

Quality Assurance Project Plan & Sampling Plan



Collection of Surface Water Samples for PFAS Analysis in Delaware Watersheds – 2026 - A WATAR Project

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ACRONYMS AND ABBREVIATIONS

CCB	Continuing Calibration Blank
CCV	Continuing Calibration Verification
DNREC	Delaware Department of Natural Resources and Environmental Control
DEL	DNREC Delaware Environmental Laboratory
DQI	Data Quality Indicators
DQO	Data Quality Objectives
DW	Division of Water
DWS	Division of Watershed Stewardship
DWHS	Division of Waste & Hazardous Substances
EDD	Electronic Data Deliverable
EPA	Environmental Protection Agency
EQulS	Environmental Quality Information System
FCA	Fish Consumption Advisory
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
HSCA	Delaware Hazardous Substance Cleanup Act
ICAL	Initial Calibration Curve
ICB	Initial Calibration Blank
ICV	Initial Calibration Verification
LCS	Laboratory Control Sample
LC-MS/MS	Liquid Chromatography with tandem mass spectrometry
LIMS	Laboratory Information Management System
LOQ	Limit of Quantitation
MDL	Method Detection Limit
MS/MSD	Matrix Spike/Matrix Spike Duplicate
NPDES	National Pollutant Discharge Elimination System
PARCC	Precision, Accuracy, Representativeness, Completeness and Comparability
PFAS	Per- and Polyfluoroalkyl Substances
PM	Project Manager
PTFE	Polytetrafluoroethylene
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RL	Reporting Limit
RPD	Relative Percent Difference
SOP	Standard Operating Procedure
SPE	Solid Phase Extraction
TMDL	Total Maximum Daily Load
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WATAR	Watershed Approach to Toxics Assessment and Restoration

1.0 PROJECT MANAGEMENT

1.1 DISTRIBUTION

Key project personnel are listed in Table 1 below.

The Project Manager (PM) will distribute this quality assurance project plan (QAPP) to the following Divisions within the Delaware Department of Natural Resources and Environmental Control: the Division of Watershed Stewardship (DNREC-DWS); the Division of Waste and Hazardous Substances (DNREC-DWHS); and the Division of Water (DNREC-DW).

Table 1. Key Project Personnel

Name & title	Phone number & email address	Mailing address
Delaware Department of Natural Resources and Environmental Control (DNREC)		
Division of Watershed Stewardship (DWS) & Division of Waste & Hazardous Substances –WATAR Program		
John Cargill, P.G. Project Manager, DWS - WAMS	302-395-2622 John.Cargill@delaware.gov	285 Beiser Blvd, Suite 102, Dover, DE
Todd Keyser, P.G. Co-Project Manager, DWHS - RS	302-395-2622 Todd.Keyser@delaware.gov	391 Lukens Drive, New Castle, DE
Amy Cannon Quality Assurance Officer, DWS - WAMS	302-739-9939 Amy.Cannon@delaware.gov	285 Beiser Blvd, Suite 102, Dover, DE

1.2 PROJECT PURPOSE AND ORGANIZATION

The purpose of this QAPP/Sampling Plan is to describe the quality system that will be used to collect and report ambient PFAS data for surface waters in Delaware watersheds (Delaware Bay Drainages and Chesapeake Bay Drainages).

This QAPP includes data quality objectives (DQOs) and quality control (QC) procedures to ensure that the data collected satisfies all applicable guidance requirements.

The organizational aspects of the program provide the framework for conducting the necessary tasks. The organizational structure and function also facilitate project performance and adherence to QC procedures and quality assurance (QA) requirements. Key project roles are filled by the personnel who are leading the various technical phases of the project and the personnel who are ultimately responsible for approving and accepting final products and deliverables. The responsibilities of these staff are described below. The project organization chart is presented in Figure 1.

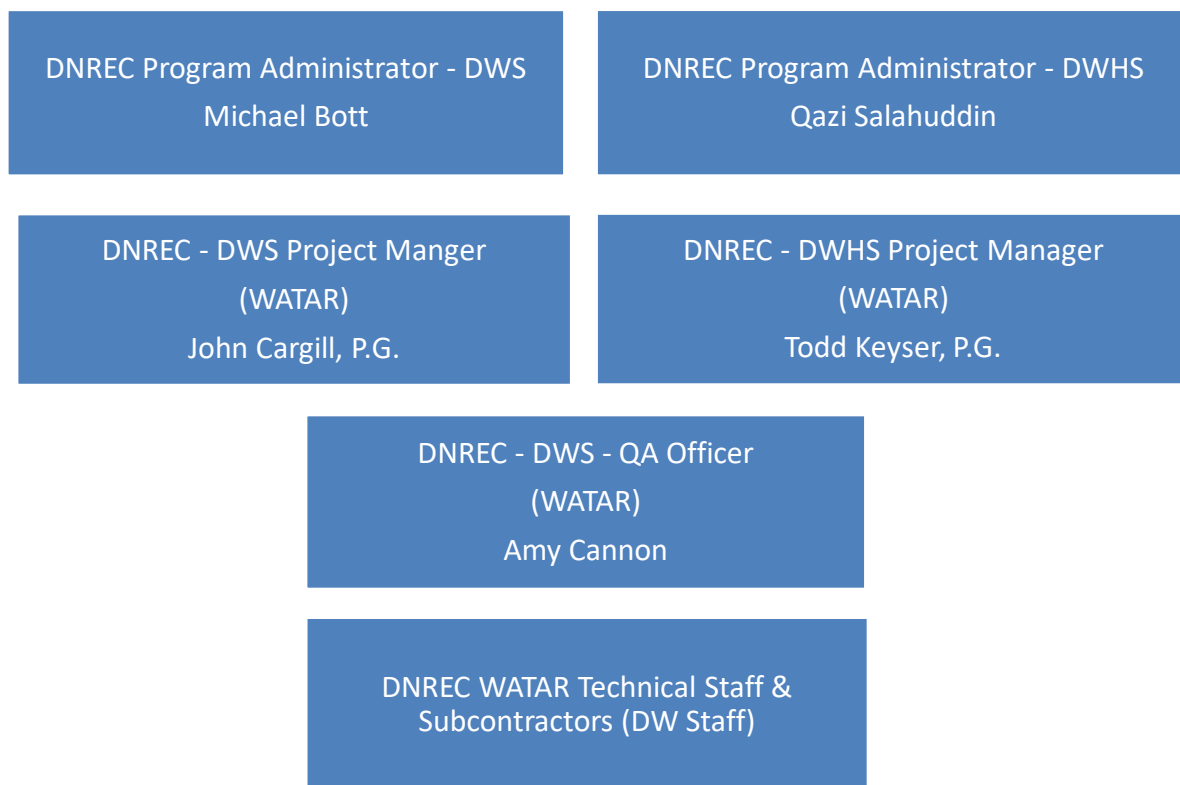


Figure 1. Project Organization

Mike Bott and Qazi Salahuddin are both DNREC Program Administrators for the project. Since the DNREC-WATAR program is a joint program between the Watershed Assessment & Management Section and the Remediation Section, both Section Administrators will provide overall administrative and oversight support to the primary DNREC PM, John Cargill, and the DNREC Co-PM, Todd Keyser, to ensure availability of resources and technical support to ensure that the project provides valid, reliable data for the programs' continuing assessment and evaluation.

John Cargill is the primary DNREC PM for the Division of Watershed Stewardship and he will provide overall project and program oversight for the project. He is coordinating the development of this plan for implementation in DNREC offices and will oversee plan implementation upon approval. Mr. Cargill will also coordinate with the DNREC Delaware Environmental Laboratory (DEL) for sample collection efforts. Either DNREC PM will also have the responsibilities listed below:

- Provide oversight for selection of analytical tools used to support data reporting
- Maintain the official approved QAPP for DNREC

-
- Coordinate with contractors, reviewers, and others to ensure technical quality and contract adherence

Amy Cannon (DWS) is the QA Officer for this project and will be the primary point of contact for DNREC in this task. Ms. Cannon will coordinate amongst DNREC WATAR and the DNREC DEL analytical laboratory, as needed.

Amy Cannon (DWS) and Mike Penzone (DWHS) will assist the PMs in all aspects of this QAPP.

1.3 PROJECT DURATION AND SCHEDULE

The work described in this QAPP covers a one-time synoptic sampling of surface waters for PFAS in the State of Delaware. Preliminary planning for this sampling event began in Spring 2026 and continued into the summer. The field sampling is scheduled to occur in Fall 2026. Data review and evaluation will be ongoing throughout the project with final interpretation scheduled to be completed in Spring/Summer 2027. The findings from the work will represent an update to a similar effort conducted in 2022 and will facilitate the creation of trends within watersheds as more data is collected over time. Finally, the data will allow for the refinement of priority lists of drainage areas that contain elevated PFAS and potential point sources/non-point sources of PFAS throughout the State.

1.4 PROJECT BACKGROUND, OVERVIEW, AND INTENDED USE OF DATA

The DNREC-WATAR team intends to measure concentrations of per- and polyfluoroalkyl substances (PFAS) in State of Delaware surface waters as part of ongoing attempts to understand the extent of PFAS impacts. The Department's intentions are to update information gathered in 2022 about PFAS distribution in Delaware surface waters, and to support the identification of potential contaminant sources (or source areas) within the state. This will be the second Surface Water PFAS Study in the State of Delaware. Therefore, the data will allow DNREC to begin monitoring PFAS trends in watersheds across the state. Tracking these chemicals' movements and eliminating their sources to the environment will have a positive impact on water quality and in maintaining healthy communities in Delaware.

The State of Delaware has a total of forty-five (45) watersheds, most of which originate in Delaware, and which drain either westward and southward into the Eastern Shore of Maryland and on into the Chesapeake Bay, or eastward into the Delaware River, Delaware Bay, or Inland Bays. A map which shows these 45 watersheds is shown below on Figure 2.

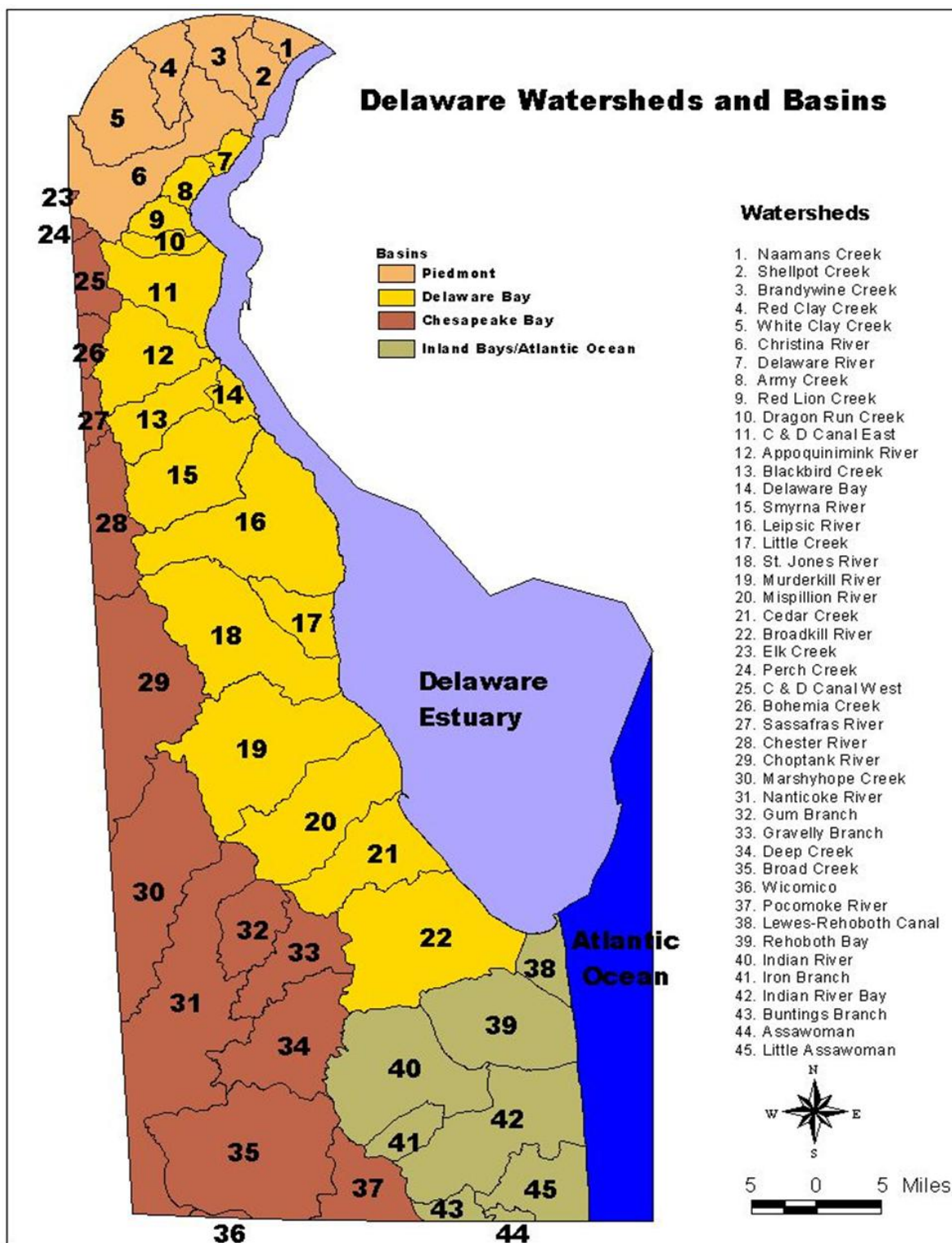


Figure 2. Delaware Watersheds

Surface water is withdrawn from a few rivers, streams and/or ponds in the state for purposes of public water supply. The remainder of domestic water supply in the state comes from groundwater. Irrigation wells for agriculture extract significant quantities of groundwater during the growing season in certain parts of the state, as well. Based on the generally sandy nature of the soils in the coastal plain of Delaware, shallow groundwater readily discharges to surface water, especially during seasonally high groundwater elevations in the winter and early spring when evapotranspiration is at a minimum. Baseflow in local streams and rivers is derived from shallow groundwater discharge. As a consequence, any contaminants that may be present in groundwater (including PFAS) can be released to surface water through groundwater-to-surface water discharge.

PFAS and other toxic contaminants can also enter surface water through overland flow (i.e., stormwater runoff) and through atmospheric deposition. Once in the surface water, contaminants can contribute to direct toxicity to fish and other aquatic life. Depending on several factors, some contaminants can also be taken up by local fish and other aquatic life in the process of bioaccumulation. This not only increases the body burden of the chemicals in the biota themselves, potentially causing impacts, but it also creates an exposure pathway to higher life forms like piscivorous birds (e.g., kingfishers, great blue heron, osprey, bald eagles), aquatic mammals (e.g., otters), and humans through the consumption of contaminated fish.

Finally, because the work being proposed geographically covers the entire state, and includes the majority of watersheds, we will be able to rank the relative degree of contamination across all sites. This in turn will help DNREC refine its priority list of watersheds, subwatersheds and sources that need additional investigation, clean-up and/or restoration.

1.5 QUALITY OBJECTIVES AND CRITERIA

This work involves the collection of new field samples and the analysis of those samples by DNREC's Delaware Environmental Laboratory (DEL). The quality indicators to be determined (precision, accuracy, representativeness, completeness, and comparability) will provide information regarding the data set and the anticipated effects of the matrices on analysis. Data outside the quality objectives and criteria will be flagged, so that data recipients can exercise discretion in determining appropriate data uses and limitations.

1.5.1 Precision

Precision is the measure of the degree to which two or more measurements agree. Precision is assessed through the collection and measurement of replicates (duplicates). Relative Percent Difference (RPD) shall be calculated for each of the replicates collected for all the parameters analyzed. Precision in the laboratory is assessed through the calculation of RPD for duplicate laboratory control samples (LCS), with one LCS being prepared at approximately twice the method limit of quantitation, called the LOQ, which is the lowest amount of PFAS that the laboratory can reliably measure and report, while the other LCS is prepared at approximately the mid-range of the calibration curve range, which shows how measured results relate to known concentrations, and the calibration curve ranges from the LOQ to the highest concentration measured. See the laboratory SOP (Appendix A) for more details. Note that replicates prepared in the laboratory from a common sampling container are considered true laboratory replicates. These types of replicates are to be distinguished from field replicates, which are samples collected

at the same time and place but into separate containers.

Regardless of the type of replicate involved, RPD is calculated using the following equation:

$$RPD = \frac{S - D}{0.5(S + D)} \times 100$$

Where:

- S = Amount in Spike 1 or concentration of parameter in original
 D = Amount in Spike 2 or concentration of parameter in replicate

RPD for laboratory replicates is a measure of laboratory precision while RPD for field replicates is a measure of potential variation associated with the ambient environment.

1.5.2 Accuracy

Accuracy is the degree of agreement between an observed value and an accepted reference value. Analytical accuracy is established through regular calibration of the LC-MS/MS instrument, including regular analysis of calibration blanks (ICB and CCB). Calibration blanks assess any background contamination within the instrument. An acceptable initial calibration curve (ICAL) must be established and verified (ICV) prior to analysis of any analytical samples. The acceptability of the initial calibration must be continuously verified (CCV) during each analytical batch. Detailed descriptions of calibration standard preparation and instrument calibration procedures are found in the laboratory SOP (Appendix A).

Accuracy in the field is assessed through the use of rinsate (field) and field blanks and through the adherence to all sample handling, preservation and holding times found in the laboratory's PFAS Aqueous Sample collection SOP (Appendix A). It is important to recognize that the methods being used under this QAPP are very sensitive and will often detect target analytes in rinsate blanks and field blanks. Here we are interested in distinguishing between extremely low-level, unavoidable background contamination from native contamination in the field samples. Making this distinction becomes increasingly more difficult the "cleaner" the sampling site becomes. A careful comparison of results for native (i.e., field) samples, rinsate blanks, field blanks, and laboratory method blanks often point to the true source of contamination and hence helps to establish the accuracy of the field data (USEPA 2009). Rinsate blanks (also commonly referred to as equipment blanks) will be prepared at a frequency of approximately one (1) per twenty samples and are produced by running high purity water over/through sampling equipment after the equipment has been subjected to decontamination procedures but prior to field sampling. A field blank, also produced at a frequency of approximately one (1) per twenty samples, includes a container that is filled with high purity water in the field at the same time that a native sample is collected. A laboratory method blank, which will be prepared at a frequency of 1 per analytical batch per matrix, is a sample of high purity water that is prepared/extracted/analyzed at the laboratory at the same time that the field samples are processed. An analytical batch consists of no more than 20 field-collected samples (not including field blanks, duplicates or laboratory-prepared QC samples) prepared for analysis at the same time using the same procedures, reagents and laboratory equipment.

A rinsate blank can be contaminated in the field by “dirty” gear or by trace contaminants in the high purity water. A field blank can be contaminated in the field by the atmosphere, other activities at the sampling site or by trace contaminants in the high purity water. A method blank can be contaminated during sample processing at the laboratory or by trace contaminants in the high purity water. Unequivocal determination of sources of unintended contamination and their significance is only possible when the same source of high purity water is used in the various blank samples, as will be done in the current work.

The other metric that helps to establish the accuracy of the field data is adherence to all sample handling, preservation and holding times. Laboratory accuracy and matrix interferences are assessed through the analysis of matrix and/or laboratory control spikes and the determination of percent recoveries. Please note that the laboratory method being used as part of this QAPP is a so-called “isotope dilution” method. This involves spiking native samples with isotopically labeled compounds (PFAS compounds labeled with carbon-13, oxygen-18, or hydrogen-2 isotopes) prior to preparing samples for analysis. The recoveries of these isotopically labeled compounds are taken into account when determining the concentrations of native analytes measured in the samples. In consequence, both matrix effects and extraction efficiencies are accounted for when using the isotope dilution method, and preparation of separate matrix spikes are unnecessary.

1.5.3 Representativeness

Representativeness expresses the degree to which data accurately and precisely represents a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition. Representativeness is dependent upon the proper design of the sampling program and will be satisfied by ensuring that the sampling and analysis plan is followed and that proper sampling techniques are used. Representativeness in the laboratory is ensured by using the proper analytical procedure, meeting sample holding times and analyzing and assessing field samples.

1.5.4 Completeness

Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount of data that was expected to be obtained under normal conditions. Field completeness is a measure of the number of valid measurements obtained from all the measurements taken in the field. Samples broken after collection may be re-sampled by the DNREC DEL. Laboratory completeness is a measure of the number of valid measurements obtained from all the measurements taken in the project.

Completeness is the ratio of the number of sample results to the total number of samples analyzed with a specific matrix and/or analysis. Following completion of the analytical testing, the percent completeness will be calculated by the following equation:

$$Completeness = \frac{V}{P} \times 100$$

V = Number of valid measurements; and
 P = Number of planned measurements.

Our numeric objective for completeness is greater than or equal to 95%.

1.5.5 Comparability

Comparability is an expression of the confidence with which one data set can be compared with another. Comparability is dependent upon the proper design of the sampling program and will be satisfied by ensuring that the field sampling plan is followed and that proper sampling techniques are used. Planned analytical data will be comparable when similar sampling and analytical methods are used and documented in the QAPP. Comparability is also dependent on similar QA objectives.

1.6 SPECIAL TRAINING REQUIREMENTS/CERTIFICATION

Sample collection must be performed by personnel who are trained in the collection of water samples intended for trace chemical analysis. DNREC's DEL, who will be conducting the sampling, has assembled a team of experienced staff to perform the work detailed in this QAPP.

Field sampling involves inherent hazards associated with the physical environment and the use of gear and equipment. The sampling team will have a designated safety officer who will provide safety briefings to the sampling crew to identify and raise awareness of hazards. The safety officer will outline procedures to follow in the event a safety issue arises in the field.

DNREC-DEL has the capabilities to perform the specialized laboratory analyses required under this QAPP. The DNREC DEL laboratory holds all appropriate approvals/certifications and are qualified to perform the work required.

The ubiquitous presence of PFAS in the environment, sampling equipment, and clothing materials, combined with low-level analytical reporting limits (parts per trillion – ppt), creates a high potential for sample contamination during collection. Therefore, sample collection for PFAS analysis will be conducted by DNREC DEL staff specifically trained to handle these types of samples and to minimize cross-contamination. In addition, certain sampling equipment, clothing, and personal care product materials (e.g. PTFE-containing or water-resistant coatings) will be restricted or banned from use during sample collection. No other specific training or certification is required.

1.7 DOCUMENTATION AND RECORDS

The DNREC PM will be responsible for maintaining all documents and records associated with this project.

Revisions to this QAPP which are made after publication will be provided to the individuals listed in Table 1 by electronic mail. If revisions are made after publication, the Revision No. at the top of the page will be changed to reflect the current Revision number. Revisions to this QAPP will be provided to all the recipients listed in Table 1, after each revision, by electronic mail.

1.7.1 Standard Data Reporting Format

Electronic data deliverables (EDDs) shall be required of all laboratories that generate data in conjunction with this QAPP. The EDD format will be specified by DNREC. Since 2012, specialty toxics data generated through DNREC-WATAR for surface water, sediment and biota has been uploaded to an EQulS database.

Laboratory Reports shall be structured to clearly present all of the required items. These reports shall be organized as follows:

Description of sample types received, tests performed, any problems encountered, and general comments shall be noted. The work order number and project shall be clearly identifiable. A table shall be presented that clearly shows all samples received that include the field sample identification, the laboratory sample identification, the sample matrix, and the tests assigned.

Data shall be reported by sample and/or by test. Pertinent information shall include, at a minimum, field sample identification, laboratory sample number, date the sample was collected, date the sample was received at the laboratory, date the sample was extracted/prepared, date the sample was analyzed, extraction/preparation/cleanup/analysis procedure(s) used, laboratory preparation batch number(s), dilution factors, all analytes tested for and their associated reporting limits (RL), matrix, units, and sample description including preservation. Any other factors that could affect the sample results shall also be noted.

All of the associated method QC information shall be reported. This information shall include method blanks, laboratory control samples, labeled isotope spikes, and other method-specific QC that is required by the analytical method. Spiking levels shall be clearly shown. This QC information must be specific to the batch that the field sample analysis was associated with. Batch numbers shall be clearly shown. Method-specific QC information must be reported with all acceptance criteria clearly shown. All method QC must meet the acceptance requirements as stated in the specified analytical methods. Any deviations from the acceptance criteria must be identified with appropriate interpretations or response actions that have been taken.

Internal standard areas shall be provided along with isotope dilution analyte (also referred to as “extracted internal standard” or “surrogate”) areas and recoveries.

This and any other information that is pertinent to the samples shall also be retained by the laboratory for a minimum of 10 years. Copies will be provided by the laboratory when requested. This may include copies of email communication, laboratory logbooks, corrective action records, instrument tune files, certificates of analysis, instrument maintenance records and/or copies of any other forms that may be necessary to reconstruct the analytical process. Copies of the original chain of custody forms will be included in analytical reports. The laboratory shall maintain on file all of the supporting data and documentation for these samples. The laboratory shall provide, upon request, copies of raw data as the WATAR Program Team deems necessary for specific methods and samples.

Sample method and associated Quality Control information shall be reported in a standard format

as a complete packet representing a batch of samples. The method quality control information should be presented in a standard order following the sample data results.

2.0 DATA GENERATION AND ACQUISITION

The elements in this section address all aspects of data generation and acquisition to ensure that appropriate methods for sampling, measurement, analysis, data collection or generation, data handling, and QC activities are employed and documented.

2.1 SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)

The surface water sampling effort will cover the entire State of Delaware, in both tidal and non-tidal portions of select watersheds. In an attempt to isolate potential PFAS sources to geographic drainage areas, surface water sampling sites were chosen at accessible locations at or just above a waterbody's head of tide. In order to characterize tidal portions of the drainage areas, surface water sampling sites were chosen at accessible locations near the waterbody's discharge point to another major waterway. DNREC considered multiple other sources of information prior to selecting final sampling sites, including: existing and readily available data on other toxic compounds in surface water, sediments, and biota; Maryland and Delaware's Clean Water Act Section 303(d) Lists of Impaired Waters for toxics and associated Total Maximum Daily Loads (TMDLs); fish consumption advisories (FCAs) in both Maryland (MDE 2026) and Delaware (DNREC 2018); the type and location of NPDES point source surface water discharges; and the location of Federal Superfund sites and Delaware waste sites. Finally, some surface water sampling sites were chosen based upon their proximity to jurisdictional boundaries (i.e., state lines). Critical factors in station selection, regardless of the watershed sampled, were accessibility and safety.

