review
LABORATORY	EDD Rejected or Approved - HGL	Not Submitted	Lab hasn't submitted
LABORATORY	EDD Rejected or Approved - Exa	Approved	Passed review
LABORATORY	EDD Rejected or Approved - Exa	Rejected	Did not pass review
LABORATORY	EDD Rejected or Approved - Exa	NA	Not applicable (not reviewed)
LABORATORY	EDD Rejected or Approved - Exa	Re-submitting	Re-submission from the lab expected
LABORATORY	EDD Rejected or Approved - Exa	Pending	Lab has submitted an EDD and it's pending review
LABORATORY	EDD Rejected or Approved - Exa	Not submitted	Lab hasn't submitted
VALIDATOR	Data Validator	CHEMVAL	ChemVal
VALIDATOR	Data Validator	PYRON	Pyron Environmental
VALIDATOR	Data Validator	JACOBS	Jacobs Engineering
VALIDATOR	EDD Rejected or Approved - DV	Approved	Approved as is (no DV input)
VALIDATOR	EDD Rejected or Approved - DV	Revised	Approved with DV input added
VALIDATOR	EDD Rejected or Approved - DV	Rejected	Did not pass review
VALIDATOR	EDD Rejected or Approved - DV	NA	Not applicable (not reviewed)

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	TrackingID	text	Tracking ID from Tracking System; incorporated during ETL procedures	No	No	Yes		X	Yes	No
Lab_EDD_Results	Lab_ID_Reported	text	LAB_ID reported by the laboratories in the EDD	Yes	No	Yes			No	No
Lab_EDD_Results	LAB_ID	text	Laboratory Name. See Valid Value list.	No	Yes	Yes		X	Yes	No
Lab_EDD_Results	lab_num	number	Lab_ID code, to keep laboratories anonymous. See Valid Value list.	No	Yes	Yes			No	Yes
Lab_EDD_Results	sdg_num	text	SDG number; incorporated during ETL procedures; extracted from Lab_Sample_ID	No	No	No			Yes	Yes
Lab_EDD_Results	SAMPLE_NO	text	For samples, these are the sample identification names (IDs) from the Chain of Custody. The Sample_No is the same, regardless of whether or not the sample is diluted or reanalyzed. For preparation batch QC, these are "MB" for the Method Blank, "OPR" for the OPR, and "LLOPR" for the LLOPR. For IDOC samples, "IPR" for the IPR samples, "MDLB" for the MDL_b samples, "MDLS" for the MDLs samples, and "LOQVER" for the LOQVER samples.	Yes	No	Yes		X	Yes	Yes
Lab_EDD_Results	LAB_SAMPLE_ID	text	The ID the laboratory assigns to the sample (which identifies the sample on the associated data files and reports). For samples that need to be re-analyzed for issues other than dilution, attach the following identifiers to the end of the lab sample identifier without a space between them (e.g., 02082022-01R): "R" for analytes, EISs & NISs reported from first re-analysis not due to dilution "R1" for analytes, EISs & NISs reported from second re-analysis not due to dilution "R2" for analytes, EISs & NISs reported from second re-analysis not due to dilution If more re-analyses not due to dilution are needed to be reported beyond three for a sample, continue on with the numbering (e.g., R3, R4, R5, etc.).	Yes	No	Yes		X	Yes	Yes
Lab_EDD_Results	ANALYSIS_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.	Yes	No	Yes			Yes	No
Lab_EDD_Results	ANALYSIS	text	Fill in "PFAS". See Valid Value list.	Yes	Yes	Yes	PFAS		Yes	No
Lab_EDD_Results	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	Yes	Yes	Yes		X	Yes	Yes
Lab_EDD_Results	lab_rep	text	Lab replicate identifier	No	No	Yes	1		Yes	Yes

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	DILUTION	number	Dilution made post extraction (e.g., extract diluted 1:10 is entered as "10"). If analyzed without dilution, enter "1".	Yes	No	Yes	1	X	Yes	Yes
Lab_EDD_Results	LAB_FLAG	text	Laboratory qualifiers. See Valid Value list.	Yes	Yes	No			Yes	No
Lab_EDD_Results	CONC_FOUND	number	Enter numeric quantitative result value only. Report to three significant figures. Do NOT enter any text string strings or symbols (e.g., "ND", "<"). For analytes that are not detected, the laboratory's sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account) is entered. Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. Report result units in "Unit" field, consistent for all sample fields.	Yes	No	Yes			Yes	No
Lab_EDD_Results	CONC_SPIKE	number	For unspiked samples enter "0" for method analytes. For spiked samples, enter the spike concentration representing the estimated concentration in the final extract (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis. For EIS and NIS, enter the spike concentration representing the concentration in the final extract in units consistent with sample result units. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	Yes			Yes	Yes
Lab_EDD_Results	PERCENT_REC	number	For unspiked samples, leave blank. No text should be included in this field (e.g. N/A). For spiked samples (OPR, LLOPR, MDLs and LOQVER), enter the spike percentage recovery as a whole number (e.g., 95 versus 0.95). Do NOT include "%" symbol. For EIS and NIS recoveries, enter the spike % recovery as a whole number (e.g., 95 versus 0.95). Report to 3 significant figures. Do NOT include "%".	Yes	No	No			Yes	Yes
Lab_EDD_Results	MDL	number	Method Detection Limit. Enter the sample specific MDL (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (µg/kg) for solid samples. Report to 3 significant figures.	Yes	No	No	-9		Yes	Yes

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	LOQ	number	Limit of Quantitation. Enter the sample specific LOQ (i.e. with extract dilution factor, sample volume/weight and final volume taken into account). Report to 3 significant figures. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (μg/kg) for solid samples. Report to 3 significant figures.	Yes	No	No	-9		Yes	Yes
Lab_EDD_Results	UNIT	text	The reporting units must be consistent for the sample record including Conc_Found, MDL, LOQ etc. The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (μg/kg) for solid samples. Ensure that all values for the sample record are reported in the same units. See Valid Value list.	Yes	Yes	Yes			No	No
Lab_EDD_Results	unit_final	text	The reporting unit, standardized	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	SAMPLE_ TRANSITION_RATIO	text	Enter the calculated Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte in the sample. Report to 3 significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g. N/A).	Yes	No	No			Yes	No
Lab_EDD_Results	EXPECTED_ TRANSITION_RATIO	text	Enter the expected Transition Ratio (Quant Ion Area/Conf Ion Area) for each analyte per the method. Report to three significant figures. For analytes this does not apply to (PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA), leave this field blank. No text should be included in this field (e.g., N/A).	Yes	No	No			Yes	No
Lab_EDD_Results	RRT	text	Enter relative retention time	Yes	No	No			Yes	No
Lab_EDD_Results	SAMPLE_SIZE	number	Enter volume (aqueous samples) or weight (solid samples) of sample extracted (in liters for aqueous samples, in kilograms for solids).	Yes	No	Yes			Yes	No
Lab_EDD_Results	SAMPLE_SIZE_UNIT	text	Will be liters (L) for aqueous samples or kilograms (Kg) for solid samples	Yes	No	Yes			Yes	No
Lab_EDD_Results	EXTRACTION_DATE	short date; mm/dd/yyyy	Use format mm/dd/yyyy (e.g. 11/20/2019) – do not include time stamp.	Yes	No	Yes			Yes	No
Lab_EDD_Results	PERC_MOISTURE	number	Percent moisture in solid samples only. Enter the percent moisture as a whole number (e.g., 73 versus 0.73). Do NOT include "%" symbol.	Yes	No	No			Yes	No
Lab_EDD_Results	MATRIX	text	Matrix analyzed. See Valid values list.	Yes	Yes	Yes			Yes	Yes

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	METHOD	text	Laboratory SOP Name in format of "name(space)revision number"	Yes	No	Yes			Yes	No
Lab_EDD_Results	STUDY_PHASE	text	Multi-Lab Validation Study Phase. See Valid Value list.	Yes	Yes	Yes			Yes	No
Lab_EDD_Results	SAMPLE_TYPE	text	See Valid Value list.	Yes	Yes	Yes		X	Yes	Yes
Lab_EDD_Results	result_type	text	Code for Result Type. See Valid Value list.	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	spike_cat	text	Code for Spike Category. See Valid Value list.	No	Yes	Yes			Yes	Yes
Lab_EDD_Results	validation_level	text	Stores information on the level of data validation that has been completed for the chemistry data.	No	No	Yes	Level 4		Yes	No
Lab_EDD_Results	validator	text	Code for Data Validator. See Valid Value list.	No	Yes	Yes			Yes	No
Lab_EDD_Results	dv_qualifier	text	Code for Data Validator qualifiers. See Valid Value list.	No	Yes	No			Yes	No
Lab_EDD_Results	dv_qualifier_reason	text	Data validation qualifier reason codes.	No	No	No			Yes	No
Lab_EDD_Results	dv_notes1	text	Include comments to distinguish the meaning of the dv_qualifier assignment (e.g., distinguishing the -J qualifier)	No	No	No			Yes	No
Lab_EDD_Results	dv_notes2	text	Additional information provided by validator deemed pertinent to their dv_qualifier assignment.	No	No	No			Yes	No
Lab_EDD_Results	dv_result	number	Validator recommended result for concentration. If this is provided, entries must be made in the dv_ResultChange_yn field and dv_ResultChange_desc fields.	No	No	No			Yes	No
Lab_EDD_Results	dv_ResultChange_yn	Logical	Enter Y or N. Indicates whether the validator made a recommendation to change the result for concentration.	No	No	Yes			Yes	No
Lab_EDD_Results	dv_ResultChange_desc	text	Description of the reasons for validator recommending a change to the result for concentration.	No	No	No			Yes	No
Lab_EDD_Results	Reviewer_qualifier	text	Code for qualifiers applied by NAVY/EPA reviewers of data validation results. See Valid Value list.	No	Yes	No			Yes	No
Lab_EDD_Results	Reviewer_notes	text	Notes from NAVY/EPA reviewers of data validation results.	No	No	No			Yes	No
Lab_EDD_Results	final_qualifier	text	Code for Final Qualifier. See Valid Value list.	No	Yes	No			No	Yes
Lab_EDD_Results	final_result	number	Final result for concentration. Combines CONC_FOUND and validator_result fields.	No	No	Yes			No	Yes
Lab_EDD_Results	lab_rep	text	Data manager assigned. Laboratory replicate number; to assist with completeness and duplicate checks.	No	No	Yes			Yes	No
Lab_EDD_Results	sample_rep	text	Data manager assigned. From lu_MatrixType.Rep field; to assist with spike_percent_rec calculation	No	No	No			No	No
Lab_EDD_Results	sample_root	text	Data manager assigned. Sample_NO without the Reg suffix; to assist with spike_percent_rec calculation	No	No	No			No	No
Lab_EDD_Results	spike_level	number	Data manager assigned. From lu_SpikeLevel table; to assist with spike_percent_rec calculation	No	No	No			No	Yes

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
Lab_EDD_Results	conc_minus_native	number	Data manager calculated. Interim value in spike_percent_rec calculation	No	No	No			No	Yes
Lab_EDD_Results	spike_percent_rec	number	Data manager calculated. Matrix spike percent recovery.	No	No	No			No	Yes
Lab_EDD_Results	spk_pct_rec_DNC	text	Data manager assigned. Determination of Calculate/DNC (do not calculate)for spike_percent_rec calculation	No	No	No			No	Yes
Lab_EDD_Results	CONC_FOUND_val	text	Data manager assigned. CONC_FOUND as a value; to assist with database-wide QAQC checks.	No	No	No			No	No
Lab_EDD_Results	LOQ_val	text	Data manager assigned. LOQ as a value; to assist with database-wide QAQC checks.	No	No	No			No	No
Lab_EDD_Results	MDL_val	text	Data manager assigned. MDL as a value; to assist with database-wide QAQC checks.	No	No	No			No	No
Lab_EDD_Results	LAB_SAMPLE_IDclean	text	Data manager assigned. Standardized LAB_SAMPLE_ID to remove re-analysis suffixes; to assist with completeness and duplicate checks.	No	No	Yes			No	Yes
Lab_EDD_Results	qaqc_dup	text	Data manager assigned. Identifies LLOPR, OPR and MB double-duty samples and exclude one of the results for the 'all in' database exports.	No	No	No			No	No
Lab_EDD_Results	DM_notes	text	Data manager notes.	No	No	No			No	No
Lab_EDD_Results	DM_notes2	text	Data manager notes - 2.	No	No	No			No	No
Lab_EDD_Results	EditDate	text	Date of append to Lab_EDD_Results table.	No	No	Yes			No	No
dicValidValues	Table	text	Valid value table name.	No	No	Yes			No	dicNo
dicValidValues	Field	text	Valid value field name.	No	No	Yes			No	dicNo
dicValidValues	Value	text	Acceptable valid value codes.	No	No	Yes			No	dicNo
dicValidValues	Description	text	Description of valid value codes, if necessary	No	No	Yes			No	dicNo
dicValidValues	VVL_match_alt_field	text	Related valid value field name.	No	No	No			No	dicNo
dicValidValues	VVL_match_alt_code	text	Matching valid value code.	No	No	No			No	dicNo
dicValidValues	Validator	text	Data validator assoicated with each LAB_ID_CODE	No	No	No			No	dicNo
LU_Compound	SORT_ORDER	number	Sort order to apply to data summary tables.	No	No	Yes			No	No
LU_Compound	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Compound	COMPOUND	text	Use the names included in the example EDD. Method analytes, and EIS and NIS compounds must be reported for each sample. See Valid Value list.	Yes	Yes	Yes			Yes	Yes
LU_Compound	CAS_NO	text	Chemical Abstract Service Registration Number	Yes	Yes	Yes			Yes	Yes
LU_Compound	result_type	text	Code for Result Type. See Valid Value list.	No	Yes	Yes			No	No
LU_Spike_Levels	PFAS_ACRONYM	text	Use acronyms included in the example EDD. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Spike_Levels	Matrix	text	Name of the matrix. See Valid Value list.	No	Yes	Yes		X	No	No
LU_Spike_Levels	Low_Spike	number	Low spike concentration	No	No	Yes			No	No
LU_Spike_Levels	High_Spike	number	High spike concentration	No	No	Yes			No	No

Appendix C – Project Database - Database Dictionary

Table	Field	Data type	Definition and Comments	Direct from EDD	Valid Value Field	Required field	Default Value	Primary Key	Include in Amended EDD for DV	Include in Exports for IDA
LU_Spike_Levels	Unit	text	Unit of spike concentration	No	No	Yes			No	No
lu_MatrixKey	Matrix Type	text	Matrix (full name)	No	No	Yes			No	No
lu_MatrixKey	Requested Name	text	Descriptive name of sample	No	No	Yes			No	No
lu_MatrixKey	Description	text	Description of sample	No	No	Yes			No	No
lu_MatrixKey	Matrix Code	text	Matrix (code)	No	No	Yes			No	No
lu_MatrixKey	Sample Identifier	text	Sample identifier (middle component of SampleID)	No	No	Yes			No	No
lu_MatrixKey	SampleID	text	SampleID (EDD SAMPLE_NO must match to this code)	No	No	Yes			No	No
lu_MatrixKey	spike_cat	text	Spike category (low, high, etc)	No	No	Yes			No	No
lu_MatrixKey	Rep	text	Sample replicate number (last component of SampleID)	No	No	Yes			No	No
lu_MatrixKey	Selected	text	Indicates if sample was selected for use in the study	No	No	Yes			No	No

Note: The Required Fields may be revised during database development; maximum field lengths will be incorporated into the database structure during development.

Appendix D – Supplemental guidance for correctly populating the EDD Template	
Item	**Guidance**
1	If possible, include all results in one worksheet.
2	We will be running QA/QC routines on the EDDs to ensure they are populated correctly. These will be delivered to you in an Excel file (e.g., LABNAME_RW_ver0_EDD_Error_Summary.xlsx).
	If you have questions/issues regarding the Error Summary report, please incorporate comments directly into the Excel file and send it back to us for review (via SharePoint).
3	Instructions for reporting CONC_FOUND for NIS compounds:
	1. Option 1: Report NIS Mass in CONC_FOUND, and in the accompanying report provide example calculation and point to where the data in the numerator (e.g., field sample) and the denominator (e.g. CCV) are found.
	2. Option2: Complete the EDDs with the NIS percent recoveries based on areas (i.e., no mass reported). CONC_FOUND does not need to be populated.
	a. If Option 2 is selected then
	i. In the report provide the formula and example calculations for one sample per batch
	ii. Point to where those areas values are found in their data packages (i.e., ensure they have reported the NIS area for the field (target) sample and the comparative area from either the mid-point of the ICAL, CCV, or equivalent.
4	Instructions for populating EDD when a single sample is serving the purpose of the MDLB and MB samples:
	1. Copy the results and code the SAMPLE_NO field as 'MDLB' for one set of results, and 'MB' for the other set of results.
	2. Ensure that the case narrative clearly identifies that the blank sample was used for multiple purposes.
5	Ensure that B flags are applied as indicated by the instructions:
	"For analytes that were detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than 1/10th the concentration in the sample, whichever is greatest. The MB must also be flagged with a "B" for all concentrations greater than ½ the LOQ."
	If the CONC_FOUND is between the MDL and LOQ <u>AND</u> it is at a concentration greater than 1/2 LOQ, the flag should be BJ or JB.
6	Although the instructions for the CONC_FOUND field indicate "For analytes that are not detected, the laboratory's sample specific MDL", the exceptions are for the following sample types, where CONC_FOUND can be less than the MDL:
	MDLS
	LOQVER
	Blanks (MB and MDLB)
7	For instances where there are re-analysis not due to dilution, all results must be reported in the EDD (i.e., the original analysis and all subseqent re-analysis).

	Appendix D – Supplemental guidance for correctly populating the EDD Template
8	The Sample Coding spreadsheet lists the mandatory batch QC samples that are required for Phase 3 and Phase 4 submittals. All mandatory batch QC samples must be present in the EDD submittals.
9	Do not add suffixes to SAMPLE_NO (e.g., IPR1, MLDB 2) - LAB_SAMPLE_ID will differentiate samples.
10	Fields that must be reported to 3 significant figures:
	CONC_FOUND
	CONC_SPIKE
	PERCENT_REC (DOES need to be reported to 3 sig figs; report as a whole number (95.1), not a fraction (0.951)
	MDL
	LOQ
	SAMPLE_TRANSITION_RATIO
	EXPECTED_TRANSITION_RATIO
	Note that the results in these fields must be rounded appropriately to 3 significant figures. Changing the display for the number of decimal places is not sufficient.
11	Solids are reported on a dry-weight basis. Tissues are reported on a wet-weight basis.
12	UNIT field must be consistent across all samples.
13	The reporting units for this project are parts per trillion (ppt) or nanograms per liter (ng/L) for aqueous samples and parts per billion micrograms per kilogram (μg/kg) for solid samples.
14	Do not include text in number fiels (e.g. N/A, %, ND, <).
15	Percent recovery must be reported in the EDD for IPR samples.

Appendix E – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code	Validator
LAB_ID_CODE	ALPHA	Alpha Analytical	lab_num	3	JACOBS
LAB_ID_CODE	BATTELLE	Battelle	lab_num	6	JACOBS
LAB_ID_CODE	CALEPA	CalEPA DTSC	lab_num	2	PYRON
LAB_ID_CODE	ELLET	Eurofins Lancaster Labs	lab_num	10	JACOBS
LAB_ID_CODE	ETA	ETA, Sacramento	lab_num	1	PYRON
LAB_ID_CODE	GEL	GEL Laboratories	lab_num	8	JACOBS
LAB_ID_CODE	MDH	Maryland Department of Health	lab_num	5	JACOBS
LAB_ID_CODE	PACE	GCAL/Pace	lab_num	9	JACOBS
LAB_ID_CODE	SGSNA	SGS North America	lab_num	7	PYRON
LAB_ID_CODE	VISTA	Vista Analytical	lab_num	4	PYRON
MATRIX	BS	Biosolids			
MATRIX	GW	Groundwater			
MATRIX	LC	Landfill Leachate			
MATRIX	OS	Ottawa sand for all soil, sediment, and biosolid MBs			
MATRIX	QC	Quality Control Sample			
MATRIX	RT	Reference Tissue for tissue MBs			
MATRIX	RW	Reagent water for all aqueous MBs			
MATRIX	SD	Sediment			
MATRIX	SS	Soil			
MATRIX	SW	Surface water			
MATRIX	TS	Tissue			
MATRIX	WW	Wastewater			
result_type	EIS	Extracted Internal Standard			
result_type	NIS	Non-Extracted Internal Standard			
result_type	TRG	Target analyte			
SAMPLE_TYPE	BLANK	method analytes in MBs			
SAMPLE_TYPE	EIS	EIS in all samples			
SAMPLE_TYPE	IPR	method analytes in IPR IDC samples			
SAMPLE_TYPE	LLOPR	method analytes in LLOPRs			
SAMPLE_TYPE	LOQVER	Method analytes in MDL LOQVER IDC samples			
SAMPLE_TYPE	MDLB	Method analytes in MDL Blank IDC samples			
SAMPLE_TYPE	MDLS	Method analytes in MDL Spike IDC samples			
SAMPLE_TYPE	NIS	NIS in all samples			
SAMPLE_TYPE	NORMAL	method analytes in field samples			
SAMPLE_TYPE	OPR	method analytes in OPRs			
spike_cat	HIGH	High			
spike_cat	LOW	Low			
STUDY_PHASE	Phase 3 - ICAL	Initial Calibration			
STUDY_PHASE	Phase 3 - IDC	Initial Demonstration of Capabilities (IDC)			
STUDY_PHASE	Phase 4.4.1	GW, SW, and WW matrices			
STUDY_PHASE	Phase 4.4.2	SS and SD matrices			
STUDY_PHASE	Phase 4.4.3	Tissue matrices			
STUDY_PHASE	Phase 4.4.4	LC and BS matrices			
validator	CHEMVAL	ChemVal			
validator	JACOBS	Jacobs Engineering			
validator	PYRON	Pyron Environmental			
Analysis	PFAS	NULL			
Lab_Flag	B	Detected in the associated MB of a sample that exceeded ½ LOQ or is at a concentration greater than 1/10th the concentration in the sample, whichever is greatest.			

Appendix E – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code	Validator
Lab_Flag	D	When the reported result is from a dilution			
Lab_Flag	I	Fail to meet ion ratio criteria			
Lab_Flag	J	At a concentration between the MDL and LOQ			
Lab_Flag	U	Not detected or were detected at a concentration less than the MDL			
UNIT	ng/L	nanograms per liter	MATRIX	GW	
UNIT	ng/L	nanograms per liter	MATRIX	LC	
UNIT	ng/L	nanograms per liter	MATRIX	SW	
UNIT	ng/L	nanograms per liter	MATRIX	WW	
UNIT	ug/kg	micrograms per kilogram	MATRIX	BS	
UNIT	ug/kg	micrograms per kilogram	MATRIX	SD	
UNIT	ug/kg	micrograms per kilogram	MATRIX	SS	
UNIT	ug/kg	micrograms per kilogram	MATRIX	TS	
dv_qualifier	I	Suspect			
dv_qualifier	J	Estimated			
dv_qualifier	J-	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J- when the %D is below acceptance criteria			
dv_qualifier	J-	If branched isomers were not included in the summed result reported, qualify associated detects as J-			
dv_qualifier	J+	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J+ when the %D is higher than acceptance criteria			
dv_qualifier	U	Values below the MDL are considered non-detects and are qualified as U at the stated MDL.			
dv_qualifier	UJ	Verify that the %Ds are within the acceptance criteria. Non-detects are qualified as UJ in all associated samples for %D outside of acceptance criteria.			
dv_qualifier	UJ	Estimated non-detect			
dv_qualifier	X	Exclusion of data is recommended			
Reviewer_qualifier	I	Suspect			
Reviewer_qualifier	J	Estimated			
Reviewer_qualifier	J-	If branched isomers were not included in the summed result reported, qualify associated detects as J-			
Reviewer_qualifier	J-	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J- when the %D is below acceptance criteria			
Reviewer_qualifier	J+	Verify that the %Ds are within the acceptance criteria. If any target analytes do not meet the acceptance criteria, qualify detects for that analyte as estimated J+ when the %D is higher than acceptance criteria			

Appendix E – Project Database – Valid Value Codes and Descriptions

VVLField	ValidValue	VV_Description	VVL_match_alt_field	VVL_match_alt_code	Validator
Reviewer_qualifier	U	Values below the MDL are considered non-detects and are qualified as U at the stated MDL.			
Reviewer_qualifier	UJ	Verify that the %Ds are within the acceptance criteria. Non-detects are qualified as UJ in all associated samples for %D outside of acceptance criteria.			
Reviewer_qualifier	UJ	Estimated non-detect			
Reviewer_qualifier	X	Exclusion of data is recommended			

Appendix F – Project Database – Valid Value Codes and Descriptions for Compounds

PFAS_ACRONYM	Compound	CAS_NO	Result_Type
13C2-4:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]hexanesulfonic acid	NA	EIS
13C2-6:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]octanesulfonic acid	NA	EIS
13C2-8:2FTS	1H,1H,2H,2H-Perfluoro-1-[1,2-13C2]decanesulfonic acid	NA	EIS
13C2-PFDoA	Perfluoro-n-[1,2-13C2]dodecanoic acid	NA	EIS
13C2-PFTeDA	Perfluoro-n-[1,2-13C2]tetradecanoic acid	NA	EIS
13C3-HFPO-DA	Tetrafluoro-2-heptafluoropropoxy-13C3-propanoic acid	NA	EIS
13C3-PFBS	Perfluoro-1-[2,3,4-13C3]butanesulfonic acid	NA	EIS
13C3-PFHxS	Perfluoro-1-[1,2,3-13C3]hexanesulfonic acid	NA	EIS
13C4-PFBA	Perfluoro-n-[13C4]butanoic acid	NA	EIS
13C4-PFHpA	Perfluoro-n-[1,2,3,4-13C4]heptanoic acid	NA	EIS
13C5-PFHxA	Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid	NA	EIS
13C5-PFPeA	Perfluoro-n-[13C5]pentanoic acid	NA	EIS
13C6-PFDA	Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid	NA	EIS
13C7-PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid	NA	EIS
13C8-PFOA	Perfluoro-n-[13C8]octanoic acid	NA	EIS
13C8-PFOS	Perfluoro-1-[13C8]octanesulfonic acid	NA	EIS
13C8-PFOSA	Perfluoro-1-[13C8]octanesulfonamide	NA	EIS
13C9-PFNA	Perfluoro-n-[13C9]nonanoic acid	NA	EIS
D3-NMeFOSA	N-methyl-d3-perfluoro-1-octanesulfonamide	NA	EIS
D3-NMeFOSAA	N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	NA	EIS
D5-NEtFOSA	N-ethyl-d5-perfluoro-1-octanesulfonamide	NA	EIS
D5-NEtFOSAA	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid	NA	EIS
D7-NMeFOSE	N-methyl-d7-perfluorooctanesulfonamidoethanol	NA	EIS
D9-NEtFOSE	N-ethyl-d9-perfluorooctanesulfonamidoethanol	NA	EIS
13C2-PFDA	Perfluoro-n-[1,2-13C2]decanoic acid	NA	NIS
13C2-PFHxA	Perfluoro-n-[1,2-13C2]hexanoic acid	NA	NIS
13C3-PFBA	Perfluoro-n-[2,3,4-13C3]butanoic acid	NA	NIS
13C4-PFOA	Perfluoro-n-[1,2,3,4-13C4]octanoic acid	NA	NIS
13C4-PFOS	Perfluoro-n-[1,2,3,4-13C4]octanesulfonic acid	NA	NIS
13C5-PFNA	Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid	NA	NIS
18O2-PFHxS	Perfluoro-1-hexane[18O2]sulfonic acid	NA	NIS
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	TRG
3:3FTCA	3-Perfluoropropyl propanoic acid	356-02-5	TRG
4:2FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid	757124-72-4	TRG
5:3FTCA	2H,2H,3H,3H-Perfluorooctanoic acid	914637-49-3	TRG
6:2FTS	1H,1H,2H,2H-Perfluorooctane sulfonic acid	27619-97-2	TRG
7:3FTCA	3-Perfluoroheptyl propanoic acid	812-70-4	TRG
8:2FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid	39108-34-4	TRG
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	756426-58-1	TRG
ADONA	4,8-dioxa-3H-perfluorononanoic acid	919005-14-4	TRG
HFPO-DA	Hexafluoropropylene oxide dimer acid	13252-13-6	TRG
NEtFOSA	N-ethyl perfluorooctanesulfonamide	4151-50-2	TRG
NEtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid	2991-50-6	TRG
NEtFOSE	N-ethyl perfluorooctanesulfonamidoethanol	1691-99-2	TRG
NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	151772-58-6	TRG
NMeFOSA	N-methyl perfluorooctanesulfonamide	31506-32-8	TRG
NMeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid	2355-31-9	TRG
NMeFOSE	N-methyl perfluorooctanesulfonamidoethanol	24448-09-7	TRG
PFBA	Perfluorobutanoic acid	375-22-4	TRG
PFBS	Perfluorobutanesulfonic acid	375-73-5	TRG

PFAS_ACRONYM	Compound	CAS_NO	Result_Type
PFDA	Perfluorodecanoic acid	335-76-2	TRG
PFDoA	Perfluorododecanoic acid	307-55-1	TRG
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	TRG
PFDS	Perfluorodecanesulfonic acid	335-77-3	TRG
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	113507-82-7	TRG
PFHpA	Perfluoroheptanoic acid	375-85-9	TRG
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	TRG
PFHxA	Perfluorohexanoic acid	307-24-4	TRG
PFHxS	Perfluorohexanesulfonic acid	355-46-4	TRG
PFMBA	Perfluoro-4-methoxybutanoic acid	863090-89-5	TRG
PFMPA	Perfluoro-3-methoxypropanoic acid	377-73-1	TRG
PFNA	Perfluorononanoic acid	375-95-1	TRG
PFNS	Perfluorononanesulfonic acid	68259-12-1	TRG
PFOA	Perfluorooctanoic acid	335-67-1	TRG
PFOS	Perfluorooctanesulfonic acid	1763-23-1	TRG
PFOSA	Perfluorooctanesulfonamide	754-91-6	TRG
PFPeA	Perfluoropentanoic acid	2706-90-3	TRG
PFPeS	Perfluoropentanesulfonic acid	2706-91-4	TRG
PFTeDA	Perfluorotetradecanoic acid	376-06-7	TRG
PFTrDA	Perfluorotridecanoic acid	72629-94-8	TRG
PFUnA	Perfluoroundecanoic acid	2058-94-8	TRG

Appendix D

PFAS MLVS Institute for Defense Analyses Report

INSTITUTE FOR DEFENSE ANALYSES

Statistical Methods in the Multi-Laboratory Validation of a PFAS Measurement Method

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IDA Product 3000051

Statistical Methods in the Multi-Laboratory Validation of a PFAS Measurement Method

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Executive Summary

Per- and polyfluoroalkyl substances (PFAS) are persistent and mobile pollutants that have drawn the attention of the scientific community and regulatory agencies concerning the potential health impacts exposure to these man-made chemicals have on humans. The Strategic Environmental Research and Development Program (SERDP) and the Environmental Security and Technology Certification Program (ESTCP), with the Environmental Protection Agency (EPA) Office of Water, Engineering and Analysis Division, developed a new method—EPA Draft Method 1633—for measuring trace contamination of 40 different PFAS in eight diverse environmental matrices: groundwater (GW), surface water (SW), wastewater (WW), soils, sediment, landfill leachate, fish tissue, and biosolids (i.e., municipal wastewater treatment plant residuals). This method uses liquid chromatography tandem mass spectrometry to quantify PFAS analytes using isotopically labeled compounds. Using a validated laboratory procedure (i.e., analytical method) to quantify PFAS provides consistent and reliable measurements that offer confidence when comparing data across different samples of the same environmental matrix type.

SERDP/ESTCP sponsored the Institute for Defense Analyses (IDA) to conduct statistical analyses, in the joint Department of Defense (DoD) and EPA multi-laboratory validation (MLV) study of EPA Draft Method 1633, to ensure objective and unbiased results. SERDP/ESTCP's study plan for the PFAS MLV closely follows the process outlined in the EPA Alternate Test Procedure (ATP) guidance, EPA 821-B-18-001, which describes the tests and procedures for developing quality control (QC) acceptance criteria from the data generated in a study. The ATP specifies the statistical formulas based on the number of labs analyzing each sample. The PFAS MLV study includes 10 participating laboratories and 3 types of datasets: initial calibration (ICAL), initial demonstration of capability (IDC), and environmental matrix samples.

