SAMPLING PLAN

Natchaug River and Quinebaug River PFAS Contamination Study:

Fish Tissue Collection & Analysis Plan

October 2019

CT DEEP WATER PROTECTION & LAND REUSE BUREAU
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1.0 INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that have been manufactured and used in the United States since the 1950s. Several thousand PFAS are known, however perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are the most extensively studied.

PFAS are characterized by strong fluorine-carbon bonds, which give them unique physical and chemical properties including extreme thermal and chemical stability, and oil, grease, and water repellency. Their use is widespread in American consumer products, including non-stick cookware, water-proof clothing, and stain-resistant carpets and upholstery. PFAS have also been widely used in industrial processes including wire manufacturing, metal plating, and the manufacturing of many industrial surfactants, resins, molds and plastics.

The downside to these unique physical and chemical properties is that PFAS are "forever chemicals"; they are resistant to degradation and are therefore persist in the environment long after they are released. PFAS are also highly mobile; they can travel long distances, move through soil, seep into groundwater, and/or be carried through air. Given their extensive use in products and industrial processes, both landfill leachate and wastewater treatment plant discharges are potential sources of PFAS release to the environment. There is also growing concern regarding the application of biosolids to agricultural lands and the use of aqueous film-forming foams (AFFF) in firefighting processes, both of which can lead to direct contamination to the soil as well as PFAS-contaminated stormwater runoff entering nearby waterbodies.

Unfortunately, there is also a growing body of evidence that suggests exposure to PFAS can lead to adverse human health effects. Because PFAS have the ability to bioaccumulate and to biomagnify, consumption of fish has been identified as a human exposure pathway of particular concern. The Connecticut Department of Energy and Environmental Protection (CT DEEP), in partnership with the Connecticut Department of Public Health (CT DPH), therefore seeks to characterize levels of PFAS in fish tissue collected from Connecticut waterbodies in order to inform fish consumption advisories.

2.0 PROJECT OVERVIEW

The sampling and analysis described in this plan will be conducted by the Connecticut Department of Energy and Environmental Protection (CT DEEP) Water Monitoring Unit in cooperation with the CT DEEP Fisheries Division and the Connecticut Department of Public Health (DPH) Environmental Health Section. The scope of this document includes the collection, handling, and shipment of the fish tissue specimens.

2.1 Study Objectives

The objective of this sampling program is to collect representative fish tissue samples from two locations in Connecticut. The samples collected will be delivered to an independent analytical laboratory for analysis of polyfluoroalkyl

substance (PFAS) levels. The results of this analysis will be used to inform local fish consumption advisories.

2.2 Sampling Locations

Fish tissue samples will be collected from the Natchaug River and the Quinebaug River at the following two locations:

1. Natchaug River (Windham, CT) – upstream of the confluence with the Willimantic River, in the vicinity of the former Eastern CT fire training facility.
2. Quinebaug River (Putnam, CT) – between the Little River confluence and Route 44

Table 1. Sampling Locations

Waterbody	Station ID	Municipality	Latitude	Longitude	Potential Source
Natchaug River	14313	Windham, CT	41.7155	-72.1941	AFFF firefighting foam
Quinebaug River	17298	Putnam, CT	41.9129	-71.9121	Industrial discharges

These two waterbodies were selected for study as a result of their site history, which indicates a high potential for PFAS contamination. Both sites are located in urbanized areas. Detailed site maps are included as Figures 1-4 in Appendix A.

2.2.1 Natchaug River Site History

The selected Natchaug River study site is located in an urbanized area of Windham, CT in the vicinity of the former Willimantic Regional Fire School. The school was constructed on leased property immediately upstream of the river's confluence with the Willimantic River in 1954 and served for decades as the primary training grounds for municipal departments in Tolland, Windham and New London Counties (49 towns). The use of aqueous film-forming foam (AFFF), a known source of PFAS, was used on the property throughout this period to support training activities. Consequently, testing was conducted in late 2018 to address concerns regarding the possibility of PFAS contamination to two public water systems and a residential well in the vicinity of the school. Testing results indicated contamination in the latter, prompting the discontinuation of its use. Testing has not been conducted to evaluate potential PFAS levels in fish tissue, despite the river being a well-used recreational fishery.

2.2.2 Quinebaug River Site History

The Quinebaug River study site is located upstream of the confluence with the Little River, in an urbanized area of Putnam, CT. Several industrial dischargers, with potential for PFAS contamination in their releases, currently and historically operated in the vicinity of this location. No known studies have been conducted to-

date to assess levels of potential PFAS contamination in the area. The river is a well-known recreational fishery as well.

2.3 Target Species, Size and Numbers

Fish consumption advisories in Connecticut have traditionally been based on analysis of the contaminant of concern within a bottom-dwelling species and a predator-species following U.S. EPA guidelines (USEPA 2000). For the latter, when possible, a gamefish species such as trout or black bass is targeted. This same strategy will be employed for this study.

White sucker (*Catostomus commersonii*) and smallmouth bass (*Micropterus dolomieu*) will be targeted for collection. In the event that these species cannot be obtained in sufficient numbers or size, largemouth bass (*Micropterus salmoides)* or yellow perch (*Perca flavescens)* may be substituted for the predator species. Fallfish (*Semotilus corporalis)* may be substituted for the bottom dwelling species.

Because the analysis will be used to support fish consumption advisories, fish of legal harvest length and comparable size will be targeted, with a minimum total length of 150 mm required to retain a fish for analysis. Maximum total length for each species should not exceed the following values if possible:

Table 2. Target Species and Total Length Ranges

Species	Total Length (Inches)		Total Length (mm)	
	Minimum	Maximum	Minimum	Maximum
Smallmouth Bass	6	20	150	500
Largemouth Bass	6	18	150	450
Yellow Perch	6	15	150	375
White Sucker	6	20	150	500
Fallfish	6	15	150	375

Field staff will be given the target of 10 fish per species at each location, to achieve an overall study goal of 40 fish. If field crews are unsuccessful at obtaining 10 fish of a single species within the ranges noted above, the project lead will determine which fish to retain, if any, for analysis.

Fish with abnormal deformities or other obvious health issues should not be included in the study.

3.0 PROJECT ORGANIZATION

Table 1 lists key personnel involved in project organization, field sampling, chemical analyses, and data quality assurance. This list also represents the project

distribution list.

Table 3. Key Project Personnel

Name	Title	Organization	Contact Information
Meghan Lally **Project Lead*	Environmental Analyst	CT DEEP Water Monitoring Unit	860-424-3061 Meghan.lally@ct.gov
Chris Bellucci **QA Officer*	Supervising Environmental Analyst	CT DEEP Water Monitoring Unit	860-424-3735 Christopher.bellucci@ct.gov
Traci Iott	Supervising Environmental Analyst	CT DEEP Water Quality Unit	860-424-3082 Traci.iott@ct.gov
Philip Trowbridge	Assistant Division Director	CT DEEP Water Planning & Management Division	860-424-3718 Philip.trowbridge@ct.gov
Brian Eltz	Fisheries Biologist	CT DEEP Fisheries Division	860-424-3406 Brian.eltz@ct.gov
Mike Beauchene	Supervising Fisheries Biologist	CT DEEP Fisheries Division	860-424-4185 Mike.beauchene@ct.gov
Pete Aarrestad	Division Director	CT DEEP Fisheries Division	860-424-4171 Peter.Aarrestad@ct.gov
Sharee Rusnak	Epidemiologist	CT DPH Environmental Health Section	860-509-7583 Sharee.Rusnak@ct.gov
Brian Toal	Interim Chief, Supervising Epidemiologist	CT DPH Public Health Section, Environmental Health Section	860-509-7741 Brian.toal@ct.gov
Nick Corso	Project Manager	SGS Axys Analytical Services	250-655-5800 nicholas.corso@sgs.com

It is the responsibility of the Quality Assurance (QA) Officer to oversee the implementation of the Sampling and Analysis Plan, including whether specified quality control (QC) procedures are being followed as described. The QA officer will discuss QA issues with the Project Lead and Project Supervisor. If necessary, the Project Manager will coordinate with CT DEEP Fisheries staff and/or CT DPH Staff to address any outstanding issues related to field sampling, sample handling, or sample preservation.

4.0 FIELD SAMPLING METHODS

CT DEEP staff will perform the collection of fish specimens. The date of collection will be dependent on the availability of field staff and suitable field conditions. Samples will be collected using backpack or barge-mounted electrofishing units. In the event that this is unsuccessful, alternative collection methods may be employed.

Standard agency electrofishing methods are described in the CT DEEP Water Monitoring Unit's standard operating procedures for electrofishing. Additional methods described herein will be employed to minimize the likelihood of PFAS contamination during collection and handling.

4.1 Standard Cross-Contamination Precautions

Among a long list of other products, PFAS is frequently used in personal care products, clothing manufactured for outdoor use, and food packaging. Therefore, the potential for PFAS cross-contamination during a routine field day of sampling is high if special precautions are not taken. As there is little to no published research documenting the use of various materials and their effect on sample results, the practices for avoiding cross-contamination are still evolving.

The following sections provide a synthesis of standard recommendations available among published PFAS studies and sampling plans. In addition, the Michigan Department of Environmental Quality has developed a useful 2-page quick reference guide summarizing these precautions (Appendix B).

IMPORTANT NOTE: These recommendations are for the collection of tissue specimens, the results of which are typically measured in parts per billion. More stringent, precautions will likely be needed if the sampling plan expands to include surface water samples, where PFAS analysis occurs at the level of parts per trillion.

4.1.1 Personal Care Products (PCPs)

The use of personal care products should be limited to the extent practical. In particular, cosmetics, moisturizers, hand creams and similar products should be avoided on the day of sampling as many of these products have been reported to contain PFAS.