Table 2 presents the watersheds to be sampled along with their respective locations. The map following Table 2 shows the spatial distribution of the sampling sites. A total of eighty-three (83) stations have been selected.

Sampling of the selected stations will be synoptic in nature and will occur in the fall under low flow conditions. Such conditions are not likely to represent a worst case for contaminant transport, but they often represent a worst case for bioavailability, toxicity and bioaccumulation. Storms of large magnitude, low duration and high frequency are less common in the fall following the Atlantic hurricane season. Stream flows tend to be lower and more steady; sediment resuspension tends to be less; and importantly, the magnitude and duration of dissolved phase contamination in the water column can be elevated due to diffusive exchange with the sediments and a high proportion of groundwater contribution which may contain PFAS. Coupled with other stressors such as higher water temperature, lower dissolved oxygen and lower water volume, the added stress associated with dissolved phase toxic contamination can make aquatic communities especially vulnerable to impact in the fall. For the above reasons, the sampling under this QAPP targets the fall.

In addition to PFAS, field measurements will be collected at each sample station using a hand-held multi-meter at the time of sampling. Measurements will be made for water temperature (T) by SM2550-2010, dissolved oxygen (DO) by ASTM D888-09 C, dissolved oxygen saturation (%) by ASTM D888-09 C, pH by SM 4500-H+ B-2011, specific conductance by SM 2510 B-2011,

and salinity by SM 2520 B-2011. These measurements provide an indication of general water quality at the site at the time of sampling.

Table 2. Sampling Stations for PFAS in Surface Water

Watershed	Description					
	Upstream Location	Latitude	Longitude	Downstream Location	Latitude	Longitude
Shellpot Creek	Shellpot Creek at Lea Blvd	39.753731	-75.515885	Shellpot Creek at Hay Rd	39.739212	-75.510841
Naamans Creek	Naamans Creek at Stone Crop Rd	39.829618	-75.505329			
Christina River	Christina River at Rt 141 (boat)	39.710368	-75.608996	Christina River Mouth (Boat)	39.720091	-75.522407
	Brandywine River at Dam 2	39.758711	-75.555171			
	Brandywine River Mouth (boat)	39.732185	-75.530461			
	Brandywine River at Smith Bridge Rd	39.837632	-75.579010			
	Hershey Run at Rt. 4 (W. Newport Pike)	39.715262	-75.626165			
	Red Clay Creek at Rt 4	39.715236	-75.640181			
	Red Clay Creek at State Line	39.813126	-75.681464			
	Burrows Run at Snuff Mill Rd	39.820202	-75.644823			
	White Clay Creek at Delaware Park Dr	39.698914	-75.675557			
	White Clay Creek at Chambers Rock Rd	39.732956	-75.759777			
	Christina River at Smalleys Dam Rd	39.655330	-75.669243			
	Collins Park at Buttonwood Ave	39.682100	-75.555334			
	Army Creek at Park Ave	39.649508	-75.617739			
	Red Lion Creek at Rt 7	39.605368	-75.664844			
Dragon Run Creek	Dragon Run at Rt 13	39.569376	-75.654725	Dragon Run at Rt 9	39.577054	-75.602082
C&D Canal				C&D Canal at Rt 9 (boat)	39.558502	-75.580263
Appoquinimink	Appoquinimink River at Rt 13	39.444241	-75.663878	Appoquinimink River Mouth (boat)	39.447014	-75.584206
	Deep Creek at Summit Bridge Rd	39.437208	-75.713219			
Blackbird Creek	Blackbird Creek at Blackbird Landing Rd	39.388678	-75.635983			
Delaware Bay				Cedar Swamp at Collins Landing	39.388734	-75.527215
Smyrna River	Duck Creek @ Duck Creek Rd	39.3088	-75.621414	Smyrna River at Flemings Landing Rd	39.353787	-75.548718
	Sawmill Branch at Walker School Rd	39.373677	-75.601144			
	Lake Como Outfall	39.29662	-75.600546			
Leipsic River	Garrisons Lake Outfall	39.244036	-75.588559	Leipsic River Mouth at Goose Point (boat)	39.244404	-75.413497
St Jones River	Silver Lake (Dover) Outfall	39.168399	-75.521536	St. Jones River Mouth (boat)	39.070007	-75.405259

Table 2 (Continued)

Watershed	Site Description					
	Upstream Location	Latitude	Longitude	Downstream Location	Latitude	Longitude
Little River Murderkill River	Moores Lake Outfall	39.126146	-75.518569	Little River Mouth (boat) S. Bowers Beach Inlet (boat)	39.159847 39.058476	-75.412825 -75.396662
	Tidbury Creek at Rising Sun Rd	39.104853	-75.523434			
	Little River at N Little Creek Rd (RT 8)	39.166458	-75.477542			
	Murderkill at Market St	39.002042	-75.465646			
	Coursey Pond Outfall	38.988849	-75.510711			
Misspillion River Cedar Creek Primehook Creek Broadkill River	McGinnis Pond Outfall	39.036228	-75.510589	Mouth of Mispillion River Cedar Creek at Bay Ave	38.952993 38.934925	-75.317679 -75.323906
	Silver Lake (Milford) Outfall	38.911225	-75.433160			
	Swiggetts Pond Outfall	38.868998	-75.377411			
	Waples Pond Outfall (Boat)	38.825437	-75.306974			
	Wagamons Pond Outfall	38.777297	-75.31300			
Nanticoke River	Red Mill Pond Outfall	38.760098	-75.203124	Nanticoke River at State Line (Boat)	38.560262	-75.700856
	Nanticoke River at Old Furnace Rd	38.66558	-75.565230			
	Nanticoke River at Rifle Range Rd	38.728410	-75.562060			
	Nanticoke River at Seaford (boat)	38.631443	-75.618019			
	Nanticoke River at Woodland Ferry (boat)	38.599028	-75.657311			
Broad Creek Deep Creek Rehoboth Bay	Broad Creek at Records Pond Outfall	38.558671	-75.567557	Broad Creek at Phillips Landing Mouth of Love Creek	38.564446 38.688932	-75.672318 -75.140548
	Broad Creek at Shell Bridge Rd	38.568782	-75.619263			
	Concord Pond Outfall	38.643126	-75.554076			
	Love Creek at Robinsonville Rd	38.717710	-75.186778			
	Munchy Branch at Beaverdam Trail	38.733869	-75.111830			
Indian River Bay	Millsboro Pond Outfall at Main Street	38.594465	-75.291195	Indian River Bay Inlet (boat) Roosevelt Inlet (boat)	38.607765 38.787731	-75.079924 -75.159316
	Indian River at Gut Point (boat)	38.589998	-75.219180			
L&R Canal Little Assawoman Bay	Dirickson Creek at Old Mill Bridge Rd (381)	38.483594	-75.11883	Little Assawoman Bay at Laws Point (boat) The Ditch/State line at Lighthouse Rd (boat)	38.483338 38.452631	-75.08046 -75.064251
	Roy Creek at Americana Parkway	38.468750	-75.115133			
	Long Branch at Steele Rd	39.55503	-75.78094			
C&D West	Sammons Pond Outfall at Old Telegraph Rd	39.51347	-75.78115			

Table 2 (Continued)

Watershed	Site Description					
	Upstream Location	Latitude	Longitude	Downstream Location	Latitude	Longitude
Bohemia	Bohemia Mill Pond Outfall at Old Telegraph Rd	39.4654	-75.77567			
	Sandy Branch at Old Telegraph Rd	39.46033	-75.77387			
Sassafras	Sassafras River at Maryland Line Rd	39.37759	-75.76649			
Chester	Cypress Branch at Delaney Church Rd	39.30068	-75.75021			
	Sewell Branch at Sewell Branch Rd	39.24678	-75.74897			
Choptank	Gravely Run at Blanco Rd	39.21861	-75.76086			
	Choptank River at Rd 287	39.03025	-75.75755			
	Cow Marsh Creek at Mahan Corner Rd	39.04548	-75.71410			
Marshyhope	Marshyhope Creek at Rt 404	38.79445	-75.69349			
Pocomoke	Pocomoke River at Bethel Rd.	38.45947	-75.34415			

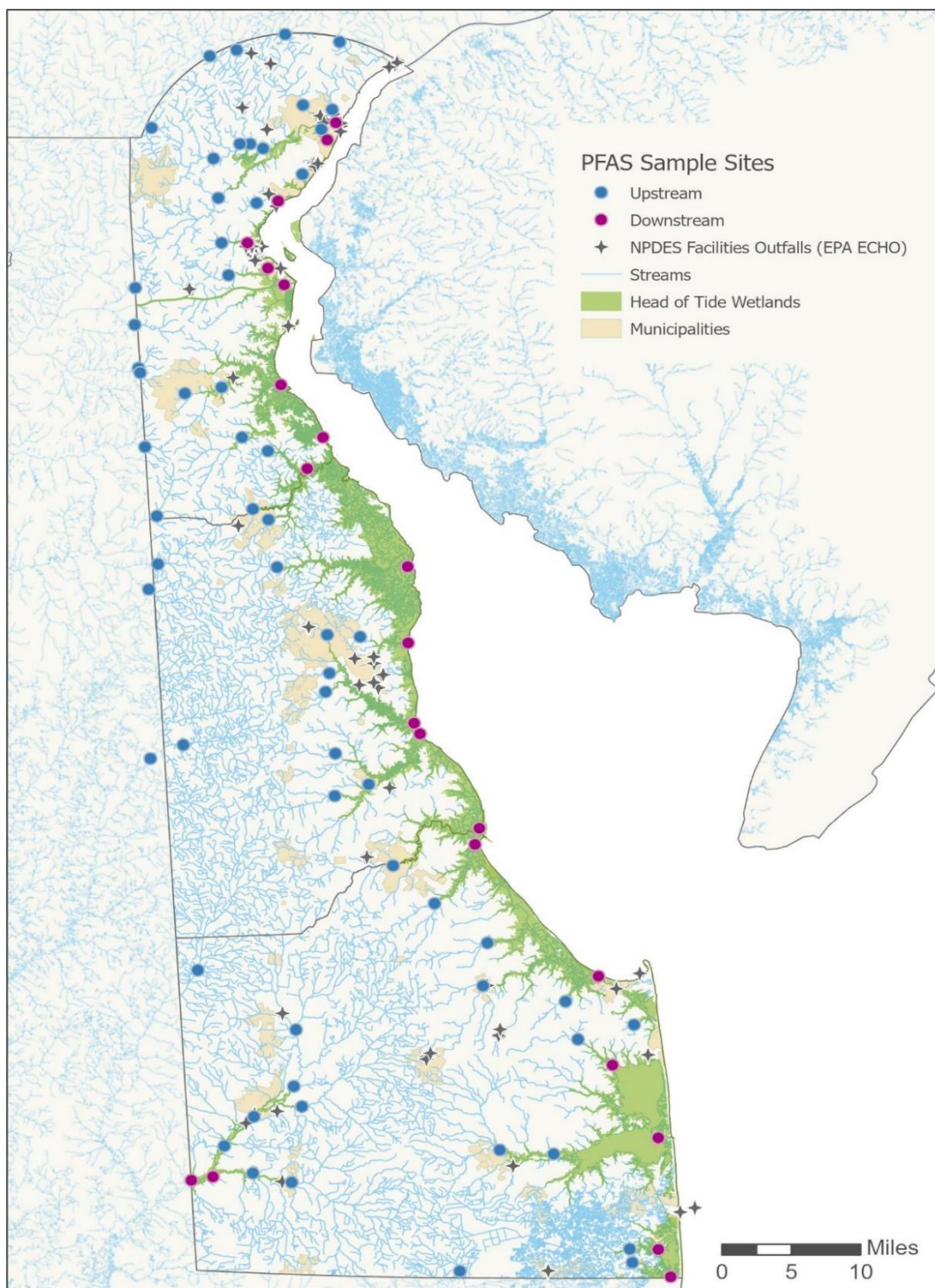


Figure 3. Proposed Sample Locations

2.2 SAMPLING METHODS

2.2.1 Surface Water

Surface water samples will be collected from land or boat depending on the site. The primary piece of equipment utilized will be a sampling pole (or a bucket at road crossings where the water surface is too far below to reach with the 24' pole). If sampling from a boat, the boat will be positioned and anchored as close as possible to the target coordinates, provided this can be done safely, as determined by the boat captain. Surface water samples will require filling of the following containers: 2 x 500ml HDPE bottles (a primary and backup sample). If high suspended solids are observed in water samples collected at a site, a smaller HDPE bottle (125 – 250ml) will also be filled to enable total suspended solids estimation in the laboratory. To avoid cross-contamination from sampling materials to sample matrix, the outside of all capped sample containers should be rinsed multiple times with the surface water being sampled before filling the containers.

The surface water sampling team will consist of 2 or more individuals. One person will be designated as “dirty hands”; one person will be designated as “clean hands”. “Clean hands” can also serve as “recorder”. “Dirty hands” will have direct contact with sampling gear and containers. “Clean hands”/“recorder” can handle containers after the samples have been collected and sealed. “Recorder” will be responsible for sample documentation.

2.3 SAMPLE HANDLING AND CUSTODY

2.3.1 Decontamination of Field Sampling Equipment

Proper decontamination ensures that contamination is not inadvertently introduced into field samples as a result of dirty equipment. For the present effort, the key pieces of equipment that need to be decontaminated include the stainless-steel bucket and the sampling pole. The bucket and the sampling pole will be decontaminated with Alconox detergent or equivalent, followed by 6 deionized (DI) water rinses, a methanol rinse, and another DI water rinse prior to going into the field. In the field, the equipment will receive 3 rinses with ambient water prior to collecting the sample.

Equipment (rinsate) blank samples will be prepared to demonstrate that all sampling gear that comes into contact with field samples is properly decontaminated.

2.3.2 Sample ID and Custody

A unique sample ID shall be assigned to each sample. The sample identification protocols will follow similar protocols developed by DNREC for other major sampling campaigns. The sample ID shall incorporate an abbreviation for the watershed, location, matrix, and month/year (mmyy). For example, a surface water sample collected from the Nanticoke River at Seaford in October 2026 shall be identified as NanSeaford-SW-1026. Field duplicates will be identified with a –DUP designator at the end of the sample ID. DNREC’s standard approach is to prepare sample IDs and labels prior to sample collection. Sample names are included in Table 3.

Table 3. Sample Names

Upstream Location		Downstream Location	
Sample Location	Sample Name	Sample Location	Sample Name
Shellpot Creek at Lea Blvd	ShellLeaBlvd-SW-mmyy	Shellpot Creek at Hay Rd	ShellHayRd-SW-mmyy
Naamans Creek at Stone Crop Rd	Naamans-SW-mmyy		
Christina River at Rt 141 (boat)	Chr141-SW-mmyy	Christina River Mouth (Boat)	ChrMouth-SW-mmyy
Brandywine River at Dam 2	BrDam2-SW-mmyy		
Brandywine River Mouth (boat)	BrMouth-SW-mmyy		
Brandywine River at Smith Bridge Rd	BrSmithBr-SW-mmyy		
Hershey Run at Rt. 4 (W. Newport Pike)	HersheyRun4-SW-mmyy		
Red Clay Creek at Rt 4	RCStanton-SW-mmyy		
Red Clay Creek at State Line	RCStateline-SW-mmyy		
Burrows Run at Snuff Mill Rd	BurrowsRn-SW-mmyy		
White Clay Creek at Delaware Park Dr	WCDEPark-SW-mmyy		
White Clay Creek at Chambers Rock Rd	WCChmbRk-SW-mmyy		
Christina River at Smalleys Dam Rd	ChrSmalleyDam-SW-mmyy		
Collins Park at Buttonwood Ave	CollinsPk-SW-mmyy		
Army Creek at Park Ave	ArmyCrkParkAve-SW-mmyy	Army Creek at Rt 9	ArmyCrk9-SW-mmyy
Red Lion Creek at Rt 7	RLC7-SW-mmyy	Red Lion Creek at Rt 9	RLC9-SW-mmyy
Dragon Run at Rt 13	DragRun13-SW-mmyy	Dragon Run at Rt 9	DragRun9-SW-mmyy
		C&D Canal at Rt 9 (boat)	CDRt9-SW-mmyy
Appoquinimink River at Rt 13	AppoR13-SW-mmyy	Appoquinimink River Mouth (boat)	AppoRMouth-SW-mmyy
Deep Creek at Summit Bridge Rd	DeepCrk-SW-mmyy		
Blackbird Creek at Blackbird Landing Rd	BBCrk-SW-mmyy		
		Cedar Swamp at Collins Landing	CedarSwp-SW-mmyy
Duck Creek @ Duck Creek Rd	DuckCrk-SW-mmyy	Smyrna River at Flemings Landing Rd	SmyrnaRiv-SW-mmyy
Sawmill Branch at Walker School Rd	SawmillBr-SW-mmyy		
Lake Como Outfall	LkComo-SW-mmyy		
Garrisons Lake Outfall	GarrisonsLk-SW-mmyy	Leipsic River Mouth at Goose Point (boat)	LeipsicMouth-SW-mmyy
Silver Lake (Dover) Outfall	SJSilverLk-SW-mmyy	St. Jones River Mouth (boat)	SJMouth-SW-mmyy
Moore's Lake Outfall	Moore'sLk-SW-mmyy		
Tidbury Creek at Rising Sun Rd	TidburyCrk-SW-mmyy		
Little River at N Little Creek Rd (RT 8)	LittleRiv8-SW-mmyy	Little River Mouth (boat)	LittleRivMouth-SW-mmyy
Murderkill at Market St	Murderkill-SW-mmyy	S. Bowers Beach Inlet (boat)	SBowerInlet-SW-mmyy
Coursey Pond Outfall	CourseyPd-SW-mmyy		
McGinnis Pond Outfall	McGinnisPD-SW-mmyy		
Silver Lake (Milford) Outfall	SilverLkMil-SW-mmyy	Mouth of Misspillion River	MisspillMouth-SW-mmyy
Swiggetts Pond Outfall	SwigPd-SW-10-mmyy	Cedar Creek at Bay Ave	CedarCrk-SW-mmyy
Waples Pond Outfall (Boat)	WaplesPd-SW-mmyy		
Wagamons Pond Outfall	WagamonPd-SW-mmyy		
Red Mill Pond Outfall	RedMillPd-SW-mmyy		
Nanticoke River at Old Furnace Rd	NanOldFurnace-SW-mmyy	Nanticoke River at State Line (Boat)	NanStateline-SW-mmyy
Nanticoke River at Rifle Range Rd	NanRifleRangeRd-SW-mmyy		
Nanticoke River at Seaford (boat)	NanSeaford-SW-mmyy		
Nanticoke River at Woodland Ferry (boat)	NanWoodlf-SW-mmyy		
Broad Creek at Records Pond Outfall	RecordsPd-SW-mmyy	Broad Creek at Phillips Landing	BroadCrkPhillips-SW-mmyy
Broad Creek at Shell Bridge Rd	BroadCrkShellBr-SW-mmyy		
Concord Pond Outfall	ConcordPd-SW-mmyy		
Love Creek at Robinsonville Rd	LoveCrk-SW-mmyy	Mouth of Love Creek	LoveCrMouth-SW-mmyy
Munchy Branch at Beaverdam Trail	MunchyBr-SW-mmyy		
Millsboro Pond Outfall at Main Street	MillsboroPd-SW-mmyy	Indian River Bay Inlet (boat)	InRivBayInlet-SW-mmyy
Indian River at Gut Point (boat)	InRivGutPt-SW-mmyy		
		Roosevelt Inlet (boat)	RoosInlet-SW-mmyy
Dirickson Creek-Old Mill Bridge Rd (381)	DirickCrk-SW-mmyy	Little Assawoman Bay at Laws Point (boat)	LitAssBay-SW-mmyy

Table 3 (Continued)

Roy Creek at Americana Parkway	RoyCrk-SW-mmyy	The Ditch/State line-Lighthouse Rd (boat)	TheDitch-SW-mmyy
Long Branch at Steele Rd	LongBR-SW-mmyy		
Sammons Pond Outfall-Old Telegraph Rd	SammonsPd-SW-mmyy		
Bohemia Mill Pond Outfall-Old Telegraph Rd	BohemMillPf-SW-mmyy		
Sandy Branch at Old Telegraph Rd	SandyBr-SW-mmyy		
Sassafras River at Maryland Line Rd	Sassafras-SW-mmyy		
Cypress Branch at Delaney Church Rd	CypressBr-SW-mmyy		
Sewell Branch at Sewell Branch Rd	SewellBr-SW-mmyy		
Gravelly Run at Blanco Rd	GravellyR-SW-mmyy		
Choptank River at Rd 287	Choptank-SW-mmyy		
Cow Marsh Creek at Mahan Corner Rd	CowMarshCrk-SW-mmyy		
Marshyhope Creek at Rt 404	MarshyCrk-SW-mmyy		
Pocomoke River at Bethel Rd.	Pocomoke-SW-mmyy		

2.3.3 Sample Preservation, Holding and Transportation

Sample containers will be supplied by the DNREC DEL and will be verified clean by lot. Table 4 lists the containers that will be used by parameter and matrix.

The sample collection team shall preserve samples on ice, at or below 6°C on site, during transport, and until receipt by the lab. The DNREC DEL laboratory shall maintain the samples at or below 6°C for up to five days post-collection until extraction or transfer to longer term storage at or below -20 °C. All samples shall be sealed inside a sealable plastic bag to prevent direct contact with melt water from ice in the coolers.

All surface water samples collected for toxics analyses will be taken to the DNREC DEL on the same day they are collected to ensure that holding times are met (90-day hold time for PFAS 1633 (M)) analysis if stored at or below -20 °C.

Table 4. Sample Containers

Parameter	Matrix	Container	Destination Laboratory
PFAS by 1633 (M)	Surface Water	Two 500ml HDPE Bottle One 125 – 250ml HDPE Bottle for TSS (if needed)	DNREC Delaware Environmental Laboratory

2.3.4 Chain of Custody Documentation

The sample packaging and shipment procedures summarized below will ensure that the samples arrive at the DNREC DEL Laboratory with the chain of custody intact.

The sample collection team is responsible for the care and custody of the samples until they are transferred or properly dispatched. As few people as possible should handle the samples. The sample collection team must complete a chain of custody form documenting the custody of each sample after collection. Samples must be accompanied by a properly completed chain of custody form. The sample IDs will be listed on the chain of custody form. When transferring the possession of samples, the

individuals relinquishing and receiving will sign, date, and note the time on the record. This record documents transfer of custody of samples from the sampler to another person, to a laboratory, or to/from secure storage.

At the end of the project all original and photocopied or triplicate copy chain of custody forms will be included in the laboratory report.

2.3.5 Field Logs

The sample collection team will record important field information in a project field logbook, on field log sheets or by another documentation method (e.g. tablets).

Entries into the logs will contain a variety of information. At the beginning of each entry, the date, start time, weather, names of all sampling team members present and the signature of the person making the entry will be entered. The date, time and location that each sample is collected will be recorded.

Measurements made and samples collected will be recorded. All entries will be made in ink, and no erasures will be made. If an incorrect entry is made, the information will be crossed out with a single strike mark and initialed by the person making the strikeout. Whenever a sample is collected, or a measurement is made, a detailed description of the location of the station and a GPS measurement shall be recorded.

2.3.6 Laboratory Custody Procedures

Upon receipt of the sample cooler(s), the DNREC DEL Laboratory shall initiate a documentation procedure to verify the custody and condition of the samples. Immediately upon opening the cooler, the lab shall measure and document the internal temperature of the cooler. Also, the Chain of Custody form shall include the date/time the samples were collected, the site identifier, name of the sampler(s) and any observations that may impact sample integrity. The Chain of Custody will be signed by the person dropping the samples off at the laboratory. The Laboratory Sample Custodian will log the date/time and sign the Chain of Custody.

2.4 ANALYTICAL METHODS

Specialty analyses of PFAS in surface water will be provided by DNREC DEL. Samples will be analyzed for the specific PFAS analytes listed in Table 5 using liquid chromatography – tandem mass spectrometry (LC-MS/MS). The laboratory's Standard Operating Procedure (SOP), presented in Appendix A, provides a detailed description of the analytical method.