IDA's role in the PFAS MLV study is to calculate statistical values using the lab-generated data to summarize the overall performance of the method. The IDA-calculated values will inform the QC acceptance criteria that the EPA establishes for the method. IDA has analyzed the ICAL, aqueous IDC, and three types of environmental aqueous matrices (WW, SW, and GW) datasets provided by the sponsor. IDA used the statistical formulas outlined in the MLV/EPA's ATP for most analysis tests and identified alternative calculations in instances when a discrepancy between the PFAS MLV dataset and formulas occurred (see table below). As an additional measure, IDA was blind to the lab names and

locations of the environmental samples and not part of the validation and verification process of the datasets. The table below provides an overview of each analysis test in the MLV, the associated performance metric defined by the EPA for a test and the range of IDA calculated values summarizing the performance of the 40 PFAS "target" analytes, and the 24 isotopically labeled compounds called extracted internal standards.

This report documents the formulas IDA used in the statistical analyses and provides some high-level observations about the aqueous datasets. A digital appendix with the summary statistic data tables generated by IDA for the ICAL, aqueous IDC, and WW, SW, and GW matrices accompanies this document.

Overview of the PFAS MLV Datasets and Analyses

PFAS MLV Dataset	Analysis Test in MLV	MLV Data Allowed Use of ATP Formula?	Performance Metric in EPA's ATP	Target Analyte Performance[1]	Extracted Internal Standard Performance[1]
ICAL	Calibration Linearity	No	pooled percent relative standard deviation (RSD)	7.31% to 13.8%	4.11% to 12.1%
Aqueous IDC	Method Detection Limit (MDL)	Yes	pooled MDL	0.315 to 9.89 ng/L	N/A
	Limit of Quantitation Verification	N/A	N/A	N/A	N/A
	Initial Precision and Recovery	Yes	mean percent recovery	95.0% to 109%	69.1% to 98.1%
			percent RSD	3.35% to 11.5%	5.36% to 17.2%
Matrix Samples	Ongoing Precision and Recovery	Yes	mean percent recovery	89.0% to 109%	53.2% to 101%
			percent RSD	7.29% to 15.9%	7.18% to 26.6%
	Low-Limit Ongoing Precision and Recovery	Yes	mean percent recovery	88.3% to 113%	50.8% to 108%
			percent RSD	8.22% to 14.3%	8.03% to 21.8%
	Matrix Spike WW[2]	No	percent RSD	8.94% to 68.0%	N/A
	Matrix Spike SW			6.50% to 104%	N/A
	Matrix Spike GW[2]			3.71% to 54.4%	N/A

[1] Nine labs reported values in most datasets.

[2] Only eight labs reported values.

Contents

1. Background

Per- and polyfluoroalkyl substances (PFAS)[1] are a persistent and mobile pollutant that have drawn the attention of the scientific community and regulatory agencies due to concerns about the potential health impact exposure to these man-made chemicals have on humans. Once championed for their heat-, oil- and water-resistant properties, attributed to a molecular structure with a short, strong bond between carbon and fluorine atoms, these substances now come as a detriment to the environment. PFAS do not easily break down and can migrate into soil, water, and air. Because of the widespread use of PFAS across the United States, including at many military installations, these chemicals are present in various regulatory environmental matrices including water, sediments, soils, and fish tissue.[2] Analysis of environmental samples help elucidate which PFAS are present and at what quantities to understand the extent of the contamination and inform decisions about cleanup for an area.

Using a validated laboratory procedure (i.e., analytical method) to quantify PFAS provides consistent and reliable measurements that offer confidence when comparing data across different samples of the same environmental matrix type. Validation of an analytical method is a process that demonstrates the method is appropriate for its intended purpose. Analytical methods can also establish performance metrics for regulatory compliance. These metrics may include accuracy, precision, specificity, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), and robustness.[3]

Since PFAS are a joint concern of the Department of Defense (DoD) and the Environmental Protection Agency (EPA), together they developed an analytical measurement method using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method offers broad applications to fulfill regulatory compliances under the

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[1] PFAS are a large group of synthetic chemicals used across the globe in consumer goods (e.g., cookware, clothing, cosmetics) and industrial applications specifically, aqueous film-forming foam used by the DoD to extinguish hazardous fires. "What are PFAS," ATSDR (Agency for Toxic Substances and Disease Registry), November 1, 2022, https://www.atsdr.cdc.gov/pfas/health-effects/overview.html; "Per- and Polyfluoroalkyl Substances (PFAS): A Nation Issue that Requires National Solutions," Department of Defense, Environmental Cleanup and Compliance, https://www.acq.osd.mil/eie/eer/ecc/pfas/.

[2] "PFAS Explained," U.S. Environmental Protection Agency, April 10, 2023, https://www.epa.gov/pfas/pfas-explained.

[3] M. Thompson, S. Ellison, and R. Wood, "Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report)," *Pure and Applied Chemistry* 74 (5) (2002): 835–855, https://doi.org/10.1351/pac200274050835.

Clean Water Act[4] as it includes 40 PFAS in 8 diverse environmental matrices: groundwater (GW), surface water (SW), wastewater (WW), soils, sediment, landfill leachate, fish tissue, and biosolids (i.e., municipal wastewater treatment plant residuals). The Strategic Environmental Research and Development Program (SERDP) and the Environmental Security and Technology Certification Program (ESTCP) are currently sponsoring a validation study for this PFAS method.[5]

The study plan for the multi-lab validation (MLV) closely follows the process outlined in an EPA document for new methods for organic and inorganic analytes used in Clean Water Act programs.[6] The EPA's Alternate Test Procedure (ATP) provides guidance for developing performance-based quality control (QC) criteria using statistical results from the data collected in the study. The EPA's ATP also includes the overall procedures for the statistical analyses and the formulas for computing the acceptance criteria as part of the evaluation of new analytical methods for approval and inclusion in the Code of Federal Regulation (40 CFR Part 136).[7] Methods that complete the laboratory validation process following specific guidance and approved by the EPA are made available to support regulatory or guidance activities.

In 2022, SERDP/ESTCP sponsored the Institute for Defense Analyses (IDA) as the independent organization to conduct the statistical analyses in the MLV of the PFAS measurement method to ensure the results were objective and unbiased.[8] The MLV study design comprises 10 laboratories that generate 3 types of datasets: initial calibration (ICAL), initial demonstration of capability (IDC), and environmental matrix samples. Prior to delivery to IDA, the datasets undergo several reviews by the sponsor, the data manager, and data validators. Additionally, the sponsor anonymized all lab names and environmental locations in the dataset as an additional measure so IDA was blind to those identities. IDA has analyzed the ICAL, aqueous IDC, and samples of three environmental matrices (WW,

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4   "Summary of the Clean Water Act," U.S. Environmental Protection Agency, June 22, 2023, https://www.epa.gov/laws-regulations/summary-clean-water-act.

5   EPA Draft Method 1633. Historically, EPA published draft methods on the Clean Water Act Methods website after completing the single-laboratory validation report. "CWA Analytical Methods for Per- and Polyfluorinated Alkyl Substances (PFAS)," U.S. Environmental Protection Agency, July 28, 2023, https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas.

6   SERDP/ESTCP, *Study Plan for Multi-Laboratory Validation of Draft EPA Method 1633 – PFAS in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS*, March 2022.

7   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.

8   IDA also supported SERDP/ESTCP in the single-laboratory validation. A. M. Buytendyk, S. C. Runkel, and S. M. Cazares, "Data Compilation in Support of Single Laboratory Validation of a Novel Per- and Polyfluoroalkyl Substances (PFAS) Detection Method for Environmental Matrices," IDA Document D-22794 (Alexandria, VA: Institute for Defense Analyses, 2021).

SW, and GW) datasets. For each dataset, IDA inspected and evaluated the analysis metrics in the MLV/EPA's ATP, and identified alternative calculations in instances with a discrepancy between the dataset and formulas. In this report, Chapter 2 documents the formulas IDA used in the statistical analyses and highlights instances where those differ from the EPA's ATP. Chapter 3 discusses high-level observations about the datasets and each statistical test. Chapter 4 provides a short summary about the datasets and overall analyses.

2.   Statistical Methods

IDA's role in the PFAS MLV study is to calculate9 the statistical values for each dataset type that summarizes the overall performance of the method for each test. These calculated values inform the QC acceptance criteria that the EPA will establish for the method. The EPA's ATP specifies three tiers of statistical formulas based on the number of labs analyzing each sample where Tier 3 requires a minimum of nine labs.[10] This chapter summarizes the statistical methods including the formulas IDA used to analyze the datasets received by the sponsor in the PFAS MLV study.

A. Initial Calibration (ICAL) Dataset

1. Calibration Linearity

Calibration establishes the relationship between the amount of an analyte (e.g., concentration) to an instrument response (e.g., signal area) by fitting a curve to data corresponding to the instrument measurements made at known analyte values. Calibration linearity refers to there being a linear relationship between the analyte concentration and the value predicted by an instrument using the calibration curve. A linear calibration curve is not required for the relationship between the actual concentration and predicted concentration to be linear, only that the calibration curve is monotonic and accurately relates the concentration to the measured signal. Internal standards or a known quantity of other compounds are often added to the sample to compare the instrument response between the standard and the analyte to determine how much of the analyte is present. When a calibration curve is proportional, a response factor (RF) expresses the ratio of the signal area to the amount (e.g., mass) of analyte compared to the signal-to-mass ratio of the standard.[11]

The metric in the EPA ATP for determining the performance of a calibration curve based on a straight line through the origin is the percent relative standard deviation

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9   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.

10   EPA, *Protocol for Review and Validation of New Methods,* G-22.

11   $Response\ Factor\ (RF) = \frac{Area_{analyte}Mass_{standard}}{Area_{standard}Mass_{analyte}}$

(RSD).[12] The percent RSD is the standard deviation divided by the mean of all the RFs multiplied by 100, for an analyte for each lab. The RSD limit is the QC acceptance criterion for the linearity test and is determined by combining or "pooling" the percent RSD from each individual lab.[13]

The PFAS MLV ICAL dataset for the linearity test includes three RSD values from each lab, for an analyte or internal standard. These three RSD values correspond to the three calibration tests performed by a lab. This dataset does not contain the necessary measured RF values to calculate an individual lab's overall percent RSD nor a pooled percent RSD for an analyte using the calculations as described in the EPA ATP. The International Union of Pure and Applied Chemistry (IUPAC) provides an alternative formula for combining the RSD of multiple series of measurements to calculate a pooled percent RSD for the PFAS MLV (Equation 1).

Equation 1: Pooled Percent RSD[14]

$$RSD_{pooled} = \sqrt{\frac{\sum(n_i-1)RSD_i^2}{\sum(n_i-1)}};$$

where n = number of RF values, RSDi = relative standard deviation of ith RF values.

B.   Initial Demonstration of Capability (IDC) Dataset

1.   Method Detection Limit (MDL)

The MDL is the lowest analyte concentration that a method can detect reliably and provides an exact procedure to evaluate the limit of detection (LOD)[15] for an analytical method.[16] The Code of Federal Regulations (CFR) defines MDL as "the minimum measured concentration of a substance that can be reported with 99% confidence that the

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[12]   Relative standard deviation (RSD) is also known as coefficient of variance (CV).

[13]   EPA, *Protocol for Review and Validation of New Methods,* G-23.

[14]   International Union of Pure and Applied Chemistry (IUPAC), *Compendium of Chemical Terminology*, 2nd ed., compiled by A. D. McNaught and A. Wilkinson (Blackwell Scientific Publications, Oxford, 1997), https://doi.org/10.1351/goldbook; "Assignment and Presentation of Uncertainties of the Numerical Results of Thermodynamic Measurements," *Pure and Applied* Chemistry 53 (9) (1981): 1805–1826, http://dx.doi.org/10.1351/pac198153091805.

[15]   The limit of detection is the lowest analyte concentration producing a response detectable above the noise level of the system, typically three times the noise level.

[16]   L. H. Keith, W. Crummett, J. Deegan, R. A. Libby, J. K. Taylor, and G. Wentler, "Principles of environmental analysis," *Analytical Chemistry* 55 (14) (1983): 2210–2218, https://doi/10.1021/ac00264a003; J. A. Glaser, D. L. Forest, G. D. McKee, S. A. Quave, and W. L. Budde, "Trace analyses for wastewaters," *Environmental Science & Technology* 15 (12) (1981): 1426–1435, https://doi.org/10.1021/es00094a002.

measured concentration is distinguishable from method results."[17] The process for determining the MDL described in the EPA ATP involves analyzing seven samples of the matrix containing a known concentration or "spike" of the analyte and seven without the analyte or "blank" samples where the samples were taken through all steps of the method. The method limit (also known as the LOD) is the QC acceptance criterion and is found using a pooled MDL, from each of the individual lab's MDL.[18]

The PFAS MLV IDC dataset for the MDL test contains seven spiked sample concentration measurements and at least seven blank sample measurements, for most labs, for an analyte. The CFR and the EPA outlines the calculations for the individual lab's MDL (Equations 2-4),19 for an analyte, as follows:

1. Find the MDL for the spiked samples, using the reported concentration values, for an analyte:

Equation 2: MDL Spiked Samples for Lab j (MDL$_{s,j}$)

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

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

2. Find the MDL for the blank samples, using the reported results, for an analyte:

   a. If none of the blank samples give a numerical result, the MDL for the blank samples does not apply.

   b. If some (but not all) of the blank samples give a numerical result, the MDL for the blank samples is the maximum value.

   c. If all of the blank samples give a numerical result, the MDL for the blank samples is:

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

[18] EPA, *Protocol for Review and Validation of New Methods,* G-23.

[19] 40 CFR Part 136, Appendix B; EPA, *Protocol for Review and Validation of New Methods,* G-9.

Equation 3: MDL Blank Samples for Lab j (MDL$_{b,j}$)

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

where $\bar{X}_j$ = mean measured concentration of the blank samples for lab j, S_b = 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.

3. Determine the MDL by comparing the calculated MDL$_s$ and MDL$_b$ values:

Equation 4: MDL

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

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.

After finding each individual lab's MDL for an analyte, Equation 5 shows the calculation for a pooled MDL for the PFAS MLV.

Equation 5: Pooled MDL[20]

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

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, $t_{(n,1-\alpha=.99)}$ = student's t-value for the one tailed test at the 99% confidence level with n degrees of freedom.

2. Limit of Quantitation Verification (LOQVER)

The limit of quantitation (LOQ) is the lowest concentration level of an analyte that produces a quantitative result with a specific degree of confidence.[21] The DoD Quality Systems Manual (QSM) for Environmental Laboratories, referenced by the MLV study

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

[21] Keith, et al., "Principles of environmental analysis," 2210–2218; The LOQ is commonly defined as ten times the noise level.

plan, describes the LOQ verification (LOQVER) procedure for analyzing four to seven samples to establish precision and relative bias for each laboratory near the LOQ.[22]

The PFAS MLV IDC dataset for the LOQVER test includes a single spiked sample concentration measurement for an analyte or internal standard from most of the labs. This dataset does not contain the necessary measured concentration data to calculate an individual lab's precision because the standard deviation of single data point is undefined.[23] Equation 6 displays the bias calculation for each lab, using the data for analytes and internals standards, for the PFAS MLV.

Equation 6: LOQ Percent Bias[24]

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

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

3. Initial Precision and Recovery (IPR)

The IPR test demonstrates whether a lab's capability to produce results are acceptable before the labs analyze the environmental samples.[25] Precision characterizes the variability that occurs in a series of experiments under similar conditions and therefore measures the reproducibility of a result.[26] Sources of random error contributing to the variability or scatter in the result include differences in the reagents and instruments used as well as different analysts conducting the experiment across labs in a study. The precision obtained for a single lab over a period of time expresses the within-lab reproducibility and the precision from results across different laboratories indicates the between-lab reproducibility. Recovery shows how the instrument response to an analyte in a sample compares to the response expected based on the calibration model.

The two metrics in the EPA ATP for determining the performance of the labs IPR are the mean percent recovery of the spiked sample measurements and a combined standard

[22] 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.

[23] IDA explored the possibility of including data from other tests (e.g., the spiked samples in the MDL test), however, the spike concentrations were less than the LOQ for some of the labs where the LOQVER test specified the spike concentrations at 1-2 times the LOQ.

[24] DoD, DOE, *DoD QSM Version 5.4*, 77.

[25] EPA, *Protocol for Review and Validation of New Methods,* G-6.

[26] M. J. Green, "Peer Reviewed: A Practical Guide to Analytical Method Validation," *Analytical Chemistry* 68 (9) (1983): 305A–309A, https://doi.org/10.1021/ac961912f; "LC-MS Method Validation," University of Tartu, https://sisu.ut.ee/lcms_method_validation.

deviation that includes the within- and between-lab standard deviations.[27] The upper- and lower-percent recovery limits are the QC acceptance criteria for recovery, which are constructed using the overall mean and a combined standard deviation of the within- and between-lab standard deviations. The percent RSD of the percent recovery is the QC acceptance criterion for precision, where the within-lab standard deviation is divided by the overall percent recovery mean multiplied by 100.

The PFAS MLV IDC dataset for the IPR test contains four spiked sample concentration measurements and the corresponding percent recoveries for a given analyte or internal standard, and lab. The EPA ATP outlines the calculations for an analyte in Equations 7–10:[28]

Equation 7: Between Lab Standard Deviation (s_b)

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

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

Equation 8: Within Lab Standard Deviation (s_w)

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

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

Equation 9: IPR Combined Standard Deviation (s_{IPR})

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

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.

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[27] EPA, *Protocol for Review and Validation of New Methods,* G-25–26.

[28] EPA, *Protocol for Review and Validation of New Methods,* G-25–26.

Equation 10: RSD

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

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

C. Environmental Matrix Dataset

1. Ongoing Precision and Recovery (OPR) and Low-Level Ongoing Precision and Recovery (LLOPR)

Both the OPR and LLOPR tests are done throughout the environmental matrix analyses and provide assurance the results produced by the labs are consistent and reproducible throughout the study. The OPR test, sometimes referred to as a QC check, demonstrates the labs' routine performance with known amounts of analytes (similar or identical to the IPR samples). The LLOPR test verifies the LOQ with samples spiked at low concentrations.

The metric in the EPA ATP for determining the performance of the labs' OPR is the mean percent recovery of the spiked sample measurements and a combined standard deviation that includes the within- and between-lab standard deviations.[29] The upper- and lower-percent recovery limits are the QC acceptance criteria for recovery, which are constructed using the overall mean and combined standard deviation.

The PFAS MLV environmental matrix datasets for the OPR and LLOPR tests contain several spiked sample concentration measurements and the corresponding percent recoveries for analytes and internal standards, for most labs. The EPA ATP outlines the same calculations for finding the between- and within-lab standard deviations in Equations 7 and 8[30] to compute the combined standard deviation for the OPR (Equation 11), for analytes in an environmental matrix dataset.

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

[30] The calculation for the within-lab standard deviations (Equation 8) excludes instances where a lab reports a single spiked sample concentration measurement as the standard deviation of a single value is undefined.

Equation 11: OPR Combined Standard Deviation (s_{OPR})[31]

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

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 10 is also the formula to calculate the RSD in the OPR test. Similarly, the calculations for the LLOPR test follow those for the OPR using Equations 7, 8, 10, and 11.

2. Matrix Spike Recovery

The matrix spike recovery tests whether the environmental matrix (e.g., WW, SW, GW) surrounding the analyte interferes in the sample preparation or instrument response affecting the ability to accurately quantify the analyte in a field sample. Structural analogs and stable isotopically labeled compounds[32] both have similar properties to the analyte and provide one technique to determine possible matrix effects. The EPA ATP describes another procedure for determining the method performance of a matrix in instances where an isotopic analog of an analyte is not available to use as an internal standard.[33] The metric defined is the relative percent difference between matrix spike and matrix spike duplicates.[34]

The PFAS MLV method is an isotopic dilution method where isotopically labeled compounds are spiked into the field samples, although, not all analytes in the study have an isotopic analog. The environmental matrix datasets for the matrix spike test contain concentration measurements from spiked field samples and the corresponding percent recoveries, for analytes and internal standards, for most labs. Although most labs made triplicate measurements of the analytes for each matrix sample, the dataset did include information to associate the matrix spike measurement with the corresponding isotopic standard measurement to calculate the relative percent difference. The calculations for the matrix spike test instead include those in Equations 7 and 8 to determine s_b and s_w as well as Equation 10 to find the RSD for the matrix test.[35]

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

[32] Compounds where an atom in the molecule is replaced by a different stable (non-radioactive) isotope of that atom (e.g., deuterium is an isotope of hydrogen).

[33] EPA, *Protocol for Review and Validation of New Methods,* G-27.

[34] EPA, *Protocol for Review and Validation of New Methods,* G-27.

[35] The WW and GW datasets ended up with only results from eight laboratories; however, IDA still analyzed these datasets as outlined at Tier 3.

3.   Discussion

The PFAS method includes 40 "target" analytes prevalent in environmental matrices that are quantified with standard or isotopically labeled compounds added to the samples. There are also 24 extracted internal standard (EIS) compounds and 7 non-extracted internal standard (NIS) compounds added to the samples.[36] EIS are isotopically labeled PFAS compounds that are added to samples prior to any preparation steps. NIS are isotopically labeled PFAS compounds added just before analyzing the prepared samples in the LC-MS/MS instrument.

In all of the datasets IDA received, the sponsor excluded one of the labs (Lab 8) for not performing the method correctly, which left nine labs for most of the datasets. One lab was missing from the WW (Lab 10) and GW (Lab 9) datasets leaving only eight labs. IDA still followed the EPA's Tier 3 formulas for the matrix spike samples as IDA's code was developed prior to receiving the matrix datasets and it allowed for comparison across datasets by using the same formula. Additionally, IDA did not include any data qualified or flagged with the letter "U," meaning the analyte was not detected or detected at a concentration less than the MDL. Appendix C provides summary figures of the lab results for each of the 40 PFAS across the ICAL, aqueous IDC, and WW, SW, GW environmental matrices datasets.

A.   Calibration Linearity

A linear calibration curve is where the instrument response is linearly proportional to the amount of analyte in the sample meaning the measured instrument signal at known amounts (or concentrations) of the analyte fits the equation of line (i.e., y=mx + b) for a range of concentrations. To assess the calibration linearity for the MLV dataset, the calibration curve is a straight line through the origin (zero response at zero concentration where b=0) and proportional with the response factor/ratio. The percent RSD of the average ratio of the instrument response to the analyte amount or RF for an analyte compared to a standard expresses the overall amount of deviation from a straight line where each point in the calibration has equal weight (i.e., measurements at low concentration have the same impact as high concentrations). The typical acceptance criterion for a linear calibration in

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[36] See Appendix A for the list of target PFAS analytes, EIS PFAS compounds, and NIS PFAS compounds. For all tests, IDA calculated values for the target analytes, and for most tests the EIS compounds. IDA only calculated values for the percent recoveries of the NIS compounds for all the aqueous matrix samples as the values were not populated for all the labs for some of the datasets.

analytical chemistry is a percent RSD of less than 15% or 20%, which is consistent with other research fields that use percent RSD as a metric of performance.[37] The MLV study plan also allowed reporting the percent RSD or the relative standard error (RSE) for calibration linearity.[38]

The PFAS MLV ICAL dataset includes, for each target analyte or EIS compound, three sets of calibration values, including number of calibration points, average RF, standard deviation, and RSD values from nine labs. Lab 3 also reported RSE values for nine of their calibrations, most of which had a percent RSD of 19.5% or above. Missing from the ICAL dataset were the individual RF values and the corresponding concentration values so IDA could not independently verify the calibration models nor the RSD/RSE values. IDA also observed several inconsistencies between the reported standard deviation values calculated by a third party compared to the lab reported RSDs and mean RFs for each calibration.

The sponsor provided another dataset with the concentrations for each calibration sample (CS) used by each lab. The number of CS reported was inconsistent with the number of calibration points reported, with the ICAL average RF and RSD values for some labs adding more ambiguity to the ICAL dataset. The heatmap in Figure 1 is a visualization of all the percent RSDs of the RFs reported for each analyte using a Z-score to better compare the individual laboratory measurements. A Z-score is a measure of how many standard deviations below or above a value is from the population mean. In this heatmap, blue shades indicate a lab's reported value is below the mean for an analyte, yellow shades depict a value is above the mean, and black or dark shades represent the value is close to the mean score (i.e., a Z-score of zero). Equation 12 shows how to compute a Z-score of a measurement value X using the mean and standard deviation for all the average RF values for a given analyte. Along the x-axis from left to right, the first 40 PFAS are the target analytes followed by the 24 PFAS EIS compounds. Most of the average RSDs reported are within 3 standard deviations from the mean for each analyte (i.e., down a column in Figure 1). The EIS compound $^{13}C_2$-PFTeDA had the highest reported RSD of 34% followed by the target analytes (7:3FTCA, NFDHA, PFMBA) and EIS compounds (D$_3$-NMeFOSAA and $^{13}C_2$-PFDoA). The median percent RSD varied from 5.35% to 11.8% for target analytes and 2.17% to 11.0% for EIS compounds.

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[37] R. Burrows and J. Parr, "Evaluating the Goodness of Instrument Calibration for Chromatography Procedures," *LCGC Supplements* 38 (11) (2020): 35–38, https://www.chromatographyonline.com/view/evaluating-the-goodness-of-instrument-calibration-for-chromatography-procedures. Generally, data with a percent RSD greater than 30% indicates a larger spread in data and could be related to an issue with the performance of the method or instrument.

[38] RSE is the standard deviation of the mean divided by the square root of the sample size and multiplied by 100.

Equation 12: Z-score

$$Zscore = \frac{X - \bar{X}}{\sigma_X};$$

where X = data point in the set, $\bar{X}$ = mean of all values in a set, σ_X = sample standard deviation of the set.

The pooled percent RSD is a way to compile percent RSDs in a series of measurements with different means and the statistical value is most meaningful when those measurements are performed under similar conditions, like a method validation, to estimate the overall precision. The computed pooled percent RSD values[39] (Equation 1) for the PFAS MLV ICAL dataset span between 7.31% to 13.8% for the target analytes and 4.11% to 12.1% for the EIS compounds. Likely due to the RSD values having outliers primarily on the high end, the pooled percent RSD for this dataset is almost always larger than the median percent RSD (by an average of 16.8%). One possible factor affecting the linearity of a calibration model in the method could be from LC-MS/MS instrument components, such as the ionization source or detector.[40]

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[39] The reference for the pooled percent RSD value cited the Bartlett Test as an option to test whether a series of measurements had the same precision or standard deviation prior to calculating a pooled value. IDA did not pursue the Bartlett Test with the PFAS MLV ICAL dataset as several inconsistencies were observed between the reported standard deviation, mean RFs, and RSDs values.

[40] "LC-MS Method Validation: 3.1. Linearity," University of Tartu, https://sisu.ut.ee/lcms_method_validation/31-linearity.
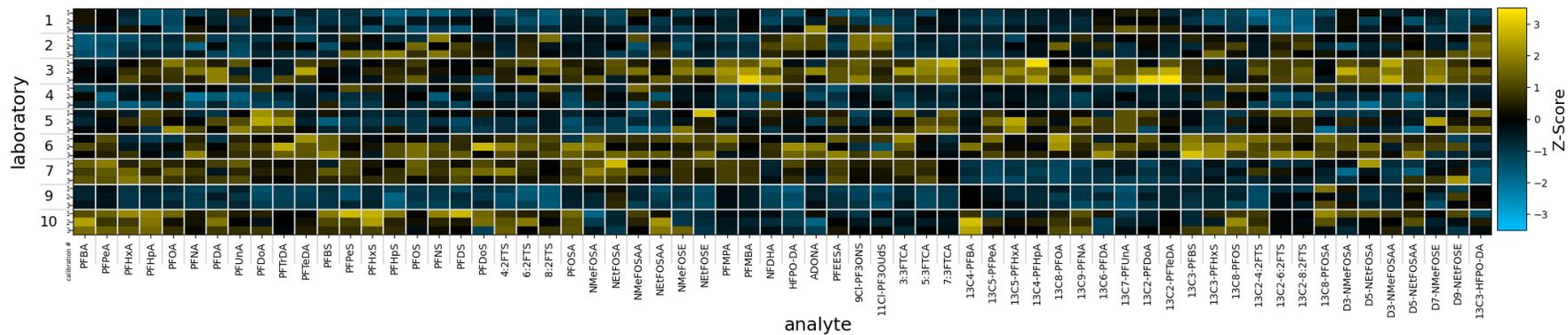
Figure 1. Z-score of the RSDs of the RFs reported for each of the three calibration tests conducted by every lab, for 40 target PFAS analytes and 24 EIS PFAS compounds.

B. Method Detection Limit (MDL)

The detection limit for an analyte is an important value to establish for an analytical method. Detection limits can also be contentious especially in low-level analyses for substances that are toxic or pose harm to the environment as regulators use values to assess risk and compliance. There are several different "detection" definitions, which can cause confusion.[41] The MDL test quantifies the lowest reliable concentration of an analyte when processing a blank or sample through the complete analytical method.[42] The MDL is theoretically derived as an error distribution associated with the operational characteristics of the method. The pooled percent MDL is a statistical value from a series of measurements performed under similar conditions by multiple laboratories.

The PFAS MLV IDC dataset for the MDL test contained seven spiked sample concentration measurements and at least seven blank sample measurements, for nine labs, for the target analytes. The scatterplot in Figure 2 is a visualization of the individual lab MDL values (dash) calculated using Equation 2 through Equation 4 and the pooled MDL value (triangle) calculated using Equation 5, for each analyte across all nine labs. The computed pooled MDL values for the PFAS MLV Aqueous IDC dataset span between 0.315 to 9.89 ng/L for the target analytes.

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[41] Keith, et al., "Principles of environmental analysis."

[42] *ACS Reagent Chemicals*, Part 1: Introduction and Definitions (Washington, DC: ACS Publications, 2017), https://pubs.acs.org/doi/book/10.1021/acsreagents; Glaser, et al., "Trace analyses for wastewaters," 1426–1435.
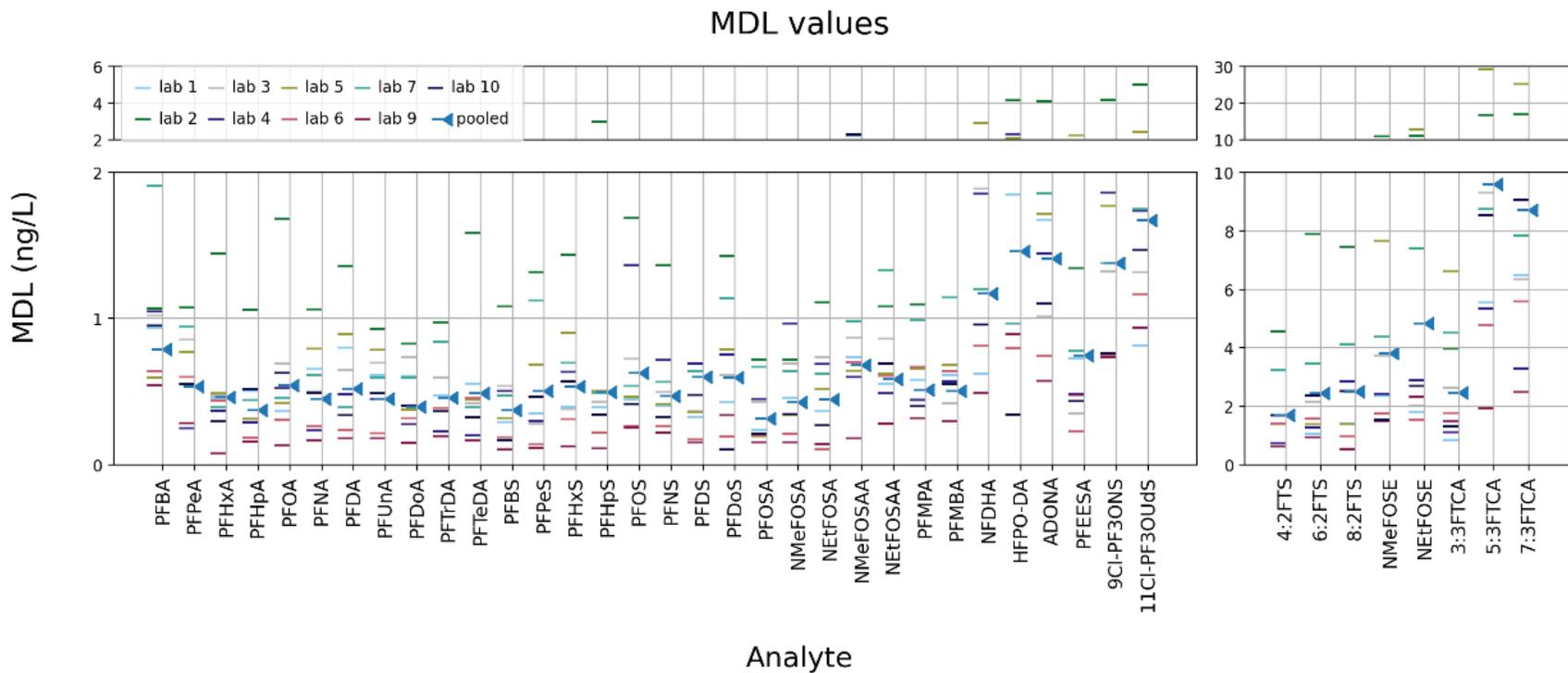
Figure 2. MDL values calculated for nine labs that went into the computed pooled MDL for 40 target PFAS analytes. The fluorotelomer sulfonic acids (4:2FTS, 6:2FTS, and 8:2FTS), perfluorooctane sulfonamide ethanols (NMeFOSE and NEtFOSE), and fluorotelomer carboxylic acids (3:3FTCA, 5:3FTCA, and 7:3FTCA) are displayed on separate axes to avoid visual suppression of smaller MDL values. A break in the y-axes of both plots avoids visual suppression of reported MDL values within each group.