Cross-contamination precautions must balance staff health and safety risks. Therefore, if bug spray and/or sunscreen are needed, staff should select products that do not contain ingredients with "fluor-" in the name. Products should be applied away from the sampling area, prior to donning waders, and hands should be washed after application.

4.1.2 Field Clothing and Personal Protective Equipment (PPE)

Staff should wear synthetic and natural fibers (e.g. cotton) when possible. Outdoor field clothing is commonly waterproof, water repellant, and/or stain resistant – properties that are often achieved through the addition of PFAS to the materials. Staff should avoid wearing clothing made from these materials if possible. Clothing materials that should be avoided include those containing Teflon or Gore-Tex, and those advertised as having been chemically treated to repel insects and provide UV protection. Staff should avoid laundering clothing in fabric softener if possible, as well as avoiding new clothing (i.e. clothing that has not been well laundered several times.)

Ideally, all clothing and PPE worn on the day of the sampling event will be PFAS-free. **However, the safety of the staff should not be compromised**. Staff are expected to wear waterproof, nonbreathable waders and rubber electrofishing gloves, to ensure proper field safety. Additionally, life jackets may be needed depending on water depth and flow velocity. If PFAS-free versions of these items are not available, then the available PPE is to be worn, and a note documenting any such limitations to cross-contamination precautions shall be recorded on the field

sheet.

If possible, the outside of gloves and waders are to be rinsed with streamwater, downstream of the sampling area, prior to sample collection.

4.1.3 Food and Beverage Consumption

PFAS are commonly found in food packaging materials, including pre-wrapped food or snacks and take out or fast food bags and containers. Staff should consume food and beverages away from the sampling area, and remove waders and gloves prior to handling any such materials. After eating, if possible, hands should be washed and gloves redonned. Water and sports beverages are safe for consumption without risk of PFAS contamination.

4.1.4 Standard Electrofishing Sampling Equipment

Standard electrofishing equipment and nets will be used to collect tissue samples. New equipment is to be avoided if possible. Equipment should be rinsed with stream water, downstream of the sampling area, prior to commencement of collection.

Field measuring boards will be limited to wooden boards. The measuring board will be rinsed with stream water in between measurements.

Field clipboards will be limited to Masonite or aluminum clipboards. Field datasheets/COCs will be printed on standard office paper (i.e. no Rite in the Rain paper). Only pencils and Ultra-Fine Point Sharpie markers are allowed as writing utensils. Refer to Appendix B for additional details.

4.1.5 Standard Precautions Documentation

All standard precautions taken to avoid cross-contamination, as well as any observed potential for cross-contamination, will be documented on the field data sheet by the project lead. If the project lead is not present during sampling, the field crew leader will be responsible for such documentation.

4.2 Fish Collection & Documentation

The general fish collection and documentation procedures outlined in the DEEP WPLR Fish Community Monitoring SOP will be followed. Expansions upon these procedures are necessary when sampling to evaluate PFAS levels; these expansions are described in the following subsections.

4.2.1 Special Fish Handling Requirements

Fish will be placed in a live car immediately upon netting. The live car is hand-constructed unit that allows ambient water continuously flows through the holding pens. The live car will be towed alongside the stream shocker during the sample collection.

Specific staff will be assigned to handling the fish (i.e. measurement, labeling, and packaging). Staff assigned to handling the fish will wear powderless nitrile gloves, which will be changed frequently. Gloves are to be changed whenever there is potential for cross-contamination, including but not limited to, the following

activities (MDEQ 2019):

- Each time sampling equipment is handled.
- Prior to sample collection.
- After handling any sample, including QA/QC samples.
- After handling of any non-dedicated sampling equipment, contact with non-decontaminated surfaces, or when judged necessary by field personnel.
- During and after decontamination of non-dedicated sampling equipment.

Staff involved in the netting of fish will be instructed to minimize contact with the fish upon collection. Staff assigned to handling the fish will assist with removing fish from the nets and placing them into the holding wells. Care will be taken to minimize contact between the fish and other surfaces prior to being placed in a sample storage bag.

4.2.2 Fish Dispatch

Fish are to be dispatched quickly via non-chemical methods and in a manner that minimizes contact with potential PFAS containing surfaces or substances. The field crew supervisor and project lead will determine the most appropriate method at the time of field sampling. Tissue damage, in particular punctures and lacerations, during dispatch should be avoided.

4.2.3 Labelling and Documentation

Fish will be removed from the live car one at a time for labelling and documentation. Fish will be removed by an assigned handler (wearing clean nitrile gloves) and placed directly into a PFAS-free sample storage bag. Air will be removed from the bag and the bag sealed. Once sealed this bag is not to be reopened. The outside of the bag will be labelled using a Sharpie Ultra-Fine marker with the field accession number of the sample.

The sealed bag containing the fish will then be placed into a second PFAS-free sample storage bag.

A label will be generated using standard white office paper and pencil (or Ultra-Fine Point Sharpie marker) and will contain the following information:

- Site ID (AWQ Station ID)
- Waterbody name
- Date of collection
- Fish species
- Fish total length in mm (see section 4.2.4)
- Field accession number

Once complete, the label will be placed inside the second bag, but outside of the first bag so as to avoid making contact with the sample. The second bag will then be sealed securely and placed on ice.

The field accession number, species, and total length will be recorded on the field datasheet/COC.

4.2.4 Field Measurements

To avoid cross-contamination, total length will be measured after the fish has been placed into the first sample bag. The fish will be placed on a measuring board and total length will be measured to the nearest millimeter and recorded on the field datasheet/COC (Appendix C). Total length is measured from the tip of the snout to the tip of the longer lobe of the caudal fins; when measuring, the caudal fin lobes are compressed along the mid-line. The project lead will supervise all total length measurements to ensure consistency and accuracy in measurements.

4.2.5 Sample Transport

Labelled and securely sealed samples will be stored on wet ice in a closed cooler.

Samples will be separated by study location, with fish collected from the Natchaug River placed in a separate cooler than those collected from the Quinebaug River. Multiple coolers may be utilized, but each cooler should contain specimens from only one study location.

The chain of custody for each sampling event is to remain with the sample(s) at all times during transport.

5.0 FIELD SAFETY

Staff are expected to follow all standard Agency field safety protocols. The field crew lead is responsible for ensuring field staff adhere to these protocols and to make any necessary corrections as needed.

Unsafe field conditions, including but not limited to very high flows, heavy rain, thunder and/or lightning will cancel sampling.

6.0 SAMPLE PROCESSING, PRESERVATION & SHIPMENT

6.1 Sample Preservation & Holding

Whole fish will be brought to the DEEP Windsor Lab (9 Windsor Avenue, Windsor, CT) at the conclusion of the collection event. Samples will be checked to ensure they are properly labelled and contained, then logged into the laboratory fish tissue log, and placed in the fish tissue freezer for freezing and short-term storage.

The freezer will be checked at least once daily during business days by the lab manager for evidence of improper operation and possible sample compromise. And such evidence will be reported to the project lead, who will determine the appropriate course of action from there.

The chain of custody is to remain with the fish samples at all times.

6.2 Sample Shipment

Samples will be frozen as whole specimens and shipped as soon as possible, but no later than 14 days after collection. Samples will be shipped overnight in coolers on dry ice or wet ice. (Chemical ice packs are to be avoided.)

The original field chain of custody (COC) is to be shipped with the fish samples; a copy of the original will be made and retained by the project lead and filed at the DEEP Windsor Lab with the field data sheets.

In addition, the SGS Axys COC (Appendix F) will be completed. A copy of the original will be made by the project lead and filed at the DEEP Windsor Lab. The original SGS Axys COC will be shipped with the samples.

The receiving laboratory will be notified of the shipment within 24 hours. Upon receipt the lab will complete the field and shipping COC and send updated copies to the project lead for electronic filing and hard copy filing at the DEEP Windsor Lab.

7.0 SAMPLE PROCESSING & ANALYSIS

7.1 Sample Summary

Samples will include a total of forty (40) fish fillet tissue samples to be analyzed for PFAS.

Ten (10) predator or game fish species and ten (10) bottom dwelling species will be collected from each location to achieve a total of twenty (20) samples per study location. No field duplicates will be collected.

7.2 Sample Processing

No field processing will occur. To minimize the potential for cross-contamination, all tissue processing will be done trained staff at the analyzing laboratory (SGS AXYS Analytical Services Ltd.)

Upon receipt of the samples, the analytical laboratory will prepare descaled, skin-on fillets for chemical analysis in accordance with USEPA guidance (USEPA, 2000) and the laboratory procedures (Appendix D).

Samples are to be analyzed as individual fish, not composite samples. The left side fillet will be used for analysis. Fillets will be homogenized using PFAS-free equipment.

The right side fillet will be archived according to SGS Axys protocols. If budget allows, the remaining carcass of the fish will also be archived for future whole fish PFAS level determination.

7.3 Laboratory Analytical Methods

Fish tissue samples will be analyzed for PFAS by SGS AXYS Analytical Services Ltd. using an in-house laboratory method (SGS AXYS MLA-110; Appendix E).

Surrogates will be added to all samples prior to QC sampling and prior to analysis.

See Appendix E for details on the analytical methods to be used and the 33 analytes to be reported by the laboratory.

7.4 Quality Control and Quality Assurance Samples

In addition, the laboratory shall prepare and analyze the following QA/QC samples for each study location:

- method blank
- laboratory control sample
- matrix spike
- matrix spike duplicate

In the event that a left-side fillet sample has a detect, and additional DPH funding is available, the right-side fillet from that fish sample will be run for comparison analysis.