Table 5. PFAS Analytes: Method 1633 (M) RLs and MDLs

Analyte	CAS Number	Reporting Limit (LOQ)	Method Detection Limit	Units
Perfluorobutanoic acid (PFBA)	375-22-4	2.17	0.119	ng/L
Perfluoropentanoic acid (PFPeA)	2706-91-4	1.08	0.163	ng/L
Perfluorohexanoic acid (PFHxA)	355-46-4	1.08	0.222	ng/L
Perfluoroheptanoic acid (PFHpA)	375-85-9	1.08	0.134	ng/L
Perfluorooctanoic acid (PFOA)	335-67-1	1.08	0.503	ng/L
Perfluorononanoic acid (PFNA)	375-95-1	1.08	0.304	ng/L
Perfluorodecanoic acid (PFDA)	335-76-2	1.08	0.252	ng/L
Perfluoroundecanoic acid (PFUnA)	2058-94-8	1.08	0.348	ng/L
Perfluorododecanoic acid (PFDoA)	307-55-1	2.17	0.455	ng/L
Perfluorotridecanoic acid (PFTrDA)	72629-94-8	1.08	0.143	ng/L
Perfluorotetradecanoic acid (PFTDA or PFTeDA)	376-06-7	1.08	0.090	ng/L
Perfluorobutanesulfonic acid (PFBS)	375-73-5	0.961	0.194	ng/L
Perfluoropentanesulfonic acid (PFPeS)	2706-91-4	1.02	0.357	ng/L
Perfluorohexanesulfonic acid (PFHxS)	355-46-4	0.990	0.301	ng/L
Perfluoroheptanesulfonic acid (PFHpS)	375-92-8	1.03	0.409	ng/L
Perfluorooctanesulfonic acid (PFOS)	1763-23-1	1.01	0.379	ng/L
Perfluorononanesulfonic acid (PFNS)	68259-12-1	1.04	0.548	ng/L
Perfluorodecanesulfonic acid (PFDS)	335-77-3	1.05	0.283	ng/L
Perfluorododecanesulfonic acid (PFDoS or PFDoDS)	79780-39-5	2.10	0.213	ng/L
NMeFOSAA	2355-31-9	2.17	0.324	ng/L
NEtFOSAA	2991-50-6	2.17	0.604	ng/L
4:2 FTS	757124-72-4	4.06	0.584	ng/L
6:2 FTS	27619-97-2	4.12	1.38	ng/L
8:2 FTS	39108-34-4	4.16	1.04	ng/L
Perfluorooctanesulfonamide (PFOSA or FOSA)	754-91-6	1.08	0.210	ng/L
N-Methylperfluorooctanesulfonamide (N-MeFOSA)	31506-32-8	2.00	0.325	ng/L
N-Ethylperfluorooctanesulfonamide (N-EtFOSA)	4151-50-2	2.00	0.409	ng/L
HFPO-DA (GenX)	13252-13-6	1.00	0.300	ng/L
9Cl-PF3ONS	756426-58-1	0.935	0.119	ng/L
11Cl-PF3OUdS	763051-92-9	0.945	0.118	ng/L
4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	919005-14-4	0.945	0.088	ng/L
5:3 FTCA	914637-49-3	10.0	2.88	ng/L
6:2 FTUCA	70887-88-6	2.00	1.10	ng/L
3-Perfluoropropyl acid (3:3 FTCA)	356-02-5	4.00	0.697	ng/L
3-Perfluoroheptyl propanoic acid (7:3 FTCA)	812-70-4	10.0	1.61	ng/L
NFDHA	151772-58-6	2.00	0.574	ng/L
PFMBA	863090-89-5	1.00	0.106	ng/L
PFMPA	377-73-1	1.00	0.081	ng/L
PFEESA	113507-82-7	0.890	0.244	ng/L
PFMOAA	674-13-5	0.892	0.233	ng/L
PFO2HxA	39492-88-1	3.67	0.601	ng/L
PPF Acid or PFPrA	422-64-0	2.01	0.687	ng/L
PFESA BP2 or Hydro-PS Acid) or Nafion BP2	749836-20-2	1.04	0.094	ng/L
N-EtFOSE	1691-99-2	10.0	1.52	ng/L
N-MeFOSE	24448-09-7	10.0	1.44	ng/L

2.5 QUALITY CONTROL

The project team will follow the policies and procedures detailed in this QAPP using standard analytical tools and protocols under the direction and oversight of the DNREC PM. Critical QC aspects for the task are handling and preserving the integrity of data and information from secondary sources. This QAPP and other supporting materials will be distributed to all project personnel. The DNREC PM will ensure that tasks described in the work plan are carried out in accordance with the QAPP.

A DNREC QC Officer will evaluate the correctness of transferred data and will review a minimum of 10 percent of all data extractions, transformations and calculations, as described further in this section of the QAPP. Any senior staff member familiar with the project, task, or specific data operation or analysis can serve as QC Officer on work performed by others. In this regard, many persons may fulfill a QC Officer function in advance of the higher-level Project QA Officer reviews. The DNREC PM will review staff performance throughout the project to ensure adherence to project protocols. Reducing uncertainty by using appropriate QC protocols is of the highest priority. The following procedures will be used to ensure the completeness and correctness of data used in the collected data deliverables:

- All original work performed by any member of the technical staff will be subject to QC checks by a different member of the technical staff who is capable of performing the QC checks;
- If applicable, 100 percent of scanned or hand entered data from hard copy documents will be reviewed by a technical staff member who did not perform the original work;
- 10 percent of transferred data from each source will be reviewed by a technical staff member who did not perform the original work;
- 10 percent of each type of data transformation will be evaluated by the DNREC QC Officer (or their designee);
- 10 percent of each type of calculation will be evaluated by the DNREC QC Officer (or their designee); and
- The DNREC PM will provide answers to specific management questions.

2.6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Key equipment used to acquire field samples in conjunction with this QAPP includes the following: sampling pole, stainless steel bucket on a rope, and boats/trailers. All equipment is visually inspected prior to use. Back-up equipment is brought into the field in the event the primary unit fails and cannot be immediately fixed. Equipment is maintained by personnel who are experienced with, and qualified to safely operate, this equipment. Qualified individuals include trained scientists from DNREC's DEL. Finally, inspection and maintenance of boats used as sampling platforms is an ongoing activity. Tire pressure and lights associated with boat trailers are checked before going on the road. Further, all required safety equipment that needs to be on the boat is checked before going on the water.

Hand-held meters and devices that will be used in conjunction with this QAPP include a YSI field multi-meter/datalogger (YSI Exo) and a hand-held GPS unit. The YSI multi-meter/logger is turned on prior to leaving for the field each day. Field personnel will ensure the instruments are fully charged prior to going

into the field. The meter is tested, inspected, and maintained per the manufacturer's instructions.

Testing, inspection, and maintenance of all laboratory instruments and equipment used in support of this effort are the responsibility of the DNREC DEL Laboratory.

2.7 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

The only field equipment that requires routine calibration is the hand-held YSI multi-meter. Calibration procedures, including calibration frequency, follow the manufacturer's recommendations. The DNREC DEL maintains a log of calibration activities associated with the multi-meters used for this project.

2.8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All field supplies and consumables for this project will be inspected by DNREC for defects and obvious signs of improper handling before use. Supplies and consumables which show signs of defects or improper handling will not be used by the sample collection team.

Supplies and consumables used at the DEL in support of this project are the responsibility of the laboratory.

2.9 NONDIRECT MEASUREMENTS

No non-direct measurements have been identified for this project.

2.10 DATA MANAGEMENT

DNREC's computer systems and network servers are specified, inventoried, and maintained (either directly or by external service contract) by a dedicated information technology staff, and all interim (working) and final electronic files used in development of project deliverables will be stored on DNREC's secure network, which is backed up daily. DNREC project scientists will store all computer files associated with the project (including all sources used to prepare deliverables) in a project subdirectory (subject to regular system backups). DNREC may use a tracking spreadsheet specifically designed for this project to document data collection, assessment and information sources compiled for this project. To maintain version control, DNREC will clearly identify files using the subject of the file and corresponding dates of preparation. The DNREC PM is responsible for ensuring that data sources are managed in accordance with this section and other relevant sections of this QAPP.

3.0 ASSESSMENT AND OVERSIGHT

The elements in this section address the activities for assessing the effectiveness of project implementation and associated QA/QC activities. The purpose of assessment is to ensure that the QAPP/Sampling Plan is implemented as described.

3.1 ASSESSMENT AND RESPONSE ACTIONS

When errors, deficiencies, or out-of-control situations exist, the Laboratory's QA program shall provide systematic procedures, called "corrective actions," to resolve problems and restore proper functioning to the analytical system(s). Laboratory personnel are alerted that response actions are necessary if:

-
- QC data are outside the acceptable windows for precision and accuracy;
 - Blanks, duplicate control samples, or single control samples contain contaminants above acceptable levels (Table 6);
 - Undesirable trends are detected in spike recoveries or RPD between duplicates;
 - There are unusual changes in method detection limits; and/or
 - Deficiencies are detected by the Laboratory QA department during internal or external audits or during performance evaluations.

Corrective action procedures may be handled by the analyst, who reviews the preparation and extraction procedure for possible errors, checks the instrument calibration, spike, and calibration mixes, and instrument sensitivity. If the problem persists or cannot be identified, the matter is referred to the Laboratory supervisor, manager, and/or QA department for further investigation. Once resolved, an explanation of the corrective action procedure and findings shall be included with the laboratory report. Full documentation of the corrective action procedure and resolution will be retained by the laboratory and copies provided at request.

The response actions and/or procedures described below are required as part of implementation of this QAPP.

3.1.1 Incoming Samples

Problems noted during sample receipt shall be documented on the Chain of Custody form. The DNREC PM shall be contacted immediately for consultation regarding problem resolution. All response actions taken shall be thoroughly documented and submitted to the DNREC PM.

3.1.2 Sample Holding Times

If samples cannot be extracted and/or analyzed within the appropriate method required holding times, the DNREC PM shall be immediately notified, such that an appropriate response action plan can be generated. All response actions taken shall be thoroughly documented. Data outside the quality objectives and criteria will be flagged, so that data recipients can exercise discretion in determining appropriate data uses and limitations.

3.1.3 Detection Limits

Appropriate sample cleanup procedures shall be employed to attempt to achieve the required detection limits. Cleanup methods employed are left to the discretion of the analyst or other appropriate Laboratory personnel, in accordance with the specified analytical method. Cleanup methods and dilutions shall be documented, with the rationale, along with revised method detection limits for those analytes directly affected. Data outside the quality objectives and criteria will be flagged, so that data recipients can exercise discretion in determining appropriate data uses and limitations.

3.1.4 Method QC

All method QC, including blanks, matrix duplicates, labeled isotope spikes, laboratory control samples, and other method specified QC, shall meet the requirements as specified in this QAPP. Failure of the method-required QC shall result in the review of all affected data. If no errors can be noted, the affected samples shall be reanalyzed and/or re-extracted then reanalyzed within method holding times to verify

the presence or absence of a matrix effect. Affected data shall be flagged accordingly using standard flagging symbols and criteria. Data outside the quality objectives and criteria will be flagged, so that data recipients can exercise discretion in determining appropriate data uses and limitations.

3.2 REPORTS TO MANAGEMENT

The reports from the DNREC DEL laboratory will serve as the Quality Assurance Reports to the QA Officer and DNREC management. The Final Reports from the laboratory must include all the items specified in this QAPP.

4.0 DATA VALIDATION AND USABILITY

Data validation and usability procedures will include, as the minimum, the elements described below:

4.1 DATA REVIEW, VERIFICATION, AND VALIDATION

All analytical data generated by the DNREC DEL Laboratory shall be reviewed prior to report generation to ensure the validity of the reported data. This internal data validation process shall consist of data generation, reduction, and a minimum of three levels of documented review. In each stage, the review process shall be documented using an appropriate form or laboratory information management software (LIMS) that is signed and dated by the reviewer. The analyst who generates the analytical data has the prime responsibility for the correctness and completeness of the data. Each step of this review process involves evaluation of data quality based on both the results of the QC data and the professional judgment of those conducting the review. This application of technical knowledge and experience to the evaluation of the data is essential in ensuring that data of high quality are generated consistently. All data generated and reduced shall follow well documented in-house protocols. Data outside the quality objectives and criteria will be flagged, so that data recipients can exercise discretion in determining appropriate data uses and limitations.

4.2 REVIEW AND VERIFICATION METHODS

Each analyst reviews the quality of their work based on an established set of guidelines. This review shall, at a minimum, ensure the following:

- Sample preparation information is correct and complete;
- Analysis information is correct and complete;
- The appropriate SOPs have been followed;
- Analytical results are correct and complete;
- QC samples are within established control limits;
- Blanks and laboratory control samples are within appropriate QC limits;
- Special sample preparation and analytical requirements have been met; and
- Documentation is complete (i.e., all anomalies in the preparation and analysis have been documented, anomaly forms are complete, holding times are documented, etc.).

Level 1 data review shall be documented using a form or LIMS and by signature and date of the reviewer.

Level 2 review shall be performed by a supervisor or data review specialist whose function is to provide an independent review of the data package. This review shall also be conducted according to an established set of guidelines and is structured to ensure that:

- All appropriate Laboratory SOPs have been followed;
- Calibration data are scientifically sound, appropriate to the method, and completely documented;
- QC samples are within established guidelines;
- Qualitative identification of sample components is correct;
- Quantitative results are correct;
- Documentation is complete and correct (e.g., anomalies in the preparation and analysis have been documented, anomaly forms are complete, holding times are documented, etc.);
- The data are ready for incorporation into the final report; and
- The data package is complete and ready for data archive.

Level 2 review shall be structured so that all calibration data and QC sample results are reviewed and all the analytical results from at least 10% of the samples are checked back to the bench sheet. If no problems are found with the data package, the review is complete. If any problems are found with the data package, an additional 10% of the sample results shall be checked back to the bench sheet. This cycle repeats until either no errors are found in the data set checked or all the data has been checked. All errors and corrections noted shall also be documented with the signature and date of the reviewer.

Level 3 review is performed by the quality assurance officer or the program administrator at the Laboratory. This review should be similar to the review as provided in Level 2 except that it should provide a total overview of the data package to ensure its consistency and compliance with this contract. All errors noted shall be corrected and documented. Level 3 data review shall also be documented with the signature and date of the reviewer.

4.3 RECONCILIATION WITH USER REQUIREMENTS

In order to ensure that all the data is consistent with the project requirements, DNREC will perform a Data Quality Assessment on the completed data set. Data Quality Indicators (DQI) specific to analytical data are described in Table 6.

DNREC will review the entire data set (100% of the data) and perform each of the following checks:

- Determine if all the requested data is present. Document any missing data. If there are no deficiencies, document that the data set is complete.
- Check the analyte specific holding times. Document any holding times which were exceeded. If there are no deficiencies, document that all holding times were met.
- Check the detection and reporting limits against those specified in the QAPP (Table 5). Document any

detection which exceeded the detection and/or reporting limits required by the QAPP. Where detection/reporting limits were exceeded, document any explanation included in the laboratory report, such as “matrix interferences.” If there are no deficiencies, document that all detection and reporting limits were met.

- Check the field, method, and calibration blanks to determine if blanks were run at the frequency required in the QAPP (Table 6). Document any deficiency in blanks. Document any blank in which a quantifiable concentration ($>$ Reporting Limit (RL), see Table 5) of the analyte was detected. If there are no deficiencies, document that sufficient blanks were analyzed, and none had quantifiable concentrations of any analyte.
- Check the initial and continuing calibration (ICAL and CCV, respectively) information to determine whether or not calibration was achieved and maintained (Table 6). Document any deficiencies. If there are no deficiencies, document that the initial and continuing calibration information is complete and acceptable.
- Check the documented quantitation processes against the process requirements described in the analytical method (Appendix A). Document any deficiencies. If there are no deficiencies, document that the quantitation process is complete and acceptable.
- Check the laboratory control samples (LCS) to determine if they were analyzed at the frequency, and for the parameters specified in the SOP and the QAPP (1 low-level laboratory control sample and 1 mid-level laboratory control sample per batch per matrix; analytical batch is no more than 20 field samples). Check the % recoveries against the acceptance criteria listed in the SOP and QAPP (Appendix A and Table 6, respectively). Document any deficiencies in frequency or % recoveries outside the acceptance criteria. If there are no deficiencies, document that the LCSs are complete and acceptable.
- Check all field and laboratory duplicates to determine if duplicates were performed at the frequency required by the QAPP. Check the RPD against the acceptance criteria listed in the QAPP. Document any deficiencies in the frequency of duplicates, or any RPDs outside the acceptance criteria. If there are no deficiencies, document that the duplicates are complete and acceptable.
- Check the labeled isotope spike recoveries to determine if they were performed at the frequency and for the parameters specified in the QAPP (labeled isotope analytes added to each sample, standard, and blank prior to analysis). Check the % recoveries against the acceptance criteria specified in the QAPP (Table 6). Document any deficiencies in frequency or any % recoveries outside the acceptance criteria. If there are no deficiencies, document that the isotope dilution spikes are complete and acceptable.
- Check for the presence and content of corrective action documentation where any deficiencies in any of the above categories exist. Document the existence and content of corrective actions.
- Check 100% of any information transcribed from field notes and logs to electronic files and databases. Document any transcription errors, verify the correct value or notation, advise the database or file manager to correct the erroneous transcription, and document the correction.

- Check related laboratory data to determine if the results are logical and reasonable. For example, if a sample is analyzed for a total and dissolved fraction of a given parameter, the results for the total fraction should be greater than (or at least equal to) the dissolved fraction. Document any deficiencies or any data which appears to be illogical or incorrect.

Table 6. PFAS Analytical Data Quality Indicators (DQI)

DQI	QC Sample or Measurement Performance Activity	QC Sample or Measurement Frequency	Measurement Performance Criteria¹
Overall precision	Field-collected replicate samples	1 per 20 field samples	$RPD \leq 30\%$ when PFAS $\geq$ RL
Overall accuracy/bias (field contamination)	Field blanks	1 per 20 field samples	$< RL$ or $< 10\%$ measured concentration in assoc. samples (whichever is higher)
Overall accuracy/bias (laboratory contamination)	Method blanks	1 per batch (≥ 1 per 20 samples)	$< RL$ or $< 10\%$ measured concentration in assoc. samples (whichever is higher)
Overall accuracy/bias (matrix interference)	MS/MSD	N/A – if isotope dilution used	N/A – if isotope dilution used
Overall accuracy/bias (matrix interference)	Isotope-dilution method	All samples and blanks	Isotope dilution analyte recoveries (Appendix A)
Analytical accuracy/bias	LCS	1 per batch (≥ 1 per 20 samples)	Approx. 70-130% recovery (analyte-specific)
Analytical accuracy/bias (instrument contamination- initial and continuing calibration blanks)	ICB/CCB	ICB: once at the start of an analytical sequence CCB: after the highest-level calibration standard and following each CV injection	ICB/CCB: $< RL$
Analytical accuracy/bias (initial calibration and verification)	ICAL	Once prior to sample analysis (repeat if needed based on CCV/CCVL results)	ICAL: $RSE/RSD \leq 20\%$; Isotope dilution analyte recoveries (Appendix A)
Analytical accuracy/bias (continuing calibration verification)	ICV/CCV	Beginning and end of analytical run and after every 10 samples in an analytical run	ICV/CCV: PFAS analyte recoveries 70-130%; Isotope dilution analyte recoveries (Appendix A)
Analytical accuracy/sensitivity (low-level continuing calibration verification)	CCVL	1 per batch (≥ 1 per 20 samples) if an ICAL is not part of the batch	CCVL ($\leq RL$): PFAS analyte recoveries 50-150% and Isotope dilution analyte recoveries (Appendix A)
Completeness	See QAPP sec. 1.5.4	Once when analytical testing is complete	$\geq 95\%$ data/information present

¹ See Laboratory SOP (Appendix A) for detailed performance criteria and corrective actions.

REFERENCES

- DNREC. 2018. Delaware Fish Consumption Advisories. <https://dnrec.alpha.delaware.gov/fish-wildlife/fishing/consumption-advisories/>
- MDE. 2026. Maryland Department of the Environment Fish Consumption Advisory – Guidelines for Recreationally Caught Fish Species in Maryland. <https://mde.maryland.gov/programs/Marylander/fishandshellfish/Pages/fishconsumptionadvisory.aspx>
- USEPA. 2009. Region III Fact Sheet Quality Control Tools: Blanks. U.S. Environmental Protection Agency, Region 3 QA Staff, Philadelphia, PA. <https://www.epa.gov/sites/production/files/2015-06/documents/blanks.pdf>

Appendix A

A.1. SOP for Aqueous Sample Collection Instructions for PFAS Analysis by LC-MS/MS at DNREC DEL Laboratory



Delaware Environmental Laboratory
60 Sunnyside Road, Smyrna, DE 19977

AQUEOUS SAMPLE COLLECTION INSTRUCTIONS FOR PFAS ANALYSIS BY LC-MS/MS

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1.0 SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes procedures to collect, transport, and store aqueous (groundwater, surface water, and wastewater) samples prior to analysis of Per- and Polyfluoroalkyl Substances (PFAS) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Department of Natural Resources and Environmental Control's Delaware Environmental Laboratory (DEL). This SOP also describes the necessary precautions that should be taken to prevent accidental sample contamination during collection, transport, and storage.

2.0 SUMMARY

2.1 Aqueous samples are collected in high-density polyethylene (HDPE) bottles with unlined HDPE or polypropylene caps.

2.1.0 Precautions must be taken to prevent accidental contamination of samples collected for PFAS analysis (see sections 6.0 and 7.0).

2.2 Samples are stored in coolers and on regular ice at all times during transportation. Samples must be stored on regular ice or refrigerated at $\leq 6^{\circ}\text{C}$ if they cannot be brought to the laboratory the same day they are collected in the field.

2.3 Once received at DEL, chain of custody documentation (COC) must be reviewed and samples stored appropriately until analysis (see section 6.1.2.2 and 6.1.2.3).

3.0 TERMS AND DEFINITIONS

3.1 Controlled document [18356](#) provides a master list of terms and definitions.

3.2 Additional terms or definitions specific to this SOP:

3.2.0 COC = chain of custody documentation

3.2.1 Cooler bottles = small HDPE or polypropylene bottles filled with PFAS-free water that may be used to monitor the temperature of coolers during sample collection and transport (see sections 6.1.2.0 and 6.1.2.1).

3.2.2 ETFE = ethylene tetrafluoroethylene

3.2.3 FEP = fluorinated ethylene propylene

3.2.4 HDPE = high-density polyethylene

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

3.2.6 LDPE = low-density polyethylene

3.2.7 PCTFE = polychlorotrifluoroethylene

3.2.8 PFA = perfluoroalkoxy alkanes

3.2.9 PFAS = per- and polyfluoroalkyl substances

3.2.10 PFPE = perfluoropolyethers

3.2.11 PPE = personal protective equipment

3.2.12 PTFE = polytetrafluoroethylene

3.2.13 PVDF = polyvinylidene fluoride

3.2.14 SDS = safety data sheet

3.2.15 PCPs = sun and insect protection and personal care products

4.0 SAFETY

4.1 At minimum, powder-free nitrile gloves must be worn at all times while handling samples and equipment.

- 4.1.0** Gloves must be changed often to prevent accidental sample contamination.
- 4.2** Read the SDSs for safe reagent and sample handling.
- 4.3** Report any injury, no matter how small, to management.

5.0 AQUEOUS SAMPLE COLLECTION INSTRUCTIONS

5.1 All bottle ware, blank sample labels, blank Field COC forms, and PFAS-free water (to be used for field, trip, and equipment blank samples) will be provided by DEL.

5.1.0 Do not allow sample labels to contact water samples during sample collection. Instead, fill all necessary sample bottles at a sampling location and affix the lids. Then, once all sample bottle lids are secure, affix sample labels to the outside of each bottle. This reduces the risk of potential PFAS-contamination from label material or adhesives.

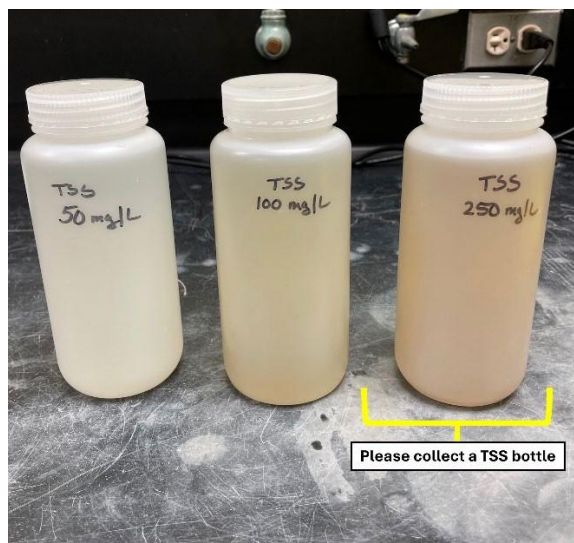
5.1.1 Two, 500 mL samples are collected at each sampling site in HDPE bottles with unlined HDPE or polypropylene caps. One bottle is the primary sample, and the other is a backup sample.

5.1.1.0 **Do not fill bottles past the shoulder.** This will allow room for sample expansion when frozen. See section 5.1.3.0. – 5.1.3.4. for pictures of proper sample filling levels and bottle ware.

5.1.1.1 An additional set of sample bottles (as pictured in section 5.1.3.1) are collected for duplicate samples, when necessary.

5.1.2 In addition, one 125 mL or 250 mL sample may be collected at a site for possible suspended solids determination (a TSS bottle). Collection of a TSS bottle is only necessary if PFAS samples appear to contain > approximately 100 mg/L of suspended solids.

5.1.2.0 Examples of approximate TSS content in PFAS sample bottles.

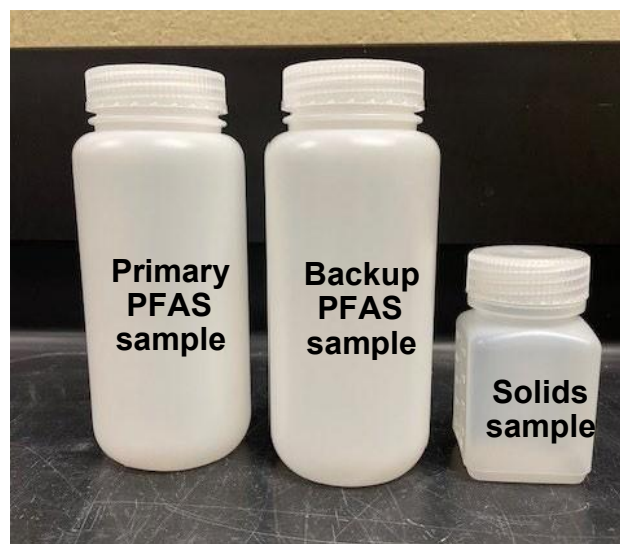


5.1.3 Examples of proper PFAS sample fill-level and bottle ware:

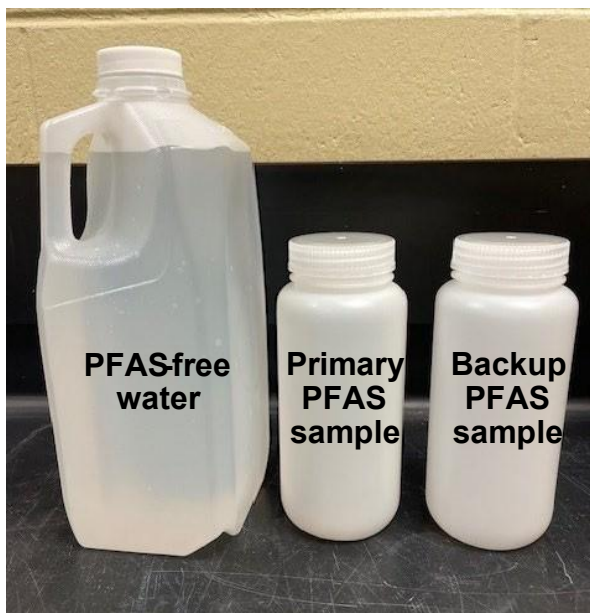
5.1.3.0 Proper sample fill-level is up to the bottle's shoulder (indicated below).