C. Limit of Quantitation Verification (LOQVER)

The LOQVER test quantifies the precision and relative bias for each lab when measuring analytes or internal standards at 1 to 2 times the LOQ. The PFAS MLV dataset for the LOQVER test is sparse with only 18 values for each analyte or internal standard with seven of the nine labs only reporting a single value and Labs 2 and 6 reporting multiple values. IDA explored whether the spiked sample MDL dataset was suitable to include in the LOQVER analysis to calculate the precision for each of the labs. The spike concentrations used by several labs for the spiked MDL measurements were less than the LOQ and did not meet the PFAS MLV study requirement so the spiked sample MDL dataset was not appropriate to use for all labs to calculate the labs' precision. IDA did calculate the percent relative bias for each lab shown in Figure 3. Relative bias is an estimate of systematic error; however, the calculations for most labs in the LOQVER test are based on a mean of one data point and not a well-represented estimate of the systematic error for those labs.

Figure 3. Percent bias of the concentrations measured in the aqueous IDC dataset reported by nine labs, for 40 PFAS target analytes and 24 EIS compounds.

D. Initial Precision and Recovery (IPR)

The IPR test establishes the variability within each lab and the reproducibility of a result between labs prior to the labs using the method with the environmental samples. The PFAS MLV dataset for the IPR includes each lab reporting four results, corresponding to measurements of the four aliquots of reference matrix spiked with analytes and standards for every lab.[43] The boxplot in Figure 4 is a visualization of the percent recoveries of the four reported measurements made by all nine labs for each target and EIS compounds in the aqueous IPR dataset and shows the spread in values across labs. A more detailed explanation of the box and whisker plot is in Appendix B. The "X" markers indicate data points outside the range defined in the box and whiskers and the colors show the lab reporting that value. The overall mean percent recovery for the target analytes range from 95.0% to 109% and EIS compounds ranged from 69.1% to 98.1%. The perfluorooctane sulfonamides (FOSA) and the perfluorooctane sulfonamide ethanols (FOSE) EIS compounds have the lowest average and median recovery values, yet the corresponding target analytes recovery values center around 100%.

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[43] SERDP/ESTCP, *Study Plan for Multi-Laboratory Validation.*
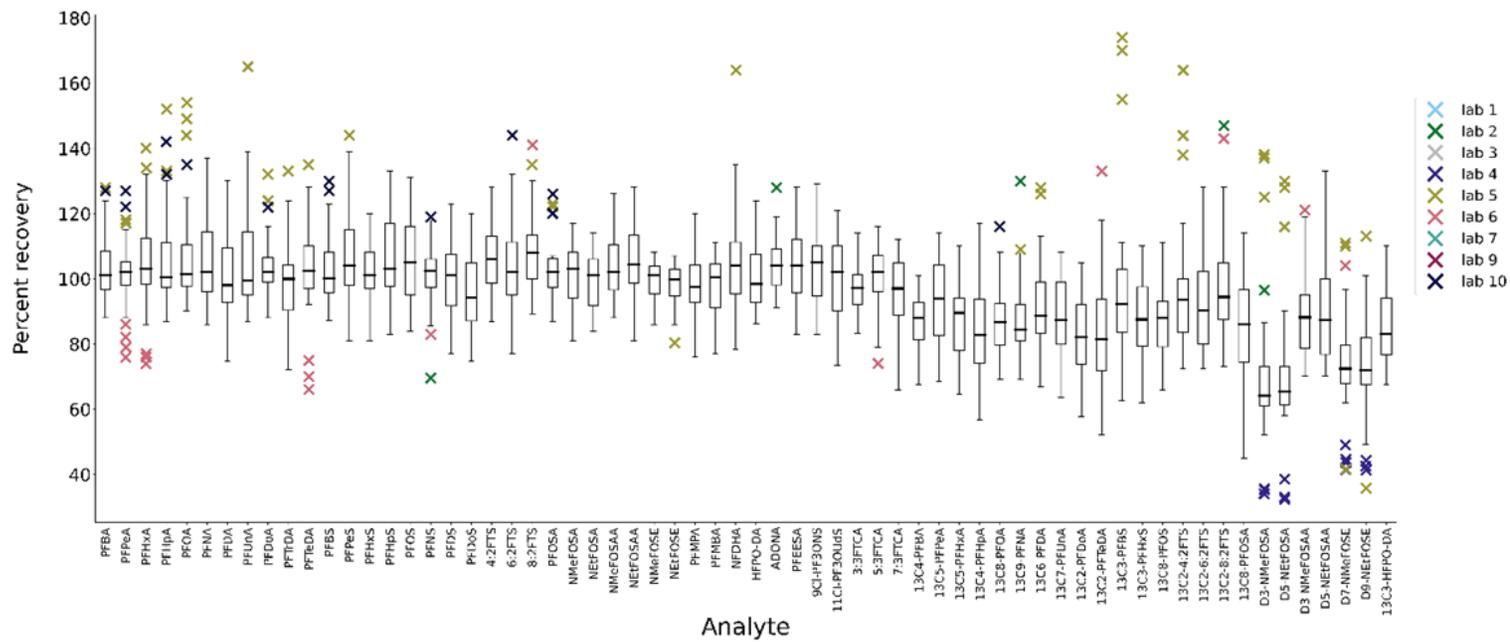
Figure 4. Percent recovery of the four measurements in the aqueous IPR dataset reported by all nine labs, for 40 PFAS target analytes and 24 PFAS EIS compounds.

The blue piecewise function (Equation 13) in Figure 5 is an empirical model known as the Horwitz curve that generalizes the relationship between the reproducibility between labs[44] for concentrations ranging from 10% to 10 parts per billion (ppb).[45] The curve was derived from studying thousands of results from interlaboratory analyses of analytes including food, pharmaceuticals, and pesticides.[46] Thompson later proposed a piecewise function in Equation 13 as the extremes of the original Horwitz curve tend to overestimate the variability.[47] In food analyses, the Horwitz curve has been used as a performance criterion and the x-axis is normally displayed with concentration units decreasing to the right.[48] An important disclaimer is the Horwitz curve provides a comparison to reproducibility results from other collaborative studies at similar concentration levels and is not an estimate of uncertainty or evaluation of performance.

Equation 13: Modified Horwitz Curve

$$\text{percent RSD}_\text{H} = \begin{cases} 22\%, \text{ if } c < 1.2 \times 10^{-7} \\ (2\ \%)c^{-0.1505}, \text{ if } 1.2 \times 10^{-7} \le c \le 0.138 \\ (1\ \%)c^{-0.5}, \text{ if } c > 0.138 \end{cases}$$

where c = dimensionless fraction of concentrations (e.g., ppb).

[44] The RSD of reproducibility, or RSD$_\text{R}$, is defined as the between-lab precision, which is the sum of the within-laboratory precision, s$_\text{r}$, and the "pure" between laboratory precision, s$_\text{L}$, expressed as variances. W. Horwitz and R. Albert, "The Horwitz ratio (HorRat): A useful index of method performance with respect to precision," *Journal of Association of Official Analytical Chemists (AOAC) International* 89, (4) (2006): 1095–1109, https://doi.org/10.1093/jaoac/89.4.1095.

[45] D. L. Massart, J. Smeyers-Verbeke, and Y. V. Heyden, "Benchmarking Analytical Methods Horwitz Curve," *LCGC Europe*, 18 (10) (2005): 528–531, https://www.chromatographyonline.com/view/benchmarking-analytical-methods-horwitz-curve.

[46] W. Horwitz, L. R. Kamps, and K.W. Boyer, "Quality Assurance in the Analysis of Foods for Trace Constituents," *Journal of Association of Official Analytical Chemists (AOAC) International* 63, (6) (1980): 1344–1354, https://doi.org/10.1093/jaoac/63.6.1344; Royal Society of Chemistry, "The amazing Horwitz function," *AMC Technical Brief* no. 17, (2004), ed. M. Thompson, https://www.rsc.org/images/horwitz-function-technical-brief-17_tcm18-214859.pdf.

[47] M. Thompson, "Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing," *Analyst* 125, (2000): 385–386, https://doi.org/10.1039/B000282H.

[48] Horwitz and Albert, "The Horwitz ratio (HorRat),"1095–1108.
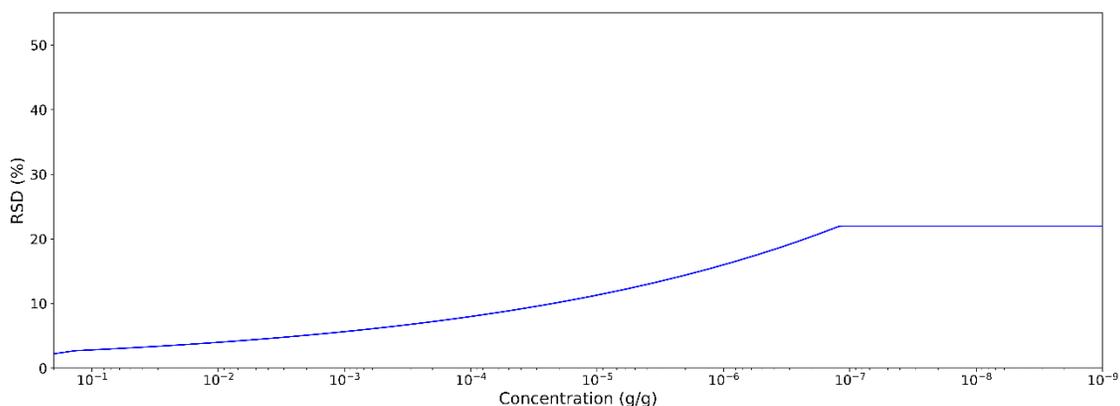
Figure 5. Plot of the modified Horwitz curve depicting the relationship between concentration and percent RSD.

The scatter plot in Figure 6 offers a visualization of the within-lab variabilities of the four measurements made by each lab (the standard deviation values for each lab used in Equation 8, for every analyte) plotted as the percent RSD verses concentrations. Most points are below 20% percent RSD with the exception of several analytes from Lab 5. The computed overall percent RSD values (Equation 10) ranged from 3.35% to 11.5% for the target analytes and 5.36% to 17.2% for the EIS compounds.
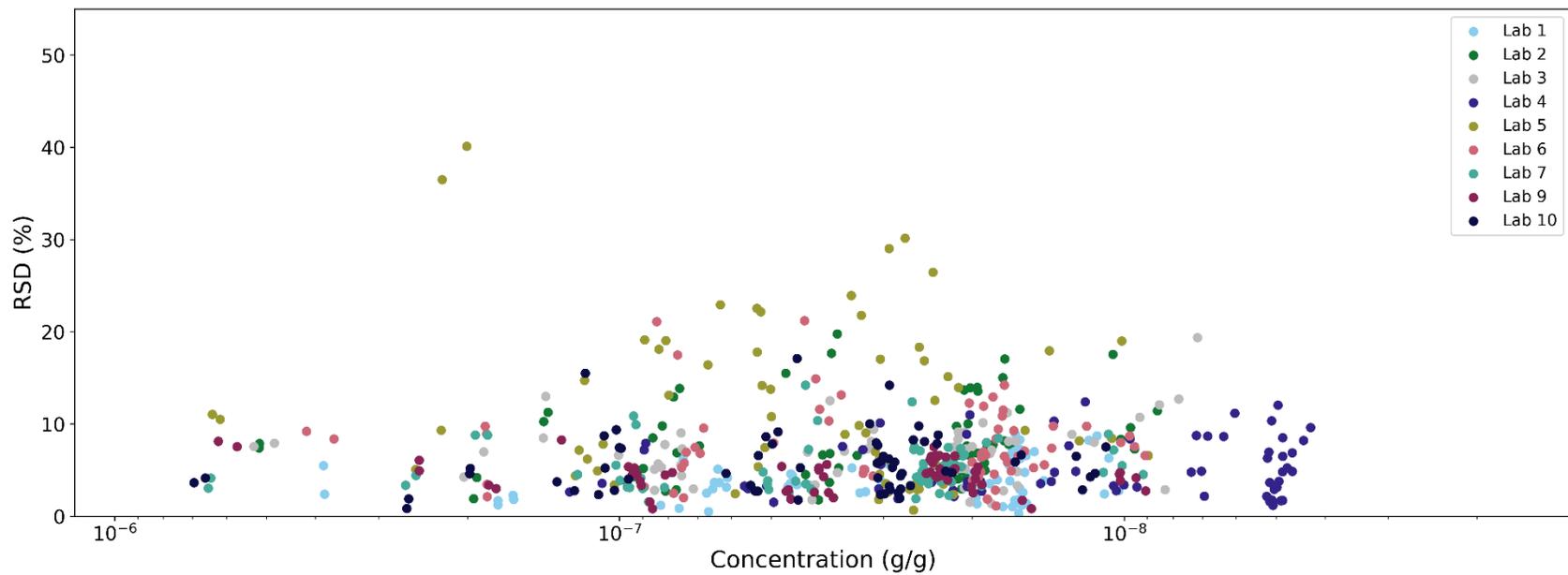
Figure 6. Percent RSD of the IPR percent recovery values reported in the aqueous IDC dataset, for each of the 40 PFAS target analytes and 24 PFAS EIS compounds, for the nine labs.

E. Matrix Spike Recovery

The matrix spike recovery test explores how the method performs with real-world environmental matrices. The aqueous matrices—WW, SW, GW—are sourced from various locations in the environment. Specific information about the source of each aqueous matrix was not provided to IDA. An independent lab added or spiked the 40 target analytes into replicate samples of all the aqueous matrices. Each of the labs received a set of six samples containing the target analytes and an unspiked sample (i.e., aqueous matrix without modifications), for each different type of aqueous matrix, totaling to 91 aqueous study samples. The laboratories were responsible for adding the EIS and NIS compounds to the samples. The number of labs in each of the aqueous matrix datasets IDA analyzed varied either because a lab chose not to participate or the sponsor deemed the results did not qualify (e.g., lab did not follow method correctly). Table 1 includes details about the number of matrices, samples, and labs reporting results for the aqueous matrices.

Table 1. Number of Real-World Aqueous Matrices and Laboratories Reporting Results

Aqueous Matrices	WW1	SW2	GW3
# of Matrices	7	3	3
# of Study Samples	49	21	21
# of Labs Reporting Results	8	9	7 to 8

[1] Labs 8 and 10 were not included.

[2] Lab 8 was not included.

[3] Labs 8 and 9 were not included. Lab 1 had results for 2 out of 3 samples.

IDA calculated the percent RSD (Equation 10) for each of the three aqueous matrix datasets for the EIS compounds and target analyte. The percent RSD for the EIS compounds included the recoveries from the unspiked samples in addition to the spiked samples. The percent RSD for the target analytes were from the six spiked samples; those results are shown in Figure 7. The computed overall percent RSD values for the matrix samples ranged from 8.94% to 68.0 % (WW), 6.50% to 104% (SW), and 3.71% to 54.4% (GW). Target analytes PFHxS and PFOS (SW, Lab 1) and 4:2FTS (WW, Lab 6) each had a single reported percent recovery greater than 1000% for a sample, which likely contributed to the high computed overall RSD value for that matrix. Additionally, one of the GW samples did not have any results for PFOS and PFHxS and another GW sample only had one lab result reported for PFHxS. About 10% of the computed percent RSDs are greater than 30% with most of the values associated with the perfluorooctane sulfonamidoacetic acids (FOSAA).
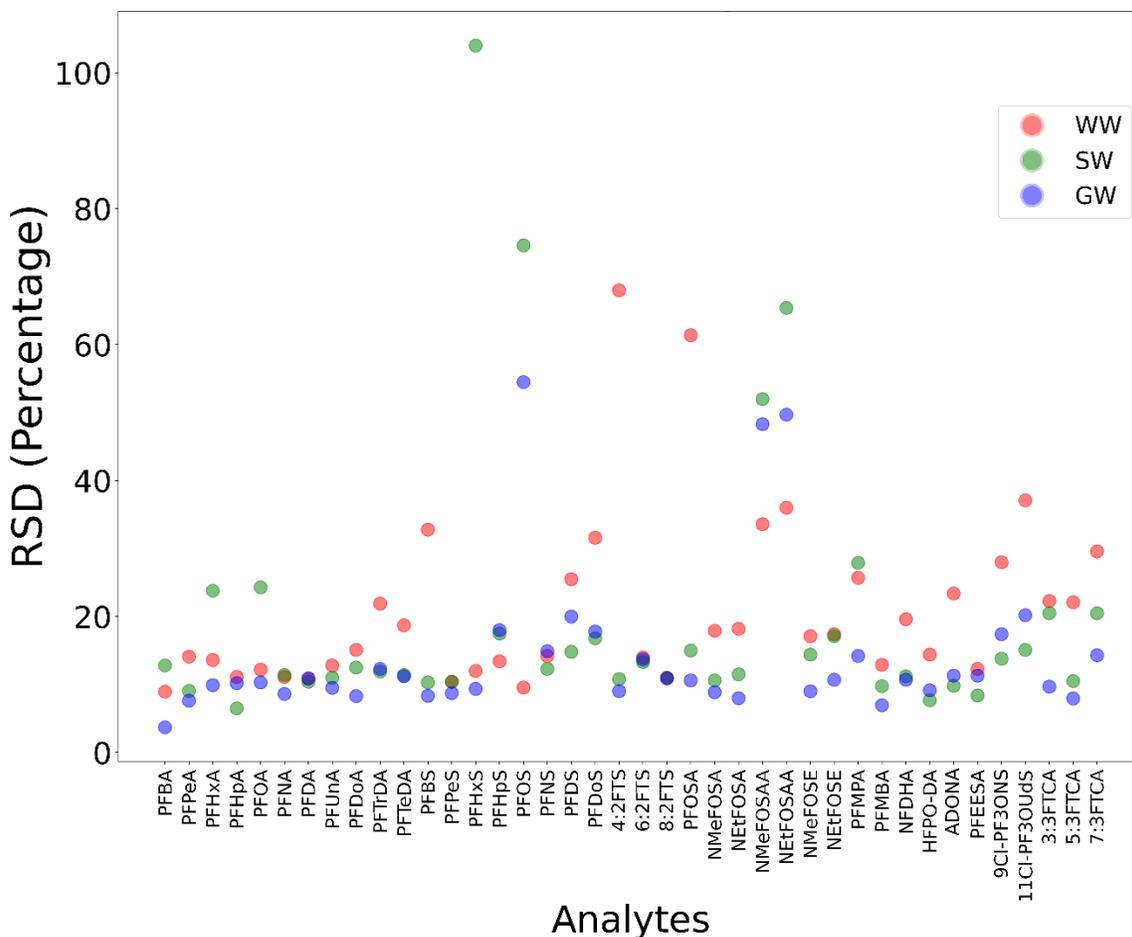
Figure 7. Percent RSD calculated from all the results across labs in each aqueous matrix spike dataset for each of the 40 PFAS analytes.

F. Ongoing Precision and Recovery (OPR)

The OPR test helps to assure the results produced by the labs when analyzing the matrix spike samples remain within the specified precision and recovery limits for the method. Labs use an aliquot of a method blank[49] spiked with analytes and standards. The OPR test also shows the variability within each lab and the reproducibility of a result between labs for the method across the aqueous environmental matrix spike samples.

The PFAS MLV OPR dataset associated with all the aqueous matrices includes nine labs. IDA received each aqueous matrix dataset with OPR values separately and computed the mean percent recovery values and the overall RSD for each matrix. Some labs performed the SW and GW matrix samples together and reported OPR measurements associated with both datasets. Later, IDA received a combined aqueous matrix dataset with

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[49] Reagent water that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, reagents, internal standards, and labeled compounds that are used with samples. SERDP/ESTCP, *Study Plan for Multi-Laboratory Validation*.

all the values for the WW, SW, and GW results with the OPR measurements and an additional column to indicate if values were associated with a single matrix (e.g., WW, SW, GW) or more than one dataset (e.g., SW and GW).

The boxplot in Figure 8 is a visualization of the percent recoveries of the reported OPR measurements in the combined aqueous matrix dataset with nine labs for each target analyte and EIS compound. The number of OPR results reported by each lab varied from 3 to 11. Because there were more WW samples, more of the results are associated with the WW dataset compared to the SW and GW datasets (Table 1). Lab 10 did not report OPR values associated with WW datasets while Lab 9 only reported OPR values related to the WW dataset. Lab 6 had the lowest percent recovery values overall, and Lab 9 had the highest percent recoveries for many of the target analytes as seen by the "X" markers in Figure 8. The overall mean percent recovery for the target analytes range from 89.0% to 109% and EIS compounds ranged from 53.2% to 101%. Similar to the IPR results, the FOSA and FOSE EIS compounds have the lowest average and median recovery values, yet the corresponding target analytes recovery values center around 100%.

The scatter plot in Figure 9 shows each lab's within-lab variability (the standard deviation values for each lab used in Equation 8) in their OPR measurements, for every analyte, where the percent RSD is plotted as a function of the concentration. Most points are below 20% percent RSD with the exception of a small number of analytes across several labs. The computed overall percent RSD values (Equation 10) ranged from 7.29% to 15.9% for the target analytes and 7.18% to 26.6% for the EIS compounds.
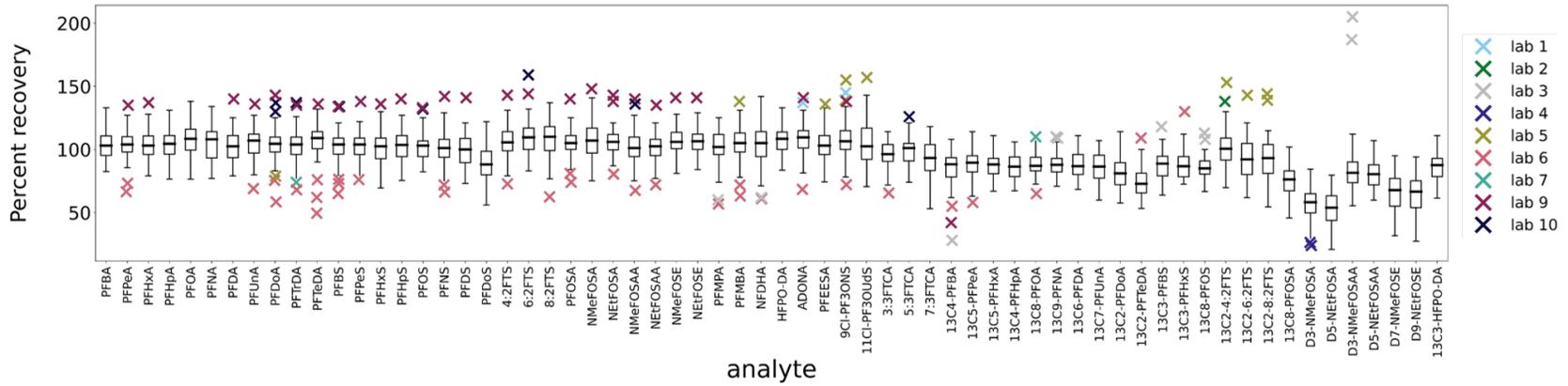
Figure 8. Percent recovery of the OPR measurements in the WW, SW, GW matrix spike datasets reported by all nine labs, for 40 PFAS target analytes and 24 PFAS EIS compounds.
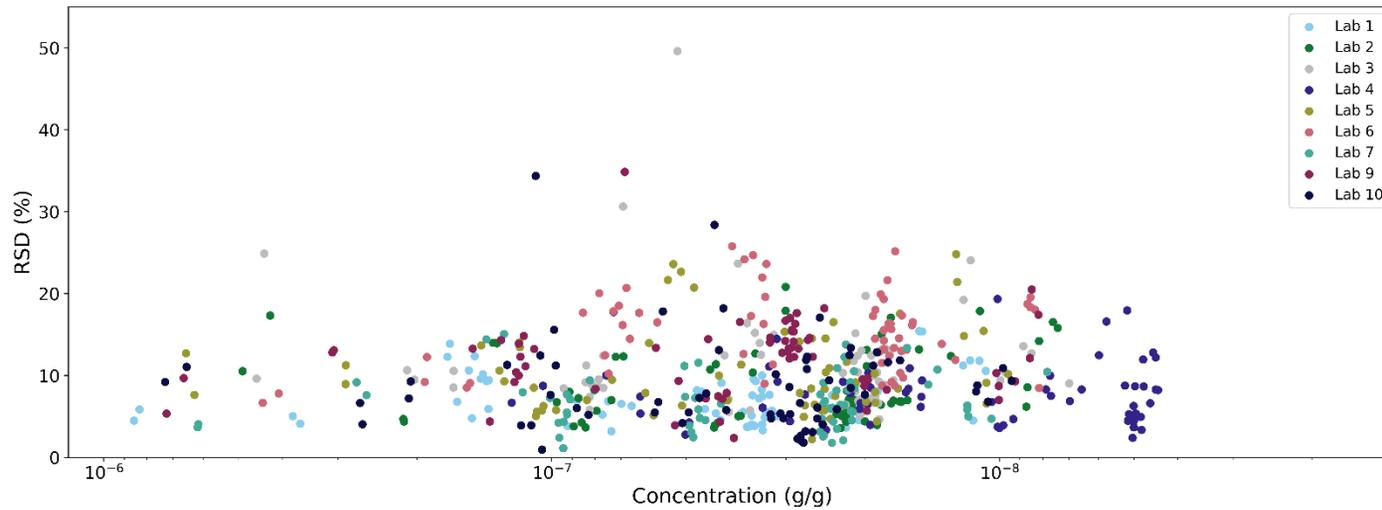
Figure 9. Percent RSD of the OPR percent recovery values reported in the WW, SW, GW matrix spike datasets calculated for every lab, for each of the 40 PFAS target analytes and 24 PFAS EIS compounds.

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G. Low-Level Ongoing Precision and Recovery (LLOPR)

The LLOPR test verifies the LOQ with samples spiked at low concentrations. Similar to the OPR test, the percent recovery measurements show the variability within each lab and the reproducibility of a result between labs for the method across the aqueous environmental matrix spike samples.

The PFAS MLV LLOPR dataset associated with all the aqueous matrices includes nine labs. Similar to the OPR dataset, IDA received each aqueous matrix dataset with LLOPR values separately and computed the mean percent recovery values and the overall RSD for each matrix. Later, IDA received a combined aqueous matrix dataset with all the values for the WW, SW, and GW results with the OPR measurements and an additional column to indicate if values were associated with a single matrix (e.g., WW, SW, GW) or more than one dataset (e.g., SW and GW).

The boxplot in Figure 10 is a visualization of the percent recoveries of the reported LLOPR measurements made by nine labs for the target and EIS compounds in the aqueous WW, SW, GW. Because there were more WW samples, more of the results are associated with the WW dataset compared to the SW and GW datasets (Table 1). The number of LLOPR results reported by each lab varied from 3 to 11 values. Lab 10 did not report LLOPR values associated with the WW dataset while Lab 9 only reported LLOPR values related to the WW dataset. Figure 10 shows Lab 9 reported the highest percent recoveries for some of the target analytes and Lab 5 reported the highest percent recoveries for some EIS compounds and a few target analytes. The overall mean percent recovery for the target analytes range from 88.3% to 113% and EIS compounds ranged from 50.8% to 108%. The range of LLOPR recovery values is slightly larger compared to the range of recovery values for the IPR and OPR. Similar to the IPR and OPR results, the FOSA and FOSE EIS compounds have the lowest average and median recovery values yet the corresponding target analytes recovery values center around 100%.

The scatter plot in Figure 11 shows each lab's within-lab variability (the standard deviation values for each lab used in Equation 8) in their LLOPR measurements, for every analyte, where the percent RSD is plotted as a function of the concentration. Most points are below 20% percent RSD with the exception of a small number of analytes across several labs. The computed overall percent RSD values (Equation 10) ranged from 8.22% to 14.3% for the target analytes and 8.03% to 21.8% for the EIS compounds.
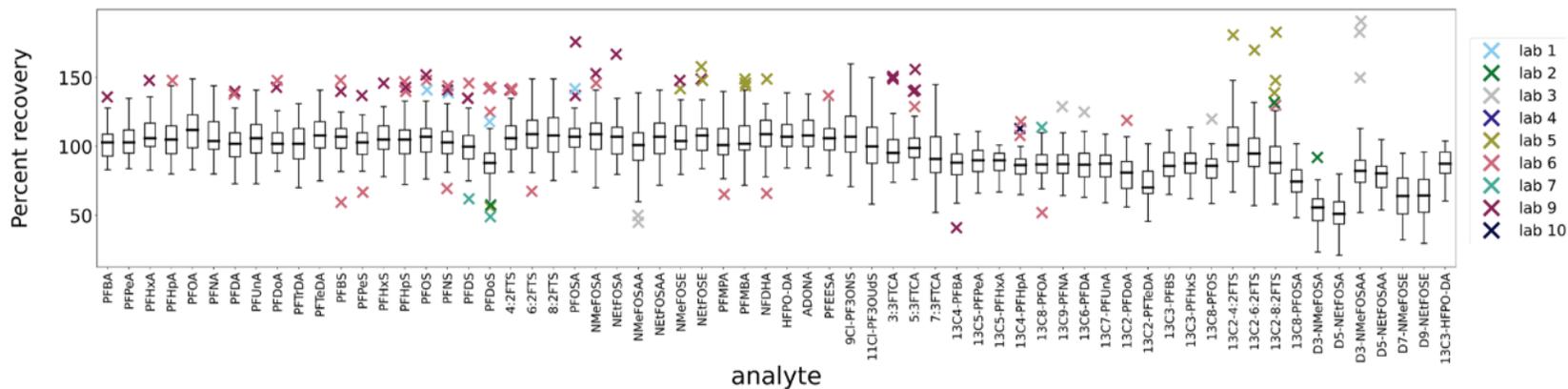
Figure 10. Percent recovery of the LLOPR measurements in the WW, SW, GW matrix spike datasets reported by nine labs, for 40 PFAS target analytes and 24 PFAS EIS compounds.
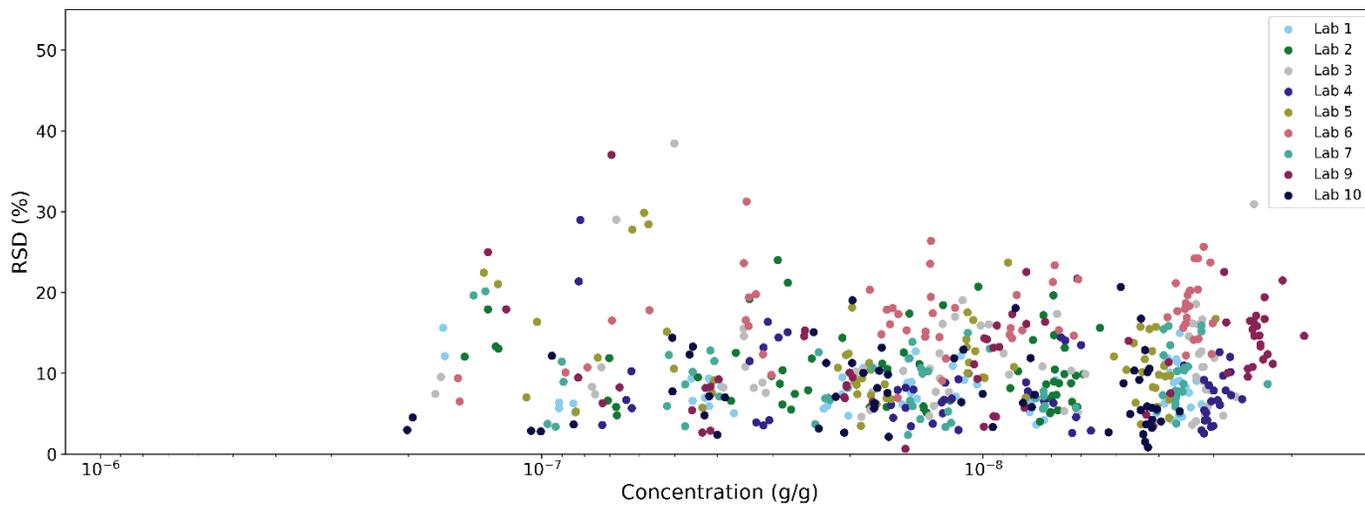
Figure 11. Percent RSD of the LLOPR percent recovery values reported in the WW, SW, GW matrix spike datasets calculated for every lab, for each of the 40 PFAS target analytes and 24 PFAS EIS compounds.