7.5 Data Review and Reporting

Data produced during the laboratory analyses described in this plan will be presented by SGS AXYS in a Level 4 data package that will be delivered to the CT Department of Public Health (CT DPH).

Following an initial review by CT DPH, data from this sampling program will be submitted to CT DEEP. CT DEEP will provide internal review and guidance to assist with interpretation of the data. The study results will be used to assess potential human health risk via fish consumption. If warranted, public reporting of the data will be coordinated by CT DPH.

8.0 REFERENCES

Connecticut Department of Energy and Environmental Protection (CT DEEP) 2013. Standard Operating Procedures for the Collection of Fish Community Data from Wadeable Streams for Aquatic Life Assessment. Bureau of Water Protection and Land Reuse, Planning and Standards Division, Hartford, CT. https://www.ct.gov/deep/lib/deep/water/water_quality_management/monitoringpubs/fishcommunitysop.pdf

Michigan Department of Environmental Quality (MDEQ). Revised 1/2019. MDEQ Fish Tissue PFAS Sampling Guidance. https://www.michigan.gov/documents/pfasresponse/Fish_Tissue_PFAS_Sampling_Guidance_644911_7.pdf

United States Environmental Protection Association (U.S. EPA) 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Vol 1, Fish Sampling and Analysis: 3rd edition. EPA 823-B-00-007. Office of Water, Washington D.C. https://www.epa.gov/sites/production/files/2018-11/documents/guidance-assess-chemical-contaminant-vol1-third-edition.pdf

APPENDIX A
Monitoring Location Maps

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Waterbody	Station ID	Location Description	Municipality	Latitude	Longitude	Potential Source
Natchaug River	14313	Between the confluence with the Willimantic River and Route 66	Windham, CT	41.7155	-72.1941	AFFF Releases

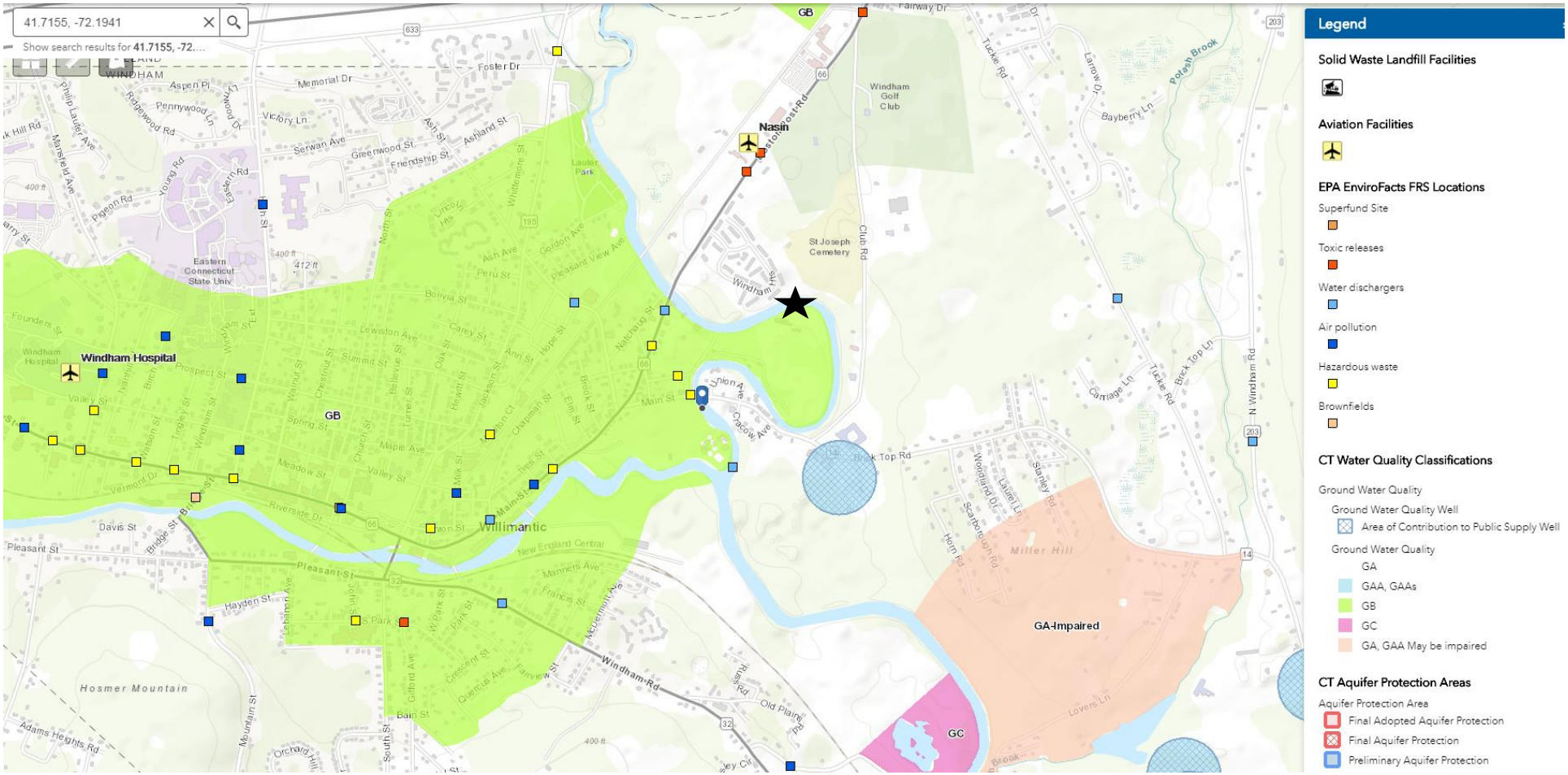

Figure 1. Map of potential sources of PFAS contaminants in the vicinity of the Natchaug River – Shetucket River confluence. The black star notes the location of the Eastern CT Fire School. Sampling will occur in the vicinity of this area.

Figure 2. Aerial photos of the lower Natchaug River at the confluence with the Shetucket River confluence. The black star notes the location of the Eastern CT Fire School. Sampling will occur in the vicinity of this area.

Waterbody	Station ID	Location Description	Municipality	Latitude	Longitude	Potential Source
Quinebaug River	17298	Between the Little River confluence and Route 44 bridge	Putnam, CT	41.9129	-71.9121	Industrial Dicharges

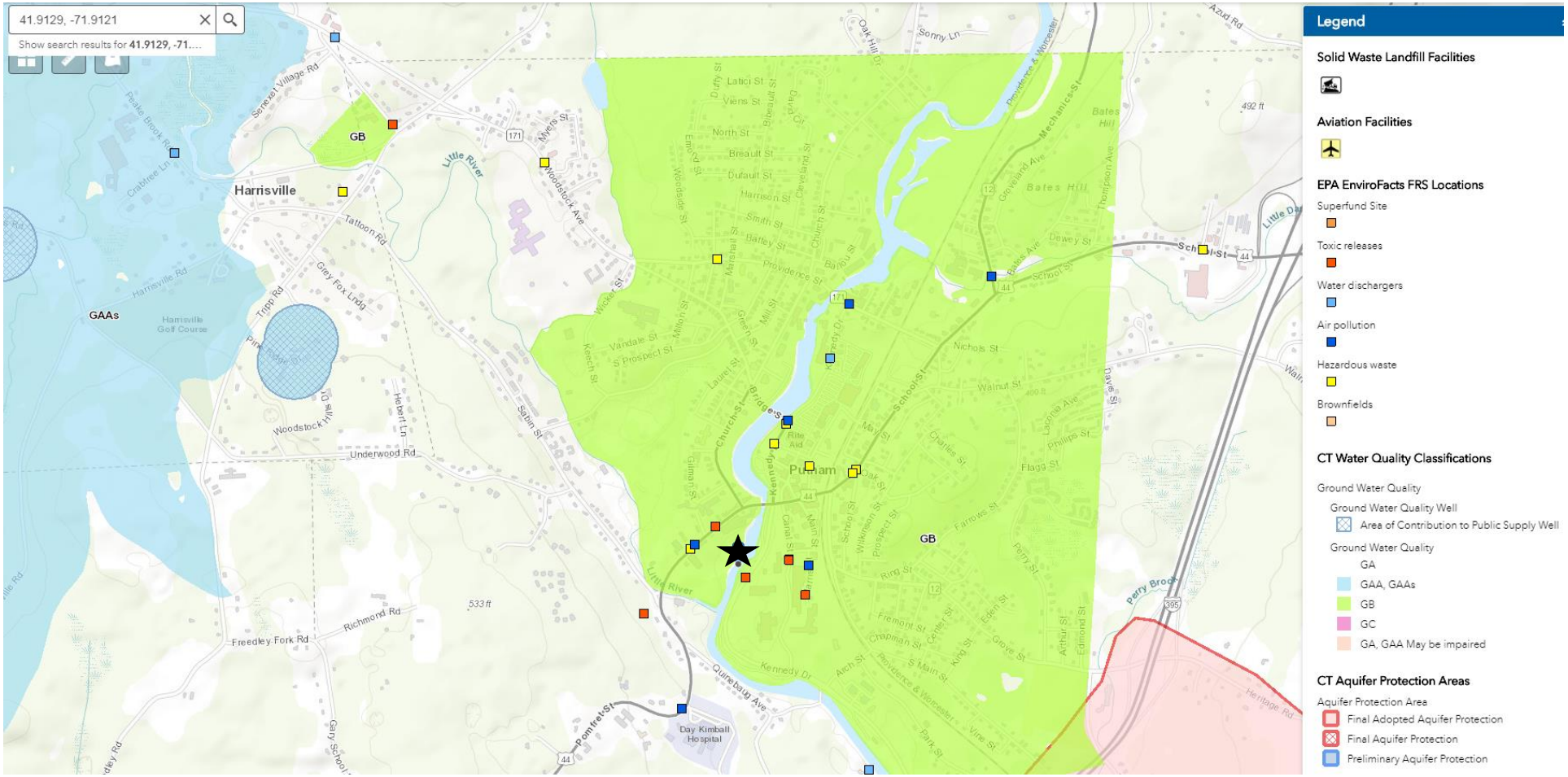

Figure 3. Map of potential sources of PFAS contaminants in the vicinity of the Quinebaug River in Putnam, CT. The black star notes the approximate location where sampling will occur.