5.1.3.1 Bottle ware required for a typical field sample: two 500 mL PFAS sample bottles and, if necessary, one smaller bottle for suspended solids determination



5.1.3.2 Bottle ware required for one Field or Equipment Blank Sample: HDPE container of PFAS-free water and two 500 mL PFAS sample bottles



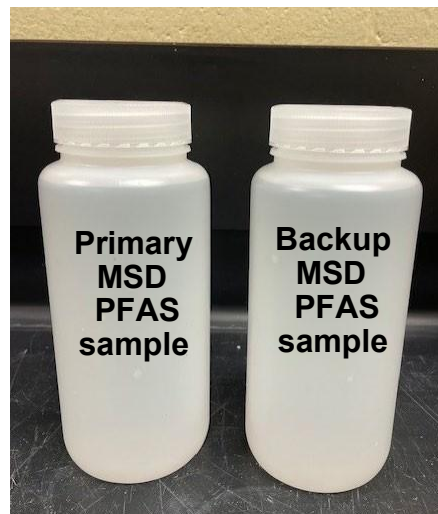
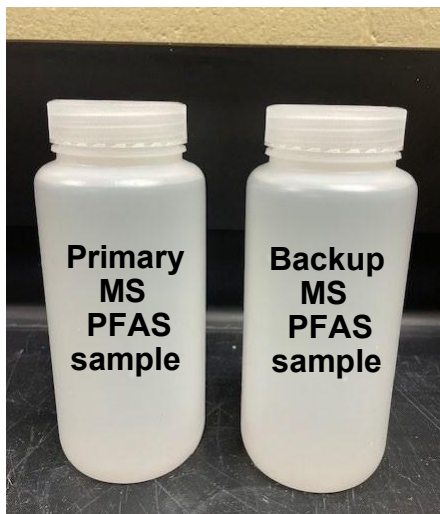
- Fill both PFAS sample bottles with the PFAS-free water **in the field**.
- Do not fill past each bottle's shoulder (see Section 5.1.3.0).
- Additional bottles and PFAS-free water will be provided by DEL if more than one Field or Equipment Blank Sample will be collected.

5.1.3.3 Bottle ware required for one Trip Blank Sample: Two 500 mL PFAS sample bottles pre-filled with PFAS-free water in the laboratory.



- **Do not open these bottles in the field.** These bottles will be transported to and from the sampling site(s), only.
- **Note: A Trip Blank Sample may not be required for every project or sampling event.** Check with project manager for clarification.

5.1.3.4 Bottle ware required for the Matrix Spike and Matrix Spike Duplicate (MS/MSD) Samples: Requires a total of four 500 mL PFAS sample bottles prefilled with PFAS-free water



- **Note: MS/MSD Samples may not be required for every project or sampling event.** Check with project manager for clarification.
- The original field sample collected with the Matrix Spike and Matrix Spike Duplicate samples must be identified on the Field COC.

5.1.4 DEL will provide a blank Field COC form with necessary bottle ware for each sampling event. Controlled document [24373](#) is the official blank DEL Field COC that will be provided.

5.1.4.0 The Field COC must be completed in ink prior to releasing samples to DEL. It is the responsibility of the sample collector(s) to complete this form.

5.1.4.1 DEL laboratory staff will confirm that all necessary information is present on the Field COC before accepting samples for analysis.

5.1.5 The U.S. Environmental Protection Agency (EPA) recommends collecting grab samples for PFAS analysis when possible.

5.1.5.0 When collecting surface water samples, collect grab samples from below the water surface.

5.1.5.1 For hard-to-reach locations, use stainless steel clamps or cable ties to attach sample bottles to a metal or wooden pole.

6.0 TRANSPORT AND STORAGE

6.1 Samples must be transported in coolers with regular ice at all times until delivered to DEL. Ice must be separated from the sample bottles as follows:

6.1.0 Place regular ice in zippered storage bags to prevent possible leaking of meltwater into sample bottles.

6.1.1 Alternatively, place sample bottles in zippered storage bags to keep them separated from loose ice in coolers.

- 6.1.2** Samples must be transported on regular ice until received by DEL. If ice is not present in coolers at the time of receipt, this will be noted on the Field COC and the Project manager will be notified.
- 6.1.2.0** Cooler bottles may be used to monitor the temperature of the coolers. If used, these bottles must remain in the coolers and on ice during sample collection and transport.
- 6.1.2.1** If used, the temperature of the cooler bottles will be measured by DEL staff upon delivery to DEL. Project managers will be notified if the measured temperatures of cooler bottles is $> 6^{\circ}\text{C}$ when delivered to DEL.
- 6.1.2.2** Once received by DEL, samples are stored refrigerated at $\leq 6^{\circ}\text{C}$ for up to 5 days following the collection date. This allows for suspended solids assessments and, ideally, sample extraction to occur in a timely manner.
- 6.1.2.3** Samples that are not extracted within 5 days of collection will be moved to frozen storage at $\leq -20^{\circ}\text{C}$ until analysis.
- 6.1.3** The “Relinquished By” and “Received By” signatures, dates, and times must be completed on the Field COC each time the samples change hands before delivery to the DEL. This includes when the samples are relinquished to and accepted by DEL.

7.0 SAMPLING PRECAUTIONS TO AVOID CONTAMINATION

7.1 PFAS may be present in materials typically used during field sample collection, which can risk sample contamination. The following are precautions that must be taken to reduce risks of sample contamination during field collection. (These precautions are summarized in Table 1):

- 7.1.0** Sampler(s) must wear powder-free nitrile gloves while collecting samples. Gloves must be changed between sample sites and each time they contact materials or surfaces that could contain PFAS.
- 7.1.1** Surfaces of sampling equipment that directly contact aqueous samples during collection and transport must not contain polymers or fluoropolymers that could potentially leach PFAS or otherwise interfere with PFAS measurements. Use sampling equipment made of stainless steel, silicone, HDPE, and/or polypropylene.
- 7.1.2** Do not use Decon 90 to decontaminate sampling equipment and coolers. Instead, use Alconox®, Liquinox®, or Citranox® to wash these items and then rinse them thoroughly with clean water.
- 7.1.3** Do not use blue ice packs to maintain the temperature of PFAS samples in the field or during transport to the DEL. Regular ice must be used and kept separate from PFAS samples per sections 6.1.0 and 6.1.1.
- 7.1.4** Field clothing and equipment can contaminate PFAS samples. Therefore, it is important to avoid contacting sampling surfaces with clothing or other equipment.
- 7.1.4.0** Samplers must not wear any PFAS-treated water-resistant or stain resistant field clothing or clothing laundered with fabric softeners.
- 7.1.4.1** Samplers must choose well-washed clothing and footwear made of PFAS-free materials. However, this does not apply to PPE if PFAS-free alternatives are not available. Sampler’s safety must be prioritized.

7.1.4.2 Samplers must not use waterproof field notebooks, plastic clipboards, sticky notes, Sharpies® or other permanent markers while sampling. Instead, use ballpoint pens to record field data and sample-related information in the field.

7.1.5 Sun and insect protection and personal care products (PCPs) can also contain PFAS, however eliminating sunscreen and insect repellent use would be a safety hazard.

7.1.5.0 Where possible, choose sunscreens and insect repellents that do not have significant PFAS background (see section 7.1.6). PCPs should not be applied in the direct sampling area, and areas that have contacted PCPs should not be allowed to contact sampling equipment or sample surfaces.

7.1.6 The “DNREC Emerging Contaminants Sampling Work Plan Focusing on PFAS” (<https://documents.dnrec.delaware.gov/dwhs/remediation/Statewide-PFAS-in-PWS-WorkPlan.pdf>) contains further guidance to avoid contamination while PFAS sampling.

7.2 *TABLE 1 – Unacceptable and acceptable items for PFAS sample collection*

UNACCEPTABLE ITEMS	ACCEPTABLE ITEMS
Sampling Equipment: Surfaces that Directly Contact Samples	
Polytetrafluoroethylene (PTFE) or Teflon™	Stainless steel
Low-density polyethylene (LDPE)	Cellulose acetate
Ethylene tetrafluoroethylene (ETFE) or Tefzel™	Silicone
Polyvinylidene fluoride (PVDF)	High-density polyethylene (HDPE)
Polychlorotrifluoroethylene (PCTFE)	Polypropylene
Fluorinated ethylene propylene (FEP)	
Perfluoroalkoxy alkanes (PFA)	
Perfluoropolyethers (PFPE)	
Non-Sampling Field Equipment	
Waterproof field books	Non-waterproof loose-leaf paper
Plastic clipboards, binders, or spiral notebooks that may have a water-resistant coating	Masonite or aluminum clipboards
Sharpies® or similar indelible markers	Ballpoint pens
Post-it® notes	
Field Clothing and Protective Gear	
Water- or stain-resistant PFAS-treated field clothing and shoes	Well-washed clothing
Clothing washed with fabric softeners	Clothing made from PFAS-free materials, such as cotton

GORE-TEX® or coated Tyvek® (unless PFASfree alternative PPE are not available - see section 7.1.4)	Rain gear made from polyurethane or wax-coated materials
Do not apply sun and insect protection and personal care products (PCPs) within the sampling area	Powder-free nitrile gloves changed often during sample collection
Areas in contact with PCPs must not contact sampling equipment or sample surfaces	Sun and insect protection and personal care products (PCPs) recommended in section 7.1.6
Sample Containers and Transport	
UNACCEPTABLE ITEMS	ACCEPTABLE ITEMS
Glass sample containers	DEL-supplied plastic containers, such as polypropylene or HDPE
Teflon™- or PTFE-lined caps	Unlined polypropylene or HDPE caps
blue ice packs	Regular ice use zippered plastic storage bags to separate ice from samples (see sections 6.1.0 – 6.1.1)
Decontamination	
Decon® 90	Alconox®
	Liquinox®
	Citranox®
	Rinse with PFAS-free water
Food and Drink	
Eating or handling pre-wrapped food, such as candy bars, granola bars, and microwave popcorn, is prohibited in sampling area	Wash hands thoroughly before handling sampling equipment, bottle ware, and samples

8.0 REFERENCES

8.1 DNREC, 2024. PFAS Sample Preparation and Analysis Standard Operating Procedure (DEL Controlled Document [29867](#)).

8.2 DNREC, 2022. Emerging Contaminants Sampling Work Plan Focusing on PFAS. Department of Natural Resources and Environmental Control Division of Waste and Hazardous Substances Remediation Section, Revision 1.5.

(<https://documents.dnrec.delaware.gov/dwhs/remediation/Statewide-PFAS-in-PWS-WorkPlan.pdf>)

8.3 USEPA, 2024. Method 1633A: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS.
(<https://www.epa.gov/system/files/documents/2024-12/method-1633a-december-5-2024508-compliant.pdf>)

A.2. SOP for PFAS Sample Preparation and Analysis by LC-MS/MS at DNREC DEL Laboratory

The following SOP is reproduced as provided by the laboratory, including its original section and appendix numbering.



Delaware Environmental Laboratory
60 Sunnyside Road, Smyrna, DE 19777

PFAS SAMPLE PREPARATION AND ANALYSIS BY LC-MS/MS

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PFAS SAMPLE PREPARATION AND ANALYSIS BY LC-MS/MS

1.0 SCOPE AND APPLICATION

- 1.1 This method is used to prepare non-potable aqueous (groundwater, surface water, and wastewater) samples for analysis of Per- and Polyfluoroalkyl Substances (PFAS) by liquid chromatography-tandem mass spectrometry (LC-MS/MS). This procedure is based on EPA Method 1633A (published December 2024).

2.0 SUMMARY

- 2.1 To determine the types and concentrations of PFAS analytes, aqueous samples are prepared by solid-phase extraction (SPE) with carbon cleanup. Isotopically labeled PFAS-compounds are added to samples prior to and after extraction to enable PFAS quantitation based on isotope dilution or extracted internal standard methods.

3.0 INTERFERENCES

- 3.1 Any reagent, glassware, supplies, solvent, tool and/or instrument used in this procedure may contain PFAS or other interferences that can cause misinterpretation of analytical results. The following outlines different types of interferences and the corresponding steps taken to account for or limit them:

Interference	Treatment or Prevention
Interferences may come from glassware, supplies, solvents, and/or reagents potentially containing PFAS.	PFAS can adhere to glass. Avoid glass containers and fluorinated plastic materials when possible. Instead, use supplies made of polypropylene (PP) and high-density polyethylene (HDPE). Any glassware that is used must be solvent rinsed and heat treated at 450-500°C before use (See Section 12.1.2 for details). Appropriate solvents, reagents, and supplies are specifically selected based on a lack of detectable PFAS concentrations. See Section 6.0 and 7.0 for equipment, supplies, and reagents demonstrated to contain low PFAS background content. Any equipment, supply, or reagent not included on these lists must be evaluated before use to ensure they do not contribute detectable levels of PFAS (> MDL) during sample preparation.
Interferences may come from the automated SPE instrumentation.	The instrument and its elution lines must be cleaned prior to and after use. See Section 12.4.2 for SPE system and in-line filter cleaning procedures.
Interferences may come from the glass wool used during the SPE procedure	Silanized glass wool must be solvent rinsed and then stored in Purge & Trap grade methanol before use. See Section 12.1.1 for details. Alternatively, multilayer frits that do not require pre-cleaning may be used in place of glass wool.

Interferences from sample matrices will vary for different sources, depending on site diversity, and may be greater than the signal produced from PFAS.	To eliminate matrix interferences prior to analysis, all samples must be processed using solid-phase extraction (SPE), and the extracts must be cleaned using graphitized carbon black (GCB) or an equivalent carbon cleanup. The SPE cartridges specified for use in this SOP contain GCB. These cartridges allow PFAS extraction and sample cleanup to occur in a single step (see Section 6.2) and have achieved the QC specifications of EPA Method 1633A.
Interference	Treatment or Prevention
Taurodeoxycholic Acid (TDCA), a bile acid, may be present in samples and may interfere with chromatography	A TDCA standard must be included in each analytical batch to demonstrate that this compound is chromatographically separated from PFOS by at least 1 minute and does not interfere with any other PFAS analyte.

TABLE 1. Potential Interferences

4.0 ABBREVIATIONS, TERMS, AND DEFINITIONS

- 4.1 Controlled document [18356](#) provides a master list of terms and definitions.
- 4.2 Controlled document [1816](#) provides a master list of abbreviations and definitions.
- 4.3 Additional abbreviations, terms, and definitions that are specific to this method appear in the following table.

Abbreviation/Term	Definition
ASVs	Autosampler vials
Cat #	Catalog number
°C	degrees Celsius
COA	Certificate of Analysis
CC/CV/ConCal	Continuing Calibration/Calibration Verification. A mid-level calibration standard analyzed periodically to ensure the continued validity of instrument calibration.
Cu	Cubic
DEL	Delaware Environmental Laboratory
DF	Dilution Factor
Double Blank	Solvent blank without internal standard added. Used to verify LC-MS/MS is clean.
DNREC	Delaware Department Natural Resources and Environmental Control
e.g.	Exempli gratia, for example

EIS	Extracted Internal Standards (sometimes referred to as Surrogates). Isotopically labeled PFAS that are added to the samples before extraction for analysis. The EIS are used to quantify PFAS analytes in a sample.
EPA	U.S. Environmental Protection Agency
ESI	Electrospray Ionization
Abbreviation/Term	Definition
GCB	Graphitized Carbon Black
HDPE	High-Density Polyethylene
hr	Hour
IAR	Ion Abundance Ratio. The peak area ratio of qualitative (secondary) and quantifier (primary) ion transitions of an analyte. Value is used to qualitatively identify analytes that produce more than one ion transition when analyzed by LC-MS/MS
ID #	Identification Number
i.e.	Id est, that is
IPR	Initial Precision and Recovery (see IDOC in Controlled document 18356)
IPA	Isopropanol
ISC	Instrument Sensitivity Check (referred to as CCVI in instrument worklists)
LC	Liquid Chromatograph/Chromatography
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry (here a UHPLC Triple Quadrupole or UHPLC-QQQ)
L	Liters
LLOPR	Low Level Ongoing Precision and Recovery. See definition of OPR. The LLOPR is prepared at approximately twice the concentration of the LOQ.
M	Molarity
MB	Method Blank
MS	Mass Spectrometer/Spectrometry
mg	Milligrams
P & T	Purge & Trap
μM	Micromolar
μL	Microliter

mm	Millimeters
mL	Milliliters
ML	Minimum Level of Quantification. This value is also equivalent to the “reporting limit”. It is calculated by multiplying the calibration standard at the LOQ by the Multiplier value of the extraction (typically 0.01 for a 500 mL sample volume).
min	Minute(s)
Abbreviation/Term	Definition
m/z	Mass to Charge Ratio
NIS	Non-Extracted Internal Standard. Isotopically labeled PFAS that are added to the samples before LC-MS/MS analysis but are not taken through sample preparation (extraction) procedures. The NIS are used to calculate recoveries of the Extracted Internal Standard (EIS).
OPR	Ongoing Precision and Recovery. A Blank Spike sample containing all PFAS analytes, EIS, and NIS at known concentrations. Two OPR samples are prepared with each extraction batch: one containing all PFAS analytes at a mid-level concentration (OPR sample) and one containing all PFAS analytes at approximately twice the LOQ (LLOPR sample).
PFAS	Per- and Polyfluoroalkyl Substances
P/N	Part Number
PP	Polypropylene
Prime	Priming Gradient. Runs the mobile phase gradient through the LC-MS/MS without simultaneously injecting a sample or standard. This provides a measure of instrument background, cleans the analytical column, and equilibrates the column prior to starting a worklist.
RT	Retention Time
SDS	Safety Data Sheets
S/N	Signal to Noise Ratio. The height of an analyte’s chromatographic peak (“signal”) divided by average height of a section of baseline away from the peak (“noise”).
SPE	Solid-phase Extraction
STD	Standard
SOP	Standard Operating Procedure
TDCA	Taurodeoxycholic Acid. A bile acid that is a potential interference in this analysis.
TSS	Total Suspended Solids
QQQ	Triple Quad Mass Spectrometer

UHPLC	Ultra-high-performance liquid chromatography
WAX	Weak Anion Exchange. Used to describe the solid-phase extraction chemistry used in this method.
WS	Working Stock
YYMMDD	Numerical date abbreviation consisting of the two-digit year, two-digit month, and two-digit day. Often used as analytical worklist or batch file names.

TABLE 2. Method-specific Abbreviations, Terms, and Definitions

5.0 SAFETY

- 5.1 Review the (19310) Safety, Waste and Chemical Hygiene Manual.
- 5.2 Safety glasses, nitrile gloves and lab coats must be worn at all times while handling samples, stocks, standards, and reagents.
- 5.3 Read the Safety Data Sheets (SDS) information for safe chemical, reagent, and sample handling.
- 5.4 Report any injury, no matter how small, to management.
- 5.5 Personnel conducting any or all the procedures described in this document must also review and follow the safety requirements and precautions described in section 5.0 of EPA Method 1633A.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Sections 6.2 and 6.3 list examples of equipment and supplies demonstrated to contain low PFAS background content. Equivalent equipment or supplies not included on these lists must be evaluated before use to ensure they do not contribute detectable levels of PFAS (> MDL) during sample preparation.

6.2 Equipment:

Instrument	Manufacturer	Model
Automated Solid-Phase Extraction System	Promochrom	SPE-03 with MOD-004 for PFAS and MOD-00P for large-volume samples
Ultrasonic Bath	Fisher Scientific	FS60
Muffle Furnace	Barnstead/Thermolyne	30400
Vortexer	ThermoFisher Scientific	Cat. No. 12-812
TurboVap LV Automated Evaporator	Biotage	415000
TurboVap LV Multi Rack (48 positions, 10-20 mm tubes)	Biotage	414964
Ultrapure Water (≥ 18.2 Mohms) System	Millipore Sigma or equivalent	SYNSVR000
Water Purification Cartridge	Millipore Sigma or equivalent	SYPKOSIX2
Freezer capable of maintaining $\leq -20^{\circ}\text{C}$	Various	Various

Refrigerator capable of maintaining $\leq 6^{\circ}\text{C}$	various	Various
Top-loading Balance	Mettler Toledo or equivalent	PR5002
Analytical Balance	Mettler Toledo or equivalent	XPE205DR
ISO/IEC 17025 Certified Class 1 Weights (10 mg - 1000 g)	Troemner or equivalent	Various
Triple Quadrupole Mass Spectrometer with MassHunter Analytical Software Package	Agilent	G6470B
Ultra-High Performance Liquid Chromatograph (modified to be PFAS -free)	Agilent	1290 Infinity II UHPLC
Nitrogen Gas Generator	Peak Scientific	Genius XE

TABLE 3. Equipment

6.3 Supplies:

Supplies/Consumables	Manufacturer/Vendor	Part Number	Notes
Borosilicate Laboratory Glassware (various sizes)	Various	Various	

Supplies/Consumables	Manufacturer/Vendor	Part Number	Notes
Class A Volumetric Flasks (various sizes)	Various	Various	Class A volumetric flasks will not be placed in the muffle furnace.
HDPE Screw-cap Bottles with HDPE or PP Closure (various sizes)	Various	Various	
Leak-resistant Wide-mouth HDPE Bottles with PP linerless cap (500 mL)	Wheaton or equivalent	209549	
Weighing Paper	Various	Various	
Silanized Glass Wool	MilliporeSigma Supelco or equivalent	20411	Fisher Scientific Cat#: 11-102-0915
Strata PFAS(WAX/GCB) SPE Cartridges (200 mg/50 mg/6 mL) or equivalent	Phenomenex or equivalent	CSO-9207	
Multilayer Frits	Promochrom	CF-06	
Promochrom High Capacity In-Line Filters	Promochrom	F-HC-30	
Anti-clogging Tips	Promochrom	F-T-M	
Centrifuge Tubes (15 mL, PP)	Falcon or equivalent	352196	
25-mm Syringe Filter, 0.2 μm Nylon Membrane	Cytiva Pall Corp. or equivalent	6750-2502 4436T	

Polypropylene Autosampler Vials with Low-Volume Inserts	FisherBrand	13-622-124	
Polypropylene 2 mL Vials	Agilent Fisherbrand	5191-8121 02-681-344 (microcentrifuge tubes) 02-681-366 (caps w/ O-rings) or equivalent	Agilent autosampler vials cannot be used to store stocks and standards.
Polypropylene Autosampler Vial Caps (9mm) with Thin Membrane Polypropylene/Silicone Septa	Agilent	5191-8151	
Borosilicate Glass (type 1) GL 45 Bottle	DWK Life Sciences or equivalent	218813653 (250 mL clear) 218865453 (1000 mL amber) 218815457 (1000 mL clear) or equivalent	
GL45 Polypropylene Screw Cap	Various	Various	
Autopipettes (pipettors) and Pipette Tips (various sizes)	Eppendorf or equivalent	various	Tips must be verified to be free of interferences
Syringe, 5mL Polypropylene, Luer Lock	ThermoFisher Scientific	50-872-581	
Indicator Paper pH 4.5-10, 0.5 Unit Readability	Whatman® or equivalent	0987618	
Gastight Syringes (25 µL – 1000 µL)	Hamilton or equivalent	Various	
Supplies/Consumables	Manufacturer/Vendor	Part Number	Notes
Polyethylene Transfer Pipets	Fisher	13-711-9AMMD	
Weigh Paper	Fisherbrand	09-898-12A	
Analytical UHPLC Column: ZORBAX Eclipse Plus C18 RRHD (2.1 x 50 mm, 1.8 µm)	Agilent	959757-902	
UHPLC Guard Column: ZORBAX Eclipse Plus C18 RRHD (2.1 x 5 mm, 1.8 µm)	Agilent	821725-901	
PFC Delay Column: InfinityLab (4.6 x 30 mm)	Agilent	5062-8100	

TABLE 4. Supplies

7.0 REAGENTS

- 7.1 Ultrapure water (≥ 18.2 Mohms): This water is produced by subjecting City of Smyrna tap water to reverse osmosis treatment followed by cation and anion

exchange. The water is then pumped to the laboratory and filtered through a mixed bed ion exchange resin.

7.1.1 Ultrapure water is used as the matrix for quality control (QC) samples prepared under this method. Detected PFAS concentrations in ultrapure water must be < MDL.

7.1.2 Ultrapure water is also used throughout the sample extraction procedure (as “Solvent 2” on the SPE-03 system) and as a constituent of some prepared reagents.

7.2 Purchased Chemicals:

7.2.1 Identifying information for any chemical purchased from an outside vendor must be recorded in the Chemical Logbook and assigned a Chemical Identification Number (ID #) when opened. Chemical Identification Numbers associated with this SOP begin with “CO” followed by the two-digit year, a dash, and then sequentially numbered beginning with 001. For example, the first Chemical ID # assigned in 2021 would be “CO21-001”. For traceability, the Chemical ID # must be written on the chemical bottle or label when assigned. The following table lists examples of chemicals used in this SOP. Alternative grades or chemicals purchased from other vendors must be evaluated before use to ensure they do not contribute detectable levels of PFAS (> MDL) during sample preparation or analysis.

Chemicals	Vendor	Grade	Part Number
Methanol	VWR or equivalent	LC-MS grade (for cleaning glassware and equipment)	BDH85800.400
Methanol	ThermoFisher Scientific or equivalent	Purge & Trap Grade	A453-1
Acetonitrile	ThermoFisher Scientific or equivalent	LC-MS grade	A955-4
Ammonium Hydroxide (28-30%)	VWR	ACS Grade	BDH3014-500MLP
Ammonium Acetate (store in desiccator)	ThermoFisher Scientific, MilliporeSigma or equivalent	LC-MS grade	A11450 or 01-100-266
Formic Acid (99%)	Thermo Scientific or equivalent		270480250
Glacial Acetic Acid	VWR ARISTAR® PLUS	Trace Metal grade	87003-239
Chemicals	Vendor	Grade	Part Number
			Note: Fisher A113-50 cannot be used for this method
Taurodeoxycholic Acid (store in desiccator)	ThermoFisher Scientific		58-022-15GM
Isopropanol (for cleaning glassware and equipment)	ThermoFisher Scientific or equivalent	LC-MS grade	A464-4
Isopropanol (CHROMASOLV)	Honeywell or equivalent	LC-MS grade	34965-1L

Nitrogen Gas, compressed	Linde		
ESI-Low Low Concentration Tuning Mix Mass Calibrant Solution	Agilent		G1969-85000

TABLE 5. Chemicals

7.3 Prepared Reagents:

7.3.1 Any reagent prepared in the laboratory must be recorded in the Reagent Logbook and assigned a Reagent ID #. Reagent ID #s associated with this SOP begin with “RO” followed by the two-digit year, a dash, and then sequentially numbered beginning with 001. For traceability, the Reagent ID # must be written on the reagent label when assigned.