4.   Summary

Method validation is a process that demonstrates that the results generated by conducting the method are reproducible and reliable for the intended purpose. A validated method to quantify PFAS is important in identifying which analytes are present in an area and to set a baseline for future monitoring. IDA analyzed five datasets provided by the sponsor in the PFAS MLV including the ICAL, aqueous IDC, and samples from three environmental matrices: WW, SW, and GW. IDA was blind to the lab identities and sample locations, and was not part of the validation/verification process of the datasets. IDA inspected and evaluated the analysis metrics in the MLV/EPA's ATP, and recommended alternative calculations in instances with a discrepancy between the dataset and formulas. IDA then calculated statistical values for the overall method performance measures including: calibration linearity, LOQVER, IPR, OPR, LLOPR, and matrix spike recovery. These values were provided to the sponsor with the intent that the statistical values will inform the QC acceptance criteria set by the EPA for the method.

Table 2 summarizes the PFAS MLV datasets, validation tests, performance measures, and range of values that IDA computed. The overall mean percent recovery and percent RSD values for the target analytes and the EIS compounds were fairly consistent across the IPR, OPR, and LLOPR tests, respectively. The percent RSD values for the matrix spike recoveries of the target analytes were much broader which is likely due to a smaller number of samples for the SW and GW matrices and specific target analytes being problematic. Additional details about the specific analyses include:

- The ICAL dataset included summary statistics for each of the labs' calibration trials and separately reported calibration concentration values from each lab. IDA was unable to independently verify the summary statistics and identified several instances where the reported average RF and standard deviation did not align with the RSD value. IDA supplied an alternative statistical formula for calculating the pooled RSD value for the method as the dataset was not structured for using the formula outlined in the EPA's ATP.

- The MDL dataset was structured to use the EPA's ATP statistical formulas and IDA calculated an aqueous pooled MDL value.

- The LOQVER test was not described in the EPA's ATP, rather the MLV study plan cited a DoD reference for calculating precision and bias. The LOQVER dataset was comprised of mostly single data point values for each lab and was

not structured to calculate a lab's precision. IDA calculated each lab's percent relative bias but was unable to evaluate the degree of systematic error.

- The IPR dataset was structured to use the EPA's ATP statistical formulas and IDA calculated a mean percent recovery value and an overall percent RSD.

- The matrix spike dataset comprised of percent recovery values of target analytes spiked prior to the delivery of the matrix samples to the labs. The EPA ATP procedure for determining the method performance of a matrix is not for an isotopic dilution method like this PFAS method. IDA calculated the overall percent RSD for each matrix.

- The OPR and LLOPR were structured to use the EPA's ATP statistical formulas. Some labs performed the SW and GW tests together and reported OPR and LLOPR measurements associated with both datasets. IDA calculated a mean percent recovery value and an overall percent RSD for each WW, SW, and GW matrix and across all aqueous matrices (e.g., WW, SW, GW) combined.

Table 2. Summary of PFAS MLV Statistical Analyses for the Aqueous Datasets

PFAS MLV Dataset	Analysis Test in MLV	MLV Data Allowed Use of ATP Formula?	Performance Metric in EPA's ATP	Target Analyte Performance[1]	Extracted Internal Standard Performance[1]
ICAL	Calibration Linearity	No	pooled percent relative standard deviation (RSD)	7.31% to 13.8%	4.11% to 12.1%
Aqueous IDC	Method Detection Limit (MDL)	Yes	pooled MDL	0.315 to 9.89 ng/L	N/A
Aqueous IDC	Limit of Quantitation Verification	N/A	N/A	N/A	N/A
Aqueous IDC	Initial Precision and Recovery	Yes	mean percent recovery	95.0% to 109%	69.1% to 98.1%
Aqueous IDC	Initial Precision and Recovery	Yes	percent RSD	3.35% to 11.5%	5.36% to 17.2%
Matrix Samples	Ongoing Precision and Recovery	Yes	mean percent recovery	89.0% to 109%	53.2% to 101%
Matrix Samples	Ongoing Precision and Recovery	Yes	percent RSD	7.29% to 15.9%	7.18% to 26.6%
Matrix Samples	Low-Limit Ongoing Precision and Recovery	Yes	mean percent recovery	88.3% to 113%	50.8% to 108%
Matrix Samples	Low-Limit Ongoing Precision and Recovery	Yes	percent RSD	8.22% to 14.3%	8.03% to 21.8%

PFAS MLV Dataset	Analysis Test in MLV	MLV Data Allowed Use of ATP Formula?	Performance Metric in EPA's ATP	Target Analyte Performance[1]	Extracted Internal Standard Performance[1]
	Matrix Spike WW[2]			8.94% to 68.0%	N/A
	Matrix Spike SW	No	percent RSD	6.50% to 104%	N/A
	Matrix Spike GW[2]			3.71% to 54.4%	N/A

[1] Nine labs reported values in most datasets.

[2] Only eight labs reported values.

Appendix A.
PFAS MLV Analytes

Table A-1. List of PFAS Analytes and Standards in MLV

Classification Type	PFAS Acronym	Quantification Reference
Target Analyte		
perfluoroalkyl carboxylic acids	PFBA	$^{13}C_4$-PFBA
	PFPeA	$^{13}C_5$-PFPeA
	PFHxA	$^{13}C_5$-PFHxA
	PFHpA	$^{13}C_4$-PFHpA
	PFOA	$^{13}C_8$-PFOA
	PFNA	$^{13}C_9$-PFNA
	PFDA	$^{13}C_6$-PFDA
	PFUnA	$^{13}C_7$-PFUnA
	PFDoA	$^{13}C_2$-PFDoA
	PFTrDA	avg. $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA
	PFTeDA	$^{13}C_2$-PFTeDA
perfluoroalkyl sulfonic acids	PFBS	$^{13}C_3$-PFBS
	PFPeS	$^{13}C_3$-PFHxS
	PFHxS	$^{13}C_3$-PFHxS
	PFHpS	$^{13}C_8$-PFOS
	PFOS	$^{13}C_8$-PFOS
	PFNS	$^{13}C_8$-PFOS
	PFDS	$^{13}C_8$-PFOS
	PFDoS	$^{13}C_8$-PFOS
fluorotelomer sulfonic acids	4:2FTS	$^{13}C_2$-4:2FTS
	6:2FTS	$^{13}C_2$-6:2FTS
	8:2FTS	$^{13}C_2$-8:2FTS
perfluorooctane sulfonamides	PFOSA	$^{13}C_8$-PFOSA
	NMeFOSA	D_3-NMeFOSA
	NEtFOSA	D_5-NEtFOSA
perfluorooctane sulfonamidoacetic acids	NMeFOSAA	D_3-NMeFOSAA
	NEtFOSAA	D_5-NEtFOSAA
perfluorooctane sulfonamide ethanols	NMeFOSE	D_7-NMeFOSE
	NEtFOSE	D_9-NEtFOSE

Classification Type	PFAS Acronym	Quantification Reference
per-and-polyfluoroether carboxylic acids	PFMPA	$^{13}C_5$-PFPeA
	PFMBA	$^{13}C_5$-PFPeA
	NFDHA	$^{13}C_5$-PFHxA
	HFPO-DA	$^{13}C_3$-HFPO-DA
	ADONA	$^{13}C_3$-HFPO-DA
ether sulfonic acids	PFEESA	$^{13}C_5$-PFHxA
	9Cl-PF3ONS	$^{13}C_3$-HFPO-DA
	11Cl-PF3OUdS	$^{13}C_3$-HFPO-DA
fluorotelomer carboxylic acids	3:3FTCA	$^{13}C_5$-PFPeA
	5:3FTCA	$^{13}C_5$-PFHxA
	7:3FTCA	$^{13}C_5$-PFHxA
EIS compounds		
perfluoroalkyl carboxylic acids	$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
	$^{13}C_5$-PFPeA	$^{13}C_2$-PFHxA
	$^{13}C_5$-PFHxA	$^{13}C_2$-PFHxA
	$^{13}C_4$-PFHpA	$^{13}C_2$-PFHxA
	$^{13}C_8$-PFOA	$^{13}C_4$-PFOA
	$^{13}C_9$-PFNA	$^{13}C_5$-PFNA
	$^{13}C_6$-PFDA	$^{13}C_2$-PFDA
	$^{13}C_7$-PFUnA	$^{13}C_2$-PFDA
	$^{13}C_2$-PFDoA	$^{13}C_2$-PFDA
	$^{13}C_2$-PFTeDA	$^{13}C_2$-PFDA
perfluoroalkyl sulfonic acids	$^{13}C_3$-PFBS	$^{18}O_2$-PFHxS
	$^{13}C_3$-PFHxS	$^{18}O_2$-PFHxS
	$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
	$^{13}C_2$-4:2FTS	$^{18}O_2$-PFHxS
	$^{13}C_2$-6:2FTS	$^{18}O_2$-PFHxS
	$^{13}C_2$-8:2FTS	$^{18}O_2$-PFHxS
perfluorooctane sulfonamides	$^{13}C_8$-PFOSA	$^{13}C_4$-PFOS
	D_3-NMeFOSA	$^{13}C_4$-PFOS
	D_5-NEtFOSA	$^{13}C_4$-PFOS
perfluorooctane sulfonamidoacetic acids	D_3-NMeFOSAA	$^{13}C_4$-PFOS
	D_5-NEtFOSAA	$^{13}C_4$-PFOS
perfluorooctane sulfonamide ethanols	D_7-NMeFOSE	$^{13}C_4$-PFOS
	D_9-NEtFOSE	$^{13}C_4$-PFOS
per-and-polyfluoroether carboxylic acids	$^{13}C_3$-HFPO-DA	$^{13}C_2$-PFHxA

Classification Type	PFAS Acronym	Quantification Reference
NIS compounds		
perfluoroalkyl carboxylic acids	$^{13}C_3$-PFBA	N/A
	$^{13}C_2$-PFHxA	N/A
	$^{13}C_4$-PFOA	N/A
	$^{13}C_5$-PFNA	N/A
	$^{13}C_2$-PFDA	N/A
perfluoroalkyl sulfonic acids	$^{18}O_2$-PFHxS	N/A
	$^{13}C_4$-PFOS	N/A

Appendix B.
Interpretation of Box and Whisker Plots

Box and whisker plots graphically present the data without making assumptions about the distribution (Figure B-1). The plots also show the spread and skewness in values across a grouping of data. The center line in the box is the median. The top and bottom of the box covers where half of the data are found from the 25th to 75th percentiles, with the length of the box defining the interquartile range. The two whisker lines outside of the box indicate the maximum and minimum of the dataset up to the interquartile (IQR) range (the range defined in the box) multiplied by 1.5. The circles are data points outside the range defined in the whiskers. [50]
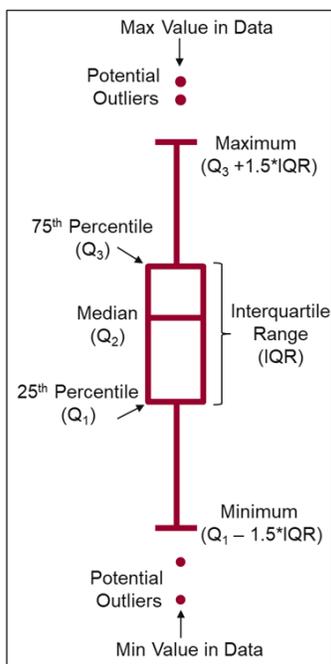
Figure B-1. Box and whisker plot description.

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[50] "Box Plot with Minitab," Lean Sigma Corporation, December 22, 2015, https://www.leansigmacorporation.com/box-plot-with-minitab/?nab=1.

Appendix C.
Data Overview Plots

IDA analyzed five datasets in the PFAS MLV including the ICAL, aqueous IDC, and samples from three environmental matrices: WW, SW, and GW. Each environmental matrix included a total of six samples spiked with the 40 PFAS analytes where three samples were spiked at a "low" concentration and three samples were spiked at a "high" concentration. The following plots provide a visualization combining data across the datasets to show the calibration concentrations, calculated MDL values, and spiked and measured concentration values for each of the 40 target analytes across the nine labs (Figures C-1–C-4).

Each plot includes the following:

- 'X' data points indicate the calibration concentrations reported by the labs.

- Blue line is the pooled MDL value calculated by IDA.

- Light blue shading is the lowest and highest lab MDL value calculated by IDA.

- Green line is the low spike concentration of the analyte added in each of the aqueous matrix samples (e.g., WW, SW, GW) reported in the dataset.

- Purple line is the high spike concentration of the analyte added in each of the aqueous matrix samples (e.g., WW, SW, GW) reported in the datasets.

- Color matched boxplots are the labs measured spike concentrations corrected for any measured analyte in the unspiked sample in the aqueous matrix samples (e.g., WW, SW, GW), reported in the datasets.
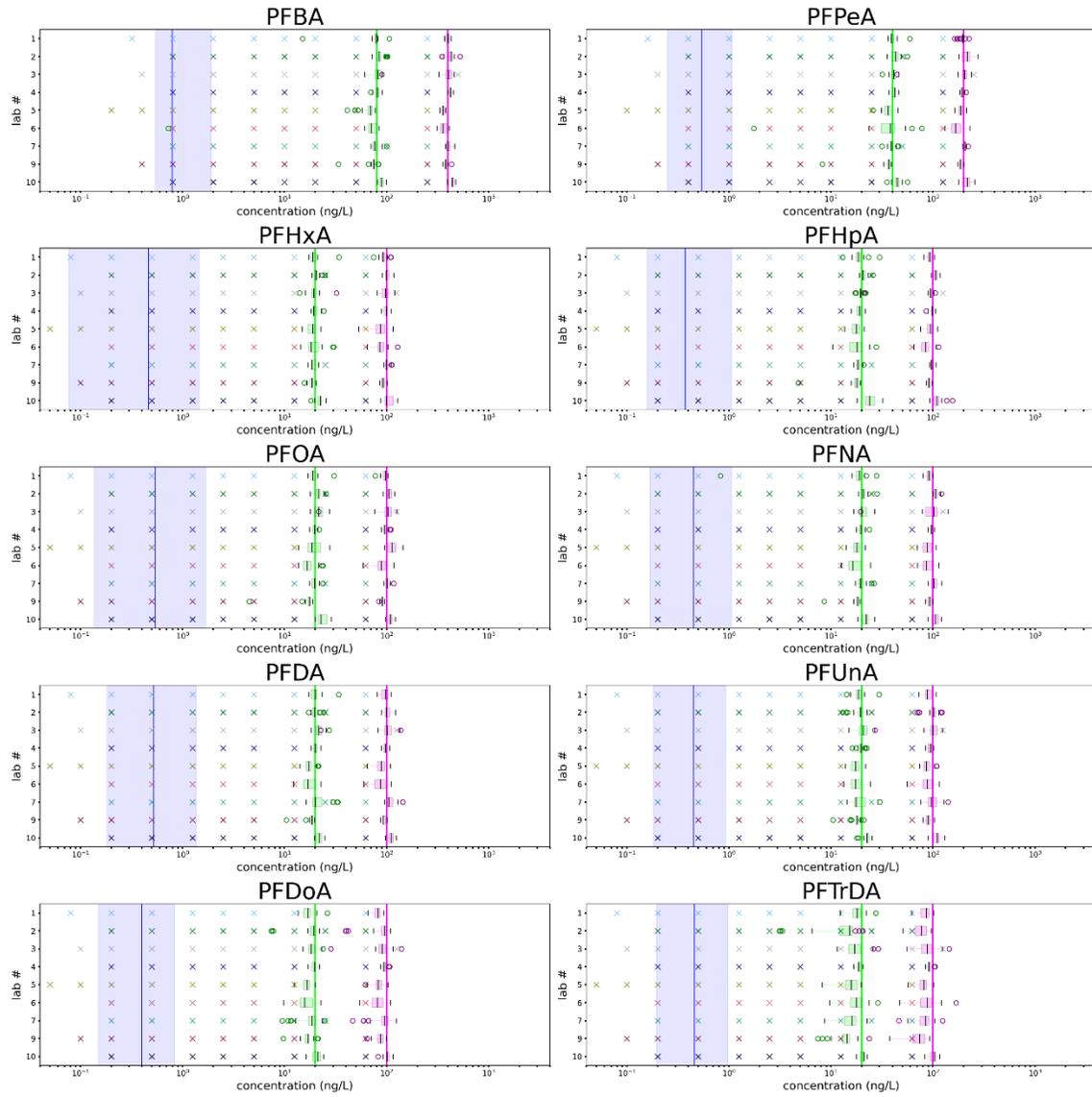
Figure C-1. Plot 1 of 4 depicting the calibration concentrations, calculated MDL values, and spiked and measured concentration values of 10 target analytes across the 9 labs.

C-2
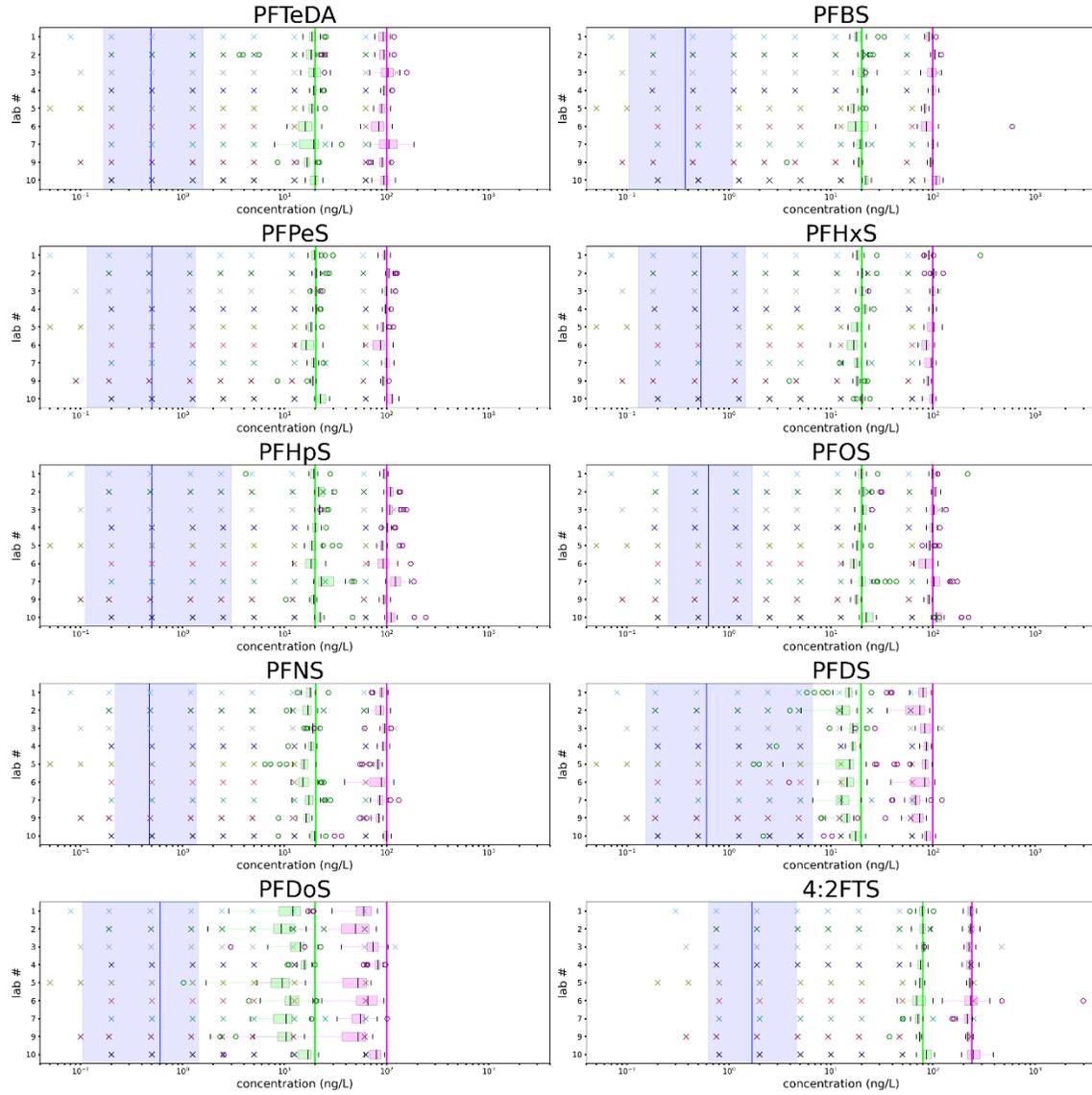
Figure C-2. Plot 2 of 4 depicting the calibration concentrations, calculated MDL values, and spiked and measured concentration values of 10 target analytes across the 9 labs.
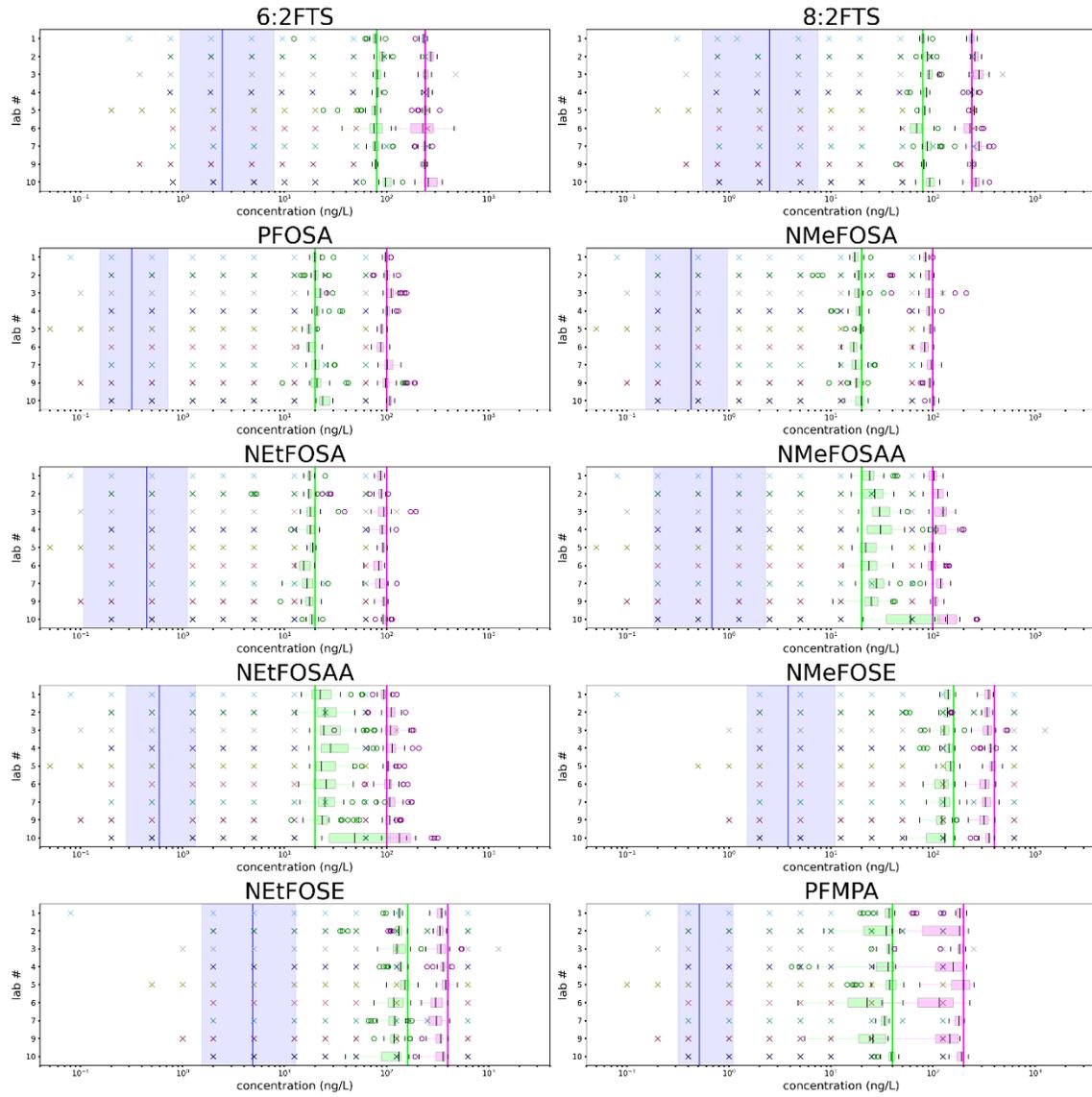
Figure C-3. Plot 3 of 4 depicting the calibration concentrations, calculated MDL values, and spiked and measured concentration values of 10 target analytes across the 9 labs.
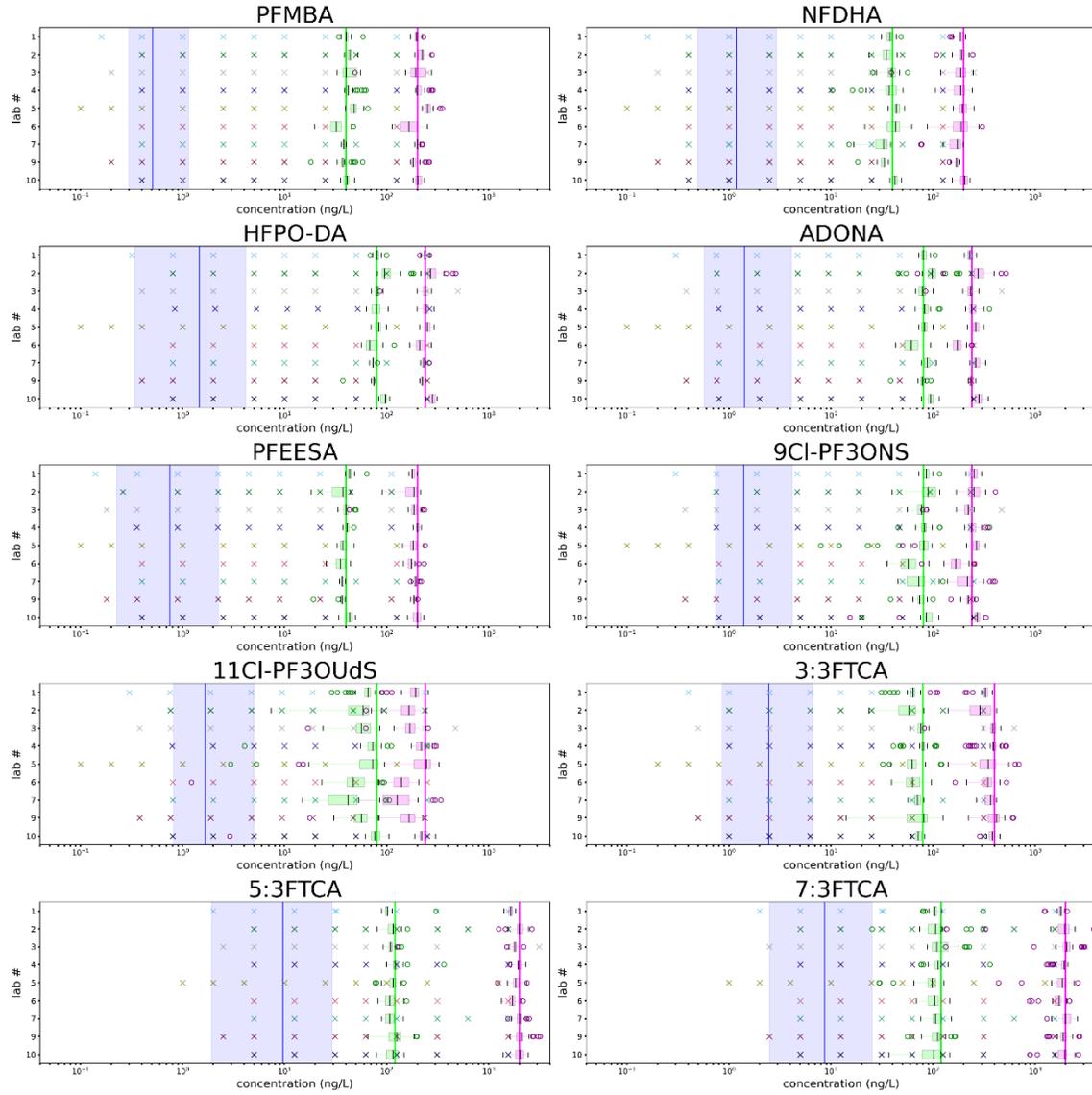
Figure C-4. Plot 4 of 4 depicting the calibration concentrations, calculated MDL values, and spiked and measured concentration values of 10 target analytes across the 9 labs.

Appendix D.
List of Tables in the Digital Appendix

Table D-1 is a list of the IDA-generated tables as CSV files for each of the listed PFAS MLV datasets included in the digital appendix. Accompanied with each table is a TXT file that includes the MLV dataset version and description of the data in each column as well as the formula or citation to any statistical equations.