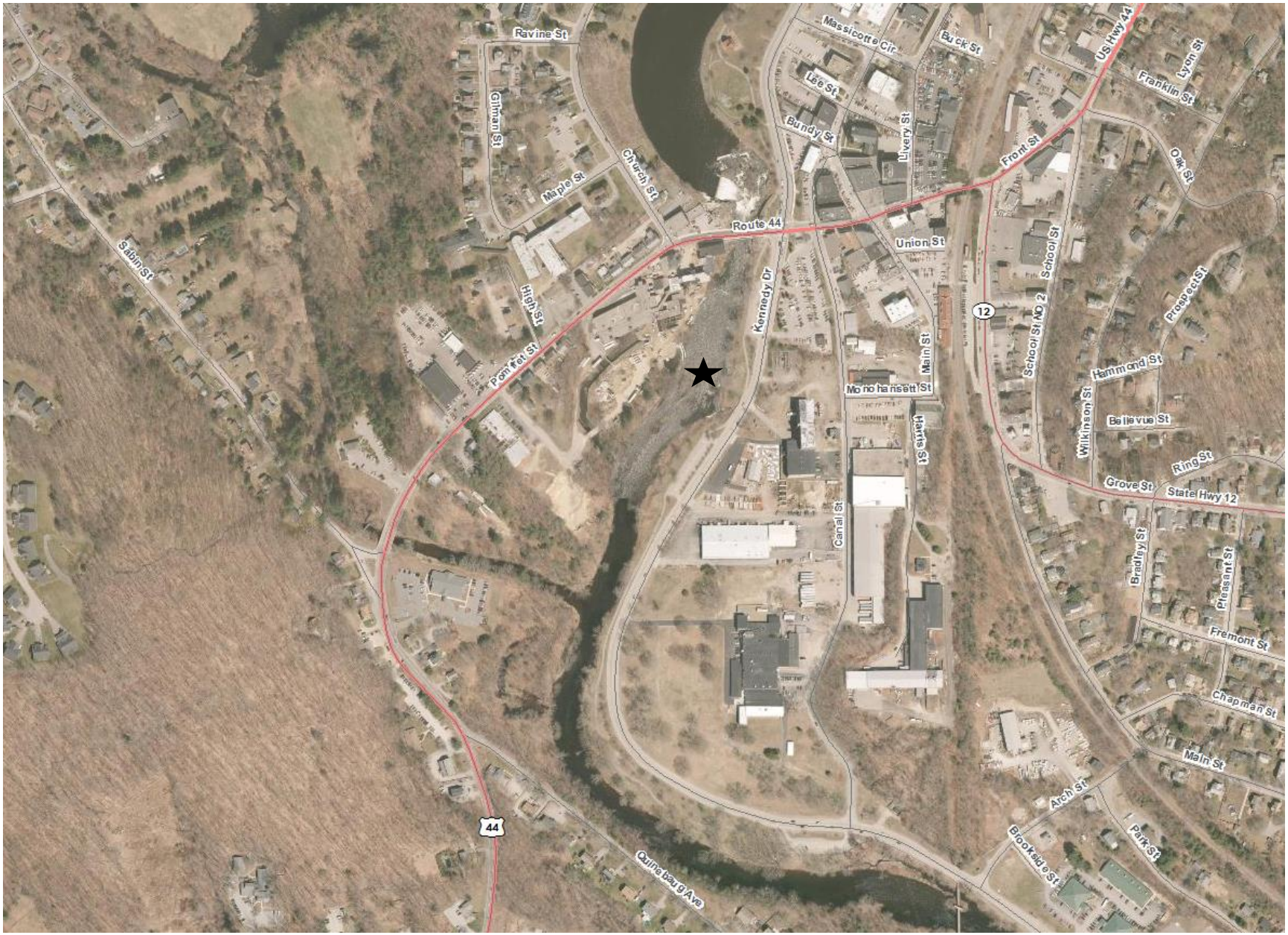

Figure 4. Aerial photos of the Quinebaug River at Putnam, CT. Sampling will occur in the reach upstream of the confluence with the Little River and downstream of Route 44, at the approximate location of the black star.

APPENDIX B
PFAS Sampling Quick Reference Field Guide

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Michigan Department of Environmental Quality

MDEQ PFAS Sampling Quick Reference Field Guide[1]

All Items Used During Sampling Event

● Prohibited

• Items or materials that contain fluoropolymers such as
  ○ Polytetrafluoroethylene (PTFE), that includes the trademarks Teflon® and Hostaflon®
  ○ Polyvinylidene fluoride (PVDF), that includes the trademark Kynar®
  ○ Polycholotrifluoroethylene (PCTFE), that includes the trademark Neoflon ®
  ○ Ethylene-tetrafluoro-ethylene (ETFE), that includes the trademark Tefzel®
  ○ Fluorinated ethylene propylene (FEP), that includes the trademarks Teflon® FEP and Hostaflon® FEP
• Items or materials that contain any other fluoropolymer

Pumps, Tubing, and Sampling Equipment

● Prohibited	■ Allowable	▲Needs Screening[2]

• Items or materials containing any fluoropolymer (potential items include tubing, valves, or pipe thread seal tape)	• High-density polyethylene (HDPE)
	• Low-density polyethylene (LDPE) tubing
	• Polypropylene
	• Silicone
	• Stainless-steel
	• Any items used to secure sampling bottles made from:
	  ○ Natural rubber
	  ○ Nylon (cable ties)
	  ○ Uncoated metal springs
	  ○ Polyethylene
		• Any items or materials that will come into direct contact with the sample that have **not** been verified to be PFAS-free
		  ○ Do not assume that any sampling items or materials are PFAS-free based on composition alone

Sample Storage and Preservation

● Prohibited	■ Allowable	▲Needs Screening[2]
• Polytetrafluoroethylene (PTFE): Teflon® lined bottles or caps	• Glass jars[4] • Laboratory-provided PFAS-Free bottles: ○ HDPE or polypropylene • Regular wet ice • Thin HDPE sheeting • LDPE resealable storage bags (i.e. Ziploc®) that will not contact the sample media[6]	• Aluminium foil[4] • Chemical or blue ice[5] • Plastic storage bags other than those listed as ■ Allowable • Low-density polyethylene (LDPE) bottles

Field Documentation

● Prohibited	■ Allowable	▲Needs Screening[2]
• Clipboards coated with PFAS • Notebooks made with PFAS treated paper • PFAS treated loose paper • PFAS treated adhesive paper products	• Loose paper (non-waterproof, non-recycled) • Rite in the Rain® notebooks • Aluminium, polypropylene, or Masonite field clipboards • Ballpoint pens, pencils, and Fine or Ultra-Fine Point Sharpie® markers	• Plastic clipboards, binders, or spiral hard cover notebooks • All markers not listed as ■ Allowable • Post-It® Notes or other adhesive paper products • Waterproof field books

Decontamination

● Prohibited	■ Allowable	▲Needs Screening[2]
• Decon 90® • PFAS treated paper towel	• Alconox®, Liquinox®, or Citranox® • Triple rinse with PFAS-free deionized water • Cotton cloth or untreated paper towel	• Municipal water • Recycled paper towels or chemically treated paper towels

Clothing, Boots, Rain Gear, and PPE

● Prohibited	■ Allowable	▲Needs Screening[2]

• New or unwashed clothing
• Anything made of or with:
  ○ Gore-Tex™ or other water-resistant synthetics
• Anything applied with or recently washed with:
  ○ Fabric softeners
  ○ Fabric protectors, including UV protection
  ○ Insect resistant chemicals
  ○ Water, dirt, and/or stain resistant chemicals
	• Powderless nitrile gloves
	• Well-laundered synthetic or 100% cotton clothing, with most recent launderings not using fabric softeners
	• Made of or with:
	  ○ Polyurethane
	  ○ Polyvinyl chloride (PVC)
	  ○ Wax coated fabrics
	  ○ Rubber / Neoprene
	  ○ Uncoated Tyvek®
		• Latex gloves
		• Water and/or dirt resistant leather gloves
		• Any special gloves required by a HASP
		• Tyvek® suits, clothing that contains Tyvek®, or coated Tyvek®

Food and Beverages

● Prohibited	■ Allowable

• No food should be consumed in the staging or sampling areas, including pre-packaged food or snacks.
  ■ If consuming food on-site becomes necessary, move to the staging area and remove PPE. After eating, wash hands thoroughly and put on new PPE.
	• Brought and consumed only outside the vicinity of the sampling area:
	  ○ Bottled water
	  ○ Hydration drinks (i.e. Gatorade®, Powerade®)

Personal Care Products (PCPs) - for day of sample collection[6]