7.3.2 Reagents are prepared and/or stored in non-fluorinated plastic containers (HDPE or PP) or glass containers that have been properly cleaned to avoid PFAS cross-contamination (see Section 12.1.2 Glassware Cleaning Procedures).

7.3.3 Below is a list of the prepared reagents used in this method. Preparation instructions for these reagents are included in Appendix A. Note that the volumes of the individual constituents and the total volume of each solution prepared may differ, so long as the ratio of the constituents remains the same as described in this SOP.

7.3.4 Sample Preparation Solutions:

- 7.3.4.1 1% ammonium hydroxide in LC-MS grade methanol = for glassware and Promochrom SPE-03 cleaning (referred to as “Solvent 1” in SPE-03 methods)
- 7.3.4.2 1% ammonium hydroxide in P & T grade methanol = for extraction (referred to as “Solvent 5” in SPE-03 methods)
- 7.3.4.3 3% ammonium hydroxide = sample pH adjustment
- 7.3.4.4 5% formic acid = sample pH adjustment
- 7.3.4.5 0.3M formic acid = SPE cartridge conditioning (referred to as “Solvent 3” in SPE-03 methods)
- 7.3.4.6 0.1M formic acid = used to prepare the SPE cartridge rinse solution
- 7.3.4.7 1:1 0.1M formic acid: P & T grade methanol = SPE cartridge rinse solution (referred to as “Solvent 4” in SPE-03 methods)
- 7.3.4.8 1633 Diluent = for standards preparation: methanol + 4% ultrapure water + 1% ammonium hydroxide + 0.625% acetic acid (See EPA 1633A Section 7.1.9)

7.3.5 LC-MS/MS Solutions:

- 7.3.5.1 1633 Mobile Phase A = 2 mM ammonium acetate in 95% ultrapure water + 5% acetonitrile (See EPA 1633A Section 7.1.11)

- 7.3.5.2 1633 Mobile Phase B = acetonitrile (LC-MS grade).
- 7.3.5.3 Seal Wash = 90% ultrapure water: 10% Isopropanol
- 7.3.5.4 Needle Wash = P & T grade methanol

8.0 STOCKS

8.1 Purchased Stocks:

8.1.1 Identifying information for any stock solutions purchased from an outside vendor must be recorded in the Chemical Logbook and assigned a Chemical ID # when opened. The stock Certificate of Analysis (COA) must reflect the assigned Chemical ID # and be scanned and saved in document management system. The original COA will be retained in the PFAS COA binder until the stock is discarded or no longer used. It is recommended that purchased stocks are not used if $< \sim 100 \mu\text{L}$ of total volume remains in the container, due to risk of stock concentration increase from solvent evaporation over time.

8.1.2 Purchased stocks received in flame-sealed ampules will be transferred to microcentrifuge tubes after opening. All purchased stocks will be stored at $\leq 6^\circ\text{C}$ after opening.

8.1.3 Purchased stocks used in the conduct of this method:

Purchased Stock ID	Manufacturer	Stock Description
Isotopically Labeled Internal Standards		
MPFAC-HIS-IS	Wellington Laboratories, Inc.	Mass-Labeled PFAS Injection Standard Solution/Mixture
MPFAC-HIS-ES	Wellington Laboratories, Inc.	Mass-Labeled PFAS Extraction Standard Solution/Mixture
MFHUEA	Wellington Laboratories, Inc.	2H-Perfluoro-2-(1,2- $^{13}\text{C}_2$)octenoic Acid
CLM-11324-A	Cambridge Isotope Laboratories	$^{13}\text{C}_3$ -Perfluoropropanoic Acid
Native PFAS		
PFAC-MXF	Wellington Laboratories, Inc.	Replacement PFAC Solution/Mixture
Purchased Stock ID	Manufacturer	Stock Description
PFAC-MXI	Wellington Laboratories, Inc.	Native N-Me/EtFOSA and N-Me/EtFOSE Solution/Mixture
PFAC-MXJ	Wellington Laboratories, Inc.	Native X:3 Fluorotelomer Carboxylic Acid Solution/Mixture

PFAC-MXH	Wellington Laboratories, Inc.	Native PFAS Solution/Mixture
PFAC-MXG	Wellington Laboratories, Inc.	Native Perfluoroalkyl Ether Carboxylic Acids and Sulfonate Solution/Mixture
FHUEA	Wellington Laboratories, Inc.	2H-Perfluoro-2-octenoic Acid
ULM-11231-S	Cambridge Isotope Laboratories	7H-Perfluoro-4-Methyl-3,6 Dioxaoctanesulfonic Acid (Nafion BP2)
ULM-11223-S	Cambridge Isotope Laboratories	Perfluoro-2-Methoxyacetic Acid (PFMOAA)
ULM-11226-S	Cambridge Isotope Laboratories	Perfluoro(3,5-Dioxahexanoic) Acid (PFO2HxA)
ULM-11323-A-S	Cambridge Isotope Laboratories	Perfluoropropanoic Acid (PFPrA)

TABLE 6. Purchased Stocks

9.0 WORKING STOCKS:

- 9.1 Working stocks prepared in the laboratory must be recorded in the Reagent Logbook and assigned a Reagent ID # (see Section 7.3.1). Working stocks are assigned expiration dates of no more than one year from the date of preparation or are determined by the earliest expiration date of a given component. Stocks are not used after the assigned expiration date, unless extended stability is confirmed.
- 9.2 All working stocks are prepared in non-fluorinated plastic containers, typically 2 mL PP microcentrifuge tubes or 15 mL PP centrifuge tubes. Use autopipettors with plastic pipette tips or gastight syringes to aliquot the constituents of each working stock. Add the dilution solvent (i.e. methanol or isopropanol) to the empty container first, followed by the remaining constituents. Cap the container, and vortex to mix well after preparation.
- 9.3 All working stocks will be stored at $\leq 6^{\circ}\text{C}$, unless required otherwise to maintain stability. Working stocks must be brought to room temperature and vortexed prior to each use. It is recommended that working stocks are not used if $< \sim 100\ \mu\text{L}$ of total volume remains in the container, due to risk of stock concentration increase from solvent evaporation over time.
- 9.4 Refer to Appendix B for a list of the working stocks used in this method and preparation instructions. Note that the volumes of the individual constituents and the total volume of each working stock may differ, so long as the ratio of the constituents remains the same as described in this SOP.

10.0 CALIBRATION AND STANDARDIZATION**10.1 Equipment Calibration and Standardization**

- 10.1.1 If auto-pipettes are used, confirm these have been calibrated within at least 12 months from current use. If necessary, submit for re-calibration before use.

10.1.2 If a balance is used, check its calibration status before use and perform a calibration check using verified weights. Document the balance calibration check in the appropriate logbook.

10.1.3 Annual maintenance and verification of the Promochrom SPE-03 automated extractor system should be conducted according to the SPE-003 (MOD-00P) Maintenance Manual.

10.2 LC-MS/MS Instrument Mass Calibration and Verification

10.2.1 Mass Calibration (referred to as “Autotuning” by Agilent) ensures the mass spectrometer assigns accurate mass to charge (m/z) values to ions, based on the ESI-L Low Concentration Tuning Mix) calibrant solution. An Autotune must be run in both negative and positive mode at least once annually. Additional Autotunes should be run in at least negative mode after instrument venting or cleaning and if a mass calibration verification (Checktune) fails acceptance criteria.

10.2.2 Mass Calibration Verification (referred to as “Checktuning” by Agilent) ensures the mass spectrometer calibration settings are still accurate, based on the ESI-L Low Concentration Tuning Mix. An acceptable Checktune must be run in at least negative mode each day samples are analyzed, prior to sample analysis. A new Autotune (Mass Calibration) should be run if the Checktune fails acceptance criteria.

10.2.3 All Autotune and Checktune results files are automatically saved on the LCMSMS1 instrument PC on the Data (D:) drive. Save a copy of each Autotune file with an equipment maintenance log entry in the document management system.

10.3 Standard Solutions for LC-MS/MS Analysis

10.3.1 Prepare all standard solutions (“standards”) in 1633 Diluent (See Section 7.3.4.8). Assign an expiration date (typically one month from preparation). The expiration date of standards may exceed the expiration date of the 1633 Diluent if the standards are stored refrigerated. Record preparation of standards in the PFAS Standards Log (Controlled Document [29314](#)).

10.3.2 Calibration standard concentrations are listed in Section 20.0, Table 12.

10.3.3 Standards acceptance criteria are described in Section 15.0. Corrective action instructions are described in Section 16.0.

10.3.4 Calibration Standard Solutions

10.3.4.1 Prior to sample analysis, prepare and analyze eight levels of calibration standards, each containing all PFAS analytes and internal standards, and one instrument blank containing only the internal standards. Preparation instructions for calibration standards and the instrument blank are described in Appendix C. Refer to Sections 7.2.1, 7.3, and 8.1.3 for details regarding standard constituents.

10.3.5 Calibration Verification Standard Solution

10.3.5.1 A mid-level calibration standard is analyzed to verify adequate instrument calibration: at the beginning of each analytical sequence before samples are analyzed, after the analysis of every ≤ 10 samples, and at the end of the analytical sequence.

10.3.5.2 Typically, calibration standard Level 5 is injected as the calibration verification standard (also referred to as a continuing calibration check or CC in some software).

10.3.5.3 A separate mid-level standard may be prepared from secondary sources to serve as the calibration verification standard. In this case the calibration verification standard would be assigned a unique identifier based on the preparation date (YYMMDD) followed by the abbreviation “-CV”. Preparation instructions can be added by appendix to this SOP.

10.3.6 Qualitative Identification Standard Solution

10.3.6.1 The qualitative identification standard solution is a mixture of linear and branched PFAS isomers that are not present in calibration standards. The qualitative standard is used to set the integration windows to sum the branched and linear isomers of these analytes. It is prepared at a concentration approximately equivalent to the highest calibration standard (Level 8).

10.3.6.2 Record preparation of the qualitative identification standard in the PFAS Standards Log. Preparation instructions are described in Appendix C. Refer to Sections 7.2.1, 7.3, 8.1.3, and 9.4.2 for details regarding standard constituents.

10.3.7 Bile Acid Interference Standard Solution

10.3.7.1 The bile acid interference standard contains TDCA and is used to demonstrate adequate chromatographic separation (≥ 1 min) between TDCA and the earliest eluting branched PFOS isomer in a standard.

10.3.7.2 The bile acid interference standard is prepared at 1 $\mu\text{g/mL}$. This standard must be analyzed after initial calibration and prior to analysis of any samples.

10.3.7.3 Record preparation of the bile acid interference standard in the PFAS Standards Log. Preparation instructions are described in Appendix C. Refer to Sections 7.2.1, 7.3, and 9.4.3 for details regarding standard constituents.

11.0 SAMPLE COLLECTION, SHIPMENT, PRESERVATION, AND STORAGE

11.1 General Sample Collection Requirements for Aqueous Samples

11.1.1 Samples are collected in HDPE containers with linerless HDPE or PP caps. Each lot of sample containers will be confirmed to be PFAS-free by preparing a method blank sample in a representative container from each lot. The PFAS analyte concentrations in the representative container must be lower than the method detection limit ($< \text{MDL}$).

11.1.2 Typical sample volume required for this method is 500 mL. Sample volumes < 500 mL may be collected if the PFAS content of the sample is expected to be above the calibration range or if characteristics of the sample matrix (e.g., high total

suspended solids) will interfere with the extraction. The volume collected must be adequate in meeting regulatory limits (Note: Extracting less than 500 mL of sample will proportionally increase the detection and quantitation limits of the method.). An appropriately sized HDPE container should be used if sample volumes lower than 500 mL are collected.

- 11.1.3 Bottles used for the collection of samples should not be filled past the shoulder. This is to allow for the expansion of ice if the samples are frozen. Unless dilution prior to extraction is required, the entire aqueous sample that is collected will be prepared for analysis, as different analytes may bind to the surface of the container. Subsampling should be avoided.

11.1.3.1 Excess sample may be poured off, if sample bottles are overfilled when received by the laboratory. Samples should be gently mixed to homogenize prior to pouring off excess, however care should be taken to avoid generating foam in the sample. Excess sample will be disposed in the PFAS-containing waste stream unless the sample is a blank.

- 11.1.4 Unless site history dictates otherwise, at least two, but possibly three, aqueous samples should be collected at each location: one 500-mL sample for extraction and analysis, one 500-mL sample for potential re-extraction and analysis (backup bottle), and one smaller volume sample may be collected for pre-screening and total suspended solids determination (TSS bottle).
- 11.1.5 Refer to the Aqueous Sample Collection Instructions for PFAS Analysis by LC-MS/MS SOP ([37277](#)) for additional instructions related to sample collection, transport, and storage.

11.2 Aqueous Sample Shipment

- 11.2.1 Field-collected aqueous samples should be kept at 0-6°C and protected from light. Samples should be stored refrigerated or on ice (not blue ice) until received by the laboratory. The Delaware Environmental Laboratory (DEL) must confirm ice is present or that the cooler temperature is $\leq 6^{\circ}\text{C}$ upon receipt.
- 11.2.2 Once received by the lab, the samples may be stored at 0-6°C (short-term) or at $\leq -20^{\circ}\text{C}$ (long-term) until extraction. The samples should be sent to the laboratory as soon as possible, and holding time will depend on storage temperature (discussed in the next section).

11.3 Aqueous Sample and Extract Holding Time

Aqueous samples should be extracted and analyzed as soon as possible but may be stored refrigerated (0-6 °C) and in the dark for ≤ 28 days after collection (i.e., “short-term storage”). Note that issues have been observed with some PFAS analytes (e.g. perfluorooctane sulfonamide ethanols and perfluorooctane sulfonamidoacetic acids) degrading/transforming into other PFAS analytes after 7 days of refrigerated storage.

- 11.3.1 Aqueous samples may be stored “long-term” (for ≤ 90 days after collection) in the dark at $\leq -20^{\circ}\text{C}$. Thaw frozen field samples prior to extraction.

11.3.1.1 Thawing should be initiated within 48 hours of extraction.

11.3.1.2 Frozen samples can be thawed in a refrigerator set to $\leq 6^{\circ}\text{C}$ for a

maximum of 48 hours prior to extraction.

11.3.1.3 Alternatively, thawing can be initiated by positioning frozen samples upright in baths containing ultrapure water at ambient temperature. Samples should not remain in ambient water for more than 7 hours before being transferred to refrigerated storage at $\leq 6^{\circ}\text{C}$.

11.3.2 Sample extracts should be analyzed as soon as possible but may be stored at $\leq 0^{\circ}\text{C}$ in the dark for up to 90 days. Note that the concentrations of some 11ClPF3OUdS and 9Cl-PF3ONS may increase in the extracts after 28 days.

12.0 SAMPLE PREPARATION PROCEDURE

12.1 Equipment And Supply Cleaning

12.1.1 Glass Wool Cleaning Procedure (Adopted from New Jersey Department of Health Lab)

- 12.1.1.1 This procedure is to be followed when multilayer frits are unavailable.
- 12.1.1.2 Rinse Scissors and metal forceps with LC-MS grade methanol 3 times.
- 12.1.1.3 Cut a sufficient amount of glass wool for the number of columns you will need.
- 12.1.1.4 Take the glass wool and add it to 250 mL glass bottle with HDPE cap. Add ultrapure water, shake well, and dispose of the rinsate. Repeat 3 times.
- 12.1.1.5 After rinsate removal, transfer the wool from the 250 mL glass using cleaned metal forceps into a glass beaker. To the glass beaker add 80% LC-MS grade methanol and 20% ultrapure water mixture to a volume that immerses the glass wool. Cover the top of the glass beaker with aluminum foil.
- 12.1.1.6 Sonicate the glass beaker with the solvent mixture and glass wool for 15 min. After, with metal forceps, turn the glass wool over in the beaker, and sonicate for an additional 15 min. After the sonication, dispose of the methanol water mixture.
- 12.1.1.7 Using a small HDPE bottle to store the clean glass wool. Fill the bottle with P & T methanol for storage. This will help to prevent PFAS contamination.

12.1.2 Glassware Cleaning Procedure

- 12.1.2.1 If necessary, remove visible residues using nylon scrub brushes, and then rinse well with ultrapure water.
- 12.1.2.2 Rinse used glassware multiple times with methanol (LC-MS grade), followed by rinsing multiple times with isopropanol (LC grade), properly discarding each rinsate. Rinse with ultrapure water

multiple times.

- 12.1.2.3 Allow glassware to dry in a fume hood or elsewhere if protected from dust. No solvent should be present on glassware placed in the muffle furnace.
- 12.1.2.4 Place all non-volumetric borosilicate glassware (i.e., beakers, graduated cylinders, bottles) in the muffle furnace. Run the available program to heat to 450 °C and hold for approximately 4 hours.
- 12.1.2.5 Allow glassware to cool and remove from muffle furnace. Rinse the outside bottom of glassware with ultrapure water if necessary. Store protected from dust or other contamination until use.
- 12.1.2.6 Rinse with an appropriate solvent immediately prior to use. If visible residues or contamination remains after standard cleaning, more aggressive cleaning options recommended by Agilent may be required.

12.2 Pre-screening Aqueous Samples for PFAS

- 12.2.1 Samples collected from sites known or suspected to contain high levels of PFAS may be pre-screened prior to extraction. Pre-screening results are used to determine if sample or extract dilutions are necessary to maintain target analytes within the calibration range and prevent contamination of the LC-MS/MS.
- 12.2.2 Sample aliquots for pre-screening should be taken from the third (small volume) sample container. Use a PP syringe and 0.2 µm nylon syringe filter to filter a few mL of sample into a clean 15-mL PP centrifuge tube. Use an auto-pipette to transfer 500 µL of filtered sample to a PP ASV. Then, spike the 500 µL of filtered sample with 20 µL of EIS+NIS WS. Using a pipettor or a glass gastight syringe, rinsed repeatedly with methanol before and after use, to add the spike. Cap and vortex the spiked sample prior to submitting for LC-MS/MS analysis.
- 12.2.3 Measured concentrations of pre-screened samples must be multiplied by a factor of 100 to be compared with nominal concentrations of calibration standard. This takes into account the concentration factor of a 500 mL sample applied during extraction. Measured concentrations in pre-screened samples > 90% of the nominal concentration of the highest calibration standard indicate the sample will require dilution prior to quantitation. Dilution factors can be applied post-extraction, as long as internal standard recovery requirements are met. If extract dilution is not an option, a smaller volume of sample should be extracted from one of the additional sample containers. The need for dilution pre- or post-extraction must be communicated to the client as method detection and quantitation limits will be impacted.

12.3 Solids Content Determination

- 12.3.1 This extraction method should be used for aqueous samples containing approximately < 50 mg of suspended solids per sample volume to be extracted (typically 500 mL) to avoid clogging the SPE-03 extraction equipment. Experienced analysts should be able to visually identify samples of low total suspended solids

(TSS). Samples with TSS visually determined to be less than approximately 100 mg/L can be extracted without more specific solids determination.

12.3.2 The solids content of aqueous samples suspected to contain >100 mg/L of suspended solids should be determined by the following procedure:

12.3.2.1 For each sample, dry a 47-mm glass fiber filter for ≥ 1 hr in a drying oven set to ≥ 100 °C. Briefly cool the filters in a desiccator and then weigh each to 0.01 mg on an analytical balance. Record the weight of the dried filter.

12.3.2.2 Select the third (small volume) sample container and shake well. Then, filter approximately 10 mL of the sample through the dried filter, using a funnel and vacuum flask or other vacuum filtering apparatus. Record the approximate volume of sample filtered. Discard the filtrate and retain the filter.

12.3.2.3 Place the filters in aluminum pans and dry ≥ 1 hr in a drying oven set to ≥ 100 °C. Briefly cool the filters in a desiccator and then reweigh each to 0.01 mg on an analytical balance. Record the weight of each dried filter + solids.

12.3.2.4 Calculate the TSS of each 10 mL sample using the following equation (adjust the sample volume in the denominator, as necessary):

$$12.3.2.5 \quad TSS \text{ (mg/L)} = \frac{\text{weight of sample aliquot after drying (mg)} - \text{weight of filter (mg)}}{0.01 \text{ L}}$$

12.3.2.6 Multiply the TSS by the volume of sample that will be extracted (500 mL). If the resulting value is greater than 50 mg of TSS, a smaller sample volume should be extracted to prevent clogging the SPE cartridge and the client should be notified.

12.4 Solid-Phase Extraction of Aqueous Samples Using the Promochrom SPE-03 Automated Extraction System

12.4.1 Data associated with sample extractions are recorded in the PFAS Extraction Log (Controlled Document [29719](#)). Refer to Appendix D for details of the Promochrom SPE-03 method settings.

12.4.2 Automated Extractor (SPE-03) Setup

12.4.2.1 Remove lower sample lines and sonicate for ~10 min in 1% ammonium hydroxide in LC-MS grade methanol (for glass cleaning).

12.4.2.2 Install freshly dispensed ultrapure water on solvent line #2 and if needed, top-off other solvent lines.

12.4.2.3 Ensure old SPE cartridges are installed at all positions as placeholders for cleaning.

-
- 12.4.2.4 Drape all 8 sample lines in large, empty glass or HDPE containers. Run the appropriate system cleaning method.
 - 12.4.2.5 Install inline filters and anti-clogging tips on sample lines that will be used in upcoming extraction. Run “clean filter” method.
 - 12.4.2.6 Remove old cartridges and rinse elution lines where cartridges connect with LC-MS grade methanol.
 - 12.4.2.7 Pack SPE cartridges with multilayer frits, placing the rough side of the frits up. Alternatively, pack with clean glass wool to half the cartridge height.
 - 12.4.2.8 Install the packed SPE cartridges in positions that will have samples, leaving old cartridges on positions that will not have samples.

12.4.3 Quality Control (QC) Sample Prep.

- 12.4.3.1 For each QC sample, use a graduated cylinder to measure 500 mL ultrapure water and transfer to a HDPE bottle.
- 12.4.3.2 Add necessary QC and Extracted Internal Standard spikes to each bottle using a pipettor or gastight syringe. Use a fresh, primed pipette tip for each sample.
 - 12.4.3.2.a. Every sample, including method blanks and field samples, is spiked with 100 µL of EIS WS.
 - 12.4.3.2.b. Method Blank sample is only spiked with 100 µL of EIS WS.
 - 12.4.3.2.c. OPR sample is spiked with 100 µL of OPR Spiking Stock.
 - 12.4.3.2.d. LLOPR sample is spiked with 25 µL of LLOPR Spiking Stock.
 - 12.4.3.2.e. MDL samples are spiked with either 50, 100, or 125 µL of MDL Spiking Stock, depending on the desired concentrations.
- 12.4.3.3 Cap samples. Swirl to mix.
- 12.4.3.4 Weigh each bottle + lid + sample. Record the weight in the PFAS Extraction Log.
- 12.4.3.5 Each extraction batch (≤ 20 field samples extracted in one day) must include at least one method blank (“MB”), one OPR sample, and one LLOPR sample.

12.4.4 Automated Sample Extraction Procedure Using Promochrom SPE-03

- 12.4.4.1 Install sample bottles on sample lines and labeled 15mL collection tubes at equivalent collection positions.
- 12.4.4.2 Position sample bottles so that sample suction lines are at the lowest point in each bottle.
- 12.4.4.3 Open nitrogen gas cylinder connected to the SPE-03 system.
- 12.4.4.4 Run “EPA 1633 DNREC” method.
- 12.4.4.5 The extraction procedure will take approximately 3 hours to complete. Periodically check that sample suction lines remain properly positioned and there are no air leaks or clogs during the sample loading portion of the method.
- 12.4.4.6 Once the extraction is complete, approximately 8 – 10 mL of sample extract should have been collected in the collection tubes. Record the approximate volume of each extract in the PFAS Extraction Log.
- 12.4.4.7 Add 25 µL of concentrated acetic acid to each extract. Cap the tubes, vortex, and store at $\leq 0^{\circ}\text{C}$ until ready to concentrate.
- 12.4.4.8 The SPE-03 lines must be cleaned before proceeding with an additional extraction set or shutting down the system for the day.
- 12.4.4.9 Remove lower sample lines and sonicate for ~10 min in 1% ammonium hydroxide in LC-MS grade methanol (for glass cleaning). Remove and discard anti-clogging tips, in-line filters and used SPE cartridges.
- 12.4.4.10 Ensure old SPE cartridges are installed at all positions as placeholders for cleaning.
- 12.4.4.11 Drape all 8 sample lines in large, empty glass or HDPE containers. Run the appropriate system cleaning method (reference Appendix D).
- 12.4.4.12 Close nitrogen gas cylinder connected to the SPE-03 system if no additional extractions will be performed that day.
- 12.4.4.13 Allow the empty sample bottles (500 mL HDPE) and lids to dry in a fume hood. When dry, record the weight of each empty bottle and lid associated with each sample in the PFAS Extraction Log. The difference between the weight of the (bottle + lid + sample) and the (bottle + lid) is the amount of sample that was extracted.

12.4.5 Extract Concentration and Filtration Procedure

- 12.4.5.1 Turn on power and nitrogen gas supply to the solvent evaporator (TurboVap LV).
- 12.4.5.2 Load the “PFAS 1633” method on the TurboVap LV. Ensure the water bath is at 60 °C and nitrogen is set to a maximum of 100 psi before proceeding.
- 12.4.5.3 Remove extracts from storage. Vortex to mix each after they have reached room temperature. Load the extract tubes into the TurboVap LV. Remove each lid and ensure it remains paired with the original extract tube after the evaporation step is complete.
- 12.4.5.4 The following evaporator method settings should be used for approximately 24 samples. Evaporation times and flow rates will need to be changed depending on the number of samples being evaporated at one time. **Avoid splashing solvent during evaporation and DO NOT evaporate to dryness.** (Both will cause analyte loss.)