Table D-1. List of IDA Generated Tables Corresponding to the PFAS MLV Datasets

Dataset	Version	IDA Table File
ICAL	ICAL Concentrations_08182022.xlsx ICAL Average RF_05182023.xlsx	ICAL_calibration_V0_220907_093746.csv AverageRF_ICAL_results_V4_230519_091739.csv
IDC	RW_DBexport_V1_20230426.csv	MDL_results_V1_230503_215159.csv LOQVER_results_V1_230503_215921.csv IPR_results_V1_230503_215140.csv
WW	WW_DBexport_V7_20230328.csv	LLOPR_results_V4_230406_212723.csv OPR_results_V4_230406_212237.csv Matrix_EIS_results_V4_230406_212819.csv Matrix_sample_results_V4_230406_211329.csv Matrix_compiled_results_V4_230406_211329.csv MB_results_V4_230406_212853.csv
SW	SW_DBexport_V4_20230407.csv	LLOPR_results_V0_230411_080130.csv OPR_results_V0_230411_080146.csv Matrix_EIS_results_V0_230411_080212.csv Matrix_sample_results_V0_230411_080232.csv Matrix_compiled_results_V0_230411_080232.csv MB_results_V0_230411_080058.csv
GW	GW_DBexport_V6_20230417b.csv	LLOPR_results_V0_230421_074935.csv OPR_results_V0_230420_183700.csv Matrix_EIS_results_V0_230420_175829.csv Matrix_sample_results_V0_230421_153930.csv Matrix_compiled_results_V0_230421_153930.csv MB_results_V0_230420_183436.csv
All Aqueous	WW_SW_GW_EXPORT_20230605.csv	LLOPR_results_V1_230607_124655.csv OPR_results_V1_230607_124749.csv Matrix_EIS_results_V1_230607_124828.csv Matrix_NIS_results_V1_230607_124909.csv

Illustrations

Tables

Figures

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Abbreviations

ATP	Alternate Test Procedure
CFR	Code of Federal Regulations
CS	calibration sample
DoD	Department of Defense
EIS	extracted internal standard
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FOSA	perfluorooctane sulfonamides
FOSE	perfluorooctane sulfonamide ethanols
GW	groundwater
ICAL	initial calibration
IDC	initial demonstration of capability
IPR	initial precision and recovery
IQR	interquartile range
IUPAC	International Union of Pure and Applied Chemistry
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
LOQVER	limit of quantitation verification
LLOPR	low-level ongoing precision and recovery
MDL	method detection limit
ML	method limit
MLV	multi-lab validation
NIS	non-extracted internal standard
OPR	ongoing precision and recovery
PFAS	per- and polyfluoroalkyl substances
ppb	parts per billion
QC	quality control
SW	surface water
RF	response factor
RSD	relative standard deviation
SERDP	Strategic Environmental Research and Development Program
WW	waste water

Appendix E

Wastewater Supporting Tables

Appendix E: List of Tables

Table E-1. Target Analytes Detected in Unspiked Wastewater Samples by Laboratory (ng/L)

Analyte	Number	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		Lab 9	
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual
WWI – Hospital																	
PFBA	8	2.64	J	3.58	J	1.04	U	1.86	J-	24.1		0.597	U	25.1		6	
PFPeA	8	5.76		6.23	JI	8.56		5.1		5.03		0.563	U	4.4		5.06	
PFHxA	8	14.2		11.8		13.1		9.82		9.7		8.69		9.4		10.4	
PFHpA	8	2.5		2.41	J	2.8		2.4		2.48		0.173	U	1.8	J	1.95	
PFOA	8	2.09		3.75	J	2.24		2.35		2.45	BJ+	2.75		1.6	J	1.62	
PFBS	8	1.84	J	2.35	J	4.16		1.57	J	1.82		0.177	U	1.8	J	2.02	
PFPeS	8	2.1		2.22	J	2.8		2.03		2.07		1.72		1.6	J	1.82	
PFHxS	8	14.1		18		21.8		18		16.1		13.6		7.8		11.7	
PFOS	8	3.04		5		5.6		4.63		3.37		3.44		2.2		1.7	J
6:2FTS	8	1.76	J	7.92	U	2.82	U	1.6	U	1.46	U	1.54	J	3.5	U	0.945	U
PFOSA	8	0.346	U	0.724	U	0.416	U	0.565	U	0.746	BJ+	0.472	J	0.67	U	0.154	U
PFMPA	8	0.578	U	1.09	U	0.48	U	0.556	U	0.745	J	0.628	U	0.99	U	0.321	U
WWJ – POTW Influent																	
PFBA	8	4.93	J	4.2	J	5.04	J	4.64	J-	4.63	J	0.597	U	12.2		5.83	
PFPeA	8	9.19		10.1		11.1		9.45		8.88		0.563	U	9.1		9.37	
PFHxA	8	21.7		21.2		21.8		18.2		17.2		20.2		18.7		19.8	
PFHpA	8	7.97		8.24		9.12		7.83		7.68		5.96		8.5		7.98	
PFOA	8	12		13.7		16.5		12.5		14.2		11.3		12.6		13.2	
PFNA	8	3.12		3.3	J	3.6		2.93		2.59		3.53		3.2		3.44	
PFDA	8	1.29	J	1.62	J	1.2	J	1.22	J	0.892	U	0.223	U	1.9	J	1.3	
PFBS	8	4.43		4.72		0.528	U	3.58		0.348	U	0.177	U	3.3		4.96	
PFPeS	8	0.351	U	1.31	U	0.272	U	0.375	U	0.729	U	0.129	U	1.1	U	1.37	I
PFHxS	8	4.2		2.92	J	3.52		3.68		3.04		2.83		2.1		3.03	
PFOS	8	7.45		6.18		7.6		7.63		7.25		7.09		6.2		7.38	J
6:2FTS	8	23.7		25.3		26.2		25.6		22.3		24.4		22.5		24.9	
8:2FTS	8	4.5	J	7.48	U	5.36	J	3.56	U	4.67	J	4.38	J	5.6	J	5.32	
PFOSA	8	0.346	U	0.724	U	0.416	U	0.565	U	0.397	BJ+	0.188	U	0.67	U	0.154	U
NMeFOSAA	8	1.44	J	3.3	U	1.68		2.19		1.49	J	0.655	U	2		1.97	
NEtFOSAA	8	0.75	J	2.26	U	0.856	U	0.61	U	0.531	JI	0.571	U	1.3	U	0.283	U
5:3FTCA	8	5.54	U	16.7	U	9.36	U	6.66	U	29.2	U	4.72	J	8.7	U	1.88	U

Table E-1. Target Analytes Detected in Unspiked Wastewater Samples by Laboratory (ng/L)

Analyte	Number	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		Lab 9	
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual
WWL – Bus Washing Station																	
PFBA	8	11.7		3.06	J	11.5		9.77	J-	8.98		0.597	U	14		11.4	
PFPeA	8	45		41		61.1		35.6		35.2		43.8		38.9		37.6	
PFHxA	8	81.6		84.1		83.9		76.9		78.6		66.5		81		79.5	
PFHpA	8	32.2		32.5		31.3		28.4		29.5		29.7		32.1		30.7	
PFOA	8	8.36		9.53		11		8.61		11.8		8.85		7.3		9	
PFNA	8	2.18		2.24	J	2.8		2.16		2.33		2.12		2.3		2.28	
PFDA	8	1.21	J	1.53	J	1.04	JI	0.899	J	--	X	0.223	U	0.77	J	1.01	
PFUnA	8	0.609	U	1.73	J	0.664	U	0.574	U	--	X	0.203	U	0.6	U	0.182	U
PFDoA	8	0.603	U	1.16	J	0.752	U	0.345	U	--	X	0.301	U	0.6	U	0.169	U
PFBS	8	0.289	U	1.45	J	0.528	U	0.628	U	0.941	J	0.177	U	1.4	J	1.03	
PFHxS	8	1.3	JI	1.43	U	0.464	U	0.789	U	0.803	J	0.291	U	0.7	U	0.171	U
PFOS	8	2.08		1.75	J	2.48	I	1.98	I	--	X	1.42	J	0.54	U	2.23	J
6:2FTS	8	6.15	J	7.92	U	5.2	J	4.42	J	4.72	J	2.63	J	35	U	4.07	
PFOSA	8	0.346	U	1.02	J	0.416	U	0.565	U	--	X	0.188	U	0.67	U	0.154	U
HFPO-DA	8	1.85	U	4.17	U	2	J	2.89	U	2.14	U	0.748	U	0.97	U	0.891	U
3:3FTCA	8	2.12	J	3.97	U	3.36	J	3.19	J	6.57	U	1.67	U	4.5	U	1.48	U
7:3FTCA	8	6.53	U	27.7	J	5.18	U	4.15	U	25.3	U	5.22	U	7.9	U	2.56	U

Table E-1. Target Analytes Detected in Unspiked Wastewater Samples by Laboratory (ng/L)

Analyte	Number	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab 7		Lab 9	
		Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual	Conc	Qual
WWM – POTW Effluent																	
PFBA	8	10		9.28	J	10.7		8.26	J-	8.56		0.597	U	14.6		12	
PFPeA	8	10.5		12.9		15		10.6		10.6		0.563	U	10.2		10.8	
PFHxA	8	38.1		41.4		41.4		36.2		39.3		32.3		36		38.5	
PFHpA	8	4.07		4.47		4.4		4.2		3.97		3.26		3.6		4.38	
PFOA	8	12		12.9		14.2		11.7		13.8		10.1		10		12.3	
PFNA	8	0.93	J	1.06	U	1.76	I	0.718	J	0.851	J	1.35	J	0.63	J	1.18	
PFDA	8	2.33		2.96	J	2.16		1.97	J	1.58	J	2.14		1.9	J	1.92	
PFUnA	8	0.609	U	1.48	J	0.664	U	0.574	U	0.786	U	0.203	U	0.6	U	0.182	U
PFDoA	8	0.603	U	1.31	J	0.752	U	0.345	U	0.412	U	0.301	U	0.6	U	0.169	U
PFTrDA	8	0.478	U	1.02	J	0.608	U	0.281	U	0.838	U	0.363	U	0.84	U	0.196	U
PFBS	8	4.53		5.25		3.44		3.83		3.8		0.177	U	3.6		5.68	
PFHxS	8	2.88	I	1.53	J	2.08		0.789	U	1.48	J	2.03		0.86	J	1.41	
PFOS	8	2.73		3.23	J	2.96		3.35		3.15		2.53		2.2		2.68	J
6:2FTS	8	6.96	J	7.92	U	9.44		6.96	J	7.92		6.75		7.8	J	7.07	
PFOSA	8	0.346	U	1.11	JI	0.416	U	0.565	U	0.359	BJ+	0.188	U	0.67	U	0.154	U
NMeFOSA	8	0.453	U	0.809	J	0.704	U	1.21	U	0.341	U	0.199	U	0.64	U	0.153	U
NMeFOSAA	8	0.739	J	3.3	U		X	1.08	J	0.84	J	0.655	U	0.98	U	0.186	U
7:3FTCA	8	6.53	U	25.4	J	5.18	U	4.15	U	25.3	U	5.22	U	7.9	U	2.56	U
WWN – Pulp and Paper Effluent																	
PFBA	8	0.941	U	3.42	J	2.64	J	--	X	5.51	J	--	X	1.9	U	--	X
PFPeA	8	0.552	U	3.42	J	13.8		0.306	U	6.7		0.563	U	3.4	J	3.19	
PFHxA	8	0.454	U	2.99	J	4.56		2.36		2.7		0.412	U	2.7		2.14	
PFHpA	8	0.501	U	1.07	J	1.12	J	0.368	U	1.7	JI	0.173	U	1.5	J	0.158	U
PFOA	8	1.1	J	1.96	J	1.68		1.29	J	1.82	BJ+	1.63		1.4	J	1.56	
PFNA	8	0.657	U	1.06	U	2.08	I	0.29	U	0.792	U	0.25	U	0.61	U	0.166	U
PFBS	8	0.289	U	1.08	U	1.2	J	0.628	U	0.864	J	0.177	U	0.95	J	0.104	U
PFHxS	8	1.65	J	1.57	J	1.36	J	1.32	JI	1.27	J	1.04	J	1.2	J	1.39	
PFOS	8	3.55		3.85		4.72		3.61		3.78		3.21		2.8		3.41	J
PFOSA	8	0.346	U	0.724	U	0.416	U	0.565	U	0.344	BJ+	0.188	U	0.67	U	0.154	U

Table E-1. Target Analytes Detected in Unspiked Wastewater Samples by Laboratory (ng/L)

Analyte	Number	Lab 1 Conc	Lab 1 Qual	Lab 2 Conc	Lab 2 Qual	Lab 3 Conc	Lab 3 Qual	Lab 4 Conc	Lab 4 Qual	Lab 5 Conc	Lab 5 Qual	Lab 6 Conc	Lab 6 Qual	Lab 7 Conc	Lab 7 Qual	Lab 9 Conc	Lab 9 Qual
WWO – POTW Effluent																	
PFBA	8	7.88	J	7.94	J	8.56		7.4	J	8.33		0.597	U	12.3		8.31	
PFPeA	8	22.6		25		27.1		23.1		23		22.8		22.4		22.4	
PFHxA	8	51.7		61		52.9		50.7		57.7		46.2		52.7		49	
PFHpA	8	8.4		8.86		9.04		8.01		7		6.78		8		8.31	
PFOA	8	9.56		12.1		12.9		9.43		11.1		7.6		10.3		10.7	
PFNA	8	1.95	J	2.64	J	3.36	J-	1.84	J	2.64		2.51		2.5		2.71	
PFDA	8	1.06	J	1.36	U	0.72	JI	0.596	U	0.892	U	0.223	U	0.69	J	0.181	U
PFBS	8	10.7		11.1		13.4		9.33		9.64		15.3		14.2		13.1	
PFPeS	8	1.66	J	1.94	J	2.08		1.96		1.75	J	0.129	U	2.2		2	
PFHxS	8	12.5		13.6		14.2		13.4		13.9		11.1		10.8		12.2	
PFHpS	8	0.874	J	3.06	U	1.6		0.633	U	0.731	J	0.204	U	0.49	U	0.112	U
PFOS	8	35.2		27.6		35.5		32.5		31.3		31.2		30.3		28.8	
6:2FTS	8	233		341		289		267		274		352		277		264	
8:2FTS	8	1.41	U	7.48	U	2.5	U	3.56	U	1.45	U	1.08	J	4.1	U	0.544	U
PFOSA	8	0.346	U	0.724	U	0.416	U	0.565	U	0.228	BJ+	0.188	U	0.67	U	0.154	U
5:3FTCA	8	9.54	J	18.5	J	11.8	J	13.1	J	29.2	U	13.8	J	8.7	U	1.88	U

Compounds undetected in all samples included: PFTeDA, PFNS, PFDS, PFDoS, 4:2FTS, NEtFOSA, NMeFOSE, NEtFOSE, PFMBA, NFDHA, ADONA, PFEESA, 9Cl-PF3ONS, 11Cl-PF3OUdS

Table E-2. Minimum and Maxium Target Analytes Concentrations in Unspiked Wastewater Samples (ng/L)

Analyte	Number of Labs	WWI		WWJ		WWL		WWM		WWN		WWO	
		min	max	min	max	min	max	min	max	min	max	min	max
PFBA	8	0.597 U	25.1	0.597 U	12.2	0.597 U	14	0.597 U	14.6	0.941 U	5.51 J	0.597 U	12.3
PFPeA	8	0.563 U	8.56	0.563 U	11.1	35.2	61.1	0.563 U	15	0.306 U	13.8	22.4	27.1
PFHxA	8	8.69	14.2	17.2	21.8	66.5	84.1	32.3	41.4	0.412 U	4.56	46.2	61
PFHpA	8	0.173 U	2.8	5.96	9.12	28.4	32.5	3.26	4.47	0.158 U	1.7 JI	6.78	9.04
PFOA	8	1.6 J	3.75 J	11.3	16.5	7.3	11.8	10	14.2	1.1 J	1.96 J	7.6	12.9
PFNA	8	0.166 U	1.06 U	2.59	3.6	2.12	2.8	1.06 U	1.76 I	0.166 U	2.08 I	1.84 J	3.36 J-
PFDA	8	0.181 U	1.36 U	0.223 U	1.9 J	0.223 U	1.53 J	1.58 J	2.96 J	0.181 U	1.36 U	0.181 U	1.06 J
PFUnA	8	0.182 U	0.928 U	0.182 U	0.928 U	0.182 U	1.73 J	0.182 U	1.48 J	0.182 U	0.928 U	0.182 U	0.928 U
PFDoA	8	0.169 U	0.822 U	0.169 U	0.822 U	0.169 U	1.16 J	0.169 U	1.31 J	0.169 U	0.822 U	0.169 U	0.822 U
PFTrDA	8	0.196 U	0.978 U	0.196 U	0.978 U	0.196 U	0.978 U	0.196 U	1.02 J	0.196 U	0.978 U	0.196 U	0.978 U
PFBS	8	0.177 U	4.16	0.177 U	4.96	0.177 U	1.45 J	0.177 U	5.68	0.104 U	1.2 J	9.33	15.3
PFPeS	8	1.6 J	2.8	0.129 U	1.37 I	0.166 U	1.31 U	0.166 U	1.31 U	0.166 U	1.31 U	0.129 U	2.2
PFHxS	8	7.8	21.8	2.1	4.2	0.171 U	1.3 JI	0.789 U	2.88 I	1.04 J	1.65 J	10.8	14.2
PFHpS	8	0.112 U	3.06 U	0.112 U	3.06 U	0.112 U	3.06 U	0.112 U	3.06 U	0.112 U	3.06 U	0.112 U	1.6
PFOS	8	1.7 J	5.6	6.18	7.63	0.54 U	2.48 I	2.2	3.35	2.8	4.72	27.6	35.5
6:2FTS	8	0.945 U	1.76 J	22.3	26.2	7.92 U	6.15 J	7.92 U	9.44	0.945 U	7.92 U	233	352
8:2FTS	8	0.544 U	7.48 U	3.56 U	5.6 J	0.544 U	7.48 U	0.544 U	7.48 U	0.544 U	7.48 U	0.544 U	1.08 J
PFOSA	8	0.154 U	0.746 BJ+	0.154 U	0.397 BJ+	0.154 U	1.02 J	0.154 U	1.11 JI	0.154 U	0.344 BJ+	0.154 U	0.228 BJ+
NMeFOSA	8	0.153 U	1.21 U	0.153 U	1.21 U	0.153 U	1.21 U	0.153 U	0.809 J	0.153 U	1.21 U	0.153 U	1.21 U
NMeFOSAA	8	0.186 U	3.3 U	0.655 U	2.19	0.186 U	9.8 U	0.186 U	1.08 J	0.186 U	3.3 U	0.186 U	3.3 U
NEtFOSAA	8	0.283 U	2.26 U	0.283 U	0.75 J	0.283 U	13 U	0.283 U	2.26 U	0.283 U	2.26 U	0.283 U	2.26 U
PFMPA	8	0.321 U	0.745 J	0.321 U	1.33 U	0.321 U	1.33 U	0.321 U	1.33 U	0.321 U	1.33 U	0.321 U	1.33 U
HFPO-DA	8	0.338 U	4.17 U	0.339 U	4.17 U	0.748 U	2 J	0.354 U	4.17 U	0.319 U	4.17 U	0.355 U	4.17 U
3:3FTCA	8	0.86 U	6.57 U	0.86 U	6.57 U	1.48 U	3.36 J	0.86 U	6.57 U	0.86 U	6.57 U	0.86 U	6.57 U
5:3FTCA	8	1.88 U	29.2 U	1.88 U	4.72 J	1.88 U	29.2 U	1.33 U	29.2 U	1.33 U	29.2 U	1.88 U	18.5 J
7:3FTCA	8	2.56 U	25.3 U	2.56 U	25.3 U	2.56 U	27.7 J	2.56 U	25.4 J	2.56 U	25.3 U	2.56 U	25.3 U

Table E-3. Summary of wastewater spike percent recoveries in low spike samples for each laboratory.

Analyte	Lab 1 spike % recovery				Lab 2 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 5 spike % recovery			
	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean
PFBA	18	91.6	132.5	101.5	18	100.6	128	110.9	17	107.2	113.6	110.6	14	87.1	112.3	103	18	51.4	96.4	80.7
PFPeA	15	90.1	148.2	103	15	100	139.9	113.1	14	102.6	116	110.2	17	92.1	116.2	103.2	18	64.5	101	87.2
PFHxA	6	87	170.5	108.8	6	92.5	124.5	102.9	6	96	110.5	104.7	9	91.4	121	101.9	9	77.5	113	94.3
PFHpA	15	81	149.5	98.1	15	95.2	129	104.1	14	95.3	110	100.4	15	89.4	108.4	97.6	15	72.5	104	87
PFOA	18	84.7	152.5	99.5	18	89.5	127.8	108.3	17	91	138	117	18	87	105.8	98.3	18	68.5	138.5	99.1
PFNA	18	80.1	139.5	96.7	18	94.5	141.5	105.6	17	96.2	133.7	113.3	18	88.5	119	98.2	18	70	106.5	89.8
PFDA	18	89.2	169.5	106.2	18	92.2	121.5	99.6	17	97.3	137.2	108.8	18	90.5	109.5	98.6	18	70.5	106	86.8
PFUnA	18	71	148	94.5	18	65	113.4	94.2	17	90.5	135	109.9	18	81	100	94.9	18	69	107	83.9
PFDoA	18	68	131	84.8	18	37	109.5	86.7	17	81	119	95.9	18	85	106.5	95.9	16	62	96.5	78.5
PFTrDA	18	62	138	86.4	18	15.6	105.5	60.8	17	57.5	129	89.9	17	84	102.5	94.6	16	41	95	68.4
PFTeDA	17	75	127	96.2	17	18	111	81.9	17	90.5	140	111.2	17	95	122	103.8	12	79.5	124.5	96.7
PFBS	18	76.4	144.5	93.9	18	98.9	129.2	105.4	17	100.5	141	115.8	18	91.1	112	101.7	18	72.7	109.5	83.7
PFPeS	18	83.2	147	101.9	18	91.6	129.6	102.8	17	95.5	116.8	102.1	18	93.9	110.1	101.7	18	81.2	114.9	92.7
PFHxS	18	81.1	139.6	93.8	18	91.7	140.3	102	14	95.1	113.9	103.6	18	89.6	130.8	106.6	18	77.6	117.4	96.1
PFHpS	18	85.5	139.5	98.2	18	100	154	112.1	17	99	129	109.1	18	87.5	127.5	102.4	18	78.5	173	102.4
PFOS	15	90.6	143.7	105.3	15	94.5	121.5	102.3	15	95.9	120.7	105.1	15	85.4	105.7	95	15	81.6	103.5	93.1
PFNS	18	66.3	133.7	88.4	18	60.4	97.5	79.7	17	81.7	109.9	96.4	18	53.5	102	89.9	18	31.8	90.6	69.6
PFDS	18	29.2	125.3	67.7	18	19.9	89.9	59.4	17	65.7	114.6	92.3	18	14.7	96	78.9	18	8.8	89.4	51.7
PFDoS	18	14.3	86.9	45.4	17	8.8	63.8	37.7	17	40.2	113.1	77.4	17	53.8	93	74.5	15	5.1	54.3	29.6
4:2FTS	18	92.2	126.7	103.9	18	97.6	120.1	105.2	17	99	111.2	104.9	18	75.2	111.3	92.8	18	84.4	103.4	93.4
6:2FTS	15	76.9	121.8	97.5	15	94	143.8	112.4	15	104	119.5	110.2	15	90.5	109	100.2	15	86.1	109.4	97
8:2FTS	18	92.8	123.7	102.2	18	100.1	136.8	110.9	17	106	151.8	123.6	18	69.8	116.7	102.9	18	87.5	110.8	100.4
PFOSA	18	91	151	103.4	18	72.5	136	100.9	17	98.5	124.5	110.1	18	96	183.5	113.9	13	73.5	104.3	86.2
NMeFOSA	18	75.5	122.5	86.8	18	32.8	108.5	84.9	17	77.5	164	98.3	18	49.8	117.5	90.2	13	69.5	104	93.3
NEtFOSA	18	78.5	125	88.8	17	23.5	92.5	74.8	17	73	168	97.3	18	57.5	110	90.9	12	82.5	99	91.4
NMeFOSAA	18	79	213	127.5	18	78.5	197	144.5	6	146.6	188.6	171.1	18	103	535	215.6	17	80.5	165	124
NEtFOSAA	18	72.5	223	119.5	18	64.5	205.5	136.8	17	106	381.5	172.1	18	100	400	188.4	15	87	177	123.8
NMeFOSE	18	72.5	94.4	85	17	34.2	95	77.2	17	47.1	126.9	83.2	17	46.3	101.9	83.9	12	66.9	113.8	87.3
NEtFOSE	18	73.8	89.4	81.6	17	21.9	96.2	71.1	17	56.4	135.6	90.6	17	53.1	101.2	84.8	10	61.6	126.9	97
PFMPA	18	64.8	102.2	89.1	18	78.2	116.8	100.4	17	62	109.5	97.4	17	15.2	107	84.5	18	36	113.5	79.9
PFMBA	18	85	146.2	101.4	18	98.8	129.8	111.2	17	115.3	139.5	127.2	17	98	156	112.8	18	99.2	163.2	123.4
NFDHA	18	78.2	121.2	93.9	18	58.8	111.5	83.5	17	64.2	141.2	99.6	18	25.8	102.8	79.8	18	90.3	131.2	113.2
HFPO-DA	18	96.4	124.8	104.7	18	110.5	228.8	151.9	17	96.1	108.6	102.8	18	88.6	128.8	105.6	18	88.1	125	102.2
ADONA	18	92.5	130.9	107.9	18	120.7	228.2	153.7	17	92.8	124.7	106.7	18	88.8	446.4	129.7	18	89.9	120.6	102
PFEESA	18	100.2	159.6	109.8	18	45.9	99	76.8	17	83	125.2	98.2	18	95	118.7	102.8	18	81.3	119.5	100.3
9Cl-PF3ONS	18	99.4	144.5	114.6	18	81.7	144.5	122.5	17	71	110.8	95.9	18	56.7	447.1	126.5	18	9.9	121.2	80.2
11Cl-PF3OUdS	18	36	113.6	74.4	18	9.3	111.1	66.3	17	44.9	106.5	82.7	18	5	313	104.6	16	3.7	95.4	55.2
3:3FTCA	18	51.7	94.3	80.1	18	58.8	102.6	79.5	17	94.1	114.2	103.2	17	51	136	101.3	18	40.4	151.2	83.9
5:3FTCA	18	80.8	253.3	94.6	18	67.4	133.8	91.9	17	77.5	116.7	93.9	18	82.6	302.5	114.6	18	64	123.3	96.6
7:3FTCA	18	81	257.5	98.9	18	61.7	255.8	98.6	17	90.8	189.2	128.6	18	63.9	301.7	103.3	18	24.6	105.8	73.9

Table E-3. Summary of wastewater spike percent recoveries in low spike samples for each laboratory.

Analyte	Lab 6 spike % recovery				Lab 7 spike % recovery				Lab 9 spike % recovery				All Labs			
	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean	n	Min	Max	Mean
PFBA	16	78.9	99.2	88.6	18	92.9	125	103.1	15	42.1	104.4	91.3	134	42.1	132.5	98.8
PFPeA	15	58.5	195	106.7	18	79	113.8	101.2	18	20.5	97	87.7	130	20.5	195	100.8
PFHxA	6	99	151.5	122.4	9	86	108	98.6	9	78	102	90.6	60	77.5	170.5	101.7
PFHpA	15	52.1	101	84	15	86	105	94	15	23.9	97	84.3	119	23.9	149.5	93.6
PFOA	18	68.8	99	82.2	18	90	109.5	99.1	18	22.5	94	83.5	143	22.5	152.5	98.2
PFNA	18	66	100	81.3	18	86	104	94.7	18	43	96	88.1	143	43	141.5	95.8
PFDA	18	61.5	107.5	80.6	18	89	116.5	101.3	18	52	98.5	90.1	143	52	169.5	96.4
PFUnA	18	66.5	119.5	88.8	18	72.5	108	84.9	18	52.5	105	87.5	143	52.5	148	92.2
PFDoA	18	49.2	105.5	74.8	18	64	116.5	93.2	18	48.7	106.5	83.5	141	37	131	86.7
PFTrDA	18	48.6	144	84.3	18	60	113	80.8	18	37.2	91.5	65.3	140	15.6	144	78.8
PFTeDA	18	53	109	79	18	88	180.5	115.6	18	42.7	110	84.7	134	18	180.5	96
PFBS	18	55.5	137.5	102.6	18	83.8	110.5	99.1	18	18.5	101.8	90.7	143	18.5	144.5	99
PFPeS	18	68.3	117.8	86	18	84.7	106.9	96.3	18	42.1	96	89.3	143	42.1	147	96.6
PFHxS	18	48.8	108.3	82	18	60.7	110.9	87	18	19.4	113.4	87.9	140	19.4	140.3	94.6
PFHpS	18	64	114	87.9	18	97.5	117	109.3	18	51.5	101.5	91.7	143	51.5	173	101.6
PFOS	15	69.8	95.8	84.1	15	79	108.5	97.5	15	76.9	99	88.4	120	69.8	143.7	96.4
PFNS	18	57.4	83.2	70.3	18	67.3	99.5	82.6	18	43	88.1	76.5	143	31.8	133.7	81.6
PFDS	18	37.4	88.4	66	18	48	90.4	68.9	18	40.2	84.8	64.6	143	8.8	125.3	68.5
PFDoS	18	22.3	66.8	50.6	18	30.2	92	56.7	18	11.7	63.8	43.5	138	5.1	113.1	52.2
4:2FTS	18	64.2	125.3	91.8	18	63.1	99.7	85.9	18	47.1	94.4	89	143	47.1	126.7	95.8
6:2FTS	15	45.3	152.8	89.6	15	78.9	143.8	104.1	15	90.2	100.4	95.6	120	45.3	152.8	100.8
8:2FTS	18	60.1	107.6	81.7	18	80.2	140.5	109.6	18	54.6	107.4	99.4	143	54.6	151.8	103.7
PFOSA	18	68	113.1	85.7	18	81	108.5	97	18	47.5	770	205.4	138	47.5	770	113.8
NMeFOSA	18	63	92	80.6	18	73	102	83.9	18	47.6	115.5	87.3	138	32.8	164	87.9
NEtFOSA	18	63	91	75.5	18	69	97.5	81.7	18	45.5	114	86.5	136	23.5	168	85.6
NMeFOSAA	18	66	202	121.9	18	100	183	140.9	18	52	194.5	128.8	131	52	535	144.7
NEtFOSAA	18	69	211.5	126.7	18	90	174	125.9	18	58	237	126.6	140	58	400	140.1
NMeFOSE	17	49.8	87.5	73.4	18	70.6	113.1	85.5	18	46.1	106.9	77.8	134	34.2	126.9	81.5
NEtFOSE	17	53.4	77.5	67.6	18	68.8	110	81.9	17	46.8	106.2	76.9	131	21.9	135.6	80.6
PFMPA	18	23.5	84	50	18	68.2	92.2	81	18	20.2	90.3	69.4	142	15.2	116.8	81.3
PFMBA	18	48.8	92.2	72.3	18	91.5	100.5	95.4	18	45.2	123.5	92.1	142	45.2	163.2	104.3
NFDHA	18	55.8	155	119.5	18	37.5	81.2	65.4	18	45.8	91.5	78.5	143	25.8	155	91.6
HFPO-DA	18	70.8	102.9	85.1	18	89.4	101.9	93.6	18	46.4	97.1	90.2	143	46.4	228.8	104.5
ADONA	18	53.4	89	70.2	18	108	134.7	117.1	18	47.9	107.5	94.9	143	47.9	446.4	110.3
PFEESA	18	64.1	106.5	86.2	18	86.8	98.8	92.7	18	47.6	97.3	89.3	143	45.9	159.6	94.5
9Cl-PF3ONS	18	44	80.4	61.8	18	67.6	155.7	107.8	18	47.8	109.2	94.1	143	9.9	447.1	100.5
11Cl-PF3OUdS	18	28.7	72.6	49.7	18	38	132.2	74.5	18	37.8	94.4	69.1	141	3.7	313	72.2
3:3FTCA	18	53.5	95.5	76.7	18	71.8	97.5	87.3	18	52.6	157.5	105.1	142	40.4	157.5	89.5
5:3FTCA	18	68.4	130	95.9	18	76.8	121.7	98.1	18	54.2	165	119	143	54.2	302.5	100.6
7:3FTCA	18	75	121.7	90.4	18	84.2	125.8	101.9	18	46.8	136.7	100.3	143	24.6	301.7	99.3

Table E-4. Summary of wastewater spike percent recoveries in high spike samples for each laboratory.

Analyte	Lab 1 spike % recovery				Lab 2 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 5 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	18	92.8	106.8	100.6	18	100.8	131.4	109.9	18	22.5	114.7	104.3	13	101.8	112.7	106.8	17	82.1	94.5	88.5
PFPeA	18	81.5	113	96.4	18	97.5	137.9	112.4	18	22.2	119.2	103.1	18	93.3	105.4	98.5	18	82	97.6	90.6
PFHxA	18	83.4	110	94.3	18	89.9	118.2	101.1	18	32.2	120.2	100	18	88.3	110.3	97.8	18	62.3	115.4	89.9
PFHpA	18	81.8	102	92.1	18	95.5	117.6	104.5	18	21.1	105.9	93.4	18	85.6	105.2	95	18	75.5	105.5	92.1
PFOA	18	87.4	104	97.7	18	100	120.2	105.8	18	21.6	125.8	108.4	18	88.2	107.3	95.8	18	96.2	132.2	108.8
PFNA	18	80.1	97.9	89.3	18	99.8	121	106.7	18	19.5	141.2	106.7	18	90.2	101.3	96.2	18	70	104.7	87.8
PFDA	18	88.6	110	101.2	18	89.2	122	99.6	18	22.7	120.8	98.6	18	88	105	96.9	18	73.9	105	88
PFUnA	18	72.5	107	89.3	18	70.1	123	98.3	18	27	124	101.8	18	84.4	101	94.2	18	75.3	109	88.6
PFDoA	18	65.7	91.6	79.7	18	39.6	101	83.2	18	28.5	140	93.1	18	86.4	107	95.3	17	63.8	90.6	79.4
PFTrDA	18	62.1	101	82.3	18	17.3	95.8	61.6	18	26	144	85.3	18	84.4	105	93.5	17	57	87	74
PFTeDA	18	77	118	93.4	18	22.9	118	84.2	17	24.6	157	113.7	18	92.9	113	101.5	16	80.5	109	91.8
PFBS	18	86.5	98.4	91.8	18	97.8	120.6	105	18	21.8	120	104.4	18	93.8	111.2	101.6	18	79.8	91.3	85.3
PFPeS	18	87.3	108	96.8	18	96.9	123.8	104.4	18	22.4	122	98.9	18	91.8	110	98	18	84.8	116.3	94.1
PFHxS	18	81.6	95.9	89.5	18	82.5	126	101.4	18	23.4	112.5	98.3	18	91.4	108	100.7	18	99.7	110.9	105
PFHpS	18	85.2	103	93.8	18	95.6	136	109.6	18	22.3	156	104.9	18	88.7	111	100	18	79.7	143.3	93.9
PFOS	18	92.4	110.3	100	18	96	118	103.7	18	25.3	134	102.1	18	85.9	110.5	96.3	18	86.9	115.7	93.9
PFNS	18	70.8	103	86.9	18	65.6	95.7	79.8	18	19.4	110	91.4	18	80.9	106	94.5	18	54.8	90.1	80.1
PFDS	18	35	95.5	69.7	18	35.6	83.3	56.6	18	17.3	112	86.7	18	73.7	96.4	84.7	18	26.9	95	68.3
PFDoS	18	17	71.7	47	18	11.3	67.4	35.3	18	3	103	70.5	18	59.3	96.9	77	18	5.3	56.7	35.6
4:2FTS	18	91.7	110.4	102.7	18	92.9	120	103.5	18	34	108.7	96.1	18	80	117.1	92.2	18	91.2	101.7	96.6
6:2FTS	18	79.6	104.6	94.8	15	100.5	123.4	109.5	15	100.4	115.3	107.3	15	91.3	112.3	101.4	15	94.6	109.6	100
8:2FTS	18	91.7	113.8	101.3	18	97.1	124.6	107.8	18	47.9	147.5	121.4	18	82.1	111.7	98.7	18	92.9	111.4	104.4
PFOSA	18	91.2	110	99.3	18	73.2	130	100.5	18	25.8	124	104.7	18	92.5	130	105.2	14	81	101.3	88.4
NMeFOSA	17	75.4	99.1	84.3	18	37.6	101	79.6	18	39	212	99.5	18	59	122	91	16	82.1	99.7	93
NEtFOSA	17	75.6	94.8	86.5	18	23.4	93.6	75	18	38.7	193	99.3	18	67.3	124	95.9	16	80.3	97.3	91
NMeFOSAA	18	81	125	101.2	18	79.7	138	116	6	126	165.3	138.2	18	89.3	200.8	134.9	18	89	115.2	101
NEtFOSAA	18	72.6	112	94.9	18	64.9	139	110.4	18	30.6	182	117.2	18	96.8	206	124.2	17	94.2	119	103.9
NMeFOSE	18	76.2	97.5	86.1	18	37	96.2	76.1	18	59.2	134.8	89.5	18	62.5	104.5	88.1	16	76.5	114.2	93.8
NEtFOSE	17	70.8	93.5	82.7	18	25.8	96	72	18	60.5	136.5	94.2	18	62.3	109.5	90.1	16	77.2	107.2	89.8
PFMPA	18	59.5	99	85.6	18	81	111.5	98.8	18	20.9	104.5	90	18	17.5	106	77.7	18	37.4	118	82.2
PFMBA	18	86	115	97.6	18	97	135.5	108.8	18	24.5	138	116.7	18	97.5	141.5	110.4	18	111.5	175	132.1
NFDHA	18	73	104.5	88.9	18	54	122.5	91.4	18	19.6	123.5	93.7	18	52.5	114.5	81.5	18	88.5	114	101.1
HFPO-DA	18	94.2	110	101.7	18	109.2	198.3	135.9	18	35.6	108.7	95.8	18	89.6	120.4	103.9	18	93.8	116.3	103.8
ADONA	18	90.8	111.7	100.3	18	109.6	216.2	141.1	18	35.1	116.7	97.3	18	94.6	132.5	110.4	18	91.2	126.2	107.9
PFEESA	18	85.5	99.5	92.4	18	44.8	96	79	18	21.4	117.5	91.1	18	91	108.5	99	18	85.5	119	100.1
9Cl-PF3ONS	18	95.4	124.2	112.1	18	71.7	170.4	116.8	18	33.1	104.2	84.6	18	94.2	143.8	109.3	18	21.1	135.8	101.1
11Cl-PF3OUdS	18	37.2	105.8	77.1	18	26.2	101.2	60.7	18	7	106.2	73.1	18	74.6	127.1	97	18	5.7	115.4	75.7
3:3FTCA	18	51.7	93.8	79.2	18	62.3	105.5	86.1	18	20.6	116	97.8	18	60	131.5	103.1	18	35.2	173.2	94.5
5:3FTCA	18	75	89.5	81.4	18	63	133	100.7	18	6.3	110.5	86.1	18	90	115	101.7	18	61	117.5	94.6
7:3FTCA	18	82.5	95	89.4	18	71	195	107.3	18	6.6	153.5	107.2	18	73.5	105	93.3	18	22	120	84.6

Table E-4. Summary of wastewater spike percent recoveries in high spike samples for each laboratory.