● Prohibited	■ Allowable	▲Needs Screening[2]
• Any PCPs[6], sunscreen, and insect repellent applied in the sampling area.	PCPs[6], sunscreens, and insect repellents applied in the staging area, away from sampling bottles and equipment followed by thoroughly washing hands: **PCPs[6]:** • Cosmetics, deodorants/antiperspirants, moisturizers, hand creams, and other PCPs[6] **Sunscreens:** • Banana Boat® for Men Triple Defense Continuous Spray Sunscreen SPF 30 • Banana Boat® Sport Performance Coolzone Broad Spectrum SPF 30 • Banana Boat® Sport Performance Sunscreen Lotion Broad Spectrum SPF 30 • Banana Boat® Sport Performance Sunscreen Stick SPF 50 • Coppertone® Sunscreen Lotion Ultra Guard Broad Spectrum SPF 50 • Coppertone® Sport High Performance AccuSpray Sunscreen SPF 30 • Coppertone® Sunscreen Stick Kids SPF 55 • L'Oréal® Silky Sheer Face Lotion 50 • Meijer® Clear Zinc Sunscreen Lotion Broad Spectrum SPF 50 • Meijer® Sunscreen Continuous Spray Broad Spectrum SPF 30 • Meijer® Clear Zinc Sunscreen Lotion Broad Spectrum SPF 15, 30 and 50 • Meijer® Wet Skin Kids Sunscreen Continuous Spray Broad Spectrum SPF 70 • Neutrogena® Beach Defense Water+Sun Barrier Lotion SPF 70 • Neutrogena® Beach Defense Water+Sun Barrier Spray Broad Spectrum SPF 30 • Neutrogena® Pure & Free Baby Sunscreen Broad Spectrum SPF 60+ • Neutrogena® UltraSheer Dry-Touch Sunscreen Broad Spectrum SPF 30 **Insect Repellents:** • OFF® Deep Woods • Sawyer® Permethrin	• Products other than those listed as ■Allowable

[1] This table is not considered to be a complete listing of prohibited or allowable materials. All materials should be evaluated prior to use during sampling. The manufacturers of various products should be contacted in order to determine if PFAS was used in the production of any particular product.

[2] Equipment blank samples should be taken to verify these products are PFAS-free prior to use during sampling.

[3] **For surface water foam samples:** LDPE storage bags may be used in the sampling of foam on surface waters. In this instance, it is allowable for the LDPE bag to come into direct contact with the sample media.

[4] **For fish and other wildlife samples:** Depending on the project objectives, glass jars and aluminum foil might be used for PFAS sampling. PFAS has been found to bind to glass and if the sample is stored in a glass jar, a rinse of the jar is required during the sample analysis. PFAS are sometimes used as a protective layer for some aluminum foils. An equipment blank sample should be collected prior to any aluminum foil use.

[5] Regular ice is recommended as there are concerns that chemical and blue ice may not cool and maintain the sample at or below 42.8°F (6°C) (as determined by EPA 40 CFR 136 – NPDES) during collection and through transit to the laboratory.

[6] Based on evidence, avoidance of PCPs is considered to be precautionary because none have been documented as having cross-contaminated samples due to their use. However, if used, application of PCPs must be done at the staging area and away from sampling bottles and equipment, and hands must be thoroughly washed after the use of any PCPs prior to sampling.

● - Prohibited ■ - Allowable ▲ - Needs Screening

APPENDIX C

PFAS Tissue Collection Field Datasheet/COC Form

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Connecticut Department of ENERGY & ENVIRONMENTAL PROTECTION

FISH TISSUE FIELD CHAIN OF CUSTODY

For questions regarding this sample contact:
CHRIS BELLUCCI 860-424-3735

Waterbody: _____ **AWQ ID#:** _____
Location: _____ **Basin ID:** _____
Latitude: _____
Town: _____ **Longitude:** _____

Sample Date: _____
Collector(s): _____
Gear: _____
Trip ID: _____

PLEASE KEEP ALL FISH ON ICE IMMEDIATELY AFTER COLLECTION

Specimen ID	Fish #	Species	Total length	Weight	Receiving Lab ID
19-F-	1				
19-F-	2				
19-F-	3				
19-F-	4				
19-F-	5				
19-F-	6				
19-F-	7				
19-F-	8				
19-F-	9				
19-F-	10				
19-F-	11				
19-F-	12				
19-F-	13				
19-F-	14				
19-F-	15				
19-F-	16				
19-F-	17				
19-F-	18				
19-F-	19				
19-F-	20				

Comments:

Relinquished By: (Print) _____	Received by: (Print) _____
(Collector) (Sign) _____	(DEEP Lab) (Sign) _____
Date/Time	Date/Time

Relinquished By: (Print) _____	Received by: (Print) _____
(DEEP Lab) (Sign) _____	(Receiving Lab) (Sign) _____
Date/Time	Date/Time

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APPENDIX D
Summarized SGS Axys Sample Preparation Procedures

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SGS AXYS Analytical Services Ltd.

SUMMARY OF SGS AXYS METHOD MLA-110 REV. 02 VER. 04

Analytical Procedure for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples, Solids, Tissues, AFFF Products and Solvent Extracts by LC-MS/MS

This method describes the analysis of per- and polyfluoroalkyl substances (PFAS) in aqueous, solid, biosolid, tissue and AFFF product samples and solvent extracts, determined as the total of linear and branched isomers. After spiking with isotopically labeled surrogate standards samples are extracted and cleaned up by Solid Phase Extraction (SPE). The extracts are then analyzed by liquid chromatography/mass spectrometry (LC-MS/MS). Final sample concentrations are determined by isotope dilution/internal standard quantification.

Typical reporting limits are shown below, for the method default sample sizes:

Analyte groups	Aqueous sample	Extract [1]	Solid [2]	Biosolid [3]	Tissues [4]	AFFF Products[5]
Typical sample size	0.5 L	0.75 mL	5 g	2.5 g	2.0 g	0.02 g
Units	ng/L	ng/mL	ng/g	ng/g	ng/g	ng/g
Perfluoroalkyl carboxylates	0.8-3.2	530-2100	0.08-0.32	0.16-0.64	0.2-0.8	10-40
Perfluoroalkyl sulfonates	0.8	530	0.08	0.16	0.2	10
Fluorotelomer sulfonates	3.2	2100	0.32	0.64	0.8	40
Perfluorooctane sulfonamides	0.8	530	0.08	0.16	0.2	10
Perfluorooctane sulfonamidoacetic acids	0.8	530	0.08	0.16	0.2	10
Perfluorooctane sulfonamide ethanols	8	5300	0.8	1.6	2.0	100
Per- and polyfluoroether carboxylates	3.2	2100	0.32	0.64	0.8	N/A
Ether sulfonates	3.2	2100	0.32	0.64	0.8	N/A

[1] Detection limits for extract samples depend on the samples size processed. The reporting limits provided here are based on the typical extract sample size listed above.

[2] A maximum of 10 g wet, or 5 g dry, solid may be analyzed.

[3] A maximum of 5 g wet, or 0.5 g dry, biosolid may be analyzed.

[4] A maximum of 2 g of tissue may be analyzed.

[5] A maximum of 0.02 g of AFFF Product is analyzed. Reporting limits are based on the calibration A point. The method is not currently validated for ether carboxylates/sulfonates in AFFF samples. Only Class B aqueous film forming foams (AFFF) or alcohol-resistant AFFF (AR-AFFF) samples are part of this scope. Other AFFF products such as fluoroprotein foams maybe be analyzed using this method, but need special handling. Consult product Safety Data Sheets (SDS) and other information before confirming. All samples will be logged in under an “AQIP” (Aqueous Industrial Product) matrix code in LIMS.

Refer to Appendix B for sample storage conditions.

PFAS Target Analytes

Perfluoroalkyl carboxylates

Perfluorobutanoic acid (PFBA, Perfluorobutanoate)
Perfluoropentanoic acid (PFPeA, Perfluoropentanoate)
Perfluorohexanoic acid (PFHxA, Perfluorohexanoate)
Perfluoroheptanoic acid (PFHpA, Perfluoroheptanoate)
Perfluorooctanoic acid (PFOA, Perfluorooctanoate)
Perfluorononanoic acid (PFNA, Perfluorononanoate)
Perfluorodecanoic acid (PFDA, Perfluorodecanoate)
Perfluoroundecanoic acid (PFUnA, Perfluoroundecanoate)
Perfluorododecanoic acid (PFDoA, Perfluorododecanoate)
Perfluorotridecanoic acid (PFTrDA, Perfluorotridecanoate)
Perfluorotetradecanoic acid (PFTeDA, Perfluorotetradecanoate)

Perfluoroalkyl sulfonates

Perfluorobutanesulfonic acid (PFBS, Perfluorobutanesulfonate)
Perfluoropentanesulfonic acid (PFPeS, Perfluoropentanesulfonate)
Perfluorohexanesulfonic acid (PFHxS, Perfluorohexanesulfonate)
Perfluoroheptanesulfonic acid (PFHpS, Perfluoroheptanesulfonate)
Perfluorooctanesulfonic acid (PFOS, Perfluorooctanesulfonate)
Perfluorononanesulfonic acid (PFNS, Perfluorononanesulfonate)
Perfluorodecanesulfonic acid (PFDS, Perfluorodecanesulfonate)
Perfluorododecanesulfonic acid (PFDoS, Perfluorododecanesulfonate)

Fluorotelomer sulfonates

1H, 1H, 2H, 2H-perfluorohexane sulfonic acid (4:2 FTS, 1H, 1H, 2H, 2H-perfluorohexane sulfonate)
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS, 1H, 1H, 2H, 2H-perfluorooctane sulfonate)
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS, 1H, 1H, 2H, 2H-perfluorodecane sulfonate)

Perfluorooctane sulfonamides

Perfluorooctanesulfonamide (PFOSA) [1]
N-Methylperfluorooctanesulfonamide (N-MeFOSA)
N-Ethylperfluorooctanesulfonamide (N-EtFOSA)

Perfluorooctane sulfonamidoacetic acids

N-Methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA, N-Methylperfluoro-1-octanesulfonamidoacetate)
N-Ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA, N-Ethylperfluoro-1-octanesulfonamidoacetate)

Perfluorooctane sulfonamidoethanols

N-Methylperfluoro-1-octanesulfonamidoethanol (N-MeFOSE)

N-Ethylperfluoro-1-octanesulfonamidoethanol (N-EtFOSE)

Ether carboxylates

2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propionic acid (HFPO-DA, 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propionoate) [2]

Decafluoro-3H-4,8-dioxanonoate (ADONA, DONA, Decafluoro-3H-4,8-dioxanonoic acid)

Ether sulfonates

9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS, 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate) [3]

11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS, 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate) [4]

[1] PFOSA also called FOSA
[2] GenX major component, not offered for AFFF products
[3] F53-B main component, not offered for AFFF products
[4] F53-B minor component, not offered for AFFF products

The carboxylic and sulfonic acid analyte concentrations can be reported as either the acid or the anion forms. The anion and corresponding acid forms and their CAS Registry Numbers are shown in Appendix A of this summary.