Settings	Nitrogen Flow Rate (L/min)	Time (min)	Estimated Volume Remaining in tube (mL)
1 st Setting	1.0	8	7
2 nd Setting	1.5	7	5.5
3 rd Setting	2.0	8	4.-4.5

Table 7. Example of evaporator method settings for TurboVap LV system

- 12.4.5.5 After extracts have been concentrated to < 5 mL, bring each to a final volume of 5 mL with 1% ammonium hydroxide in P & T grade methanol using the tube graduations. Vortex to mix.
- 12.4.5.6 Using a pipettor or methanol-rinsed glass gastight syringe, add 20 µL of MPFAC-HIF-IS (non-extracted internal standard stock) to a separate clean 15-mL collection tube for each sample.
- 12.4.5.7 Take the plunger out of a 5-mL PP syringe and install a syringe filter (25-mm filter, 0.2-µm nylon membrane). Carefully decant the sample extract into the syringe barrel.
- 12.4.5.8 Replace the plunger and filter the entire extract into the new collection tube containing the non-extracted internal standard stock.
- 12.4.5.9 Vortex to mix and transfer a portion of the extract into an autosampler vial for LC-MS/MS analysis.
- 12.4.5.10 Cap the collection tube containing the remaining extract and store at ≤ 0°C.

12.4.6 Extract and Sample Dilution

- 12.4.6.1 Sample extracts with area counts for any PFAS analyte that exceed

the calibration range should be diluted within the calibration range using 1633 Diluent and reanalyzed. If a sample extract cannot be diluted and reanalyzed, the reported results must be qualified.

- 12.4.6.2 Sample extracts with EIS recoveries that are outside of acceptable range due to matrix effects may be corrected by diluting the extract with 1633 Diluent.
- 12.4.6.3 Results may be reported from a diluted extract as long as the following criteria are met:
 - 12.4.6.3.a. Extracted Internal Standard (EIS) peaks must have $S/N \geq 3$ for quantitation and qualifier transitions, if applicable.
 - 12.4.6.3.b. EIS recoveries in diluted extracts must meet requirements in Section 20.0, Table 14.
- 12.4.6.4 If either of the above criteria are not met, and the PFAS analyte response is sufficient, a smaller volume of sample can be taken from the second sample container. The sample is gently mixed, then the desired volume transferred to a new 500 mL HDPE container. This subsampling must follow EPA Method 1633A Appendix B policy on sample handling and recording requirements if foam formation occurs. (Note: A reference bottle marked with approximate graduations may be used to aid volume estimations.) This total weight must be recorded. The sample is then diluted to approximately 500 mL with ultrapure water before extraction, and final weight must be recorded. Typical extraction protocols are followed and post-extraction weight is recorded. The extracted sample volume weight pre-dilution is used to calculate the Multiplier.
- 12.4.6.5 If either of the above criteria are not met and the PFAS sample cannot be diluted due to low analyte response or insufficient hold time, report appropriately qualified data.
- 12.4.6.6 Adjust detection and quantitation limits for analytes reported from diluted samples to account for dilution. Extract, sample, or sample and extract dilution must be differentiated to the client. If reanalysis with dilution was conducted to correct EIS recovery, original as well as reanalysis recovery, must be provided to the client.

13.0 SAMPLE ANALYSIS BY LC-MS/MS

- 13.1 All functions related to sample analysis on the LC-MS/MS are conducted within the Agilent MassHunter Data Acquisition software unless noted otherwise.
- 13.2 LC-MS/MS Setup and Conditioning (Conducted daily, prior to standard and/or sample analysis).
 - 13.2.1 Open Agilent MassHunter Data Acquisition software on the instrument PC and make sure communication with the instrument is established.

13.2.2 Confirm sufficient volumes of mobile phases and washes are installed. Ensure the “Actual Volumes” in the Bottle Fillings menu of the Binary Pump reflect the solution volumes installed on the lines. Make sure the Binary Pump is set to prevent analysis if solution levels fall below a minimum of 0.04 L and the pump is set to turn off if solutions are too low.

13.2.2.1 Mobile phases or solutions containing particles or evidence of algal growth must be removed prior to starting the instrument and replaced with fresh solutions prepared in clean bottles.

13.2.2.2 Do not use LC-MS/MS solutions past their assigned expiration dates.

13.2.3 Load the “1633_DNREC_dMRM_Acq.m” acquisition method file from the Method Editor window. Acquisition method settings for PFAS analysis by EPA Method 1633A are found in Controlled Document [29847](#). Turn on the mass spectrometer portion (QQQ) of the instrument and allow it to warm up.

13.2.4 Pump Purge, Needle Prime, and Analytical Column Conditioning

13.2.4.1 Pump Purge = Select “Prepare Pump” under the Binary Pump menu. Select “Purge” and draw solution on the necessary lines through the pumps at 5 mL/min for 4 min. This clears air bubbles from the lines and the pumps.

13.2.4.1.a. Pump purge should be conducted at least every time fresh mobile phases are installed, or air bubbles are observed in lines.

13.2.4.1.b. Mobile phases pump to waste (not through the analytical column) during pump purging. Do not turn the column heater on until pump purging is finished, and mobile phases are pumping through the analytical column.

13.2.4.1.c. If necessary, turn the Binary Pump on after purging is finished to start pumping mobile phase through the column.

13.2.4.1.d. Allow the column to reach set temperature and the column backpressure to stabilize before initiating an analytical run.

13.2.4.2 Needle Prime = Select “Prime Solvents” under the Multisampler menu to start pumping Needle Wash through the needle wash port.

13.2.4.3 Analytical Column Conditioning = Select “Prepare Pump” under the Binary Pump menu. Select “Conditioning” to remove tiny air bubbles trapped in the pump heads by pumping mobile phases at starting conditions for 15 mins.

13.2.4.3.a. Analytical Column Conditioning is necessary only when column backpressure is not stabilized in Section 13.2.4.1.d.

13.2.5 Mass Spectrometer Tuning (Mass Calibration)

13.2.5.1 Follow the instrument manufacturer's instructions to conduct either an Autotune or a Checktune as described in Section 15.0 and Section 10.2.

13.2.5.2 A tune report will be automatically generated and saved. If instrument tuning fails, refer to Section 16.0 for guidance.

13.2.6 Recommended Retention Time Window Verification

13.2.6.1 After completing Sections 13.1.1 through 13.1.5, it is recommended that the analyst verifies all analytes and internal standard peaks elute within assigned retention time (RT) windows.

13.2.6.2 To do so, inject the highest-level calibration standard on the LCMS/MS and assess the data using the Agilent Mass Hunter Qualitative analysis software.

13.2.6.2.a. Visually confirm that all peaks are approximately centered within their respective RT windows.

13.2.6.2.b. Update the RT window in the acquisition method for any analytes that do not fall sufficiently within the assigned RT window.

13.2.6.2.c. Resave the acquisition method if any changes are made.

13.3 Initial Calibration (ICAL)

13.3.1 Prepare calibration standards as described in Section 10.3.3. and Appendix C.

13.3.2 Typically, 8 levels of calibration standards are prepared for this method. Fewer may be used in the ICAL as long as all method requirements are met. See Section 15.0 for ICAL requirements and acceptance criteria.

13.3.3 An acceptable ICAL must be established before samples can be analyzed.

13.4 Instrument Sensitivity Check (ISC)

13.4.1 An ISC is performed to confirm analyte quantification at the LOQ prior to sample analysis. It is a standard or series of standards prepared at the LOQ but not necessarily the lowest concentration calibration standard.

13.4.2 Unless a single ISC standard is prepared to contain all analytes at their respective LOQ concentrations, multiple standard levels may be injected as the ISC. (See Section 20.0, Table 12 for clarification of the analyte concentrations used to set the ML of each analyte.)

13.4.3 For this method, calibration standard levels 1 - 3 may be injected as ISC standards if an ICAL is not also included in the analytical worklist.

13.4.3.1 For this method, ISC standards may be labeled as CCVIL (the lowest level standard) or CCVI in analytical worklists.

13.4.4 If an ICAL is included in the analytical worklist and analyzed immediately prior to samples in that worklist, calibration standard levels 1 – 3 from the ICAL confirm analyte quantification at the LOQ.

13.4.5 Sample analysis may proceed if the ISC meets all acceptability requirements described in Section 15.0.

13.5 Calibration Verification (CV or CC)

13.5.1 Prepare a calibration verification (CV) standard as described in Appendix C. The CV standard is sometimes referred to as a continuing calibration (CC or ConCal) standard in the Agilent Mass Hunter software.

13.5.2 The CV standard is prepared at approximately the middle of the calibration curve range and may be prepared from secondary sources, if available.

13.5.3 Sample analysis may proceed if the CV standard meets the acceptability requirements described in Section 15.0.

13.6 Analytical Worklist Setup

13.6.1 General Worklist Setup

13.6.1.1 Create a new worklist from appropriate worklist template file.

13.6.1.1.a. Use “PFAS_ICAL_Template.wkl” if an ICAL will be run within the worklist.

13.6.1.1.b. Use “PFAS_CV_Template.wkl” if a previous ICAL will be applied to the run. This worklist will include ISC and CV standards, only.

13.6.1.1.c. Appendix E shows an example of a worklist that includes an ICAL and an example that does not include an ICAL.

13.6.1.2 Confirm the Root file Folder is set to save the new worklist in D:\MassHunter\Worklists\PFAS Data.

13.6.1.3 Immediately save the worklist as the date of analysis in YYMMDD format to prevent accidental editing of the template file. The worklist name is also referred to as the analytical batch name.

13.6.1.3.a. Avoid using spaces or underscores in file names. These characters can interfere with data upload to the LIMS.

13.6.1.4 Run Parameters

13.6.1.4.a. The analyst who is conducting the analysis must enter their initials in the “Operator Name” text box.

13.6.1.4.b. Confirm that the "SCP_InstrumentStandby(){MH_Acq_Scripts.exe}" script is selected as BOTH the "Acquisition clean-up" script AND the “Post-worklist script”. This script must be included for every worklist so that the instrument will automatically shut down if there is an error or when the worklist is finished. Without this script, the instrument may pump dry if an error

occurs.

13.6.1.5 Data File Settings

- 13.6.1.5.a. Confirm the Root file Folder is set to save collected data in D:\MassHunter\Data\PFAS Data
- 13.6.1.5.b. Under Sub-folder 1, select User Text and enter the analytical batch name (YYMMDD) in the text box. This will be the subfolder where data files from this analytical batch are saved.
- 13.6.1.5.c. Avoid using spaces or underscores in file names. These characters can interfere with data upload to the LIMS.

13.6.1.6 Edit the worklist as necessary.

- 13.6.1.6.a. Ensure the correct acquisition method file, Sample Names, Sample Positions, Sample Types, and Level Names are reflected.
- 13.6.1.6.b. The same injection volume must be used for all samples and standards throughout the worklist. To ensure this, “As Method” should be listed in the “Inj Vol (µl)” for all rows.
- 13.6.1.6.c. Update Data File names as necessary. Note that each data file in the worklist must be unique and have the “.d” designation included in the “Data File” column.
 - 13.6.1.6.c.1 Avoid using spaces or underscores in file names. These characters can interfere with data upload to the LIMS.
- 13.6.1.6.d. If necessary, add additional description information in the “Comment” column.

13.7 Initiate Sample Analysis

- 13.7.1 Verify that a check mark appears next to each row intended for analysis in the worklist and worklist changes are saved.
- 13.7.2 Select the “play” symbol  to begin analysis. Data will automatically save according to worklist settings.

13.8 Record necessary data in the PFAS Analysis Run Log (see Controlled Document [29669](#)).

14.0 DATA ANALYSIS, CALCULATIONS, AND REPORTING

14.1 Data Analysis Procedure (conducted in the Agilent MassHunter Quantitative Analysis software).

14.1.1 Creating a Batch File

- 14.1.1.1 Select “New Batch” in Agilent MassHunter Quantitative Analysis

and select “Browse”.

- 14.1.1.2 Open the subfolder (named YYMMDD) containing the data files to be quantified.
- 14.1.1.3 Enter a file name for the new batch (for consistency, this should be the same as the worklist name: YYMMDD). Avoid using spaces or underscores in file names. These characters can interfere with data upload to the LIMS.
- 14.1.1.4 Click “create” to create the batch file and begin processing data.
- 14.1.1.5 Add samples to the batch when prompted or by choosing “Add Samples”. Select the data files you want to process in the batch.
- 14.1.1.6 The batch file should display the “Batch Table”, “Compound Information”, and “Calibration Curve” windows. In the Batch Table window, select the “Flat Table” view, which lists the injections in horizontal rows and analytes in the vertical columns.
- 14.1.1.7 Confirm that all “Sample Types” and “Levels” are correctly entered.
- 14.1.1.8 Enter the following information for all extracted samples in the Batch Table:
 - 14.1.1.8.a. “Amt.” Column = Enter the total mass of the sample that was extracted (in L), which is determined by subtracting the mass of empty bottle and lid after extraction from the mass of the sample in the bottle and lid before extraction. This is typically between 0.400 and 0.500 L for this method.
 - 14.1.1.8.b. “Tot. Amt.” Column = Enter the final volume of each extract (after the concentration step) in L. For this method, the final extract volume is typically 0.005 L.
 - 14.1.1.8.c. “Info.” Column = Enter “LCSA” into this cell for each extracted QC sample. This allows the software to properly evaluate QC sample results against the acceptable recovery limits for each analyte.
- 14.1.1.9 Save the batch file before proceeding.

14.1.2 Applying a Quantitation Method to the Batch

- 14.1.2.1 Select the “Method” tab near the top left of the computer screen and expand the “Open” dropdown menu.
- 14.1.2.2 If an ICAL was analyzed in the analytical batch (Section 13.6.1.1.a), choose the “Open Method from Existing File” option, and select the appropriate quantitation method file from the list.

-
- 14.1.2.2.a. Record the name of the quantitation method file in the PFAS Analysis Run Log (Controlled Document [29669](#)).
 - 14.1.2.3 If an ICAL was not analyzed in the analytical batch (Section 13.6.1.1.b), choose the “Open Method from Existing Batch” option, and select the most recent batch that included an ICAL from the list.
 - 14.1.2.3.a. Record the name of the batch ID in the space for “Quantitation Method” in the PFAS Analysis Run Log (Controlled Document [29669](#)).
 - 14.1.2.4 The Method Editor window will automatically open once a quantitation method is selected.
 - 14.1.2.5 For now, close the Method Editor by choosing “Exit” (the red X symbol) along the left side of the computer screen.
 - 14.1.2.5.a. If an ICAL was analyzed in the analytical batch (Section 13.6.1.1.a), select the “Analyze” option, and choose “Yes” to apply the selected quantitation method to the batch and analyze the data based on the new ICAL.
 - 14.1.2.5.b. If an ICAL was not analyzed in the analytical batch (Section 13.6.1.1.b), select the “Quantitate” option, and choose “Yes” to apply the selected quantitation method to the batch and analyze the data based on the existing ICAL.
 - 14.1.2.6 Save the batch file before proceeding.

14.1.3 Reviewing a Batch

- 14.1.3.1 “Surrogate Dil.” Column = Once steps in Sections 14.1.1.7.a. and 14.1.1.7.b. are complete, copy the “Multiplier” value (with five decimal places displayed) for each extracted sample and paste it into the corresponding “Surrogate Dil.” cell for that sample. This ensures proper calculation of surrogate (EIS) recoveries.
- 14.1.3.2 Use the acceptance criteria listed in Section 15.0 when conducting Batch review.
- 14.1.3.3 Check to make sure all peaks are integrated appropriately. If manual integration is necessary, ensure that the chromatography is set to display both pre- and post-manual integration. The MassHunter Quantitative Analysis software will automatically identify any peak that is manually integrated.
 - 14.1.3.3.a. If manual integrations are applied, include a justification and initials and date in the “UserAnnotation” column.
- 14.1.3.4 Check to make sure the integration settings for analytes with isomers appropriately integrate all quantifier and qualifier peaks in

the highest-level calibration standard and the qualitative ID standard. This must be completed before RTs and IARs are updated (see sections 14.1.3.5 and 14.1.3.6).

- 14.1.3.4.a. This applies to Nafion BP2, N-EtFOSAA, N-MeFOSAA, PFHxS, PFNA, PFOA, and PFOS in this method.

14.1.3.5 Check that all RTs are acceptable.

- 14.1.3.5.a. Update the target RT for all analytes in the Batch Table. First, select either the level 5 calibration standard (if ICAL was included) or the first CCV (if no ICAL was included). Then choose “Update Retention Times” from the “Method” tab.
- 14.1.3.5.b. Once updated, the RT of each analyte in each injection will be compared to the RT of that analyte in the selected standard.
- 14.1.3.5.c. Check that the RT separation of TDCA and branched PFOS isomers is acceptable.

14.1.3.6 Check that all Ion Abundance Ratio (IAR) requirements are met.

- 14.1.3.6.a.
$$IAR = \left(\frac{\text{Peak Area of Qualifier Ion Transition}}{\text{Peak Area of Quantifier Ion Transition}} \right) \times 100$$
- 14.1.3.6.b. IAR criteria only apply to analytes that have both a quantifier and qualifier ion transition. This does not apply to EIS or NIS compounds or PFMOAA, PFBA, PFMBA, PFMPA, PFPeA, N-MeFOSE, N-EtFOSE, and PFO2HxA.
- 14.1.3.6.c. Update the target IAR for all analytes in the Batch Table. First, select either the level 5 calibration standard (if ICAL was included) or the first CCV (if no ICAL was included). Then choose “Update Qualifier Ratios” from the “Method” tab.
- 14.1.3.6.d. Once updated, the IAR of each analyte in each injection will be compared to the IAR of that analyte in the selected standard.

14.1.3.7 Check that the ICAL (if applicable), ISC, and CV standards meet all acceptability criteria.

14.1.3.8 Check that all surrogate recoveries are acceptable.

14.1.3.9 Check that the QC sample (LLOPR, OPR, CRM, MS and MSD) recoveries are within acceptable limits.

14.1.3.10 Check that all blanks meet acceptability criteria.

- 14.1.3.11 Check to see if any dilutions are needed.
- 14.1.3.12 Save the batch file before proceeding.
- 14.1.3.13 Consult the Corrective Action section of this SOP (Section 16.0) if any acceptance criteria are not met.
 - 14.1.3.13.a. Qualify sample data or conduct reanalyses as necessary.

14.2 Calculations Used for Sample Quantitation

14.2.1 Multiplier, Dilution Factors (DF), and Final Concentration:

14.2.1.1
$$\text{Multiplier} = \frac{\text{Final extract volume (in L)}}{\text{Mass of water sample prior to extraction (in kg)}}$$

- 14.2.1.2 Note that the Multiplier only applies to extracted samples. For most samples this value will be approximately 0.01, as calculated from a 5 mL final extract volume (0.005 L) and 500 mL water sample mass (approximately 0.5 kg).

14.2.1.3
$$DF = \frac{\text{Final volume of dilution}}{\text{Initial sample volume that was diluted}}$$

- 14.2.1.3.a. Note that the volumes used in this equation must be in the same units.
- 14.2.1.3.b. The DF does not need to be accounted for when determining a sample's Final Concentration (see sec. 14.2.1.4), as it will be either accounted for in the Calculated Concentration (if the dilution is done post-extraction) or in the Multiplier (if the dilution is done by extracting less sample volume).

14.2.1.4
$$\text{Final Concentration} = \text{Calculated Concentration} \times \text{Multiplier}$$

- 14.2.1.5 Note that "Calculated Concentration" is the concentration measured in the sample extract.

14.2.2 Response Ratios (RR) and Response Factors (RF):

- 14.2.2.1 These values are calculated for each target PFAS analyte or EIS compound in each calibration standard.
- 14.2.2.2 A RR is calculated for each target PFAS analyte quantified by "isotope dilution", meaning the target PFAS analyte has an equivalent isotopically labeled analog adding as the EIS compound.
- 14.2.2.3 A RF is calculated for each target PFAS analyte quantified by "extracted internal standard quantification", meaning an isotopically labeled analog of a different analyte is used as the EIS compound for a target PFAS analyte that has a similar RT and

chemical characteristics.

14.2.2.4 RR or RF =

$$\frac{\text{Peak Area of Target} \times \text{Mass of EIS Compound in Calibration Standard}}{\text{Peak Area of EIS Compound} \times \text{Mass of Target Compound in Calibration Standard}}$$

14.2.3 Quantitation:

$$\text{Final concentration measured } \left(\frac{ng}{L} \right) =$$

$$14.2.3.1 \frac{\text{Target Peak Area} \times \text{EIS Mass added (ng)}}{\text{EIS Peak Area} \times \overline{RR} \text{ or } \overline{RF}} \times \text{Multiplier}$$

14.2.4 Relative Standard Error (RSE) for Calibration Curve Linearity Assessment:

14.2.4.1 For this method, correlation coefficient (r) and coefficient of determination (r^2) are not appropriate measures of assessing curve linearity. Instead, the Relative Standard Error (RSE) for each target PFAS analyte is used.

14.2.4.2

$$RSE = 100 \times \sqrt{\sum_{i=1}^n \frac{\left[\frac{x'_i + x_i}{x_i} \right]^2}{n - p}}$$

14.2.4.3 Where:

14.2.4.4 x_i = true concentration of the calibration standard

14.2.4.5 x'_i = Measured concentration of the calibration standard

14.2.4.6 n = Number of calibration standard levels used in curve

14.2.4.7 p = Curve type (2 for linear, 3 for quadratic)

14.2.5 Recovery:

$$14.2.5.1 \% \text{ Recovery} = \left(\frac{\text{Concentration measured in sample or standard}}{\text{Concentration added to sample or standard}} \right) \times 100$$

14.2.5.2 $\text{Matrix Spike \% Recovery} =$

$$\frac{(\text{Conc. measured in matrix spike sample} - \text{Conc. measured in unspiked sample})}{\text{Conc. added to matrix spike sample}} \times 100$$

14.2.6 Relative Percent Difference (RPD) in duplicate samples:

$$14.2.6.1 \quad RPD = \left[\frac{|a-b|}{\left(\frac{a+b}{2}\right)} \right] \times 100$$

where: a = concentration measured in initial sample

14.2.6.2 b = concentration measured in duplicate sample

14.3 Data Reporting Procedures

14.3.1 Generate Excel and PDF reports for the Batch

14.3.1.1 Once data review is complete in the MassHunter Quantitation software, select “Generate report”.

14.3.1.2 Ensure the report folder is set to save the report files within the “QuantReports” subfolder of the Batch folder named YYMMDD.

14.3.1.3 Ensure the “DNREC 1633 Report Method” file is selected as the report method.

14.3.1.3.a. The report method will generate an Excel report (as a .csv file) for upload to the LIMS.

14.3.1.3.b. The report method will also generate a pdf report(s) displaying sample chromatography and calibration curves.

14.3.1.4 Select “Choose samples...” to select which injections to include in the report.

14.3.1.4.a. All lines in the batch are automatically selected for reporting.

14.3.1.4.b. Unselect any injections that are not necessary for reporting.

14.3.1.4.c. Click “Ok” to generate the reports.

14.3.1.5 Copy all raw data files (.d), QuantResults, and QuantReports folders from the D drive of the instrument computer and paste them into the appropriate subfolder in “L:\LCMSMS\LCMSMS1\PFAS Raw Data Files”.

14.3.1.6 Copy the Excel .csv file and paste it in the “Unparsed” subfolder in ["L:\LCMSMS\LCMSMS1"](L:\LCMSMS\LCMSMS1).

14.3.2 Parse the Excel .csv report and upload to the LIMS

14.3.2.1 Open the universal parser file “ELS Parser.exe” saved on the L Drive.

- 14.3.2.2 Click and drag the unparsed Excel .csv file as directed and then click “Parse”.
- 14.3.2.3 A parsed Excel file will be automatically saved as “pYYMMDD-1.xls” in the “Parsed” subfolder in ["L:\LCMSMS\LCMSMS1"](#).
- 14.3.2.4 Upload the parsed Excel file to the LIMS.

14.3.3 Data review and approval

- 14.3.3.1 Follow Controlled Document [1814](#) “Data Review and Approval Checklist” once data are successfully uploaded to the LIMS.
 - 14.3.3.1.a. The analyst who reviewed the data in MassHunter is typically responsible for reviewing the LIMS data to ensure all quality criteria are met.
 - 14.3.3.1.b. Qualify and/or write a narrative to accompany any data that do not meet quality criteria. Refer to Controlled Document [1645](#) for guidance.
 - 14.3.3.1.c. Validate the LIMS data once data review is complete.
 - 14.3.3.1.d. A second party (not the analyst who validated the data) will conduct a secondary review and then approve of the data, indicating data are ready for reporting through the LIMS.

14.3.4 Save accompanying data logs

- 14.3.4.1 Scan necessary PFAS Analysis Run log, PFAS Extraction log pages and Chain of Custody logs as pdf files. Save to the L drive.
- 14.3.4.2 Submit the completed data package to the designated data approver for review and approval through the document management system or appropriate LIMS workflow.

15.0 QUALITY CONTROL (METHOD PERFORMANCE AND ACCEPTANCE CRITERIA)

15.1 The Quality Control section of this SOP is derived from the metrics and criteria discussed in EPA Method 1633A.

Quality Control Checks	Preparation Frequency	Acceptance Criteria
Mass Calibration (Autotune)	At least annually and as needed after instrument, vented, cleaned, or if mass calibration verification fails. Instrument manufacturer recommends conducting an Autotune monthly during regular use. When an Autotune is conducted, record it as routine equipment maintenance in the document	Calibration must be performed using ESI-L Low Concentration Tuning Mix and metrics set by the instrument manufacturer (i.e. Agilent Technologies). For mass calibration see Section 10.1 EPA Method 1633A.

	management system.	
Mass Calibration Verification (Checktune)	Checktune is performed each day a worklist is run, before the analysis of standards and/or samples.	Mass calibration verification is performed using ESI-L Low Concentration Tuning Mix and is
	Checktune report does not need to be documented in the document management system.	determined by observing the amount of peak drift from expected masses. If the drift (determined by shift of peak apex) is larger than 0.2 Da, the mass axis must be recalibrated (as per manufacturer's instructions). Further details are outlined in Section 10.1.7 EPA Method 1633A.