Analyte	Lab 1 spike % recovery				Lab 2 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 5 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	18	92.8	106.8	100.6	18	100.8	131.4	109.9	18	22.5	114.7	104.3	13	101.8	112.7	106.8	17	82.1	94.5	88.5
PFPeA	18	81.5	113	96.4	18	97.5	137.9	112.4	18	22.2	119.2	103.1	18	93.3	105.4	98.5	18	82	97.6	90.6
PFHxA	18	83.4	110	94.3	18	89.9	118.2	101.1	18	32.2	120.2	100	18	88.3	110.3	97.8	18	62.3	115.4	89.9
PFHpA	18	81.8	102	92.1	18	95.5	117.6	104.5	18	21.1	105.9	93.4	18	85.6	105.2	95	18	75.5	105.5	92.1
PFOA	18	87.4	104	97.7	18	100	120.2	105.8	18	21.6	125.8	108.4	18	88.2	107.3	95.8	18	96.2	132.2	108.8
PFNA	18	80.1	97.9	89.3	18	99.8	121	106.7	18	19.5	141.2	106.7	18	90.2	101.3	96.2	18	70	104.7	87.8
PFDA	18	88.6	110	101.2	18	89.2	122	99.6	18	22.7	120.8	98.6	18	88	105	96.9	18	73.9	105	88
PFUnA	18	72.5	107	89.3	18	70.1	123	98.3	18	27	124	101.8	18	84.4	101	94.2	18	75.3	109	88.6
PFDoA	18	65.7	91.6	79.7	18	39.6	101	83.2	18	28.5	140	93.1	18	86.4	107	95.3	17	63.8	90.6	79.4
PFTrDA	18	62.1	101	82.3	18	17.3	95.8	61.6	18	26	144	85.3	18	84.4	105	93.5	17	57	87	74
PFTeDA	18	77	118	93.4	18	22.9	118	84.2	17	24.6	157	113.7	18	92.9	113	101.5	16	80.5	109	91.8
PFBS	18	86.5	98.4	91.8	18	97.8	120.6	105	18	21.8	120	104.4	18	93.8	111.2	101.6	18	79.8	91.3	85.3
PFPeS	18	87.3	108	96.8	18	96.9	123.8	104.4	18	22.4	122	98.9	18	91.8	110	98	18	84.8	116.3	94.1
PFHxS	18	81.6	95.9	89.5	18	82.5	126	101.4	18	23.4	112.5	98.3	18	91.4	108	100.7	18	99.7	110.9	105
PFHpS	18	85.2	103	93.8	18	95.6	136	109.6	18	22.3	156	104.9	18	88.7	111	100	18	79.7	143.3	93.9
PFOS	18	92.4	110.3	100	18	96	118	103.7	18	25.3	134	102.1	18	85.9	110.5	96.3	18	86.9	115.7	93.9
PFNS	18	70.8	103	86.9	18	65.6	95.7	79.8	18	19.4	110	91.4	18	80.9	106	94.5	18	54.8	90.1	80.1
PFDS	18	35	95.5	69.7	18	35.6	83.3	56.6	18	17.3	112	86.7	18	73.7	96.4	84.7	18	26.9	95	68.3
PFDoS	18	17	71.7	47	18	11.3	67.4	35.3	18	3	103	70.5	18	59.3	96.9	77	18	5.3	56.7	35.6
4:2FTS	18	91.7	110.4	102.7	18	92.9	120	103.5	18	34	108.7	96.1	18	80	117.1	92.2	18	91.2	101.7	96.6
6:2FTS	18	79.6	104.6	94.8	15	100.5	123.4	109.5	15	100.4	115.3	107.3	15	91.3	112.3	101.4	15	94.6	109.6	100
8:2FTS	18	91.7	113.8	101.3	18	97.1	124.6	107.8	18	47.9	147.5	121.4	18	82.1	111.7	98.7	18	92.9	111.4	104.4
PFOSA	18	91.2	110	99.3	18	73.2	130	100.5	18	25.8	124	104.7	18	92.5	130	105.2	14	81	101.3	88.4
NMeFOSA	17	75.4	99.1	84.3	18	37.6	101	79.6	18	39	212	99.5	18	59	122	91	16	82.1	99.7	93
NEtFOSA	17	75.6	94.8	86.5	18	23.4	93.6	75	18	38.7	193	99.3	18	67.3	124	95.9	16	80.3	97.3	91
NMeFOSAA	18	81	125	101.2	18	79.7	138	116	6	126	165.3	138.2	18	89.3	200.8	134.9	18	89	115.2	101
NEtFOSAA	18	72.6	112	94.9	18	64.9	139	110.4	18	30.6	182	117.2	18	96.8	206	124.2	17	94.2	119	103.9
NMeFOSE	18	76.2	97.5	86.1	18	37	96.2	76.1	18	59.2	134.8	89.5	18	62.5	104.5	88.1	16	76.5	114.2	93.8
NEtFOSE	17	70.8	93.5	82.7	18	25.8	96	72	18	60.5	136.5	94.2	18	62.3	109.5	90.1	16	77.2	107.2	89.8
PFMPA	18	59.5	99	85.6	18	81	111.5	98.8	18	20.9	104.5	90	18	17.5	106	77.7	18	37.4	118	82.2
PFMBA	18	86	115	97.6	18	97	135.5	108.8	18	24.5	138	116.7	18	97.5	141.5	110.4	18	111.5	175	132.1
NFDHA	18	73	104.5	88.9	18	54	122.5	91.4	18	19.6	123.5	93.7	18	52.5	114.5	81.5	18	88.5	114	101.1
HFPO-DA	18	94.2	110	101.7	18	109.2	198.3	135.9	18	35.6	108.7	95.8	18	89.6	120.4	103.9	18	93.8	116.3	103.8
ADONA	18	90.8	111.7	100.3	18	109.6	216.2	141.1	18	35.1	116.7	97.3	18	94.6	132.5	110.4	18	91.2	126.2	107.9
PFEESA	18	85.5	99.5	92.4	18	44.8	96	79	18	21.4	117.5	91.1	18	91	108.5	99	18	85.5	119	100.1
9Cl-PF3ONS	18	95.4	124.2	112.1	18	71.7	170.4	116.8	18	33.1	104.2	84.6	18	94.2	143.8	109.3	18	21.1	135.8	101.1
11Cl-PF3OUdS	18	37.2	105.8	77.1	18	26.2	101.2	60.7	18	7	106.2	73.1	18	74.6	127.1	97	18	5.7	115.4	75.7
3:3FTCA	18	51.7	93.8	79.2	18	62.3	105.5	86.1	18	20.6	116	97.8	18	60	131.5	103.1	18	35.2	173.2	94.5
5:3FTCA	18	75	89.5	81.4	18	63	133	100.7	18	6.3	110.5	86.1	18	90	115	101.7	18	61	117.5	94.6
7:3FTCA	18	82.5	95	89.4	18	71	195	107.3	18	6.6	153.5	107.2	18	73.5	105	93.3	18	22	120	84.6

Table E-5. Summary of wastewater EIS percent recovery for each laboratory.

Analyte	Lab 1 % recovery				Lab 2 % recovery				Lab 3 % recovery				Lab 4 % recovery				Lab 5 % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
$^{13}C_4$-PFBA	42	22.4	130	74.2	42	39.1	92.6	73.2	42	13	291	83.5	42	1	84.9	43.5	42	8.44	83.8	45.4
$^{13}C_5$-PFPeA	42	72	127	89.3	42	55.4	105	85.5	42	46	244	79.5	42	2.1	87.8	69	42	45.4	85.8	68.7
$^{13}C_5$-PFHxA	42	80.6	127	91.8	42	58.8	96.3	85.4	42	66	306	98.8	42	28.3	86.3	75.6	42	48	97.2	74.7
$^{13}C_4$-PFHpA	42	75.5	123	88.3	42	49.4	113	80.9	42	67	350	110.3	42	64.7	85.4	75.4	42	31.1	90.8	72.6
$^{13}C_8$-PFOA	42	77.7	135	93.4	43	55.9	100	82.2	42	68	268	95.4	42	66	86.1	77.2	42	25.9	105	77.6
$^{13}C_9$-PFNA	42	79.5	134	91.8	42	49.2	97.4	81.6	42	58	266	94.6	42	68.5	87.2	77.8	42	12.2	110	73.6
$^{13}C_6$-PFDA	42	74.2	134	91.1	42	43.9	94.6	77.9	42	47	302	90	42	60.1	84.7	72.7	42	2.68	92	67.6
$^{13}C_7$-PFUnA	42	43.7	101	79.5	42	29.3	82.6	65.7	42	26	278	81.9	42	38	82.6	71	42	0.617	96.8	60.7
$^{13}C_2$-PFDoA	42	26.2	97.5	67.3	42	12.3	75.3	52.8	42	15	282	90.8	42	10.6	77.2	63	42	0.563	85.6	53.6
$^{13}C_2$-PFTeDA	42	8.9	85	44.9	42	0.242	56.7	32.3	42	1	130	52.1	42	0.5	69.2	51.9	42	0.0848	57.5	27.8
$^{13}C_3$-PFBS	42	82	119	92.4	42	54.4	94.1	83.8	42	58	273	86.4	42	19	88.6	75.6	42	40.9	85	69.4
$^{13}C_3$-PFHxS	42	82.5	134	93.6	42	51	97.7	85.4	42	59	337	99.2	42	66.8	88.5	75.8	42	11.8	83.1	68.5
$^{13}C_8$-PFOS	42	70.1	120	84.2	42	34.3	89.1	77.1	42	48	288	94.2	42	61.1	82.7	73.1	42	0.251	83.8	56.9
$^{13}C_2$-4:2FTS	42	112	175	136.8	42	128	546	298.4	42	111	550	165.5	42	45.7	155	105.5	42	78.3	194	136.7
$^{13}C_2$-6:2FTS	42	65.7	187	136.4	42	68	426	199	42	58	328	103.8	42	69.8	125	91	42	18.6	132	95.5
$^{13}C_2$-8:2FTS	42	93.9	378	157.9	42	48.3	439	218.3	42	54	428	130.1	42	61.8	390	139.6	42	2.52	285	118.1
$^{13}C_8$-PFOSA	42	9.96	91.2	66.2	42	27.6	98.4	70.9	42	57	282	88.2	42	49.6	72.7	59.8	42	0.122	64	43.2
D_3-NMeFOSA	42	7.4	67.2	44.6	42	11.9	65.3	46.1	42	24	225	69.8	42	28.1	61.2	46.2	42	1.43	52.4	28.2
D_5-NEtFOSA	42	9.07	63.8	39.5	42	7.44	50.8	34.6	42	18	220	68.2	42	12.8	66.4	45.8	42	0.948	50.8	25.4
D_3-NMeFOSAA	42	54.7	92.5	72.3	42	40.4	140	99.9	42	24	599	143.3	42	46.6	95.6	72.8	42	1.07	122	59.1
D_5-NEtFOSAA	42	48.4	110	71.7	42	34.7	178	117.3	42	15	279	97	42	29.6	88.4	72.4	42	0.846	90.8	53.5
D_7-NMeFOSE	42	8.13	72.4	42.4	42	8.7	94	46.7	42	35	299	89.1	42	0.1	70.4	54.7	42	0.396	65.2	29.3
D_9-NEtFOSE	42	7.81	66	37.8	42	5.44	76.8	43.6	42	24	289	79.5	42	0.2	68.4	51.2	42	0.331	44	21.1
$^{13}C_3$-HFPO-DA	42	72.9	126	84.7	42	25.5	80.3	54.2	42	68	305	98.9	42	17.7	89.5	68	42	48.7	98	72.2

Table E-5. Summary of wastewater EIS percent recovery for each laboratory.

Analyte	Lab 6 % recovery				Lab 7 % recovery				Lab 9 % recovery				All Labs			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
$^{13}C_4$-PFBA	42	3.54	88.9	38	42	30	87	66.9	42	3	161	47.7	336	1	291	59.1
$^{13}C_5$-PFPeA	42	54	149	90.9	42	48	91	76.8	42	38	156	73.3	336	2.1	244	79.1
$^{13}C_5$-PFHxA	42	71.5	120	87.4	42	57	98	82	42	62	158	83.1	336	28.3	306	84.8
$^{13}C_4$-PFHpA	42	69.5	142	97.5	42	62	105	84.5	42	59	152	79.5	336	31.1	350	86.1
$^{13}C_8$-PFOA	42	64.5	115	86.8	42	62	107	84.3	42	63	157	84.4	337	25.9	268	85.1
$^{13}C_9$-PFNA	42	62.1	111	86	42	62	97	82.2	42	59	154	83.8	336	12.2	266	83.9
$^{13}C_6$-PFDA	42	66	132	88.6	42	59	90	78.6	42	52	148	80.7	336	2.68	302	80.9
$^{13}C_7$-PFUnA	42	57.1	119	81.1	42	48	78	64.3	42	31	126	75.9	336	0.617	278	72.5
$^{13}C_2$-PFDoA	42	40.4	134	79.7	42	44	71	57.1	42	11	109	63.1	336	0.563	282	65.9
$^{13}C_2$-PFTeDA	42	22.7	105	63.2	42	22	53	37.8	42	2	108	42.1	336	0.0848	130	44
$^{13}C_3$-PFBS	42	74.7	153	114	42	63	95	83	42	61	157	81.2	336	19	273	85.7
$^{13}C_3$-PFHxS	42	64.7	141	95.5	42	73	104	85.2	42	58	159	82.8	336	11.8	337	85.8
$^{13}C_8$-PFOS	42	64.6	108	82.9	42	57	89	77.1	42	49	150	79.9	336	0.251	288	78.2
$^{13}C_2$-4:2FTS	42	113	323	167.1	42	62	150	106.3	42	156	327	220.7	336	45.7	550	167.1
$^{13}C_2$-6:2FTS	42	68.7	282	145.2	42	56	135	91	42	92	226	148.6	336	18.6	426	126.3
$^{13}C_2$-8:2FTS	42	57.6	362	128.8	42	54	140	91.3	42	91	441	165.5	336	2.52	441	143.7
$^{13}C_8$-PFOSA	42	61	96	79.1	42	53	98	73.2	42	47	139	75.5	336	0.122	282	69.5
D_3-NMeFOSA	42	38.4	80.5	59.8	42	40	65	50.3	42	19	83	52.2	336	1.43	225	49.7
D_5-NEtFOSA	42	33	75.5	55.3	42	31	61	47.2	42	9	81	43.6	336	0.948	220	44.9
D_3-NMeFOSAA	42	65.8	116	91.4	42	65	143	95.9	42	46	140	81.9	336	1.07	599	89.6
D_5-NEtFOSAA	42	63	142	89.9	42	66	138	95.2	42	26	132	80.1	336	0.846	279	84.6
D_7-NMeFOSE	42	2.82	92	56	42	34	59	45.6	42	0.252	86	39.7	336	0.1	299	50.4
D_9-NEtFOSE	42	2.65	80	51.4	42	25	59	43.9	42	0.145	68	29.3	336	0.145	289	44.7
$^{13}C_3$-HFPO-DA	42	75.6	171	111.5	42	44	84	68.6	42	59	156	79.3	336	17.7	305	79.7

Appendix F

Surface Water Supporting Tables

Appendix F: List of Tables

Table F-1. Target Analytes Detected in Unspiked Surface Water Samples by Laboratory (ng/L)

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

Compounds undetected in all samples included: PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFHpS, PFNS, PFDS, PFDoS, 4:2FTS, 8:2FTS, NMeFOSA, NEtFOSA, NMeFOSAA, NMeFOSE,
NEtFOSE, PFMPA, PFMBA, NFDHA, HFPO-DA, ADONA, PFEESA, 9Cl-PF3ONS, 11Cl-PF3OUdS, 3:3FTCA, 5:3FTCA, 7:3FTCA

Table F-2. Minimum and Maximum Detected Values Reported by all Laboratories for Unspiked Samples

Analyte	Number of Labs	SWD1		SWF1		SWG1	
		Min	Max	Min	Max	Min	Max
PFBA	9	0.597 U	6.5 J	0.941 U	1.93 J	0.545 U	1.9 U
PFPeA	9	0.306 U	1.7 JI	0.306 U	0.726 J	0.289 U	1.08 U
PFHxA	9	0.298 U	2.02 J	0.298 U	0.8 J	0.298 U	0.509 J
PFHpA	9	0.173 U	1.66 J	0.173 U	0.415 J	0.158 U	1.06 U
PFOA	9	1 J	1.89 J	0.29 U	2.93 J	0.29 U	0.189 J
PFNA	9	0.25 U	1.28 J	0.166 U	1.06 U	0.166 U	1.06 U
PFBS	9	0.177 U	1.36 J	0.177 U	1.04 JI	0.104 U	1.08 U
PFPeS	9	0.116 U	1.1 U	0.129 U	0.502 JI	0.116 U	1.31 U
PFHxS	9	0.291 U	0.363 J	1.43 U	1.04 J	0.291 U	0.189 J
PFOS	9	0.415 U	1.43 JI	0.248 U	0.64 J	0.248 U	1.7 U
6:2FTS	9	0.945 U	2.39 J	0.945 U	7.92 U	0.945 U	7.92 U
PFOSA	9	0.188 U	11.1	0.188 U	12.3	0.188 U	13.6
NEtFOSAA	9	0.283 U	1.3 U	0.283 U	0.88 J	0.283 U	2.26 U

Table F-3. Summary of surface water spike percent recoveries in low spike samples for each laboratory.

Analyte	Lab 1 spike % recovery				Lab 2 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 5 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	9	18.8	101	85.9	6	90.3	98.8	94.5	9	75.8	103.3	94.3	6	91.2	102.5	97.0	9	78.7	91.2	86.6
PFPeA	9	93.2	100	97.4	9	89.2	98.2	93.6	9	92.8	104.5	100.0	9	96.8	108.2	103.1	9	81.4	101.2	90.0
PFHxA	9	86	373.8	123.4	9	92.9	103	98.9	9	87	101	95.1	9	93	108.2	98.1	9	74	99.5	91.1
PFHpA	9	65.3	97.5	88.8	9	101.2	113	107.1	9	94	99	96.7	9	87	103	93.3	9	77.3	97.5	90.0
PFOA	9	86	388	126.1	6	98.8	113	105.5	9	89.5	111	99.4	9	95.4	109	99.8	9	86.6	115.6	97.6
PFNA	9	4.1	106.5	83.2	9	93	106	100.7	9	87	112.1	97.4	9	91	105.3	98.4	9	77	108.5	92.6
PFDA	8	86	100.5	90.0	9	86	101	94.7	9	91.5	128.5	113.3	9	92.5	113	104.2	9	73.5	99.5	84.4
PFUnA	8	82	102.5	92.3	8	91	98.5	95.2	9	74	121	95.9	9	96	112.5	100.6	9	84	110	94.3
PFDoA	8	73.5	103	85.5	8	84.5	95.5	90.1	9	75.5	93	86.2	9	90.5	101	96.8	9	65.5	88.5	81.8
PFTrDA	8	85.5	105.5	92.6	8	63	83	74.2	9	68.5	101	82.2	9	86.5	94	91.4	9	49.6	95.5	82.7
PFTeDA	8	82.5	102.5	91.9	8	75.5	84	80.6	9	75	100	88.3	9	91.5	99.5	94.8	9	76	105	90.7
PFBS	9	81	166.3	95.1	9	91.6	105	97.3	9	82.3	100.2	90.8	9	94.3	108.5	101.6	9	75.9	89.5	82.8
PFPeS	9	84	147	96.4	9	93.1	135.6	100.5	9	88.1	107.9	97.9	9	98	112.4	102.5	9	81.2	91.6	85.9
PFHxS	9	86.1	1447.8	239.9	9	94.5	104	98.9	9	92	112.4	99.9	9	100.5	113.1	107.2	9	73.2	92.5	81.7
PFHpS	9	20.8	100.5	83.7	5	97.5	121.5	109.7	9	101	134.5	114.8	9	87.5	114.5	99.8	9	82	103	90.7
PFOS	9	86	1095.1	204.0	8	98	125.5	109.5	9	85	116.6	100.1	9	88.5	108.5	98.9	9	83.9	97	89.2
PFNS	8	77.7	94.6	87.1	8	57.4	99.5	83.2	9	76.7	100.5	91.3	9	80.7	98	89.0	9	70.3	85.6	76.8
PFDS	8	74.2	84.3	79.8	5	36.1	89.4	65.8	9	48.9	85.9	75.0	9	74.2	97	84.3	9	74.7	91.4	81.1
PFDoS	8	59.8	76.9	67.1	8	14.6	66.8	51.8	9	34.2	76.9	64.1	9	73.9	100	80.8	9	44	66.3	52.6
4:2FTS	8	74.4	99.2	89.1	9	77	93.5	87.1	9	87.1	108.2	98.3	9	80.9	108	93.5	9	91.8	99.4	94.8
6:2FTS	9	15.4	101	86.8	9	106.4	130	115.4	9	94.9	102.8	97.8	9	90.4	111.5	103.4	9	93.1	106.5	101.1
8:2FTS	8	92.5	104	98.0	9	85.9	120.8	105.3	9	97.6	121.1	110.8	9	101.1	117.2	110.6	9	95.6	105.4	102.8
PFOSA	8	87	103.5	96.0	9	91	106	98.8	9	85	148	113.3	9	93.5	117.5	103.5	9	83	98	89.4
NMeFOSA	8	79	91.5	84.6	8	85.5	102.5	95.7	9	75	103	89.1	9	74.5	101.5	91.8	9	90.5	99.5	96.3
NEtFOSA	8	77	97	86.1	8	85	101	89.7	9	71	101.5	86.8	9	78.5	104	88.7	9	86	95.5	92.1
NMeFOSAA	8	95	222.5	132.8	9	89.5	205.5	138.5	9	92.5	280.5	168.4	9	97	253.5	156.0	9	89.5	198	126.2
NEtFOSAA	8	85.5	289	142.8	8	91	242.5	153.7	9	87	321.5	169.8	9	93.5	302.5	170.6	9	92	283	153.3
NMeFOSE	8	74.4	91.2	84.5	8	73.1	88.1	82.3	9	62.1	88.8	77.8	9	75.6	97.5	86.7	9	76.2	112.5	95.7
NEtFOSE	8	57.8	88.1	78.2	5	82.5	87.5	84.5	9	50.9	91.9	72.7	9	65	92.5	80.0	9	70.6	110.6	91.6
PFMPA	8	48.5	98.8	77.5	9	21.2	59	40.7	9	78.5	92	87.3	9	10.2	79.8	51.7	9	68.5	114.5	92.3
PFMBA	8	90.5	106.5	99.1	9	95.7	112.5	103.3	9	83.5	100.7	93.4	9	98	130	108.1	9	106.8	124	115.5
NFDHA	8	83	98.8	91.5	9	70.8	102.8	86.0	9	84.5	101.8	90.9	9	85.2	125.7	105.8	9	93.5	123.8	109.2
HFPO-DA	8	86.2	101.8	95.3	9	101.8	122.8	110.8	9	90.8	114.1	102.1	9	79	116.1	93.4	9	90	116.6	104.2
ADONA	8	94.1	99	96.6	9	97.5	127.2	111.9	9	85.7	105.4	95.2	9	93.9	109.2	100.7	9	90.3	124.2	106.0
PFEESA	8	99	112.7	104.1	9	91.3	100.7	97.4	9	93.3	121.9	105.0	9	93.5	112	103.7	9	84	101	91.7
9Cl-PF3ONS	8	90	103.7	96.4	8	53.2	113	91.2	9	69.6	101.9	89.8	9	91	106.7	98.9	9	89.4	118.7	105.1
11Cl-PF3OUdS	8	78.6	86.5	82.8	8	18.8	83.2	61.7	9	29.4	80.9	63.4	9	74.1	93	84.8	9	81.2	110.8	98.4
3:3FTCA	8	38.9	86.8	66.0	9	29.1	72.5	53.3	9	74.8	95	88.3	9	59.2	102.1	86.6	9	46.8	83.4	70.2
5:3FTCA	8	74.5	94.2	82.6	9	87.5	108.3	99.6	9	85	96.7	92.0	9	85	106.7	97.4	9	85	101.7	94.0
7:3FTCA	8	66.1	90.8	79.4	9	68.8	102.5	92.0	9	73.5	98.3	84.0	9	64.2	101.7	87.3	9	66.6	91.7	80.9

Table F-3. Summary of surface water spike percent recoveries in low spike samples for each laboratory.

Analyte	Lab 6 spike % recovery				Lab 7 spike % recovery				Lab 9 spike % recovery				Lab 10 spike % recovery				All Labs			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	6	0.9	83	67.0	9	87.9	98.9	93.5	6	88.8	95.5	93.5	9	104.7	116.6	111.2	69	0.9	116.6	92.1
PFPeA	9	4.4	101.5	74.5	9	89.5	100.5	94.5	9	88.2	96.2	93.5	9	88.5	118	106.6	81	4.4	118	94.8
PFHxA	9	72	91.5	83.6	9	85.1	96.5	90.9	9	89.5	97.5	93.2	9	97.5	128	111.9	81	72	373.8	98.5
PFHpA	9	70.5	90	79.2	9	83	91	87.7	9	85.4	94	90.3	9	91.5	124	111.3	81	65.3	124	93.8
PFOA	9	75	84.5	78.9	9	88.3	99.5	92.7	9	83.8	90.6	86.9	9	99.6	142	117.1	78	75	388	100.3
PFNA	9	63	82.5	74.4	9	89.5	125	104.3	9	86.5	96.5	92.3	9	92	133	110.2	81	4.1	133	94.8
PFDA	9	75.5	100	83.9	9	81	163.5	114.7	9	88.5	97	92.5	9	98.5	124.5	111.2	80	73.5	163.5	98.9
PFUnA	9	68	87.5	76.2	9	86	151	110.8	9	81	92	88.6	9	90	125.5	110.6	79	68	151	96.1
PFDoA	9	69.5	91	77.8	9	47.5	123.5	88.2	9	71.5	86.5	83.2	9	81	121.5	100.6	79	47.5	123.5	87.8
PFTrDA	9	66	106.5	86.4	9	43.5	100	74.4	9	63.5	81.5	75.1	8	89	109.5	99.1	78	43.5	109.5	84.1
PFTeDA	9	65.5	79.5	71.7	9	59	93.5	74.7	9	68	87.5	80.8	8	80	118.5	93.4	78	59	118.5	85.1
PFBS	9	57.5	87	72.8	9	85.2	103	92.5	9	89.4	98.6	93.9	9	100.5	116.5	108.2	81	57.5	166.3	92.8
PFPeS	9	62.4	96.5	74.3	9	81.7	102	94.3	9	82.2	101	95.6	9	96.5	135.6	112.3	81	62.4	147	95.5
PFHxS	9	63.3	93.5	77.2	9	86.6	102	92.8	9	83	95.7	90.5	9	83	119.9	99.9	81	63.3	1447.8	109.8
PFHpS	9	60.5	96	79.2	9	105.5	239.5	146.3	9	88	103	98.3	9	98.5	231.5	123.6	77	20.8	239.5	104.9
PFOS	9	65	86	76.3	9	92.5	218	132.6	9	83.7	95.2	90.3	9	98	139	109.8	80	65	1095.1	112.4
PFNS	9	62.4	82.2	72.0	9	58.9	121.8	90.0	9	78.2	92.6	85.7	9	42.8	102.5	89.4	79	42.8	121.8	84.9
PFDS	9	59.1	75.8	65.9	9	33.3	78.8	55.8	9	65.2	82.3	75.7	9	10.9	89.4	73.3	76	10.9	97	73.3
PFDoS	9	31.2	63.8	53.6	9	31.7	55.3	41.6	9	51.8	78.4	61.2	8	58.3	82.9	68.6	78	14.6	100	60.1
4:2FTS	9	55.5	121.2	83.5	9	75.7	95.6	86.8	9	91.2	96.9	94.8	9	85.6	128	104.7	80	55.5	128	92.6
6:2FTS	9	69.2	133.8	98.1	9	79.6	106.4	91.8	9	97.2	103.2	99.7	9	73.6	146.2	117.3	81	15.4	146.2	101.3
8:2FTS	9	65.2	113.8	83.4	9	103.3	204.5	129.6	9	98.2	105.6	102.6	9	110.8	145.5	124.5	80	65.2	204.5	107.6
PFOSA	9	71.5	85	80.3	9	92.5	153	112.6	9	88	211	123.2	9	101.5	141.5	120.7	80	71.5	211	104.3
NMeFOSA	9	68.5	93	79.4	9	75	135.5	101.6	9	86.5	96.5	89.7	9	68	112.5	95.7	79	68	135.5	91.6
NEtFOSA	9	65	86	71.1	9	64	133	94.8	9	76	96.5	88.4	9	81.5	118	96.1	79	64	133	88.2
NMeFOSAA	9	88	191	122.2	9	86	375.5	186.5	9	83	211.5	135.1	9	152.5	665	368.6	80	83	665	170.9
NEtFOSAA	9	74	235.5	127.8	9	70.5	479.5	203.9	9	75	269.5	146.1	9	116.5	785	366.7	79	70.5	785	182.5
NMeFOSE	7	56	80.6	69.4	9	53.5	104.4	78.3	9	64.4	93.1	74.5	8	34.1	87.5	65.4	76	34.1	112.5	79.7
NEtFOSE	6	46.9	96.9	74.9	9	41.2	78.8	61.0	9	59	94.4	73.1	8	24.8	86.2	63.0	72	24.8	110.6	75.1
PFMPA	9	11.7	66	40.2	9	72.2	88.8	79.8	9	13.8	68	46.2	9	66.2	115	89.6	80	10.2	115	67.1
PFMBA	9	65.3	102	81.9	9	93	98.2	95.2	9	92	145.5	105.7	9	88.5	116.8	101.9	80	65.3	145.5	100.5
NFDHA	9	53.8	115	90.1	9	82	94.8	88.8	9	70.5	90	84.3	9	94.8	114.8	104.4	80	53.8	125.7	94.6
HFPO-DA	9	71.9	93.1	82.0	9	84.4	97.2	91.9	9	90.6	96.4	93.6	9	106.4	130	117.4	80	71.9	130	99.0
ADONA	9	65.7	80.3	73.9	9	95.5	112.3	103.2	9	92.3	118.8	99.0	9	103.6	135.9	117.3	80	65.7	135.9	100.5
PFEESA	9	70.3	92.3	81.6	9	86.3	94.3	89.4	9	88.5	96.3	91.3	9	99.3	122.4	108.0	80	70.3	122.4	96.8
9Cl-PF3ONS	9	68.6	82.8	74.8	9	57	87.3	73.1	9	78.8	124.5	91.6	9	24.9	130.8	100.8	79	24.9	130.8	91.2
11Cl-PF3OUdS	9	47.9	67.7	60.1	9	18.6	42.8	28.5	9	55.1	105.5	70.6	8	76.6	94.9	84.7	78	18.6	110.8	70.3
3:3FTCA	9	49.1	79.9	66.8	9	70.4	89.2	79.6	9	17.5	80.5	60.9	9	76.2	104.2	90.1	80	17.5	104.2	73.6
5:3FTCA	9	72.1	84.2	78.8	9	74.3	90	82.6	9	54.2	90.8	80.1	9	66.3	111.7	89.2	80	54.2	111.7	88.6
7:3FTCA	9	57	74.1	66.3	9	63.4	85	75.9	9	51.4	92.5	75.6	9	30.8	96.7	68.6	80	30.8	102.5	78.9

Table F-4. Summary of surface water spike percent recoveries in high spike samples for each laboratory.