1.0 EXTRACTION AND CLEANUP PROCEDURES

Aqueous samples size may be up to 1000 mL for aqueous samples analyzed by this method, and up to 0.75 mL for extracts/solvents. Samples are stored in HDPE (high density polyethylene) containers. All samples are spiked with surrogate standards. Aqueous samples are extracted by solid phase extraction (SPE) using weak anion exchange cartridges; wash and elution procedures are chosen to meet various analysis requirements. Sample extracts may also be treated with carbon powder. The extracts are spiked with recovery standards and analyzed by LC-MS/MS.

Extract/solvent samples don't undergo solid phase extraction. The samples are spiked with surrogate and recovery standards, and analyzed by LC-MS/MS.

Solid and biosolid sample size may be up to 5 g dry weight for solid samples or up to 5 g wet weight (max. 0.5 g dry weight) for biosolid samples. After addition of isotopically labelled surrogate standards the sample is extracted by shaking three times with methanolic ammonium hydroxide solution, each time collecting the supernatants. The supernatants are combined and treated with ultra pure carbon powder. The resulting solution is diluted with water and cleaned up by solid phase extraction (SPE) using disposable cartridges containing a weak anion exchange sorbent. The eluate is spiked with recovery standards and analyzed by LC-MS/MS.

Tissue sample size may be up to 2 g. After addition of isotopically labelled surrogate standards the sample is extracted with methanolic potassium hydroxide solution, with acetonitrile, and finally with methanolic potassium hydroxide solution, each time collecting the supernatants. The supernatants are combined, diluted with water and cleaned up by solid phase extraction (SPE)

on a weak anion exchange sorbent. Sample extracts may also be treated with carbon powder. The eluate is spiked with recovery standards and analyzed by LC-MS/MS.

All AFFF samples are pre-screened before analysis to determine the appropriate amount of sample to analyze. A suitable subsample is dissolved in water, spiked with surrogate standards and extracted by solid phase extraction (SPE). Sample extracts may optionally be treated with carbon powder. The extracts are spiked with recovery standards and analyzed by LC-MS/MS

2.0 INSTRUMENTATION

Analysis of the sample extract is performed on a UPLC (ultrahigh performance liquid chromatography) reversed phase C18 column using a solvent gradient. The column is coupled to a triple quadrupole mass spectrometer run at unit mass resolution in the Multiple Reaction Monitoring (MRM) in negative electrospray ionization mode.

3.0 CALIBRATION

Initial calibration of the LC-MS/MS instrument is performed by the analysis of five or more calibration solutions. A mid-level calibration standard is analyzed to verify the initial calibration and injected after:

- at least every 12 hours.
- For DoD accredited work after every 10 client samples or every 12 hours, whichever occurs first, and at the end of the instrumental run sequence.

List of Surrogate and Recovery Standards

Surrogate Standards	Recovery Standards
$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	$^{13}C_4$-PFOA
$^{13}C_4$-PFHpA	$^{13}C_5$-PFNA
$^{13}C_8$-PFOA	$^{13}C_2$-PFDA
$^{13}C_9$-PFNA	$^{18}O_2$-PFHxS
$^{13}C_6$-PFDA	$^{13}C_4$-PFOS
$^{13}C_7$-PFUnA	
$^{13}C_2$-PFDoA	
$^{13}C_2$-PFTeDA	
$^{13}C_3$-PFBS	
$^{13}C_3$-PFHxS	
$^{13}C_8$-PFOS	
$^{13}C_2$-4:2 FTS	
$^{13}C_2$-6:2 FTS	

$^{13}C_2$-8:2 FTS	
$^{13}C_8$-PFOSA	
D_3-N-MeFOSA	
D_5-N-EtFOSA	
D_3-N-MeFOSAA	
D_5-N-EtFOSAA	
D_7-N-MeFOSE	
D_9-N-EtFOSE	
$^{13}C_3$-HFPO-DA	

4.0 QUANTIFICATION PROCEDURES

Calculations

Target compounds are quantified using the isotope dilution/internal standard method, comparing the area of the quantification ion to that of the ^{13}C-labelled or deuterium labeled standard and correcting for response factors. Linear quantification equations are determined from a multi-point calibration series with 1/X weighting fit and expressed as below:

$$Y = \text{slope} \times X + \text{intercept}$$

where: $Y = \text{response ratio} = \left(\frac{\text{area of Target}}{\text{area of Surr}} \times \text{weight of Surr (ng)} \right)$, and

X = weight of target (ng)

The slope and intercept are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Sample Conc.} = \left(\frac{\text{area of Target}}{\text{area of Surr}} \times \text{weight of Surr (ng)} - \text{intercept (ng)} \right) \times \left(\frac{1}{\text{slope}} \right) \times \left(\frac{1}{\text{sample size (L or g)}} \right)$$

where Surr is the surrogate standard

The recovery of the surrogate standard is calculated (by internal standard quantification against the recovery standard using an average RRF) and monitored as an indication of overall data quality. Final target concentrations are recovery corrected by this method of quantification.

4.1 Reporting Limits

Sample Specific Detection Limits (SDL) are determined by converting the area equivalent to 3.0 times the estimated chromatographic noise height to a concentration in the same manner that target peak responses are converted to final concentrations. The SDL accounts for any effect of matrix on the detection system and for recovery achieved through the analytical work-up.

Results are reported to the greater of the SDL or the concentration equivalent to the lowest calibration standard analyzed.

SGS AXYS Analytical Services Ltd.

Analytes and Quantification References

Target analytes	Quantified using
PFBA	$^{13}C_4$-PFBA
PFPeA	$^{13}C_5$-PFPeA
PFHxA	$^{13}C_5$-PFHxA
PFHpA	$^{13}C_4$-PFHpA
PFOA	$^{13}C_8$-PFOA
PFNA	$^{13}C_9$-PFNA
PFDA	$^{13}C_6$-PFDA
PFUnA	$^{13}C_7$-PFUnA
PFDoA	$^{13}C_2$-PFDoA
PFTrDA	$^{13}C_2$-PFTeDA
PFTeDA	$^{13}C_2$-PFTeDA
PFBS	$^{13}C_3$-PFBS
PFPeS	$^{13}C_3$-PFHxS
PFHxS	$^{13}C_3$-PFHxS
PFHpS	$^{13}C_8$-PFOS
PFOS	$^{13}C_8$-PFOS
PFNS	$^{13}C_8$-PFOS
PFDS	$^{13}C_8$-PFOS
PFDoS	$^{13}C_8$-PFOS
4:2 FTS	$^{13}C_2$-4:2 FTS
6:2 FTS	$^{13}C_2$-6:2 FTS
8:2 FTS	$^{13}C_2$-8:2 FTS
PFOSA	$^{13}C_8$-PFOSA
N-MeFOSA	D_3-N-MeFOSA
N-EtFOSA	D_5-N-EtFOSA
N-MeFOSAA	D_3-N-MeFOSAA
N-EtFOSAA	D_5-N-EtFOSAA
N-MeFOSE	D_7-N-MeFOSE
N-EtFOSE	D_9-N-EtFOSE
HFPO-DA	$^{13}C_{-3}$-HFPO-DA
ADONA	$^{13}C_{-3}$-HFPO-DA
9Cl-PF3ONS	$^{13}C_{-3}$-HFPO-DA
11Cl-PF3OUdS	$^{13}C_{-3}$-HFPO-DA

SGS AXYS Analytical Services Ltd.

Surrogate standards	Quantified using
$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	$^{13}C_4$-PFOA
$^{13}C_8$-PFOA	$^{13}C_4$-PFOA ($^{13}C_2$-PFHxA for AFFF products)
$^{13}C_9$-PFNA	$^{13}C_5$-PFNA
$^{13}C_6$-PFDA	$^{13}C_2$-PFDA
$^{13}C_7$-PFUnA	$^{13}C_2$-PFDA
$^{13}C_2$-PFDoA	$^{13}C_2$-PFDA
$^{13}C_2$-PFTeDA	$^{13}C_2$-PFDA
$^{13}C_3$-PFBS	$^{18}O_2$-PFHxS
$^{13}C_3$-PFHxS	$^{18}O_2$-PFHxS
$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
$^{13}C_2$-4:2 FTS	$^{18}O_2$-PFHxS
$^{13}C_2$-6:2 FTS	$^{18}O_2$-PFHxS
$^{13}C_2$-8:2 FTS	$^{18}O_2$-PFHxS
$^{13}C_8$-PFOSA	$^{13}C_4$-PFOS
D_3-N-MeFOSA	$^{13}C_4$-PFOS
D_5-N-EtFOSA	$^{13}C_4$-PFOS
D_3-N-MeFOSAA	$^{13}C_4$-PFOS
D_5-N-EtFOSAA	$^{13}C_4$-PFOS
D_7-N-MeFOSE	$^{13}C_4$-PFOS
D_9-N-EtFOSE	$^{13}C_4$-PFOS
$^{13}C_3$-HFPO-DA	$^{13}C_2$-PFHxA
Recovery standards	**Quantified using**
$^{13}C_3$-PFBA	External
$^{13}C_2$-PFHxA	External
$^{13}C_4$-PFOA	External
$^{13}C_5$-PFNA	External
$^{13}C_2$-PFDA	External
$^{18}O_2$-PFHxS	External
$^{13}C_4$-PFOS	External
$^{13}C_3$-PFBA	External

5.0 QUALITY ACCEPTANCE CRITERIA

Samples are analyzed in batches consisting of a maximum of twenty samples, one procedural blank and one spiked matrix (OPR) sample. A duplicate is analyzed, provided there is sufficient sample, with batches containing 7-20 samples. Matrix spike/matrix spike duplicate (MS/MSD) pairs may be analyzed on an individual contract basis. The batch is carried through the complete analytical process as a unit. For sample data to be reportable, the batch QC data must meet the established acceptance criteria presented on the analysis reports.