Initial Calibration (ICAL)	Initial calibration is required when: • the chromatographic conditions are changed • the existing calibration criteria are altered or affected • the instrument sensitivity check (ISC) or calibration verification (CV) do not meet criteria. 8 different levels of calibration standards are prepared for this method but fewer may be used in the initial calibration.	The ICAL must contain all EIS and NIS compounds and target analytes. Calibration models should be applied in the following order: 1) Average Response Factor 2) Linear (1/x weighted, not forced through origin) 3) Quadratic (1/x weighted, not forced through origin) Minimum of 6 standards must be used for average response factor or linear models, with at least 5 $\geq$ LOQ. Minimum of 7 standards must be used for non-linear (quadratic) models, with at least 6 $\geq$ LOQ. The lowest concentration standard included in the ICAL must meet the Instrument Sensitivity Check (ISC) S/N requirements. Instrument Linearity = relative standard error (RSE) of EIS compounds and target analytes must be $\leq 20\%$. % accuracy of ICAL standards should be 70 – 130%.
Calibration Verification (CV)	The ICAL must be verified before the analysis of samples. A mid-level calibration standard serves as the CV standard. The CV standard is analyzed following the ICAL, after every 10 injections of field sample extracts, and at the end of the analytical worklist.	The % recovery of PFAS analytes must be within 70-130%.

Instrument Sensitivity and Instrument Sensitivity Check (ISC)	The ISC is a standard or series of standards prepared at the LOQ but not necessarily the lowest concentration calibration standard.	In all samples and standards, the signal-to-noise ratio (S/N) must be ≥ 3 for both the quantitation and
	The ISC must be conducted at the beginning of each analytical worklist unless the worklist also includes an ICAL. For this method, calibration standard levels 1 - 3 may be injected as ISC standards if an ICAL is not also included in the analytical worklist.	confirmation ion transitions or ≥ 10 for the quantitation ion, if the analyte does not have a confirmation ion. % recovery of each target analyte must be 70 - 130% in the ISC. Further information is in Section 7.3.4, 14.1 EPA Method 1633A.
Retention Time (RT) Window	Set at the start of an analytical worklist, and after initial calibration.	RTs of all EIS compounds, NIS compounds, and target PFAS analytes must fall within ± 0.4 min of the predicted RTs from the ICAL midpoint standard or the CV standard (i.e. whichever criteria was used to establish the RT window for the analytical batch). RTs of analytes with exact matching isotopically labeled internal standards must fall within ± 0.1 min of each other. For further information see Section 15.1.2 EPA Method 1633A.
Isomer Integration Windows	Set at the start of an analytical worklist, using either the highest level calibration standard or the qualitative identification (QID) standard.	Integration windows must be set for analytes that have multiple (branched and linear) isomers. The summed results of all isomers

		are reported for each analyte.
Ion Abundance Ratio (IAR)	Set at the start of an analytical worklist, using either the mid-level calibration standard (level 5; when an ICAL is included in the analytical worklist) or the ICV standard (when an ICAL is not included in the analytical worklist).	IAR of each target PFAS analyte must be between 50-150% of the IAR set from the selected standard. IAR criteria do not apply to EIS, NIS, or target analytes that do not have a qualifier ion transition (PFMOAA, PFBA, PFMBA, PFMPA, PFPeA, N-MeFOSE, NEtFOSE, and PFO2HxA).
Extracted Internal Standard (EIS)	Spiked into all samples, including quality control samples (i.e. OPR, LLOPR, and method blank), prior to extraction . EIS are also spiked into all calibration standards.	The acceptable % recovery for extracted internal standards is reported in Section 20.0, Table 14.
Non-extracted Internal Standard (NIS)	Spiked into all samples, including quality control samples (i.e. OPR, LLOPR, and method blank), after all sample preparation steps are complete NIS are also spiked into all	Compare the NIS response (peak area) in each sample/standard with the mean response for each NIS compound in the ICAL standards.
	calibration standards.	The NIS response must be between 50-200% of the mean response in the corresponding ICAL standards. (Section 20.0, Table 14.)
Bile Salt Interference Check (TDCA)	For each analytical sequence, after an initial calibration.	RT of the TDCA bile salt must be $\geq$ one min from the beginning of the RT window for branched or linear PFOS isomers. For further information see Sections 7.5 and 14.2. EPA Method 1633A.

Initial Precision and Recovery (IPR), also referred to as Initial Demonstration of Capability (IDOC)	A method blank and four replicates of a mid-level quality control (QC) sample must be prepared. IPR must be performed after all method parameters are set for sample preparation and analysis. In addition, each analyst must perform an IPR/IDOC prior to analyzing any samples by this method.	The mean % recoveries and relative standard deviation (RSD) must meet the criteria in Section 20.0, Table 13. See also Section 9.2 in EPA Method 1633A for further discussion.
Ongoing Precision and Recovery Spike (OPR)	A mid-level quality control (QC) sample prepared once per preparation batch per day.	The acceptable criteria for OPR % recoveries is reported in Section 20.0, Table 13.
Low Level Ongoing Precision and Recovery Spike (LLOPR)	A low-level quality control (QC) sample prepared once per preparation batch per day to verify the LOQ. The LLOPR must be prepared at approximately 2x the LOQ.	The acceptable criteria for LLOPR % recoveries is reported in Section 20.0, Table 13.
Instrument Blank	The instrument blank is a solvent blank containing the EIS and NIS internal standards. It is analyzed at the start of an analytical sequence, after the highest-level calibration standard, and following each CV injection.	The instrument blank must not contain any analyte of interest at or above the method detection limit (MDL). See Section 14.6 EPA Method 1633A. Current MDLs are reflected in the LIMS and Controlled Document 29854.
Method Blank (MB)	An ultrapure water sample, spiked only with EIS (pre-extraction) and NIS (post-sample preparation), prepared once per preparation batch per day. Analyze the method blank before the LLOPR and OPR samples from the same preparation batch.	1) Concentration of MB is < th Minimum Level of Quantitation (ML). Current MLs are reflected in the LIMS and Controlled Document 29854. 2) Concentration < 1/3 of the regulatory compliance limit. 3) Concentration < 1/10 of a sample

		in the preparation batch. See also Section 9.5 EPA Method 1633A.
Method Detection Limit (MDL)	MDLs must be established before samples can be analyzed. MDL samples cannot be analyzed before the mass spectrometer undergoes mass calibration, chromatographic conditions are established, and an ICAL is conducted. Established MDLs are verified annually.	MDLs must be established according to the procedure in 40 CFR Part 136, Appendix B. Follow the MDL SOP. Controlled Document 1704 Current MDLs are reflected in the LIMS and Controlled Document 29854 .
Qualitative Identification Standard (QID)	Analyze at the start of an analytical sequence, and after initial calibration.	Used to set isomer integration windows for some analytes (see above). See Section 7.3.5 EPA Method 1633A.
Matrix Spike and Matrix Spike Duplicate (MS/MSD)	MS and MSD may be requested but are not required for this method because it is an isotope dilution method.	If requested, refer to projectspecific acceptance criteria.

Table 8. Quality Control (Method Performance and Acceptance Criteria)

16.0 CORRECTIVE ACTION

16.1 The Corrective Action section of this SOP is derived from the metrics and criteria discussed in EPA Method 1633A.

Quality Control Check Failure	Corrective Action
Mass Calibration (Autotune) fails QC criteria	First, verify there is enough ESI-L Low Concentration Tuning Mix in the reservoir and rerun an Autotune. Ensure Tuning Mix is not expired, degraded, or contaminated. If Autotune still fails, clean spray chamber and/or capillary and rerun an Autotune. If Autotune fails again, contact outside instrument support.
Mass Calibration Verification (Checktune) fails QC criteria	First, verify there is enough ESI-L Low Concentration Tuning Mix in the reservoir and rerun a Checktune. If Checktune still fails, conduct a mass calibration (Autotune).

Initial Calibration (ICAL) fails QC criteria.	If ICAL criteria are not met, the analysis must be halted. Inspect the LC-MS/MS for proper operation and cleanliness. Either reanalyze existing ICAL standards or prepare and analyze a fresh set of calibration standards. The new ICAL must meet criteria before any samples or blanks are analyzed.
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Quality Control Check Failure	Corrective Action
Instrument Sensitivity or Instrument Sensitivity Check (ISC) fails QC criteria	If S/N criteria fail due to elevated background noise in the ISC, stop analysis, and inspect the LCMS/MS for proper operation and cleanliness. Repeat ICAL with fresh calibration standards, if necessary. If S/N criteria fail for the qualifier transition of a PFAS analyte in a sample, the result is considered as estimated and must be appropriately qualified. If S/N criteria fail for the quantifier transition of a PFAS analyte in a sample, the result should not be reported.
Calibration Verification (CV) fails QC criteria	Typically, if the CV is not within 70-130% recovery, any extracts analyzed between the failed CV need to be reanalyzed. Reanalysis will not be necessary if the CV recovery for an analyte is > 130%, but that analyte was not detected in any extracts bracketed by the failed CV. If CV failure continues, however, the LC-MS/MS should be recalibrated until the CV criteria are met.
Retention Time (RT) Window fails QC criteria	If RT window fails the QC criteria in a standard, QC sample, or for the EIS or NIS compounds in any sample/standard, analysis needs to be halted and the issue corrected. Modify chromatographic conditions and repeat ICAL. If the RT window of a target PFAS analyte fails the QC criteria in a sample, the data should not be reported.

Ion Abundance Ratio (IAR)	If the result of a target PFAS analyte in a sample does not meet IAR acceptance criteria and all sample extract cleanup procedures have been applied, the result must be qualified as an estimated value due to the inability to positively confirm identification of the analyte.
Quality Control Check Failure	Corrective Action
Extracted Internal Standard (EIS) fails QC criteria	If EIS % recovery is outside acceptance criteria in a field sample extract, the result for the associated PFAS analyte(s) in that sample extract cannot be reported. An additional extract cleanup or dilution procedure, to bring the EIS recovery within specifications is required. If the EIS recovery in the sample extract cannot be improved, a smaller volume of sample taken from the second sample bottle may be diluted to 500 mL with ultrapure water and then re-extracted. If EIS recovery cannot be corrected by dilution or reextraction, the data must be reported with an appropriate qualifier indicating the result is estimated. The client must be consulted before a sample is re-extracted for this reason. If EIS % recovery is outside of acceptance criteria in a QC sample, consider the impact to sample results from that preparation batch and inform the client. The results for the associated PFAS analyte(s) in samples from the preparation batch may not be able to be reported or the data may be qualified.
Non-extracted Internal Standard (NIS) fails QC criteria	If NIS analytes are observed at lower or higher thresholds outlined in Section 20.0, Table 14, analysis of the samples needs to be halted and the issue corrected. Instrument sensitivity must be improved if all samples in the analytical batch are below acceptable criteria. If only some samples in the analytical batch are below acceptable criteria, reinject the impacted samples to assess for possible injection errors.

Bile Salt (TDCA) Interference Check fails QC criteria	If this check fails, modify chromatographic conditions and repeat ICAL. This must be resolved before any field samples are analyzed for PFOS. For further information, see Section 14.2 EPA Method 1633A.
Ongoing precision and recovery spike (LLOPR and OPR) fails QC criteria	If any PFAS is observed outside of the acceptable criteria, analysis must be halted. Correct the issue causing the unacceptable recovery, re-prepare, reextract, and reanalyze the samples in the extraction batch. For further discussion see Section 14.5 EPA Method 1633A.

Quality Control Check Failure	Corrective Action
Instrument Blank fails QC criteria	The instrument blank must not contain any analyte of interest $\geq$ MDL. If the instrument blank fails the criteria, analyze additional instrument blanks until the response is $<$ MDL or troubleshoot the LCMS/MS until the contamination issue is corrected. Reanalyze any samples bracketed by a failed instrument blank.
Method Blank (MB) fails QC criteria	When PFAS is found in the method blank exceeding specified quality control limits, analysis of the samples will be halted, and the issue corrected. If PFAS contamination is found in the extraction batch, evaluate all consumables to isolate the contamination source(s). Inform the client that samples associated with the contaminated method blank must be reextracted reanalyzed alongside a new method blank and QC samples. Also inform the client if samples will not be able to be reextracted within holding time or if there is not a sufficient volume of the field samples remaining for additional extraction/analysis. In the event the new blank exceeds PFAS limits, or if an insufficient volume remains to perform an analysis, the laboratory must record and disclose the failures.

Sample Contamination	Inform the client and correct the issue causing the contamination. Re-prepare, re-extract, and reanalyze the samples in the extraction batch (including QC samples). Document contamination in a nonconformance report and forward to the Quality Manager.
Reagent contamination	Remake the reagent and confirm it is free of contamination. Re-prepare, re-extract, and/or reanalyze the samples in the extraction/analysis batch, including QC samples if affected. Inform the client before any samples are re-extracted. Document contamination in a nonconformance report and forward to the Quality Manager.
Quality Control Check Failure	Corrective Action
Sample analyte peak response is above the highest calibration standard	Dilute the sample extract using 1633 diluent and reanalyze for analytes whose concentration are above the calibration curve range. If the EIS responses in the diluted extract are within acceptable limits, the analytes corresponding to the EIS compounds may be quantified in the diluted extract. Additionally, the S/N criteria described for the ISC and RT window criteria must also be met in the diluted extract. Method detection limits, analyte concentration calculated in the sample, and minimum levels of quantitation, must be adjusted based on the dilution factor applied. If the above criteria are not met in the diluted extract, the affected analytes cannot be quantified in the dilution. Another extraction, using a smaller volume of sample (taken from an extra sample bottle) and diluted to 500 mL with ultrapure water, may be conducted or results from the original extract may be reported with an appropriate qualifier. The client must be consulted before a sample is reextracted for this reason.

Table 9. Corrective Actions

17.0 WASTE MANAGEMENT AND POLLUTION PREVENTION

17.1 All wastes should be disposed of in accordance with the DEL Safety, Waste and Chemical Hygiene Manual.

17.2 Take all necessary actions to minimize pollution in the laboratory. Recycle noncontaminated paper, vials, and plastic bottles.

Waste	EPA Hazard Waste No.	Label	Description
Dilute Formic Acid in Water	U123	Corrosive pH < 3.5	Neutralize with sodium carbonate unless combined with an organic solvent. If combined with an organic solvent, put into container with organic nonhalogenated liquid waste for removal/disposal
Waste	EPA Hazard Waste No.	Label	Description
Methanol, Isopropanol, Acetonitrile, Dilute Ammonium Hydroxide, Dilute Formic Acid, Dilute Acetic Acid	F003, U123	Flammable, toxic, carcinogenic, and corrosive substances	Place in non-halogenated organic liquid waste receptacle for removal/disposal. Keep separated from waste that is likely to contain PFAS residues.
PFAS residues in Water, Methanol, Isopropanol, Acetonitrile, Dilute Ammonium Hydroxide, Dilute Formic Acid, Dilute Acetic Acid	F003, U123	Flammable, toxic, carcinogenic, and corrosive substances	Place in separate, nonhalogenated organic liquid waste receptacle for removal/disposal. Keep separated from waste that does not contain PFAS residues.
Dilute Ammonium Hydroxide in Water	N/A	Corrosive 11 > pH	Neutralize with dilute acetic acid unless combined with an organic solvent. If combined with an organic solvent, put into container with organic non-halogenated liquid waste for removal/disposal
Solid PFAS Waste (Used SPE cartridges, vials, pipette tips, syringe filters, or other materials containing residual PFAS stocks, standards, or concentrated extracts that cannot be decontaminated)	F003, U123	Solid waste containing, flammable, toxic, carcinogenic, and corrosive substances	Some containers (sample containers, etc.) can be rinsed and then disposed as non-hazardous solid wastes. Place solid waste containing PFAS waste that cannot be decontaminated into a solid waste receptacle for appropriate removal/disposal

Table 10. Waste Management

18.0 REFERENCES

- 18.1 EPA Method 1633A Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS (published December 2024).
- 18.2 Aqueous Sample Collection Instructions for PFAS Analysis by LC-MS/MS (Controlled Document [37277](#)).
- 18.3 Promochrom Technologies- SPE-03 MOD-00P User Manual.
- 18.4 Promochrom Technologies- SPE-03 MOD-00P Maintenance Manual.
- 18.5 Quality Manual for DNREC Environmental Laboratory and Field Operations (Controlled Document [861](#)).

19.0 RESOURCES

Time (Hours)	30
Number of FTEs	2
Average Batch Size	20
Average # Batches/day	1

20.0 ADDITIONAL TABLES

Table 11**Target PFAS Analyte Names, Abbreviations, CAS Numbers, and Assigned Internal Standards**

Analyte Name	Abbreviation	CAS #	Assigned Internal Standard
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	13C3-HFPO-DA
3-Perfluoropropyl propanoic acid	3:3FTCA (FPrPA)	356-02-5	13C5-PFPeA
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4	13C2-4-2 FTSA
2H,2H,3H,3H-Perfluorooctanoic acid	5:3FTCA (FPePA)	914637-49-3	13C5-PFHxA
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2	13C2-6-2 FTSA
3-Perfluoroheptyl propanoic acid	7:3FTCA (FHpPA)	812-70-4	13C5-PFHxA
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4	13C2-8-2 FTSA
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	13C3-HFPO-DA
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	13C3-HFPO-DA
6:2 fluorotelomer alpha, beta-unsaturated carboxylate *	FHUEA (6:2 FTUCA) *	70887-88-6 *	13C2-FHUEA *
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	13C3-HFPO-DA
2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]- 1,1,2,2-tetrafluoro-ethanesulfonic acid *	NafionBP2 (Hydro-PS Acid or PFESA-BP 2) *	749836-20-2 *	13C4-PFHpA
N-ethylperfluoro-1-octanesulfonamide ("Sulfuramid")	N-EtFOSA	4151-50-2	2H5-N-EtFOSA
N-ethyl perfluorooctanesulfonamidoacetic acid	N-EtFOSAA	2991-50-6	2H5-N-EtFOSAA
2(N-ethylperfluoro-1-octanesulfonamido)-ethanol	N-EtFOSE	1691-99-2	2H9-EtFOSE
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA (3,6-OPFHpA)	151772-58-6	13C5-PFHxA

N-methylperfluoro-1-octanesulfonamide	N-MeFOSA	31506-32-8	2H3-N-MeFOSA
N-methyl perfluorooctanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	2H3-N-MeFOSAA
2(N-methylperfluoro-1-octanesulfonamido)-ethanol	N-MeFOSE	24448-09-7	2H7-MeFOSE
Perfluorobutanoic acid	PFBA	375-22-4	13C4-PFBA
Perfluorobutanesulfonic acid	PFBS	375-73-5	13C3-PFBS
Perfluorodecanoic acid	PFDA	335-76-2	13C6-PFDA
Perfluorododecanoic acid	PFDoA	307-55-1	13C2-PFDoA
Analyte Name	Abbreviation	CAS #	Assigned Internal Standard
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	13C8-PFOS
Perfluorodecanesulfonic acid	PFDS	335-77-3	13C8-PFOS
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	13C5-PFHxA
Perfluoroheptanoic acid	PFHpA	375-85-9	13C4-PFHpA
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	13C8-PFOS
Perfluorohexanoic acid	PFHxA	307-24-4	13C5-PFHxA
Perfluorohexanesulfonic acid	PFHxS	355-46-4	13C3-PFHxS
Perfluoro-4-methoxybutanoic acid	PFMBA (PF5OHxA)	863090-89-5	13C5-PFPeA
Perfluoro-2-methoxyacetic acid or 2,2-difluoro-2(trifluoromethoxy)-acetic acid *	PFMOAA *	674-13-5 *	13C4-PFBA
Perfluoro-3-methoxypropanoic acid	PFMPA (PF4OPeA)	377-73-1	13C5-PFPeA
Perfluorononanoic acid	PFNA	375-95-1	13C9-PFNA
Perfluorononanesulfonic acid	PFNS	68259-12-1	13C8-PFOS
Perfluoro-3,5-dioxahexanoic acid or 2-[Difluoro(trifluoromethoxy)methoxy]-2,2-difluoroacetic acid *	PFO2HxA *	39492-88-1 *	13C5-PFPeA

Perfluorooctanoic acid	PFOA	335-67-1	13C8-PFOA
Perfluorooctanesulfonic acid	PFOS	1763-23-1	13C8-PFOS
Perfluoro-1-octanesulfonamide	PFOSA (FOSA)	754-91-6	13C8-PFOSA
Perfluoropentanoic acid	PFPeA	2706-90-3	13C5-PFPeA
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	13C3-PFHxS
2,2,3,3,3-Pentafluoropropionic acid *	PFPrA (PPF Acid) *	422-64-0 *	13C3-PFPrA *
Perfluorotetradecanoic acid	PFTeDA (PFTA)	376-06-7	13C2-PFTDA
Perfluorotridecanoic acid	PFTrDA	72629-94-8	Average of 13C2-PFTDA and 13C2-PFDoA
Perfluoroundecanoic acid	PFUnDA (PFUnA)	2058-94-8	13C7-PFUnDA

* Analyte is not part of the 40 PFAS included in EPA Method 1633A. This analyte has been validated for inclusion in the analytical method described in this SOP.

Table 12

Calibration Standard Levels and Concentrations

Analyte Abbreviation	Cal. Std. Level 1 (ng/L) ^b	Cal. Std. Level 2 (ng/L) ^b	Cal. Std. Level 3 (ng/L) ^b	Cal. Std. Level 4 (ng/L) ^b	Cal. Std. Level 5 (ng/L) ^b	Cal. Std. Level 6 (ng/L) ^b	Cal. Std. Level 7 (ng/L) ^b	Cal. Std. Level 8 (ng/L) ^b
11Cl-PF3OUdS	47.3	94.5 ^a	189	662	1890	4725	9450	18900
3-3 FTCA	NA	200	400 ^a	1400	4000	10000	20000	40000
4-2 FTSA	203	406 ^a	813	2844	8125	20313	40625	81250
5-3 FTCA	500	1000 ^a	2000	7000	20000	50000	100000	200000
6-2 FTSA	206	412 ^a	823	2882	8233	20583	41167	82333

7-3 FTCA	NA	1000 ^a	2000	7000	20000	50000	100000	200000
8-2 FTSA	208	416 ^a	832	2912	8320	20800	41600	83200
9CI-PF3ONS	NA	93.5 ^a	187	655	1870	4675	9350	18700
ADONA	NA	94.5 ^a	189	662	1890	4725	9450	18900
FHUEA	NA	100	200 ^a	700	2000	5000	10000	20000
HFPO-DA	NA	100 ^a	200	700	2000	5000	10000	20000
Nafion BP2	52.2	104 ^a	209	731	2088	5220	10440	20880
N-EtFOSA	NA	100	200 ^a	700	2000	5000	10000	20000
N-EtFOSAA	NA	108	217 ^a	758	2167	5417	10833	21667
N-EtFOSE	500	1000 ^a	2000	7000	20000	50000	100000	200000
NFDHA	NA	100	200 ^a	700	2000	5000	10000	20000
N-MeFOSA	NA	100	200 ^a	700	2000	5000	10000	20000
N-MeFOSAA	NA	108	217 ^a	758	2167	5417	10833	21667
N-MeFOSE	500	1000 ^a	2000	7000	20000	50000	100000	200000
PFBA	217 ^a	433	867	3033	8667	21667	43333	86667
PFBS	NA	96.1 ^a	192	673	1922	4805	9609	19218

Analyte Abbreviation	Cal. Std. Level 1 (ng/L) ^b	Cal. Std. Level 2 (ng/L) ^b	Cal. Std. Level 3 (ng/L) ^b	Cal. Std. Level 4 (ng/L) ^b	Cal. Std. Level 5 (ng/L) ^b	Cal. Std. Level 6 (ng/L) ^b	Cal. Std. Level 7 (ng/L) ^b	Cal. Std. Level 8 (ng/L) ^b
PFDA	NA	108 ^a	217	758	2167	5417	10833	21667
PFDoDA	NA	108	217 ^a	758	2167	5417	10833	21667
PFDoS	NA	105	210 ^a	736	2102	5254	10508	21017
PFDS	NA	105 ^a	209	732	2091	5227	10454	20908

PFEESA	NA	89.0 ^a	178	623	1780	4450	8900	17800
PFHpA	NA	108 ^a	217	758	2167	5417	10833	21667
PFHpS	NA	103 ^a	206	723	2065	5162	10324	20648
PFHxA	NA	108 ^a	217	758	2167	5417	10833	21667
PFHxS	NA	99 ^a	198	693	1980	4951	9902	19803
PFMBA	50.0	100 ^a	200	700	2000	5000	10000	20000
PFMOAA	NA	89.2 ^a	178	624	1784	4460	8920	17840
PFMPA	NA	100 ^a	200	700	2000	5000	10000	20000
PFNA	NA	108 ^a	217	758	2167	5417	10833	21667
PFNS	NA	104 ^a	208	730	2084	5211	10422	20843
PFO2HxA	NA	184	367 ^a	1285	3672	9180	18360	36720
PFOA	NA	108 ^a	217	758	2167	5417	10833	21667
PFOS	NA	101 ^a	201	704	2011	5027	10053	20107
PFOSA	NA	108 ^a	217	758	2167	5417	10833	21667
PFPeA	108 ^a	217	433	1517	4333	10833	21667	43333
PFPeS	NA	102 ^a	204	714	2039	5097	10194	20388
PFPrA	NA	100	200 ^a	700	2000	5000	10000	20000
PFTDA	NA	108 ^a	217	758	2167	5417	10833	21667
PFTTrDA	NA	108 ^a	217	758	2167	5417	10833	21667
PFUnDA	NA	108 ^a	217	758	2167	5417	10833	21667

^a Calibration standard concentration used to set the LOQ and ML. The ML = the standard concentration x Multiplier of the extraction (0.01). See Controlled Document [29854](#) for current MDL and ML values.

^b Concentration units are in ng/L extract. The extraction process concentrates aqueous samples (by a factor of 100) prior to analysis. To be quantified, aqueous samples (concentration units in ng/L sample) are extracted, and the resulting sample extract responses must fall within the calibration response ranges for the standards listed in Table 12.