Analyte	Lab 1 spike % recovery				Lab 2 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 5 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	6	92.8	99.8	95.9	5	87.3	104	93.4	9	81	101.1	94.6	6	100.2	106.2	103.0	9	86.2	93.9	89.4
PFPeA	9	92	104	97.8	9	90	96.2	93.2	9	90	102	98.3	9	91	96.5	93.8	9	88.9	105	96.8
PFHxA	9	86.6	96.1	91.4	9	96	103	98.5	9	81.2	103.2	93.7	9	92.1	109	99.9	9	75.2	110.4	88.5
PFHpA	9	85.3	103	92.4	9	107	116	111.3	9	89.4	97.3	94.5	9	97.4	106	101.2	9	86.8	111	96.8
PFOA	9	89.3	98.5	94.1	8	102.1	118	110.9	9	91.8	109.1	100.0	9	93.8	110	98.9	9	101.9	126.8	117.3
PFNA	9	85.8	100	92.7	8	100	112	104.9	9	79.6	120	93.5	9	89.3	102	98.1	9	79.1	107	93.6
PFDA	9	86	98.2	91.4	8	93.1	102	98.1	9	93.5	135	107.2	9	90.2	106	97.1	9	74.7	100	90.8
PFUnA	9	79.5	97.6	90.0	8	93.8	101	97.8	9	81	115	94.2	9	84.1	96.3	91.5	9	77	92	84.3
PFDoA	9	72.5	93.5	81.2	8	88.9	98.7	93.5	9	78.1	99.8	86.9	9	88.1	99.7	94.0	9	61.9	101	84.7
PFTrDA	9	75.9	92.5	83.6	8	70.5	90.2	79.8	9	71.4	94.2	84.8	9	85.1	93.6	89.1	9	73.4	93.8	83.0
PFTeDA	9	76	95.7	86.9	8	79.1	94.4	85.5	9	84.1	109	95.9	9	86.5	95.8	92.1	9	74.1	101	85.9
PFBS	9	82.8	94	87.4	9	94.2	102.8	98.0	9	84.1	96.8	88.8	9	94.7	109.1	103.1	9	78.8	85.5	81.8
PFPeS	9	86.3	102.6	94.3	8	97	105	100.4	9	91.5	102	96.0	9	91.1	98.8	95.9	9	85.5	92.1	88.7
PFHxS	9	88.6	101	93.8	8	96.1	107	99.9	9	92.8	101	97.0	9	94.6	102	98.5	9	85.5	89.4	87.6
PFHpS	9	87.7	102	96.2	8	93.1	115	104.5	9	101	146	113.6	9	95.3	122	106.5	9	84.5	95.8	90.7
PFOS	9	90.3	101	97.8	8	102	117	110.5	9	89.9	118	99.9	9	91.2	109	99.0	9	86.7	98.2	92.5
PFNS	9	85.5	97.2	90.5	8	75.4	101	90.0	9	89.8	105	92.9	9	88.6	102	92.6	9	74.2	85.2	80.2
PFDS	9	70.4	86.9	81.8	8	63.2	88	77.4	9	66.6	92.6	77.3	9	79.8	92	85.4	9	80.8	94.7	87.4
PFDoS	9	50	78.9	65.5	8	42	79.3	60.0	9	62.2	98.6	73.2	9	73.3	97.4	83.5	9	42.6	69.5	58.5
4:2FTS	9	82.5	95.8	88.3	9	80.4	100.4	89.1	9	85.8	107.5	93.1	9	89.2	110.8	98.6	9	89.6	100.4	94.7
6:2FTS	9	90.9	101.3	96.7	8	113.4	130.5	119.5	9	95.5	99.2	96.8	9	99.2	114.6	107.0	9	97	137.8	107.6
8:2FTS	9	88.8	108.3	96.4	8	107.1	123.3	115.7	9	95.8	135	111.1	9	97.5	119.6	103.9	9	100.8	109.6	104.5
PFOSA	9	93.2	99.4	96.9	8	94.6	104	98.1	9	91.8	150	118.6	9	93.9	107	100.2	9	87.5	96	90.7
NMeFOSA	9	73.9	91.4	82.1	8	82.1	102	92.7	9	72.4	118	91.7	9	81.5	103	92.0	9	87.9	103	95.7
NEtFOSA	9	75.9	93.6	84.7	8	83.2	96.3	89.4	9	77	104	89.9	9	74.8	104	90.4	9	85.7	102	92.5
NMeFOSAA	9	91.7	117	102.8	8	95.8	134	108.9	9	90.5	151	119.3	9	89.8	128	107.5	9	87.2	116	98.8
NEtFOSAA	9	80.9	126	96.1	8	89.4	153	109.8	9	95.2	135	110.0	9	92.5	146	114.8	9	95.1	150	112.2
NMeFOSE	9	68.5	92.5	83.5	8	75.8	89.5	84.8	9	71.5	87.5	79.2	9	84	93.5	89.4	9	76.5	109.5	97.6
NEtFOSE	9	66	93	81.2	8	74.2	90.2	84.9	9	65.5	93.2	81.7	9	78.2	92	86.1	9	88.5	114	102.2
PFMPA	9	31.2	95	72.7	9	19.9	65.5	39.8	9	80.5	91	87.4	9	13.2	74	46.9	9	74	115.5	95.3
PFMBA	9	90.5	112	100.1	9	93.5	122	107.3	9	81	96.5	89.8	9	95.5	130.5	104.9	9	113.5	142.5	125.6
NFDHA	9	90	98	92.7	9	87	103	95.9	9	68.5	99	84.2	9	85	121	105.6	9	85.5	126.5	97.7
HFPO-DA	9	87.9	101.2	95.9	9	89.6	117.5	105.5	9	83.3	105.4	99.2	9	82.5	105.4	94.5	9	87.1	112.5	106.2
ADONA	9	84.6	99.6	93.0	9	29.4	123.8	100.3	9	82.9	101.2	95.5	9	87.1	102.5	93.4	9	101.7	121.2	112.2
PFEESA	9	84	94.5	87.9	9	47.8	106.5	93.5	9	85.5	103	95.5	9	94.5	106	101.4	9	78.5	109	85.4
9Cl-PF3ONS	9	87.5	101.2	95.9	8	70	117.5	93.9	9	85	97.9	92.1	9	85	103.3	93.4	9	102.1	118.3	111.9
11Cl-PF3OUdS	9	67.1	87.9	78.1	8	49.2	90.4	70.0	9	52.5	87.9	67.5	9	73.3	92.9	82.2	9	97.5	114.6	107.1
3:3FTCA	9	23.4	86.8	63.3	9	40.2	79.5	53.9	9	77.5	103.2	92.8	9	52.2	96.2	81.5	9	51.7	88.2	72.9
5:3FTCA	9	70.5	92	83.7	9	73	118	101.5	9	81	96	89.9	9	80.5	103.5	93.7	9	84.5	118.5	93.3
7:3FTCA	9	62	103.5	83.5	9	6.8	115.5	90.4	9	79.5	112.5	98.8	9	65	101.5	87.4	9	80	131.5	95.4

Table F-4. Summary of surface water spike percent recoveries in high spike samples for each laboratory.

Analyte	Lab 6 spike % recovery				Lab 7 spike % recovery				Lab 9 spike % recovery				Lab 10 spike % recovery				All Labs			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	6	95.8	99.8	97.5	9	95	116	99.5	6	94	98	96.4	9	107.4	119.8	112.1	65	81	119.8	98.2
PFPeA	9	80	103.5	87.2	9	99.5	105.5	102.3	9	93.6	100	97.2	9	95.5	129	109.0	81	80	129	97.3
PFHxA	9	84.3	100	94.2	9	93.7	99.7	96.5	9	94	101.5	97.1	9	87.6	128	104.4	81	75.2	128	96.0
PFHpA	9	82.5	90	85.9	9	92	99	94.1	9	92.9	98.6	95.1	9	92.9	114	105.7	81	82.5	116	97.5
PFOA	9	79.8	103.7	92.7	9	93.3	105	100.3	9	90.1	95	92.4	9	100.7	118.9	107.6	80	79.8	126.8	101.5
PFNA	9	85.9	103	97.2	9	93.2	113	101.1	9	92.8	99.5	97.1	9	89	121	105.5	80	79.1	121	98.1
PFDA	9	86.7	112	96.9	9	98	144	112.5	9	96.4	102	98.6	9	106	124	113.7	80	74.7	144	100.7
PFUnA	9	88.3	110	99.1	9	75.9	141	102.8	9	88.3	97.6	93.0	9	95.1	127	111.2	80	75.9	141	96.0
PFDoA	9	67.4	102	84.5	9	46.6	125	83.7	9	76.9	94.7	87.8	9	82.4	115	102.3	80	46.6	125	88.7
PFTrDA	9	86	109	94.3	9	46.1	102	70.6	9	67.7	87.4	80.3	9	83.6	117	101.3	80	46.1	117	85.3
PFTeDA	9	68.8	113	91.8	9	70.3	113	92.1	9	74.7	92.6	85.3	9	71.5	121	95.0	80	68.8	121	90.1
PFBS	9	89.6	113	99.4	9	88.9	104.2	96.9	9	94.7	100.1	98.0	9	83.6	125.8	108.3	81	78.8	125.8	95.7
PFPeS	9	82.5	115	105.0	9	89.5	114	98.6	9	95.2	105	99.2	9	98.7	116	107.4	80	82.5	116	98.4
PFHxS	9	80	103	91.4	9	83.3	107.2	95.9	9	89.8	97.1	93.0	9	78.5	107	97.4	80	78.5	107.2	94.9
PFHpS	9	78.4	109	98.2	9	101	167	129.7	9	95.6	109	101.7	9	95	122	105.1	80	78.4	167	105.1
PFOS	9	80.3	112	97.8	9	96.8	146	114.8	9	90.9	98.5	94.9	9	96.9	114	105.1	80	80.3	146	101.3
PFNS	9	92.2	117	103.5	9	69.2	109	83.7	9	81.7	92.3	86.8	9	91.9	106	97.2	80	69.2	117	90.8
PFDS	9	77.3	104	91.5	9	39.6	63.5	52.3	9	66.2	85.4	76.0	9	78.9	93.4	84.7	80	39.6	104	79.3
PFDoS	9	65	86.7	76.1	9	32.4	56.7	42.4	9	46.1	66.4	58.2	9	60.6	86	72.8	80	32.4	98.6	65.7
4:2FTS	9	72.5	114.2	88.0	9	79.2	101.2	91.9	9	94.2	103.8	98.7	9	92.9	161.2	121.0	81	72.5	161.2	95.9
6:2FTS	9	71.3	168.4	109.0	9	87.1	103.4	98.3	9	100.9	104.2	102.4	9	78.8	135.9	110.5	80	71.3	168.4	105.1
8:2FTS	9	67.9	107.9	90.5	9	95.8	132.9	115.4	9	104.2	108.7	106.7	9	98.8	119.2	107.5	80	67.9	135	105.6
PFOSA	9	83.1	107	92.9	9	92.4	120	103.3	9	95.4	105.9	99.4	9	103	114	109.0	80	83.1	150	101.1
NMeFOSA	9	86	101	94.4	9	77.1	120	100.9	9	90.3	101	95.5	9	96.8	114	104.3	80	72.4	120	94.4
NEtFOSA	9	77.1	94.5	87.4	9	58.6	126	89.8	9	87.8	103	95.0	9	91.4	113	101.0	80	58.6	126	91.1
NMeFOSAA	9	91.1	144	113.2	9	85.1	149	117.4	9	96.1	127	108.8	9	104	272	175.8	80	85.1	272	117.0
NEtFOSAA	9	93.2	161	114.8	9	81.4	177	125.7	9	90.3	139	111.3	9	94	317	184.4	80	80.9	317	120.0
NMeFOSE	9	76.8	102.8	87.5	9	53.8	110.5	76.8	9	67	88	79.4	9	58	97	80.8	80	53.8	110.5	84.3
NEtFOSE	9	68.8	98.5	83.2	9	40.5	86.5	61.6	9	60.8	90.5	81.2	9	47.5	99	79.4	80	40.5	114	82.4
PFMPA	9	15.7	78.5	47.5	9	78	92	84.9	9	12	75	50.1	9	71	105	90.3	81	12	115.5	68.3
PFMBA	9	82.5	117	93.7	9	97.5	103	100.4	9	92.5	131	102.3	9	91	114.5	103.8	81	81	142.5	103.1
NFDHA	9	66.5	123.5	90.5	9	90	98.5	94.5	9	84.5	91.5	87.8	9	77	113	97.3	81	66.5	126.5	94.0
HFPO-DA	9	89.2	113.8	100.3	9	89.6	95	92.5	9	94.2	99.6	96.9	9	103.3	129.6	117.3	81	82.5	129.6	100.9
ADONA	9	62.1	87.1	75.7	9	95	110	103.7	9	102.5	105.8	104.4	9	101.7	141.2	116.5	81	29.4	141.2	99.4
PFEESA	9	76.5	94.5	85.4	9	91	97.5	94.6	9	90.5	100	94.9	9	90.5	116.5	104.2	81	47.8	116.5	93.7
9Cl-PF3ONS	9	67.5	91.2	75.5	9	65.8	98.8	80.6	9	77.9	92.5	86.7	9	108.3	130.8	117.9	80	65.8	130.8	94.2
11Cl-PF3OUdS	9	57.5	72.1	65.8	9	22.4	55	35.1	9	57.1	77.1	66.9	9	78.8	102.1	89.4	80	22.4	114.6	73.6
3:3FTCA	9	66.8	91	81.1	9	66.8	83.8	74.4	9	58.8	97.5	82.9	9	71.8	112.8	94.2	81	23.4	112.8	77.5
5:3FTCA	9	66.5	84	75.8	9	77.5	97.5	90.2	9	81.5	95	89.2	9	69.5	122.5	100.6	81	66.5	122.5	90.9
7:3FTCA	9	44	85.5	69.4	9	65.5	100	87.4	9	65.5	92.5	82.4	9	44.4	132.5	86.1	81	6.8	132.5	86.7

Table F-5. Summary of surface water EIS percent recovery for each laboratory.

Analyte	Lab 1 % recovery				Lab 2 % recovery				Lab 3 % recovery				Lab 4 % recovery				Lab 5 % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
$^{13}C_4$-PFBA	21	6.95	85	43	21	6.16	24.9	12.9	21	85	113	102	21	1.7	23.6	12.8	21	20.1	68.6	40.4
$^{13}C_5$-PFPeA	21	64	98.7	85.9	21	60.9	106	84.5	21	83	132	99.2	21	56	101	73.9	21	40.8	91.2	70.9
$^{13}C_5$-PFHxA	21	83.2	99.3	88.5	21	39.5	96.2	86.5	21	88	112	100.2	21	63.9	100	75.2	21	45.2	90.4	73.3
$^{13}C_4$-PFHpA	21	83.2	101	88.8	21	12.3	94	79.6	21	87	112	95.4	21	59.6	101	72	21	44	90	72
$^{13}C_8$-PFOA	21	75.4	94.5	87.2	21	2.37	93	74.2	21	86	124	99.6	21	66.6	103	76	21	49.2	99.6	83.1
$^{13}C_9$-PFNA	21	73.8	101	85.8	21	0.35	99.8	78.1	21	78	118	97.3	21	65.8	95.1	74.4	21	35.4	95.2	76.9
$^{13}C_6$-PFDA	21	70.4	91.9	80.5	21	0.35	100	71.9	21	65	103	85.3	21	61.5	88.3	70.1	21	39.4	94.4	78.5
$^{13}C_7$-PFUnA	21	65.6	90.8	79.3	21	0.01	91.7	63.1	21	50	97	78.5	21	59.1	87.2	69	21	35.4	90.4	76.8
$^{13}C_2$-PFDoA	21	57.2	82.9	70.2	21	0.02	87.2	56.9	21	44	91	72.4	21	54.5	82.4	63.9	21	32.7	87.2	73.3
$^{13}C_2$-PFTeDA	21	50	81.4	62	21	0.02	68.9	47	21	27	78	61.1	21	53	78.3	62.4	21	23.3	72.6	58.3
$^{13}C_3$-PFBS	21	78.3	105	90.6	21	17.7	97.2	83.7	21	94	115	104.9	21	65.8	99.9	76.6	21	39	79.4	66.5
$^{13}C_3$-PFHxS	21	79.6	96.4	87.6	21	0.75	95.2	78.1	21	83	117	100.6	21	63	101	74.6	21	39.7	82.7	74.6
$^{13}C_8$-PFOS	21	75.1	92.8	83.3	21	0.04	95.7	68.4	21	61	105	86.3	21	58.2	104	72.5	21	37.8	88.8	79.1
$^{13}C_2$-4:2FTS	21	86.9	120	102.2	21	48.4	187	133.7	21	104	165	131.1	21	59.7	112	76.2	21	45.4	168	122.1
$^{13}C_2$-6:2FTS	21	79.7	93.5	87.4	21	2.61	116	90.1	21	90	114	100.5	21	65.6	105	76.3	21	37.4	157	107.8
$^{13}C_2$-8:2FTS	21	73.2	98.2	86.5	21	0.11	178	96.8	21	70	127	94.5	21	54.7	103	72.8	21	33.3	149	106.9
$^{13}C_8$-PFOSA	21	73.8	91.1	81.9	21	0.35	111	74.6	21	59	100	78.6	21	44.9	87.5	63.5	21	36	80	63
D_3-NMeFOSA	21	59.2	77.9	69.2	21	0.11	85.6	60.1	21	54	82	69.4	21	24.1	59.8	40	21	26.3	66.4	48.8
D_5-NEtFOSA	21	52	73.5	63.9	21	0.03	74	52	21	48	85	67.5	21	20.5	59.4	37.3	21	23	64.4	45.6
D_3-NMeFOSAA	21	69.3	87.8	78.1	21	0.01	125	77.2	21	57	97	82.8	21	55.1	94.1	67.7	21	35.8	89.8	76.3
D_5-NEtFOSAA	21	65.5	84.4	77.2	21	0.04	108	68.4	21	44	94	74.6	21	49.4	96.1	64.2	21	33.6	85.8	74.1
D_7-NMeFOSE	21	57.2	81.3	67.2	21	0.01	69	46.7	21	50	87	70.4	21	34.5	81.1	50.3	21	26.4	68.8	52.9
D_9-NEtFOSE	21	56.4	76.3	64.6	21	0.01	67.2	43.6	21	48	92	69.1	21	31.9	95.8	51	21	17.8	60.8	44.2
$^{13}C_3$-HFPO-DA	21	84	103	90.8	21	42.6	97.7	84.4	21	85	127	99.7	21	65.9	109	77.8	21	39.3	80.7	67

Table F-5. Summary of surface water EIS percent recovery for each laboratory.

Analyte	Lab 6 % recovery				Lab 7 % recovery				Lab 9 % recovery				Lab 10 % recovery				All Labs			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
$^{13}C_4$-PFBA	21	2.49	28.6	13.1	21	46	88	60.2	14	14	27	21.2	21	31.7	76.1	54.4	182	1.7	113	40.7
$^{13}C_5$-PFPeA	21	51.2	91	74.2	21	79	100	90.3	21	19	79	65.3	21	74.8	114	89.6	189	19	132	81.5
$^{13}C_5$-PFHxA	21	68.5	104	80.4	21	90	106	97.3	21	58	81	74.7	21	79.3	99.2	87.5	189	39.5	112	84.8
$^{13}C_4$-PFHpA	21	70.5	92.5	80	21	91	108	98.2	21	65	76	71.1	21	72.6	103	86.8	189	12.3	112	82.7
$^{13}C_8$-PFOA	21	56.5	106	80.9	21	77	105	92.2	21	73	85	78.8	21	66.1	97.1	80.4	189	2.37	124	83.6
$^{13}C_9$-PFNA	21	63.9	86.4	75.4	21	62	97	83	21	70	80	74.3	21	64.8	97.9	83.4	189	0.35	118	80.9
$^{13}C_6$-PFDA	21	61.6	88.3	76.7	21	40	92	68.5	21	64	79	71.4	21	51	91.3	82.2	189	0.35	103	76.1
$^{13}C_7$-PFUnA	21	61.6	97.9	76	21	29	76	51	21	57	73	67.8	21	38.1	90.7	77.2	189	0.01	97.9	71
$^{13}C_2$-PFDoA	21	58.2	91.2	71.5	21	24	57	41.8	21	53	70	63.5	21	19.1	85.9	71.7	189	0.02	91.2	65
$^{13}C_2$-PFTeDA	21	36.1	80.7	61.6	21	22	52	36.4	21	48	71	58.3	21	2.2	71.3	61.8	189	0.02	81.4	56.5
$^{13}C_3$-PFBS	21	70.4	108	90.6	21	84	107	97.9	21	56	81	76.1	21	68.9	103	85.6	189	17.7	115	85.8
$^{13}C_3$-PFHxS	21	61.6	102	79	21	74	109	93.5	21	69	79	74.8	21	69.6	98.9	86.4	189	0.75	117	83.3
$^{13}C_8$-PFOS	21	62.5	96.9	76.8	21	33	94	68.2	21	67	80	72.9	21	30.6	94.7	84.4	189	0.04	105	76.9
$^{13}C_2$-4:2FTS	21	67.5	150	105.4	21	88	119	98.4	21	66	116	88.2	21	69.5	116	87.5	189	45.4	187	105
$^{13}C_2$-6:2FTS	21	46.3	99.7	74.9	21	87	115	100.3	21	67	85	76.4	21	63.7	122	88.8	189	2.61	157	89.2
$^{13}C_2$-8:2FTS	21	60.4	91.4	72	21	40	109	75.9	21	57	78	68.2	21	51.4	114	85	189	0.11	178	84.3
$^{13}C_8$-PFOSA	21	64	91.5	78	21	47	88	74.9	21	22	74	63.8	21	60.3	99.5	83.5	189	0.35	111	73.6
D_3-NMeFOSA	21	50.5	83	66.5	21	27	64	50.1	21	35	69	56.5	21	37.4	82.6	68.2	189	0.11	85.6	58.8
D_5-NEtFOSA	21	52.5	81.5	62.9	21	26	57	44.9	21	35	68	52	21	27	79.3	64.4	189	0.03	85	54.5
D_3-NMeFOSAA	21	63.2	91	75.9	21	42	79	64.8	21	57	70	63.8	21	40.1	85.8	76.2	189	0.01	125	73.6
D_5-NEtFOSAA	21	62.2	92.2	75.2	21	32	69	55.2	21	55	69	62.9	21	29	82.6	70	189	0.04	108	69.1
D_7-NMeFOSE	21	0.339	86	56.6	21	20	48	33.2	21	33	64	47.6	21	8.33	77	63.2	189	0.01	87	54.2
D_9-NEtFOSE	21	0.53	79	54.1	21	18	51	33.5	21	24	61	44.9	21	3.13	72.3	59.8	189	0.01	95.8	51.7
$^{13}C_3$-HFPO-DA	21	70.1	97.4	81.3	21	80	108	90.7	21	52	78	71.5	21	70.7	101	84.7	189	39.3	127	83.1

Appendix G

Groundwater Supporting Tables

Appendix G: List of Tables

Table G-1. Target Analytes Detected in Unspiked Groundwater Samples by Laboratory (ng/L)

Analyte	Number of Labs	Lab 1 Conc	Lab 1 Qual	Lab 2 Conc	Lab 2 Qual	Lab 3 Conc	Lab 3 Qual	Lab 4 Conc	Lab 4 Qual	Lab 5 Conc	Lab 5 Qual	Lab 6 Conc	Lab 6 Qual	Lab 7 Conc	Lab 7 Qual	Lab 10 Conc	Lab 10 Qual
GWA – GW #1, midwest																	
PFBA	8	10.3		12.6	J	10		6.29	J	9.26		12.1		9.7		11.4	
PFPeA	8	11.4		12.7		11.8		11.7		10.7		16.6		10		9.3	
PFHxA	8	22.9		24.1		20		22		25		24		19.8		22.6	
PFHpA	8	6.59		6.39		6.08		5.86		5.75		7.09		5.5		8.31	
PFOA	8	5.55		5.09		4.72		5.6		6.36		5.64		4.2		6.79	
PFNA	8	0.657	U	1.06	U	0.504	U	0.29	U	1.24	J	0.25	U	0.61	U	0.493	U
PFBS	8	41.9		48		39.3		47.9		36.6		43.1		45.6		58.7	
PFPeS	8	33.4		35.2		30.1		34.1		27.1		36.3		32.2		47.1	
PFHxS	8	139		139		123		138		98.9		126		126		131	
PFHpS	8	2.96		3.06	U	4.32		2.97		3.31		5.44		4.7		3.17	
PFOS	8	78.8		78.5		70.6		62.3		75.8		80		78.4		0.415	U
6:2FTS	8	1.07	U	7.92	U	2.16	U	1.6	U	53.3		1.48	U	3.5	U	2.36	U
NMeFOSA	8	0.453	U	0.822	J	0.696	U	1.21	U	0.341	U	0.199	U	0.64	U	0.35	U
GWB – GW #2, southwest																	
PFBA	8	--	X	17		15.1		14.2		13.7		16.2		13.3		16.6	
PFPeA	8	--	X	37.7		35.8		33.6		31.9		39.6		33.4		36.7	
PFHxA	8	--	X	95.5		73.4		87.2		85.8		93.8		85.6		108	
PFHpA	8	--	X	15.3		14.1		14.1		11.3		15.9		12.5		14	
PFOA	8	--	X	83		75.8		76.2		78.3		90.1		76.3		96	
PFNA	8	--	X	1.06	U	1.12	J	0.72	J	0.792	U	0.922	J	0.61	U	0.849	J
PFBS	8	--	X	37.4		31.3		36.6		27.6		29.5		34		34.5	
PFPeS	8	--	X	33.5		30.6		29.6		26.4		32.7		31.4		35.3	
PFHxS	8	--	X	369		322		324		244		316		341		308	
PFHpS	8	--	X	5.65		6.4		4.29		4.22		8.77		6.9		4.48	
PFOS	8	--	X	246		197		200		198		246		240		212	
6:2FTS	8	--	X	13.8	J	10.9		13.4		37.3		9.94		11		12	
GWC – GW #13																	
PFPeA	8	0.552	U	1.39	JI	0.856	U	0.533	JI	0.772	U	0.563	U	0.94	U	0.549	U
PFHxA	8	1.22	J	--	X	0.88	JI	1	J	0.944	JI	0.412	U	0.63	J	0.298	U
PFOA	8	1.15	J	--	X	0.696	U	0.651	U	0.547	J	0.29	U	0.46	U	1.03	J
PFBS	8	1.57	J	--	X	1.92		1.53	J	1.36	J	2.04		1.5	J	2.46	
PFPeS	8	0.361	J	--	X	0.56	J	0.422	J	0.729	U	0.542	J	1.1	U	0.72	JI
PFHxS	8	1.62	J	--	X	2.4		1.67	J	1.61	J	1.83		1.3	J	1.81	J
PFOS	8	0.441	U	--	X	0.728	U	1.7	U	0.631	U	0.548	J	0.54	U	0.415	U
6:2FTS	8	1.07	U	--	X	2.16	U	1.6	U	3.02	BJ+	1.48	U	3.5	U	2.36	U
PFOSA	8	0.346	U	--	X	0.432	U	1.58	JI	2.17		0.188	U	0.67	U	0.212	U
NEtFOSA	8	0.365	U	--	X	0.736	U	1.07	J	0.521	U	0.0998	U	0.62	U	0.273	U
NEtFOSAA	8	0.554	U	--	X	0.88	J	0.61	U	0.531	U	0.571	U	1.3	U	0.693	U

Compounds undetected in all samples included: PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFNS, PFDS, PFDoS, 4:2FTS, 8:2FTS, NMeFOSAA, NMeFOSE, NEtFOSE, PFMPA, PFMBA, NFDHA, HFPO-DA, ADONA, PFEESA, 9Cl-PF3ONS, 11Cl-PF3OUdS, 3:3FTCA, 5:3FTCA, 7:3FTCA.

Table G-2. Minimum and Maximum Detected Values Reported by all Laboratories for Unspiked Samples

Analyte	Number of Labs	GWA1		GWB1		GWC1	
		Min	Max	Min	Max	Min	Max
PFBA	8	6.29 J	12.6 J	13.3	17	0.597 U	1.9 U
PFPeA	8	9.3	16.6	31.9	39.6	0.549 U	1.39 JI
PFHxA	8	19.8	25	73.4	108	0.298 U	1.22 J
PFHpA	8	5.5	8.31	11.3	15.9	0.173 U	0.76 U
PFOA	8	4.2	6.79	75.8	96	0.29 U	1.15 J
PFNA	8	0.25 U	1.24 J	0.61 U	1.12 J	0.25 U	0.792 U
PFBS	8	36.6	58.7	27.6	37.4	1.36 J	2.46
PFPeS	8	27.1	47.1	26.4	35.3	0.729 U	0.72 JI
PFHxS	8	98.9	139	244	369	1.3 J	2.4
PFHpS	8	3.06 U	5.44	4.22	8.77	0.204 U	0.633 U
PFOS	8	0.415 U	80	197	246	0.415 U	0.631 J
6:2FTS	8	1.07 U	53.3	9.94	37.3	1.07 U	3.02 BJ+
NMeFOSA	8	0.199 U	0.822 J	0.199 U	1.21 U	0.199 U	1.21 U
PFOSA	8	0.188 U	0.724 U	0.188 U	0.724 U	0.188 U	2.17
NEtFOSA	8	0.0998 U	1.11 U	0.0998 U	1.11 U	0.0998 U	1.07 J
NEtFOSAA	8	0.531 U	2.26 U	0.531 U	2.26 U	0.531 U	0.88 J

Table G-3. Summary of groundwater spike percent recoveries in low spike samples for each laboratory.