SGS AXYS Analytical Services Ltd.

QC Specifications for Aqueous, Solid, AFFF and Tissue Samples: Procedural Blank Levels and OPR Recovery Ranges

Compound	Procedural Blank Level (ng/sample)	OPR Recovery Range [1] for Aqueous, Solid and AFFF Samples (%)	OPR Recovery Range [1] for Tissue Samples (%)
PFBA	≤ 1.6	70-130	70-130
PFPeA	≤ 0.8	70-130	70-130
PFHxA	≤ 0.4	70-130	70-130
PFHpA	≤ 0.4	70-130	70-130
PFOA	≤ 0.4	70-130	70-130
PFNA	≤ 0.4	70-130	70-130
PFDA	≤ 0.4	70-130	60-130
PFUnA	≤ 0.4	70-130	70-140
PFDoA	≤ 0.4	70-130	70-130
PFTrDA	≤ 0.4	70-130	70-130
PFTeDA	≤ 0.4	70-130	70-130
PFBS	≤ 0.4	70-130	60-130
PFPeS	≤ 0.4	70-130	70-130
PFHxS	≤ 0.4	70-130	70-130
PFHpS	≤ 0.4	70-130	70-130
PFOS	≤ 0.4	70-130	70-140
PFNS	≤ 0.4	70-130	60-150
PFDS	≤ 0.4	70-130	40-150
PFDoS	≤ 0.4	60-130	70-140
4:2 FTS	≤ 1.6	70-130	40-150
6:2 FTS	≤ 5	70-130	70-130
8:2 FTS	≤ 1.6	70-130	70-170
PFOSA	≤ 0.4	70-130	70-130
N-MeFOSA	≤ 0.4	70-130	50-140
N-EtFOSA	≤ 0.4	70-130	70-130
N-MeFOSAA	≤ 0.4	70-130	60-140
N-EtFOSAA	≤ 0.4	70-130	60-140
N-MeFOSE	≤ 4	70-130	70-150
N-EtFOSE	≤ 4	70-130	70-130
HFPO-DA	≤ 1.6	70-130	70-130
ADONA	≤ 1.6	70-130	70-130
9Cl-PF3ONS	≤1.6	70-130	70-130
11Cl-PF3OUdS	≤ 1.6	70-130	60-130

[1] Marginal exceedance allowance – results for one compound may fall outside of these limits by a maximum of 10% of the value. Note that for AFFF products, these are interim specifications and data outside the specifications may be acceptable based on application and professional judgment.

SGS AXYS Analytical Services Ltd.

DoD QSM Rev. 5.3 Recovery Acceptance Limits for OPRs

Compound	OPR Recovery Range for Aqueous Samples (%)	OPR Recovery Range for Solid Samples (%)
PFBA	73-129	71-135
PFPeA	72-129	69-132
PFHxA	72-129	70-132
PFHpA	72-130	71-131
PFOA	71-133	69-133
PFNA	69-130	72-129
PFDA	71-129	69-133
PFUnA	69-133	64-136
PFDoA	72-134	69-135
PFTrDA	65-144	66-139
PFTeDA	71-132	69-133
PFBS	72-130	72-128
PFPeS	71-127	73-123
PFHxS	68-131	67-130
PFHpS	69-134	70-132
PFOS	65-140	68-136
PFNS	69-127	69-125
PFDS	53-142	59-134
4:2 FTS	63-143	62-145
6:2 FTS	64-140	64-140
8:2 FTS	67-138	65-137
PFOSA	67-137	67-137
N-MeFOSA	68-141	n.a.
N-MeFOSAA	65-136	63-144
N-EtFOSAA	61-135	61-139

SGS AXYS Analytical Services Ltd.

QC Specifications for Aqueous, Solid, AFFF and Tissue Samples: Surrogate Standard Recoveries, OPR and Samples

Surrogate Standard	OPR and Sample Recovery Range [1] for Aqueous, Solid And AFFF Samples (%)	OPR and Sample Recovery Range [1] for Tissue Samples (%)
$^{13}C_4$-PFBA	50-150	50-150
$^{13}C_5$-PFPeA	50-150	50-150
$^{13}C_5$-PFHxA	50-150	50-150
$^{13}C_4$-PFHpA	50-150	50-150
$^{13}C_8$-PFOA	50-150	50-150
$^{13}C_9$-PFNA	50-150	50-150
$^{13}C_6$-PFDA	50-150	50-180
$^{13}C_7$-PFUnA	50-150	50-150
$^{13}C_2$-PFDoA	50-150	50-150
$^{13}C_2$-PFTeDA	50-150	50-150
$^{13}C_3$-PFBS	50-150	50-150
$^{13}C_3$-PFHxS	50-150	50-150
$^{13}C_8$-PFOS	50-150	50-150
$^{13}C_2$-4:2 FTS	50-150	50-220
$^{13}C_2$-6:2 FTS	50-150	50-180
$^{13}C_2$-8:2 FTS	50-150	50-300
$^{13}C_8$-PFOSA	50-150	50-150
D_3-N-MeFOSA	30-150	[2]
D_5-N-EtFOSA	20-150	[2]
D_3-N-MeFOSAA	50-150	[2]
D_5-N-EtFOSAA	50-150	[2]
D_7-N-MeFOSE	30-150	[2]
D_9-N-EtFOSE	30-150	[2]
$^{13}C_3$-HFPO-DA	50-150	50-150

[1] Lower surrogate recoveries may be accepted based on application and professional judgment. Note that for AFFF products, these are interim specifications and data outside the specifications may be acceptable based on application and professional judgment.

[2] These surrogates used only to quantify the analogous native compounds. Formal surrogate recovery limits are under review.

SGS AXYS Analytical Services Ltd.

QC Specification Table: Other Parameters

QC Parameter	Specification
MS Acquisition Rate	Minimum acquisition rate for every native analyte and labeled compound peak: At least 10 data points per peak.
Instrument Sensitivity	Daily, S:N ≥ 10:1 for the primary transition product ion for all analytes for the lowest calibration standard (CAL B) and S:N ≥ 3:1 for the secondary ion. *Additional requirement for DoD QSM 5.1.1/5.3: Instrument sensitivity checks with analyte concentrations at the LOQ levels (refer to VER-004 "DL, LOD and LOQ Plan for DoD Work") shall be performed prior to analysis and then at least once every 12 hours. The found analyte concentrations must be within ±30% of the true values.*
Mass Calibration	Instrument must have a valid mass calibration following the manufacturer specified procedure prior to any sample analysis. The mass calibration is updated on an as-needed basis (e.g. QC failures, ion masses fall outside of the ±0.5 amu of the true value, major instrument maintenance, or if the instrument is moved.) Refer to SIN-033. The entire range shall be mass calibrated. The maximal allowed residual error is ≤ 0.1 Da for each mode with no more than two calibration points missed.
Mass Calibration Verification	Mass calibration must be verified to be ±0.5 amu of true value by acquiring a full scan continuum mass spectrum of a PFAS stock standard. Mass calibration is verified after each mass calibration, prior to initial ICAL.
Initial Calibration (I-CAL)	Run initially, and as required to maintain calibration verification and instrument sensitivity. CAL B is the default lowest calibration standard. (1/x) weighted linear fit, do not force through origin. Calculated conc. 75-125 % of actual (lowest cal may be 70-130%). R^2 ≥ 0.990. *Alternate specification for DOD QSM 5.1.1 / 5.3: Calculated conc. 70-130%.* R^2 ≥ 0.990. Surrogate recoveries must fall within 50-150%. Peak Asymmetry: 0.8-1.5 for PFBA and PFPeA measured in Cal E (mid cal point) at 10% of the peak height. If this is not achieved, perform instrument maintenance and re-run I-CAL.
Initial Calibration Verification (ICV):	Prior to sample analysis; analyze a second source standard (similar concentration to the CAL E); quantify against I-Cal, results must meet Cal/Ver accuracy specifications. *Alternate specification for DOD QSM 5.1.1 / 5.3: Calculated conc. 70-130%.*
Calibration Verification (Cal/Ver or CCV)	Continuing Calibration Verification using CAL E Run every 12 hours, quantify against I-CAL.