Table 13**Quality Control Sample Acceptable Recovery and RSD Criteria**

	IPR		
Analyte Abbreviation	Mean Recovery (%)	RSD (%)	OPR / LLOPR Recovery (%)
11CI-PF3OUdS	50 - 150	35	55 - 160
3:3FTCA	70 - 130	23	65 - 130
4:2FTS	70 - 135	27	70 - 145
5:3FTCA	70 - 130	24	70 - 135
6:2FTS	70 - 135	32	65 - 155
7:3FTCA	55 - 130	34	50 - 145
8:2FTS	70 - 140	33	60 - 150
9CI-PF3ONS	70 - 145	30	70 - 155
ADONA	70 - 135	23	65 - 145
FHUEA *	70 - 130	20	70 - 140
HFPO-DA	70 - 135	23	70 - 140
Nafion BP2 *	70 - 130	20	70 - 140
NEtFOSA	70 - 130	26	65 - 145
NEtFOSAA	70 - 135	28	70 - 145
NEtFOSE	70 - 130	21	70 - 135
NFDHA	65 - 140	37	50 - 150
NMeFOSA	70 - 135	30	60 - 150
NMeFOSAA	65 - 140	32	50 - 140
NMeFOSE	70 - 135	29	70 - 145
PFBA	70 - 135	21	70 - 140
PFBS	70 - 140	23	60 - 145
PFDA	65 - 140	26	70 - 140
PFDoA	70 - 130	21	70 - 140
PFDoS	45 - 135	35	50 - 145
PFDS	70 - 135	30	60 - 145
PFEESA	70 - 135	25	70 - 140
PFHpA	70 - 135	28	70 - 150
PFHpS	70 - 140	30	70 - 150
PFHxA	70 - 135	24	70 - 145
PFHxS	70 - 135	27	65 - 145

PFMBA	65 - 145	27	60 - 150
PFMOAA *	70 - 130	20	70 - 140
PFMPA	60 - 140	23	55 - 140
PFNA	70 - 140	28	70 - 150
PFNS	70 - 135	29	65 - 145
PFO2HxA *	70 - 130	20	70 - 140
PFOA	65 - 155	27	70 - 150
PFOS	70 - 140	29	55 - 150
PFOSA	70 - 135	22	70 - 145
PFPeA	70 - 135	23	65 - 135
PFPeS	70 - 135	25	65 - 140
PFPrA *	70 - 130	20	70 - 140
PFTeDA	70 - 145	27	60 - 140
PFTrDA	60 - 145	29	65 - 140
PFUnA	70 - 135	29	70 - 145

* Analyte is not part of the 40 PFAS included in EPA Method 1633A. This analyte has been validated for inclusion in the analytical method described in this SOP.

Table 14**Internal Standard Acceptable Recovery Criteria**

Extracted Internal Standard Abbreviation	Recovery (%)
13C2-4-2 FTSA	40 - 200
13C2-6-2 FTSA	40 - 200
13C2-8-2 FTSA	40 - 300
13C2-FHUEA *	40 - 130
13C2-PFDoA	10 - 130
13C2-PFTDA	10 - 130
13C3-HFPO-DA	40 - 130
13C3-PFBS	40 - 135
13C3-PFHxS	40 - 130
13C3-PFPrA *	40 - 130
13C4-PFBA	5 - 130
13C4-PFHpA	40 - 130
13C5-PFHxA	40 - 130
13C5-PFPeA	40 - 130
13C6-PFDA	40 - 130
13C7-PFUnDA	30 - 130
13C8-PFOA	40 - 130
13C8-PFOS	40 - 130
13C8-PFOSA	40 - 130
13C9-PFNA	40 - 130
2H3-N-MeFOSA	10 - 130
2H3-N-MeFOSAA	40 - 170
2H5-N-EtFOSA	10 - 130
2H5-N-EtFOSAA	25 - 135
2H7-MeFOSE	10 - 130

2H9-EtFOSE	10 - 130
Non-extracted Internal Standard Abbreviation	Response %^a
13C2-PFHxA	50 - 200
13C4-PFOA	50 - 200
13C5-PFNA	50 - 200
13C2-PFDA	50 - 200
18O2-PFHxS	50 - 200
13C4-PFOS	50 - 200
13C3-PFBA	50 - 200

* Analyte is not part of the 40 PFAS included in EPA Method 1633A. This analyte has been validated for inclusion in the analytical method described in this SOP.

^a Calculated by comparing the NIS response (peak area) in each sample/standard with the mean response for each NIS compound in the ICAL standards.

21.0 APPENDICES

Appendix A

Reagent Preparation Instructions for PFAS Analysis by EPA Method 1633A

- **1% ammonium hydroxide in LC-MS grade methanol (1 L) = for glassware cleaning:**
 - 970 mL LC-MS grade methanol
 - 33 mL ammonium hydroxide
 - Each measured by graduated cylinders or equivalent and combined in an appropriately rinsed glass or HDPE plastic bottle.
 - Store ambient. Recommend discarding if not used 3 months after preparation.
 - Documentation in Reagent Prep Logbook is not required if used only for cleaning.
- **1% ammonium hydroxide in P & T grade methanol (1 L) = for extraction** (referred to as “Solvent 5” in SPE-03 methods):
 - 970 mL P & T grade methanol
 - 33 mL ammonium hydroxide
 - Each measured by graduated cylinders or equivalent and combined in an appropriately rinsed glass or HDPE plastic bottle.
 - Store ambient. **Discard 1 month after preparation.**
- **3% ammonium hydroxide (100 mL) = sample pH adjustment:**
 - 10 mL ammonium hydroxide
 - 90 mL ultrapure water
 - Each measured by graduated cylinders or equivalent and combined in an appropriately rinsed glass or HDPE plastic bottle.
 - Store ambient. **Discard 3 months after preparation.**
- **5% formic acid (100 mL) = sample pH adjustment:**
 - 95 mL ultrapure water
 - 5 mL formic acid measured by pipettor or volumetric pipette
 - Combined in an appropriately rinsed glass or HDPE plastic bottle.
 - Store ambient. **Discard 2 years after preparation.**
- **0.3 M formic acid (1 L) = SPE cartridge conditioning** (referred to as “Solvent 3” in SPE-03 methods):

-
- 11.3 mL (**density = 1.2200 g/mL**) formic acid (measured by pipettor or equivalent) added to < 900 mL ultrapure water in graduated cylinder
 - Bring to 1000 mL final volume with ultrapure water
 - Transfer to an appropriately rinsed glass or HDPE plastic bottle. Mix well.
 - Store ambient. **Discard 2 years after preparation.**
 - **0.1 M formic acid (1 L) = used to prep. SPE cartridge rinse:**
 - 3.8 mL (**density = 1.2200 g/mL**) formic acid (measured by pipettor or equivalent) added to < 900 mL ultrapure water in graduated cylinder
 - Bring to 1000 mL final volume with ultrapure water
 - Transfer to an appropriately rinsed glass or HDPE plastic bottle. Mix well.
 - Store ambient. **Discard 2 years after preparation.**
 - **1:1 0.1 M formic acid: P & T grade methanol (1 L) = SPE cartridge rinse** (referred to as “Solvent 4” in SPE-03 methods):
 - 500 mL 0.1M formic acid
 - 500 mL methanol (P & T grade)
 - Combined in an appropriately rinsed glass or HDPE plastic bottle.
 - Store ambient. **Discard 2 years after preparation.**
 - **1633 Diluent (100 mL) = to prep. standards and dilute extracts:**
methanol + 4% water + 1% ammonium hydroxide + 0.625% acetic acid (See EPA 1633A Section 7.1.9)
 - 92 mL methanol (P & T grade) measured by graduated cylinder
 - 1.7 mL ultrapure water (measured by pipettor)
 - 3.3 mL ammonium hydroxide measured by gastight syringe, or pipettor, or volumetric pipette
 - 625 µL acetic acid (measured by pipettor)
 - Combine in an appropriately rinsed glass or 125 mL HDPE plastic screw-cap bottle. Mix well.
 - Store ambient. **Discard 1 month after preparation.**

LC-MS/MS Solutions:

- **1633 Mobile Phase A (1 L) = for LC-MS/MS analysis:** 2 mM ammonium acetate in 95% ultrapure water + 5% acetonitrile (See EPA 1633A Section 7.1.11, labeled “Eluent B” in text)
 - Prepare in an appropriately rinsed amber glass 1000 mL mobile phase bottle.

-
- 950 mL ultrapure water (measured by graduated cylinder)
 - 0.154 g ammonium acetate (LC-MS grade) measured using verified analytical balance
 - 50 mL acetonitrile (LC-MS grade) measured by graduated cylinder
- Add ammonium acetate to the ultrapure water in the bottle before adding acetonitrile to the bottle.
- Swirl to mix well.
 - Sonicate in water bath for 10 - 15 mins to dissolve and degas.
 - Store ambient. **Assign 3-month expiration per EPA 1633A.**
 - **Best if prepared at least 1 day in advance of use on LC-MS/MS to avoid bubble formation in LC line!**
- **1633 Mobile Phase B (1 L) = for LC-MS/MS analysis:** 100% acetonitrile (LC-MS grade).
- Prepare in an appropriately rinsed clear glass 1000 mL mobile phase bottle.
 - Sonicate in water bath for 5 – 10 mins to degas before use.
- **Seal Wash (500 mL) = for washing LC-MS/MS pump seals:** 90% ultrapure water: 10% Isopropanol
- Prepare in an appropriately rinsed amber glass 1000 mL mobile phase bottle.
 - 450 mL ultrapure water (measured by graduated cylinder)
 - 50 mL Isopropanol (LC-MS grade) measured by graduated cylinder
 - Swirl to mix well. Sonicate in water bath for 5 – 10 mins to dissolve and degas.
 - Store ambient. **Assign 3-month expiration.**
- **Needle Wash = for rinsing LC-MS/MS needle prior to each injection:** 100% P & T grade methanol
- Prepare in methanol-rinsed clear glass 1000 mL mobile phase bottle.

Appendix B

Working Stock Preparation Instructions

Record preparation of working stocks (WS) in the PFAS Reagent Log. Prepare/store each in polypropylene (PP) centrifuge tubes of appropriate volumes. Store refrigerated. Different volumes may be used, so long as the ratio of constituents remains the same as written, below:

Internal Standard Working Stocks

Intermediate MFHUEA WS (1 mL final volume):

- 990 µL Isopropanol
- 10 µL MFHUEA (Wellington Laboratories purchased stock)

500 ng/mL 13C3-PFPrA WS (1 mL final volume):

- 990 µL Methanol
- 10 µL CLM-11324-1.2 (Cambridge Isotope purchased stock)

Combo EIS + NIS WS: Combine all labeled compounds in Methanol (1 mL final volume)
=

- 650 µL Methanol
- 50 µL Isopropanol
- 100 µL MPFAC-HIF-ES
- 100 µL MPFAC-HIF-IS
- 50 µL Intermediate MFHUEA WS
- 50 µL 500 ng/mL 13C3-PFPrA WS
 - **Add 40 µL of EIS + NIS WS to 1000 µL of each calibration standard**

EIS WS: Combine all labeled compounds in Methanol (2.50 mL final volume) =

- 1250 µL Methanol
- 250 µL Isopropanol
- 500 µL MPFAC-HIF-ES
- 250 µL Intermediate MFHUEA WS
- 250 µL 500 ng/mL 13C3-PFPrA WS
 - **Add 100 µL of EIS WS to every 500 mL water sample**

Qualitative ID Working Stock

QID WS (1 mL final volume):

-
- 980 µL Methanol
 - 10 µL T-PFOA (Wellington purchased stock)
 - 10 µL ipPFNA (Wellington purchased stock)

TDCA (Taurodeoxycholic acid) Working Stocks:**Verify analytical balance** (using certified weight set)

- Check mass reading of 10 mg and 20 g weights
 - Pass if each is within $\pm 0.2\%$ of given weight
 - Record balance verification in appropriate logbook

1000 µg/mL TDCA WS =

- Weigh 10 mg of TDCA sodium salt on analytical balance and directly into 10 mL volumetric flask
- Bring to volume with ultrapure water and mix well.
- Assign expiration date = 3 months from date of preparation
- Transfer solution to 15 mL PP centrifuge tube for storage

(Optional) 10 µg/mL TDCA WS =

- Transfer 100 µL of 1000 µg/mL TDCA WS to a volumetric flask or polypropylene tube
- Bring to 10 mL final volume with ultrapure water and mix well
- Assign expiration date = 3 months from date of preparation
- Store in 15 mL PP centrifuge tube

Quality Control Working Stocks**LLOPR Combo WS** (1 mL final volume):

- 860 µL Methanol
- 20 µL FHUEA (Wellington Labs purchased stock)
- 20 µL Nafion BP2 (ULM-11231-S)
- 20 µL PFMOAA (ULM-11223-S)
- 40 µL PFO2HXA (ULM-11226-S)
- 40 µL PFPrA (ULM-11323-S)

LLOPR Spiking Stock (1 mL final volume):

- 725 µL Methanol
- 40 µL LLOPR Combo WS
- 20 µL PFAC-MXF
- 60 µL PFAC-MXI
- 30 µL PFAC-MXJ
- 50 µL PFAC-MXG
- 75 µL PFAC-MXH

○ **Add 25 µL of LLOPR Spiking Stock to 500 mL water sample**

PFAS+ WS (1 mL final volume):

- 500 µL Methanol
- 200 µL Isopropanol
- 50 µL Nafion BP2 (ULM-11231-S)
- 50 µL PFMOAA (ULM-11223-S)
- 100 µL PFO2HXA (ULM-11226-S)
- 50 µL PFPrA (ULM-11323-S)
- 50 µL FHUEA (Wellington Labs purchased stock)

OPR Spiking Stock (1 mL final volume):

- 310 µL Methanol
- 100 µL PFAC-MXF
- 100 µL PFAC-MXI
- 100 µL PFAC-MXJ
- 100 µL PFAC-MXG
- 210 µL PFAC-MXH
- 80 µL PFAS+ WS

○ **Add 100 µL of OPR Spiking Stock to 500 mL water sample**

Method Detection Limit (MDL) PFAS+ WS (1 mL final volume):

- 475 µL Methanol
- 50 µL Isopropanol
- 50 µL Nafion BP2 (ULM-11231-S)
- 50 µL PFMOAA (ULM-11223-S)
- 100 µL PFO2HXA (ULM-11226-S)
- 75 µL PFPrA (ULM-11323-S)
- 200 µL FHUEA (Wellington Labs purchased stock)

MDL Intermediate Stock (1 mL final volume):

- 310 µL Methanol
- 100 µL PFAC-MXF
- 100 µL PFAC-MXI
- 100 µL PFAC-MXJ
- 100 µL PFAC-MXG
- 210 µL PFAC-MXH
- 80 µL MDL PFAS+ WS

MDL Spiking Stock (3 mL final volume):

- 2925 µL Methanol
 - 75 µL MDL Intermediate Stock
-
- Add 50 µL of MDL Spiking Stock to 500 mL water sample for Spike 1
 - Add 100 µL of MDL Spiking Stock to 500 mL water sample for Spike 2
 - Add 125 µL of MDL Spiking Stock to 500 mL water sample for Spike 3

Appendix C

1633 Standard Preparation Instructions

Prepare all standards in 1633 diluent.

Record preparation of Standard Level 8 in the PFAS Reagent Log and assign a reagent ID # in addition to the YYMMDD-8 identifier. Record preparation of remaining standards and instrument blank in the PFAS Standards Log. Prepare/store each in polypropylene (PP) centrifuge tubes of appropriate volumes. Store refrigerated. Assign expiration dates based on the shortest expiration date of any standard constituent. Different volumes may be used, so long as the ratio of constituents remains the same as written, below:

Calibration standards will be uniquely identified by the preparation date (YYMMDD) followed by the calibration level (-1, -2, -3, etc.). The instrument blank will be identified by the preparation date (YYMMDD) followed by the abbreviation “-Blank”.

Prepare 3 mL of Level 8 Calibration Standard in 1633 diluent:

- **2 mL + 761 µL** of diluent into 15-mL polypropylene tube
- 65 µL PFAC-MXH
- 30 µL PFAC-MXF
- 30 µL PFAC-MXG
- 60 µL PFAC-MXI
- 30 µL PFAC-MXJ
- **24 µL PFAS+ WS**
 - **Vortex to Mix**
- Transfer 1000 µL of Level 8 standard to a microcentrifuge tube. Use remaining to prepare other standards.

Prepare Remaining Standards in 1633 diluent: prepare in microcentrifuge tubes, unless noted

-
- Level 7 = (1 mL final volume): 500 µL Level 8 + 500 µL diluent
 - Level 6 = (1 mL final volume): 250 µL Level 8 + 750 µL diluent
 - Level 5 = (**3 mL final volume**): 300 µL Level 8 + 2 mL + 700 µL diluent
 - **prepare in 15-mL centrifuge tube**
 - Transfer 2000 µL of Level 5 to a separate 15-mL centrifuge tube
 - Level 4 = (1 mL final volume): 35 µL **Level 8** + 965 µL diluent
 - Level 3 = (1 mL final volume): 100 µL Level 5 + 900 µL diluent
 - Level 2 = (1 mL final volume): 50 µL Level 5 + 950 µL diluent
 - Level 1 = (1 mL final volume): 25 µL Level 5 + 975 µL diluent
 - Transfer 2000 µL of diluent to a **15-mL centrifuge tube**. This will be the instrument blank.

Add Internal Standards:

- Add 40 µL of **Combo EIS + NIS Working Stock** to each 1000 µL of standard. Proportionally increase the amount of **Combo EIS + NIS Working Stock** added to more than 1000 µL of standard, if necessary.
- **Vortex to mix each.**

Qualitative Identification Standard Prep.

The Qualitative Identification Standard (QID Standard) will be identified by the preparation date (YYMMDD) followed by the abbreviation “-QID”.

QID and TDCA Standards do not have Internal Standards added to them.

- **QID Standard** (1 mL final volume):
 - 960 µL 1633 Diluent
 - 40 µL QID WS

EPA 1633A TDCA Interference Check Standard Prep.

The TDCA Interference Check Standard (TDCA Standard) will be identified by the preparation date (YYMMDD) followed by the abbreviation “-TDCA”.

- **TDCA Standard (1 µg/mL):** Prepare in 15 mL centrifuge tube
 - Add ~9 mL of 1633 Diluent to the centrifuge tube
 - Add 10 µL of 1000 µg/mL TDCA WS
 - Bring to final volume of 10 mL with additional 1633 Diluent using the tube graduations.

OR

- **TDCA Standard (1 µg/mL):** Prepare in microcentrifuge tube
 - 900 µL 1633 Diluent
 - 100 µL 10 µg/mL TDCA WS

Appendix D

Promochrom SPE-03 Automated Solid-Phase Extraction System Method Settings

Solvent 1 = 1% ammonium hydroxide in LC-MS grade methanol (cleaning only)

Solvent 2 = ultrapure water

Solvent 3 = 0.3 M formic acid

Solvent 4 = 1:1 0.1 M formic acid/P & T methanol

Solvent 5 = 1% ammonium hydroxide in P & T methanol

Solvent 6 = LC-MS grade methanol (cleaning only)

1633 system cleaning method – use before extraction:

Action	Inlet1	Inlet2 (ratio)	Flow	Volume
Rinse	Solvent 2	--	5	10.0
Rinse	Solvent 2	--	5	10.0
Air-Purge R	Air	--	5	5.0
Rinse	Solvent 1 or 5	--	5	5.0
Rinse	Solvent 1 or 5	--	5	5.0
Air-Purge R	Air	--	5	5.0
Clean	Solvent 1 or 5	Air (20%)	30	5.0
Clean	Solvent 1 or 5	Air (20%)	30	5.0
Air-Purge S	Air	--	30	2.0
Elute W2	Solvent 1 or 5	--	10	5.0

Elute W2	Solvent 1 or 5	--	10	5.0
Air-Purge W2	Air	--	10	5.0

Filter cleaning method – use after system cleaning but before extraction:

Action	Inlet1	Inlet2 (ratio)	Flow	Volume
Clean	Solvent 5	--	10	5.0
Add Samp W2	Sample	--	10	9.0
Air-Purge S	Air	--	10	0.1

“EPA 1633 DNREC” – 500mL right side up loading method settings:

Action	Inlet1	Inlet2 (ratio)	Flow	Volume
Elute W2	Solvent 5	--	8	15.0
Elute W1	Solvent 3	--	8	5.0
Add Samp W1	Sample	--	5	550.0
Rinse	Solvent 2	--	10	10.0
Air-Purge R	Air	--	10	3.0
Add Samp W1	Sample	--	5	35.0
Rinse	Solvent 4	--	70	5.0
Air-Purge R	Air	--	10	3.0
Add Samp W2	Sample	--	5	30.0
Blow N2	--	Time based	--	1 min
Rinse	Solvent 5	--	70	10.0
Air-Purge R	Air	--	10	3.0

Collect 1	Sample	--	5	1.0
Collect 1	Sample	--	5	25.0

“EODcleansys1633” - use after extraction at the end of the day

Action	Inlet1	Inlet2 (ratio)	Flow	Volume
Rinse	Solvent 1	--	5	5.0
Rinse	Solvent 1	--	5	5.0
Air-Purge R	Air	--	5	5.0
Clean	Solvent 1	Air (20%)	30	5.0
Clean	Solvent 1	Air (20%)	30	5.0
Air-Purge S	Air	--	30	2.0
Elute W2	Solvent 1	--	10	5.0
Elute W2	Solvent 1	--	10	5.0
Air-Purge W2	Air	--	10	5.0
Rinse	Solvent 2	--	5	10.0
Rinse	Solvent 2	--	5	10.0
Rinse	Solvent 2	--	5	10.0
Rinse	Solvent 2	--	5	10.0
Air-Purge R	Air	--	5	5.0
Elute W2	Solvent 2	--	10	5.0
Elute W2	Solvent 2	--	10	5.0
Air-Purge W2	Air	--	10	5.0
Rinse	Solvent 6	--	5	5.0

Rinse	Solvent 6	--	5	5.0
Air-Purge R	Air	--	5	5.0
Clean	Solvent 6	Air (20%)	30	5.0
Clean	Solvent 6	Air (20%)	30	5.0
Air-Purge S	Air	--	30	2.0
Elute W2	Solvent 6	--	10	5.0
Elute W2	Solvent 6	--	10	5.0
Air-Purge W2	Air	--	10	5.0

Appendix E

Analytical Worklist Examples

Example Analytical Worklist Including ICAL

Injections	Description
Prime(s)	Clears analytical column after instrument idle
Double Blank	Solvent blank w/out internal standard verifies clean LC-MS/MS
ICB	Instrument blank = solvent contains internal standards
Cal 1	Calibration Standard level 1
Cal 2	Calibration Standard level 2
Cal 3	Calibration Standard level 3
Cal 4	Calibration Standard level 4
Cal 5	Calibration Standard level 5
Cal 6	Calibration Standard level 6
Cal 7	Calibration Standard level 7
Cal 8	Calibration Standard level 8
CCB-1	Instrument blank = contains internal standards
ICV	Initial Calibration Verification Standard

QID	Qualitative ID Standard
CCB-2	Instrument blank = contains internal standards
Method Blank	Method Blank
LLOPR	Low-level laboratory control sample = confirms LOQ
OPR	Mid-level laboratory control sample
CRM	Certified Reference Material
TDCA	TDCA Interference Check Standard
Sample 1	Field Sample
Sample 2	Field Sample
Sample 3	Field Sample
Sample 4	Field Sample
Sample 5	Field Sample
Sample 6	Field Sample
Sample 7	Field Sample
Sample 8	Field Sample
Sample 9	Field Sample
Sample 10	Field Sample
MS	Matrix Spike Field Sample (if needed)
MSD	Duplicate Matrix Spike Field Sample (if needed)
CCV-1	Continuing Calibration Verification Standard
CCB-3	Instrument blank = contains internal standards
Sample 11	Field Sample
Sample 12	Field Sample
Injections	Description
Sample 13	Field Sample
Sample 14	Field Sample
Sample 15	Field Sample
Sample 16	Field Sample
Sample 17	Field Sample

Sample 18	Field Sample
Sample 19	Field Sample
Sample 20	Field Sample
MS	Matrix Spike Field Sample (if needed)
MSD	Duplicate Matrix Spike Field Sample (if needed)
CCV-2	Continuing Calibration Verification Standard
CCB-4	Instrument blank = contains internal standards

Example Analytical Worklist Without ICAL

Injections	Description
Prime(s)	Clears analytical column after instrument idle
Double Blank	Solvent blank w/out internal standard verifies clean LC-MS/MS
ICB	Instrument blank = solvent contains internal standards
CCVIL 1	Instrument sensitivity check (ISC) = calibration standard level 1
CCVIL 2	Instrument sensitivity check (ISC) = calibration standard level 2
CCVIL 3	Instrument sensitivity check (ISC) = calibration standard level 3
ICV	Initial Calibration Verification Standard
QID	Qualitative ID Standard
CCB-1	Instrument blank = contains internal standards
Method Blank	Method Blank
LLOPR	Low-level laboratory control sample = confirms LOQ
OPR	Mid-level laboratory control sample
CRM	Extracted Certified Reference Material
TDCA	TDCA Interference Check Standard
Sample 1	Field Sample
Sample 2	Field Sample
Sample 3	Field Sample
Sample 4	Field Sample
Sample 5	Field Sample

Sample 6	Field Sample
Sample 7	Field Sample
Sample 8	Field Sample
Sample 9	Field Sample
Sample 10	Field Sample
Injections	Description
MS	Matrix Spike Field Sample (if needed)
MSD	Duplicate Matrix Spike Field Sample (if needed)
CCV-1	Continuing Calibration Verification Standard
CCB-2	Instrument blank = contains internal standards
Sample 11	Field Sample
Sample 12	Field Sample
Sample 13	Field Sample
Sample 14	Field Sample
Sample 15	Field Sample
Sample 16	Field Sample
Sample 17	Field Sample
Sample 18	Field Sample
Sample 19	Field Sample
Sample 20	Field Sample
MS	Matrix Spike Field Sample (if needed)
MSD	Duplicate Matrix Spike Field Sample (if needed)
CCV-2	Continuing Calibration Verification Standard
CCB-3	Instrument blank = contains internal standards