Analyte	Lab 1 spike % recovery				Lab 2 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 5 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	6	97.1	100.8	99.5	7	100.8	107.5	104.2	9	93.1	99	95.6	9	96.5	105.4	101.9	9	83.7	94.2	88.2
PFPeA	6	97.3	107	101.4	9	105.3	114.2	109	9	79.5	102.7	95.1	9	95.2	114.5	104	9	86.5	112.8	97.6
PFHxA	3	90.9	101.4	97.7	3	106	116.5	111	6	70	98	86.8	3	91.5	109	100.2	3	82.8	98.8	92.1
PFHpA	6	83	104.5	92	9	101	114	104.6	9	86	97	92.4	9	79.5	104.5	91.4	9	68.2	105.7	90.2
PFOA	6	89.8	97.8	93.7	3	102	111	105.3	6	90.5	109.9	99.4	6	90	100	95.5	6	83.7	124.2	99.2
PFNA	6	87.5	98	94.5	7	99.5	111.5	105.6	9	83.9	109.5	92.2	9	93.5	107.9	99.4	9	74.8	95	86.7
PFDA	6	90.5	108.5	99.4	7	97	111.5	102	9	95.5	115.5	104.2	9	93.5	113	101.2	9	71	107.5	86.6
PFUnA	6	89	99	95.1	7	96.5	103.5	99.9	9	90.5	113.5	101.4	9	95.5	108.5	102.1	9	74	103.5	87.1
PFDoA	6	76	96.5	87	7	99.5	109.5	102.8	9	76.5	103.5	90.8	9	96.5	110	100.9	9	84	100.5	92.6
PFTrDA	6	90.5	111.5	98.1	7	48.4	97.5	83.1	9	70.5	101	85.3	9	90.5	102	94.8	9	78	99.5	88.6
PFTeDA	6	89	107	96.5	6	90.5	94.5	93.1	9	72	113	84.1	9	87.5	100	93.9	9	85	97	90.8
PFBS	3	94.7	99.7	97.9	3	104.5	122	113.8	3	85.4	95.4	88.9	3	99.8	112.4	105.9	3	77.7	86.7	82.7
PFPeS	3	90.8	100.7	94.9	2	103	111.4	107.2	3	89.8	99.7	96.2	3	96.9	107.3	101	3	83.2	90.1	86.6
PFHxS	3	88.5	96.4	93.8	2	106	107.5	106.8	3	86.6	97.5	92.2	3	100.6	116.6	107.3	3	74.6	89	79.7
PFHpS	6	92.5	104.2	96.9	7	99	146.5	123.7	9	101.4	117	108.8	9	85.2	110.5	97.6	9	83.5	119	95.6
PFOS	3	91	100.5	95.2	0	--	--	--	3	99	113	107.7	3	92.5	96	94	3	89.3	123.3	101
PFNS	6	80.7	93.1	86	5	51.5	104	87.4	9	89.6	97	93.2	9	82.7	98	89.5	9	73.3	100	82.9
PFDS	6	71.2	87.4	78.4	5	25.9	91.4	69.5	9	73.7	85.9	79.7	9	75.3	91.4	83.2	9	73.7	111.6	89.7
PFDoS	6	65.8	83.9	76.1	6	44.3	81.9	67	9	51.3	79.4	63.6	9	71.4	92	80.6	9	46	80.9	63.1
4:2FTS	6	84.1	100.5	93.3	9	92.8	106.5	99.9	9	88	99.2	94.5	9	91.7	104.6	98.2	9	92	104	96.7
6:2FTS	6	90.4	100.9	94.7	8	101.9	120.3	111	9	91.5	101.5	94.9	9	89.6	119.3	102.5	9	30	99.6	71.6
8:2FTS	6	98.9	104.6	102.2	5	97.2	117.6	108.7	9	95.2	135.5	111.8	9	97.4	111.2	105.4	9	102.3	115.9	107
PFOSA	6	97	103.5	99.9	5	104.5	113	107	9	95	131	105.3	9	98	115.1	104.4	9	79.6	98	87.8
NMeFOSA	6	87.5	104.5	95.2	5	85.4	97	91.8	9	82.5	98	88.2	9	87	101	96.1	9	91.5	103	97.9
NEtFOSA	6	88	92.5	89.8	5	87.5	104.5	95.7	9	81	94	87.3	9	79.1	98.5	88.6	9	90	101	95.2
NMeFOSAA	6	102	129	112.3	7	94	147	113.9	9	94	215	141.9	9	91	171.5	135	9	82.5	129.5	107.3
NEtFOSAA	6	79.5	160.5	124.3	5	96.5	111.5	105.5	9	89.5	210.1	135.2	9	86.5	210.5	146.3	9	89	180	126.5
NMeFOSE	6	87.5	103.1	94.7	4	92.5	97.5	94.9	9	76.2	88.8	81	9	84.4	98.1	90.6	9	86.9	110.6	98.4
NEtFOSE	6	83.8	89.4	87.1	4	89.4	100	94.4	9	71.2	86.2	76.9	9	78.8	88.1	83.8	9	73.1	116.9	94.3
PFMPA	6	96	105.2	101.2	9	34.8	108.5	67.3	9	85.5	95	89.9	9	78.5	106.5	94.1	9	95.5	130	111.8
PFMBA	6	100.7	106	104.5	9	101.2	122.2	113.1	9	82	98.2	90.1	9	97.2	105.2	101.6	9	106.5	138.8	119.9
NFDHA	6	94.5	109	102.8	9	81.8	110.2	97.7	9	75.5	118.2	100.5	9	92.2	117.5	106	9	99.5	128.2	109
HFPO-DA	6	95.9	102.8	98.9	9	112.4	135	119.9	9	88.9	102.6	95.6	9	82.6	109.4	92.6	9	87.4	112.4	104.7
ADONA	6	90.6	97.9	92.8	9	56.9	132.2	102.4	9	84.4	91.5	88.2	9	83.3	112.3	98.2	9	90.8	114.1	103.6
PFEESA	6	112	121.2	114.7	9	45.6	110.7	90.1	9	86.8	110.5	98.6	9	92.8	114.2	104.3	9	80.8	95.3	87.9
9Cl-PF3ONS	6	95.9	112.7	103.7	7	49.4	129.5	99.2	9	83.8	102.1	92.8	9	83.9	105.4	97.8	9	94.5	117.8	105.9
11Cl-PF3OUdS	6	70.8	92.5	81.6	7	9.5	95.3	68	9	59.7	79.4	68.7	9	71.1	93	84.6	9	91.3	118.8	105.3
3:3FTCA	6	74.9	79.3	77.1	9	46.8	97.9	73.2	9	84.2	95.2	89.6	9	92	102.9	98.9	9	73.6	85.5	81.7
5:3FTCA	6	80.2	85.8	82.7	9	78.7	104.2	93.7	9	86.7	95	89.8	9	90	107.5	101.1	9	84.2	99.2	92.8
7:3FTCA	6	81.8	91.7	85.9	8	21.1	101.7	85.4	9	79.7	90.8	83	9	85	100.8	94.7	9	72.3	100.8	83.8

Table G-3. Summary of groundwater spike percent recoveries in low spike samples for each laboratory.

Analyte	Lab 6 spike % recovery				Lab 7 spike % recovery				Lab 10 spike % recovery				All Labs			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	9	99.2	105.5	102.9	9	89.5	93	91.5	9	101.7	123.2	112.1	67	83.7	123.2	99.3
PFPeA	9	74.8	122.2	99.6	9	93.5	101.3	97.3	9	106.8	141.2	118.2	69	74.8	141.2	102.9
PFHxA	3	90.5	106.5	100.5	6	89.9	103	96.5	3	90.5	116	105.8	30	70	116.5	97.4
PFHpA	9	91.1	139	110.1	9	88.5	102.5	94	9	90	160.5	128.6	69	68.2	160.5	100.8
PFOA	6	99.5	118.8	108.2	6	91	119	100.3	6	102.8	144	121.2	45	83.7	144	102.7
PFNA	9	85.9	122.4	106.7	9	89.5	132	102.1	9	92.8	124	108.7	67	74.8	132	99.5
PFDA	9	91	113.5	104.3	9	88	166.5	113.9	9	98	119.5	106.8	67	71	166.5	102.4
PFUnA	9	89	121	99.4	9	82	143	109	9	93.5	126.5	115	67	74	143	101.4
PFDoA	9	81	113.5	103.5	9	54	102	87.3	9	85.5	120.5	106.8	67	54	120.5	96.7
PFTrDA	9	76	118	98.8	9	44	90.5	74.4	9	94	114	104.9	67	44	118	90.9
PFTeDA	9	73.5	115.5	95.6	9	39.5	114.5	70.6	9	91.5	111	103.6	66	39.5	115.5	90.7
PFBS	3	92.3	96.8	95	3	84.5	97	92	3	110.2	124.7	119	24	77.7	124.7	99.4
PFPeS	3	93.4	117.1	106.1	3	89.1	118.8	100	3	108.8	132.6	120.5	23	83.2	132.6	101.3
PFHxS	3	88.9	107.8	98.4	3	79.6	111.9	93.4	3	97.5	107.4	101.8	23	74.6	116.6	96.2
PFHpS	9	88.1	126.3	112.1	9	96	226.5	156.3	9	103.5	121.6	110.3	67	83.5	226.5	113
PFOS	3	92.8	95.8	94.6	3	94	189.5	127.5	6	94.5	575	318.9	24	89.3	575	157.2
PFNS	9	88.6	120.3	105	9	77.2	139.6	97.1	9	86.6	125.2	100.3	65	51.5	139.6	93.3
PFDS	9	73.7	114.6	98.3	9	48	100.5	69.7	9	81.8	110.6	97.3	65	25.9	114.6	84.3
PFDoS	9	76.4	103.5	91.1	9	23.1	90.5	55.6	9	84.4	107.5	95.4	66	23.1	107.5	74.3
4:2FTS	9	71.3	117.6	94	9	82.7	99	91.1	9	92.7	129.2	113	69	71.3	129.2	97.8
6:2FTS	9	88.9	137.6	114.6	9	86.6	111.9	95.3	9	115	178.8	133	68	30	178.8	102.4
8:2FTS	9	87	140.5	106	9	103.4	154.3	119.4	9	84.7	134.3	109.7	65	84.7	154.3	109.1
PFOSA	9	99	116	103.7	9	90	155.5	112.4	9	101	148.5	123.8	65	79.6	155.5	105.7
NMeFOSA	9	83.5	98	91.6	9	61	115	94.8	9	76	116	96.6	65	61	116	94.1
NEtFOSA	9	90	98.5	94.2	9	47	95	79.6	9	74	102	90.7	65	47	104.5	89.8
NMeFOSAA	9	96.5	149.5	124.9	9	102	237.5	140	9	102.5	570	328.3	67	82.5	570	153.3
NEtFOSAA	9	92	190	137.7	9	79	230	139.1	9	115.5	655	309.1	65	79	655	157.2
NMeFOSE	9	88.1	101.9	95.7	9	54.4	87.5	76.5	9	50.9	101.9	78.5	64	50.9	110.6	88
NEtFOSE	9	83.8	108.1	98.1	9	43.5	78.1	64.6	9	49	102.5	80.5	64	43.5	116.9	84.1
PFMPA	9	73.2	125.7	100.2	9	88.2	93.2	91.5	9	93.5	113.2	102.1	69	34.8	130	94.5
PFMBA	9	81.8	117.8	104.3	9	89.2	94.8	92.5	9	89	121.8	103.9	69	81.8	138.8	103.7
NFDHA	9	57.8	119	90.6	9	83.5	96.2	88.4	9	95.5	121.8	107.7	69	57.8	128.2	100.2
HFPO-DA	9	93.4	147.5	110.1	9	88.2	94.8	91.4	9	104.4	132.5	118.1	69	82.6	147.5	104.1
ADONA	9	65.7	118	97	9	100.6	107.1	102.8	9	96.1	143.4	119.5	69	56.9	143.4	100.9
PFEESA	9	83.5	109.5	101	9	89.3	94	91.2	9	85.3	122.4	107.5	69	45.6	122.4	98.7
9Cl-PF3ONS	9	89.7	113.6	100.4	9	56.4	92.3	74.8	9	100.4	142	116	67	49.4	142	98.6
11Cl-PF3OUdS	9	82.9	117.3	99.4	9	26.6	59.2	39	9	91	130.9	108	67	9.5	130.9	82.3
3:3FTCA	9	91.2	120.8	102.7	9	90	101	94.5	9	81	104	91.2	69	46.8	120.8	89.1
5:3FTCA	9	88.3	105.8	96.7	9	83.3	95	89.8	9	80.6	124.2	101.9	69	78.7	124.2	94
7:3FTCA	9	83.3	98.3	91.9	9	72.6	90.8	84.7	9	60.4	122.5	93.2	68	21.1	122.5	87.9

Table G-4. Summary of groundwater spike percent recoveries in high spike samples for each laboratory.

Analyte	Lab 1 spike % recovery				Lab 2 spike % recovery				Lab 3 spike % recovery				Lab 4 spike % recovery				Lab 5 spike % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	6	93.8	102.7	98.1	6	99.8	107	104.6	9	92.7	100.2	95.6	9	102.4	109.2	106.5	9	83.6	93.4	89.4
PFPeA	6	92	100.3	97.1	9	106.8	111.2	108.6	9	88.1	107	98	9	92.2	96.7	94.7	9	90.2	106	96.5
PFHxA	6	88.5	96.9	93	9	98.9	110.5	105.6	9	81	97.3	89.8	9	90.8	105	98.3	9	53.2	101.1	84.6
PFHpA	6	88	96.8	93	9	99.4	106.7	102.4	9	88.5	98.5	92.3	9	95.9	103	100.1	9	86.8	101	92.4
PFOA	6	87	94.4	90.9	6	93	117	102.8	9	76.2	107.2	91.2	9	89.8	102	96.1	9	95.4	144.7	120.8
PFNA	6	85.6	98.9	92	8	102	119	108.3	9	79.1	114	90.4	9	89.9	103	96	9	72.8	93	84.4
PFDA	6	80.8	105	91.4	8	105	112	108.5	9	86.8	137	104.4	9	93.1	107	98.5	9	64.7	109	87.5
PFUnA	6	83.2	98.7	87.5	8	103	120	108.1	9	91.8	123	100.6	9	84	99.8	93.1	9	74.1	109	89
PFDoA	6	78.1	91.9	85.5	8	97.8	109	103.2	9	78.4	102	89.7	9	92	105	97.1	9	78.7	103	88.5
PFTrDA	6	86	96.3	92.1	8	80.7	95.3	87.6	9	67.8	97.5	86.8	9	84.7	98	91.6	9	78.8	101	87.6
PFTeDA	6	82.9	105	94.9	8	91.9	97.3	94.9	9	68.6	113	86.9	9	89.6	94.6	92.2	9	79.6	99.5	91.1
PFBS	6	84.8	107.1	94.2	9	100	113.6	107.9	9	75.7	97.7	89	9	90.1	114.1	100	9	76.4	91.4	83
PFPeS	6	82.6	106.6	94.6	8	106	126.5	113.1	9	89.9	102.4	96.1	9	92.9	106.9	99.5	9	81.4	105.6	92.4
PFHxS	3	83.1	97.3	90.2	3	102	104	103.3	3	92.7	104.6	98	3	94.9	100.3	97.3	6	81	123.1	96.9
PFHpS	6	92.5	102	98.8	8	107	133.4	117.5	9	91	131	105.8	9	96.7	108	102	9	87	100.8	93
PFOS	6	95.3	111.2	104.2	5	30.5	106	75.6	6	85.4	112	96.6	6	89.7	116.7	100.9	6	78.2	106.2	91.9
PFNS	6	82.7	94.1	89.2	8	86.6	109	96.4	9	87.2	101	94.2	9	89.2	97.8	93.3	9	78.6	89.9	83.1
PFDS	6	75.1	90.1	83	8	74.6	92.6	83.3	9	75.4	88.1	81.9	9	81.2	92.4	87.9	9	80.9	97.8	90.4
PFDoS	6	68.2	81.5	74.8	8	61.6	77.8	72	9	57	82.4	70.2	9	77.1	88.6	83.6	9	54.5	69.9	63.9
4:2FTS	6	86.7	106.7	95.4	9	91.7	100.8	95.3	9	83.3	90.8	87.4	9	87.5	108.3	97	9	88.8	98.8	93.8
6:2FTS	6	88	99.2	95.2	9	89.2	121.3	111.7	9	85.1	102.5	92.5	9	99.6	116.3	107.5	9	73.2	105	91.2
8:2FTS	6	93.8	104.2	99.3	5	101.2	117.5	107.5	9	94.2	131.7	107.2	9	99.6	119.2	103.7	9	97.5	109.6	105.6
PFOSA	6	95.2	102	98.8	8	101	108	103.4	9	91.4	156	109.4	9	94	110.4	101.8	9	84.2	96	90.5
NMeFOSA	6	82.8	86.5	85.2	8	93.4	101	97.5	9	82	124	96.8	9	83.8	98.6	91.6	9	90.8	101	95
NEtFOSA	6	80.9	92.5	86.4	8	86.1	104	92.7	9	76.3	112	92.1	9	78.3	91.6	83.9	9	89.6	100	93.6
NMeFOSAA	6	88.6	108	97.5	8	95	115	105.6	9	94.9	154	113.7	9	92.3	106	100.3	9	82.9	102	93
NEtFOSAA	6	84.1	97.1	92.2	5	94.9	110	101.8	9	92	164.1	114.7	9	95.9	119	104.7	9	91.8	119	104.6
NMeFOSE	6	87.5	97.5	92.7	5	91.5	96.2	94.7	9	76.5	99.5	89.4	9	89.2	95.5	92.7	9	85.8	118.8	96.4
NEtFOSE	6	86.8	91.8	89.3	5	90.5	96.5	93.9	9	70.5	100.5	81.4	9	86.2	92.8	90.2	9	82.8	123.8	97.7
PFMPA	6	99	106	101.8	9	25.7	100.5	64.8	9	84.5	93	89.4	9	65	93.5	81.6	9	99.5	127	114.7
PFMBA	6	90.5	105.5	99.8	9	106.5	139	116.6	9	76.5	90	85.8	9	95.5	99.5	97.4	9	111.5	134.5	123.9
NFDHA	6	92.5	104.5	100.8	9	90	111	99	9	75.5	113	94.2	9	85.5	112	98	9	79	102	91.2
HFPO-DA	6	89.6	106.2	95.7	9	92.1	116.3	105.7	9	83.3	115	95.9	9	93.8	119.6	103.6	9	90.4	122.1	104.1
ADONA	6	84.6	95	89.3	9	52.5	113.3	98	9	81.7	100.4	88.8	9	92.5	106.7	97.3	9	90	130.4	111.3
PFEESA	6	82	99	90.2	9	61	106.5	96.6	9	85.5	113	93.1	9	98	105.5	102.6	9	71.5	93.5	84.9
9Cl-PF3ONS	6	91.3	104.6	97.8	8	91.7	107.9	99.6	9	80.4	97.1	87.6	9	93.3	110.4	98.9	9	95.8	130	111.1
11Cl-PF3OUdS	6	70.8	89.6	79.4	8	68.3	80.8	74.8	9	62.5	76.7	70.3	9	85	95.8	90.5	9	91.7	136.7	111.4
3:3FTCA	6	79	94.8	84.8	9	35	91.5	68.5	9	87.8	98.2	92.1	9	95	98.8	96.8	9	72.2	96.8	86.4
5:3FTCA	6	79.5	86	83.6	9	87.5	105.5	98.7	9	86.5	104	91.1	9	93	103.5	98.5	9	79	105	94.1
7:3FTCA	6	82.5	95.5	88.6	9	16.4	106	90.5	9	88.5	108	96.1	9	91.5	103	97.4	9	86	111	98.4

Table G-4. Summary of groundwater spike percent recoveries in high spike samples for each laboratory.

Analyte	Lab 6 spike % recovery				Lab 7 spike % recovery				Lab 10 spike % recovery				All Labs			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
PFBA	9	96.4	103.2	99.4	9	94.6	99.7	97.1	9	98.5	118.3	108.7	66	83.6	118.3	99.8
PFPeA	9	91.2	115.5	98.8	9	97.5	104.8	101.9	9	89.2	122	107	69	88.1	122	100.5
PFHxA	9	64.2	128.2	91.3	9	94.2	112.4	100.9	6	92.4	123.4	106.7	66	53.2	128.2	95.9
PFHpA	9	86.7	113.9	100.2	9	92.3	101.5	96.8	9	94	155	116.2	69	86.7	155	99.4
PFOA	9	76.9	118	99.7	9	96.7	117.7	104.6	9	98	121	111.2	66	76.2	144.7	102.6
PFNA	9	84.4	114	98.3	9	90.1	122	107.2	9	89.2	118	104.7	68	72.8	122	97.8
PFDA	9	87.4	109	98.2	9	99.7	144	117.3	9	97.3	123	107.8	68	64.7	144	102.1
PFUnA	9	88.4	115	98.5	9	96.5	127	110.6	9	90	130	104.5	68	74.1	130	99.4
PFDoA	8	88.1	109	99.8	9	69	103	90.1	9	91.6	108	100.8	67	69	109	94.5
PFTrDA	8	80.3	168	106.5	9	62.7	86	77.7	7	86.5	115	100.8	65	62.7	168	90.8
PFTeDA	8	74.8	107	91.5	9	69	92.7	78	7	84.5	109	95.3	65	68.6	113	90.2
PFBS	9	72.9	96.9	83.5	9	96.3	103	98.9	9	84.3	120.5	103.3	69	72.9	120.5	95
PFPeS	9	67.7	99.3	87.3	9	94.6	118	108.9	9	95.9	131.9	114.6	68	67.7	131.9	100.9
PFHxS	3	78.3	88	84.6	3	99.7	105.7	102	3	92.4	103.2	97	27	78.3	123.1	96.2
PFHpS	9	94	171.2	108.7	9	100.3	185	144	9	103.8	242	136.7	68	87	242	113.9
PFOS	6	82	107.5	97.3	6	91.6	172.6	136.6	6	105	223	157.5	47	30.5	223	108.2
PFNS	9	38.6	105	87.7	9	82.1	131	94.2	9	30.9	111	87.6	68	30.9	131	90.7
PFDS	9	3.9	96.1	79.7	9	57.6	123	74.5	9	8.5	106	77.9	68	3.9	123	82.3
PFDoS	8	48.8	94	75.5	9	43.2	101	60.8	8	2.5	95.7	78.2	66	2.5	101	72.1
4:2FTS	9	87.1	115.8	98	9	82.9	99.2	90.5	9	79.2	123.3	95.8	69	79.2	123.3	94.1
6:2FTS	9	71.7	140.1	107.2	9	83.8	108	96.9	9	78.8	147.1	111.7	69	71.7	147.1	102
8:2FTS	9	86.7	129.6	101	9	108.3	162.9	124.6	9	81.7	148.3	114.1	65	81.7	162.9	108.3
PFOSA	9	85.2	105	94.7	9	92.4	138	114.7	9	98.7	119	106.4	68	84.2	156	102.6
NMeFOSA	9	80.6	95.3	86	9	86.2	103	96	9	82.9	108	98.6	68	80.6	124	93.6
NEtFOSA	8	88.4	94.8	91.7	9	61.2	87.1	78.9	9	77.5	93.7	89.5	67	61.2	112	88.6
NMeFOSAA	8	88.9	128	103.6	9	106	135	114.9	9	106	183	136.1	67	82.9	183	108.7
NEtFOSAA	8	106	132	115.4	9	100	134	110.4	9	89.6	192	127.9	64	84.1	192	110.1
NMeFOSE	6	88.8	97	92	9	60	80.8	75.8	9	66	99.8	87.1	62	60	118.8	89.6
NEtFOSE	6	86.8	99.2	92.6	9	51.5	71.5	64	8	64.5	94	84.5	61	51.5	123.8	85.8
PFMPA	9	55.5	114.5	85.8	9	93.5	101.5	98.8	9	85	111.5	97.1	69	25.7	127	91.3
PFMBA	9	99	124.5	106.1	9	94.5	101	98.8	9	93.5	117	100.5	69	76.5	139	103.8
NFDHA	9	69	111.5	89.6	9	87	95.5	91.6	9	93	115.5	104.4	69	69	115.5	95.9
HFPO-DA	9	74.2	111.2	97.4	9	88.8	99.2	94	9	100	131.2	116.2	69	74.2	131.2	101.8
ADONA	9	63.7	100.4	84	9	97.5	107.5	102.1	9	106.7	144.2	121.7	69	52.5	144.2	99.5
PFEESA	9	82	116.5	93	9	89	100	95.1	9	80	108.5	93.3	69	61	116.5	93.7
9Cl-PF3ONS	9	32.2	106.7	82.4	9	57.9	85.4	71.1	9	6.4	137.5	90.2	68	6.4	137.5	92
11Cl-PF3OUdS	9	0.5	85.4	70.3	9	34.1	47.9	40.2	8	1.2	125	93.4	67	0.5	136.7	78.6
3:3FTCA	9	93	131	105.9	9	97.2	104.5	101	9	85.5	106	94.3	69	35	131	91.5
5:3FTCA	9	76.5	108.5	87.9	9	98.5	103.5	101.2	9	90.5	121.5	102.6	69	76.5	121.5	95.2
7:3FTCA	9	74.5	90.5	84.3	9	82.5	103	94.9	9	78	124	96.1	69	16.4	124	93.5

Table G-5. Summary of groundwater EIS percent recovery for each laboratory.

Analyte	Lab 1 % recovery				Lab 2 % recovery				Lab 3 % recovery				Lab 4 % recovery				Lab 5 % recovery			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
$^{13}C_4$-PFBA	21	57.2	95.7	86.1	21	4.73	62.4	24.4	21	93	108	99.3	21	18.8	79.3	40.1	21	58.3	79.5	71.6
$^{13}C_5$-PFPeA	21	78	97.8	88.8	21	34.1	92	79.5	21	86	116	99.2	21	71.7	84.6	78.1	21	62.4	88.2	72.7
$^{13}C_5$-PFHxA	21	80.1	102	88.9	21	4.55	95.1	82.5	21	85	111	97.8	21	64.7	80.7	74	21	66.8	83.6	75.7
$^{13}C_4$-PFHpA	21	75.3	97.1	84.3	21	0.832	94.9	77.5	21	86	107	95.2	21	65	83.4	71.9	21	63.2	92	75.1
$^{13}C_8$-PFOA	21	79.9	94.7	87.6	21	0.0857	95.9	73.7	21	91	112	100.7	21	69.1	88.4	75.7	21	66.8	97.6	81.3
$^{13}C_9$-PFNA	21	73.9	99.5	87.3	21	0.157	97.2	72.5	21	78	110	96.2	21	66.8	81.9	73.8	21	60.2	88	76.6
$^{13}C_6$-PFDA	21	78.7	102	90.3	20	0.133	94.8	73.6	21	62	116	86.3	21	57.2	79.5	70.5	21	72.7	99.2	84.4
$^{13}C_7$-PFUnA	21	72.9	101	84.2	20	0.0556	81.4	64.8	21	46	104	78.3	21	51.6	80.8	70	21	66.3	99.2	82.9
$^{13}C_2$-PFDoA	21	58.9	90	76.6	20	0.0292	75.6	56.4	21	51	88	70.7	21	52.3	74.1	64.2	21	59	94.4	75.9
$^{13}C_2$-PFTeDA	21	57.3	84.1	70.5	20	0.003	74.9	52.3	21	46	87	63.6	21	51.5	69.6	62.3	21	52	86.4	66.3
$^{13}C_3$-PFBS	21	78.5	89.2	84.2	21	1.47	96.9	79	21	94	112	103.3	21	63.9	87	76.1	21	59.2	79.4	69.5
$^{13}C_3$-PFHxS	21	81.1	91.5	85.5	24	0.0497	90.1	72.8	21	88	111	98.4	21	65.4	85.1	74.2	21	70	82.7	76.9
$^{13}C_8$-PFOS	21	75.1	95.2	84	21	0.00469	90.1	65.7	21	74	101	89.9	21	63.8	87.4	73.9	21	74.2	86.7	80.2
$^{13}C_2$-4:2FTS	21	81	112	98.4	21	5.22	149	111	21	95	136	116	21	62.5	82.3	72.6	21	81.7	158	119.2
$^{13}C_2$-6:2FTS	21	79	111	96.9	21	0.112	97.8	74.7	21	88	110	99.2	21	64.1	84.3	74	21	69.3	143	108.2
$^{13}C_2$-8:2FTS	21	78.4	107	92.2	21	0.0158	242	116.3	21	73	97	88.1	21	54.9	88.3	72.5	21	69	146	106.5
$^{13}C_8$-PFOSA	21	67.3	82.8	76.1	21	0.0465	110	71.3	21	56	93	82.5	21	56.2	70.5	63.3	21	57.2	83.6	67.3
D_3-NMeFOSA	21	51	74.1	61	21	0.143	83.1	59.6	21	44	77	65.5	21	30.6	41.3	35.5	21	38.8	60.4	49.8
D_5-NEtFOSA	21	45.8	71.2	57.2	21	0.0502	73.6	50.7	21	44	80	65	21	28.6	39	33.1	21	35.4	58	47.2
D_3-NMeFOSAA	21	68.2	90.9	77.8	21	0.0681	101	65.3	21	59	97	79.4	21	59.9	74.8	68	21	72.2	88.2	79.6
D_5-NEtFOSAA	21	62.2	94.7	77	21	0.0885	82.8	60.4	21	52	83	70.5	21	55.8	75.4	64.6	21	67	86.6	78.4
D_7-NMeFOSE	21	51.2	80	64.9	21	0.016	68.4	46.2	21	52	85	72.3	21	42.8	60.4	51.2	21	43.2	80.8	63.9
D_9-NEtFOSE	21	49.7	74.6	63.5	21	0.0149	75.8	47.2	21	56	87	73.6	21	38.1	71.6	50.9	21	35.6	67.6	53.6
$^{13}C_3$-HFPO-DA	21	84	106	94.1	21	5.91	96.9	77.9	21	91	119	98.6	21	67.6	90.7	77.9	21	55.8	84.8	69.8

Table G-5. Summary of groundwater EIS percent recovery for each laboratory.

Analyte	Lab 6 % recovery				Lab 7 % recovery				Lab 10 % recovery				All Labs Combined			
	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg	n	Min	Max	Avg
$^{13}C_4$-PFBA	21	11	39	27.1	21	91	101	97.3	21	54.3	92.5	80.9	168	4.73	108	65.9
$^{13}C_5$-PFPeA	21	53	78	65.7	21	89	98	92.7	21	73.8	110	92.6	168	34.1	116	83.7
$^{13}C_5$-PFHxA	21	58	85	71.6	21	89	100	94.5	21	78.4	105	89.1	168	4.55	111	84.3
$^{13}C_4$-PFHpA	21	61	80	67.7	21	87	100	94	21	68.2	110	86.2	168	0.832	110	81.5
$^{13}C_8$-PFOA	21	56	83	68.7	21	67	102	87.5	21	75.1	98.5	84.4	168	0.0857	112	82.4
$^{13}C_9$-PFNA	21	58	96	73.7	21	49	93	76.9	21	74.9	106	87.9	168	0.157	110	80.6
$^{13}C_6$-PFDA	21	37	78	68.7	21	33	98	64.9	21	51.6	103	85.5	167	0.133	116	78.1
$^{13}C_7$-PFUnA	21	10	83	67.2	21	29	77	51.1	21	26.1	102	81.6	167	0.0556	104	72.6
$^{13}C_2$-PFDoA	21	1	74	65.5	21	33	59	44.4	21	10.3	94.9	78.4	167	0.0292	94.9	66.6
$^{13}C_2$-PFTeDA	21	0.3	81	58.8	21	29	62	48.5	21	0.138	95.9	75.3	167	0.003	95.9	62.3
$^{13}C_3$-PFBS	21	66	120	87.3	21	89	105	96.5	21	68.7	117	85.2	168	1.47	120	85.1
$^{13}C_3$-PFHxS	21	69	108	81.2	21	68	102	88.7	21	59.4	112	88.2	171	0.0497	112	83.1
$^{13}C_8$-PFOS	21	37	80	70	21	32	96	61.8	21	14	98.3	85.3	168	0.00469	101	76.4
$^{13}C_2$-4:2FTS	21	69	116	82.4	21	85	112	97.5	21	71.3	113	91.3	168	5.22	158	98.6
$^{13}C_2$-6:2FTS	21	49	101	75.4	21	77	114	99.2	21	63.3	131	90.2	168	0.112	143	89.7
$^{13}C_2$-8:2FTS	21	36	114	84	21	40	98	70.7	21	50.5	134	92.4	168	0.0158	242	90.4
$^{13}C_8$-PFOSA	21	51	85	72.8	21	48	101	74.5	21	49.8	104	87.4	168	0.0465	110	74.4
D_3-NMeFOSA	21	11	70	57.1	21	32	66	43.2	21	28.6	90.7	70.5	168	0.143	90.7	55.3
D_5-NEtFOSA	21	2	67	52.3	21	30	60	43.3	21	18	90.6	68.6	168	0.0502	90.6	52.2
D_3-NMeFOSAA	21	9	77	64.2	21	33	82	57.5	21	30.6	103	84.5	168	0.0681	103	72
D_5-NEtFOSAA	21	5	83	63	21	36	70	51.9	21	18.7	116	80.5	168	0.0885	116	68.3
D_7-NMeFOSE	21	0.3	82	57	21	26	49	39.5	21	12.3	99	78.3	168	0.016	99	59.2
D_9-NEtFOSE	21	0.4	80	53.8	21	26	51	40.3	21	4.71	98.9	75.4	168	0.0149	98.9	57.3
$^{13}C_3$-HFPO-DA	21	60	95	72.9	21	82	96	88.9	21	66.5	120	87.6	168	5.91	120	83.5