QC Parameter	Specification
	Calculated conc. 80 -120 % of actual allowing 70-130% of actual for a maximum of three compounds. *Alternate requirement for DoD QSM 5.1.1/5.3: Every 10 client samples or every 12 hours whichever occurs first, and at the end of the analytical sequence; quantify against I-CAL. Calculated conc. 70- 130 % of actual.* Surrogate recoveries must fall within 50-150%. For internal purposes monitor Peak Asymmetry for every Cal/Ver
Instrumental Carryover and Instrument Background	Every Initial Calibration, Cal/Ver, or OPR: ≤ 0.3 % carryover. *Additional requirement for DoD QSM 5.1.1/5.3: Instrument blanks shall be run immediately following the highest standard analyzed and daily prior to sample analysis. The concentration of each analyte must be ≤ ½ of the LOQ (C CAL, refer to VER-004 "DL, LOD and LOQ Plan for DoD Work") which is equivalent to < LMCL (B CAL).*
Duplicate Samples	If conc. ≥ 5 times R.L., RPD ≤ 40% If conc. < 5 times R.L., guideline RPD ≤ 100%
MS/MSD	Optional test, client must request If conc. ≥ 5 times R.L., RPD ≤ 40% If conc. < 5 times R.L., guideline RPD ≤ 100% *Alternate requirement for DoD QSM 5.1.1 For aqueous and solid/biosolid samples under DoD accreditation one Matrix Spike and one Matrix Spike Duplicate shall be included with every analysis batch. MS/MSD recoveries are evaluated against project acceptance limits if prescribed by the client, otherwise MS/MSD recoveries are evaluated against limits of 70-130%. RPDs are evaluated against project limits if prescribed by the client, otherwise RPDs are evaluated against the DoD specific limit of <30%.* *Alternate requirement for DoD QSM 5.3:* For aqueous and solid/biosolid samples under DoD accreditation one Matrix Spike and one Matrix Spike Duplicate shall be included with every analysis batch. MS/MSD recoveries are evaluated against project limits if prescribed by the client, otherwise MS/MSD recoveries are evaluated against the DOD specific acceptance ranges listed in Table 6b of this document, or against the MLA-110 OPR method recovery limits for analytes not listed in Table 6b. RPDs are evaluated against project limits if prescribed by the client, otherwise RPDs are evaluated against the DoD specific limit of <30%.

Appendix A: Naming Convention and CAS Numbers

PFAS: Naming Convention and CAS Numbers

Abbreviation	***Name - Anion Form***	***CAS#***
PFBA	Perfluorobutanoate	45048-62-2
PFPeA	Perfluoropentanoate	45167-47-3
PFHxA	Perfluorohexanoate	92612-52-7
PFHpA	Perfluoroheptanoate	120885-29-2
PFOA	Perfluorooctanoate	45285-51-6
PFNA	Perfluorononanoate	72007-68-2
PFDA	Perfluorodecanoate	73829-36-4
PFUnA	Perfluoroundecanoate	196859-54-8
PFDoA	Perfluorododecanoate	171978-95-3
PFTrDA	Perfluorotridecanoate	862374-87-6
PFTeDA	Perfluorotetradecanoate	365971-87-5
PFBS	Perfluorobutanesulfonate	45187-15-3
PFPeS	Perfluoropentanesulfonate	175905-36-9
PFHxS	Perfluorohexanesulfonate	108427-53-8
PFHpS	Perfluoroheptanesulfonate	146689-46-5
PFOS	Perfluorooctanesulfonate	45298-90-6
PFNS	Perfluorononanesulfonate	474511-07-4
PFDS	Perfluorodecanesulfonate	126105-34-8
PFDoS	Perfluorododecanesulfonate	343629-43-6
4:2 FTS	4:2 fluorotelomersulfonate	414911-30-1
6:2 FTS	6:2 fluorotelomersulfonate	425670-75-3
8:2 FTS	8:2 fluorotelomersulfonate	481071-78-7
N-MeFOSAA	N-Methylperfluorooctanesulfonamidoacetate	n.a.
N-EtFOSAA	N-Ethylperfluorooctanesulfonamidoacetate	n.a.
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoate	122499-17-6
ADONA	Dodecafluoro-3H-4,8-dioxanonanoate	2127366-90-7
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	1621485-21-9
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	2196242-82-5

SGS AXYS Analytical Services Ltd.

PFAS: Naming Convention and CAS Numbers

Abbreviation	***Name - Acid Form***	***CAS#***
PFBA	Perfluorobutyric acid	375-22-4
PFPeA	Perfluoropentanoic acid	2706-90-3
PFHxA	Perfluorohexanoic acid	307-24-4
PFHpA	Perfluoroheptanoic acid	375-85-9
PFOA	Perfluorooctanoic acid	335-67-1
PFNA	Perfluorononanoic acid	375-95-1
PFDA	Perfluorodecanoic acid	335-76-2
PFUnA	Perfluoroundecanoic acid	2058-94-8
PFDoA	Perfluorododecanoic acid	307-55-1
PFTrDA	Perfluorotridecanoic acid	72629-94-8
PFTeDA	Perfluorotetradecanoic acid	376-06-7
PFBS	Perfluorobutanesulfonic acid	375-73-5
PFPeS	Perfluoropentanesulfonic acid	2706-91-4
PFHxS	Perfluorohexanesulfonic acid	355-46-4
PFHpS	Perfluoroheptanesulfonic acid	375-92-8
PFOS	Perfluorooctanesulfonic acid	1763-23-1
PFNS	Perfluorononanesulfonic acid	68259-12-1
PFDS	Perfluorodecanesulfonic acid	335-77-3
PFDoS	Perfluorododecanesulfonic acid	79780-39-5
4:2 FTS	4:2 fluorotelomersulfonic acid	757124-72-4
6:2 FTS	6:2 fluorotelomersulfonic acid	27619-97-2
8:2 FTS	8:2 fluorotelomersulfonic acid	39108-34-4
N-MeFOSAA	N-Methylperfluorooctanesulfonamidoacetic acid	2355-31-9
N-EtFOSAA	N-Ethylperfluorooctanesulfonamidoacetic acid	2991-50-6
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	13252-13-6
ADONA	Dodecafluoro-3H-4,8-dioxanonanoic acid	919005-14-4
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	756426-58-1
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9

SGS AXYS Analytical Services Ltd.

PFAS: Naming Convention and CAS Numbers

Abbreviation	***Name - Sulfonamide***	***CAS#***
PFOSA	Perfluorooctanesulfonamide	754-91-6
N-MeFOSA	N-Methylperfluorooctanesulfonamide	31506-32-8
N-EtFOSA	N-Ethylperfluorooctanesulfonamide	4151-50-2

Abbreviation	***Name - Sulfonamidoethanol***	***CAS#***
N-MeFOSE	N-Methylperfluorooctanesulfonamidoethanol	24448-09-7
N-EtFOSE	N-Ethylperfluorooctanesulfonamidoethanol	1691-99-2

Appendix B: Sample Storage Conditions

Sample Storage Requirements

Matrix	Sample Size (per analysis)	Sample Container [1]	Sample Condition Upon Receipt	Storage Condition [2]	Sample Hold Time [3]	Extract Hold Time [4]	Preservation
Aqueous	Up to 1000 mL, but typically 500 mL or less (max. 50 mg solids)	High density polyethylene (HDPE)	0-4°C, dark	≤ -20°C, dark	90 days	30 days	None Required
Solvent extracts	Typically 0.75 mL	High density polyethylene (HDPE)	0-4°C, dark	0-4°C, dark	60 days	30 days	None Required
Solid	Up to 5 g dry but not more than 10 g wet.	High density polyethylene (HDPE)	0-4°C, dark	≤ -20°C, dark	1 Year	30 days	None required
Biosolid	Up to 0.5 g dry but not more than 5 g wet.	High density polyethylene (HDPE)	0-4°C, dark	≤ -20°C, dark	1 Year	30 days	None required
Tissue	Up to 2 g (wet)	High density polyethylene (HDPE) or amber glass jar	0-4°C, dark	≤ -20°C, dark	1 Year	30 days	None Required
AFFF	Up to 0.02 mL	High density polyethylene (HDPE)	Room temperature.	0-4°C, dark	90 days	30 days	None Required

[1] HDPE containers are preferred; amber glass containers are also acceptable. All containers should be organically clean; i.e. solvent-rinsed or purchased 'certified' clean. All containers should be tightly sealed with screw cap lids.

[2] Storage temperatures quoted are nominal temperatures.

[3] Hold times are from time of sampling. Client negotiated requests for specific holding times or other method-specific holding times are adhered to. This 90 day holding time on freezing of aqueous samples is based on SGS AXYS storage stability studies. For aqueous samples under DoD accreditation, the sample hold time is ≤14 days.

[4] Hold times for sample extracts are from time of extraction with storage at 4°C. This 30-day holding time is a guideline, longer hold times may be accepted based on professional judgement. For samples under DoD accreditation the extract hold time is ≤28 days with storage at room temperature.

SGS AXYS Analytical Services Ltd.

Stability of PFAS in Aqueous Samples

SGS AXYS has completed an extended-time storage study for the 29 PFAS listed in the table below. The study was conducted in reagent water, surface water and two wastewater treatment plant (WWTP) effluents. PFAS concentrations were measured at 0, 7, 14, 30, 90, and 180-day timepoints with data analysis of a 180-day timepoint in progress. Data analysis up to the 90-day timepoint shows that precursors present in samples containing matrix and biological activity can transform under room temperature and cold storage conditions within 7 days. At this time, only freezing of aqueous samples was demonstrated to stabilize the analytes over a period of up to 90 days. We are recommending freezing non-potable aqueous samples as soon as practicable if not analyzed within 3-4 days.

Summary of Analyte Stability in Aqueous Samples by Storage Condition

	Stability (days)		Remarks
Analyte	**4°C**	**-20°C**	
C_4-C_{14} Perfluorinated carboxylates including PFOA	90	90	
C_4-C_{10} perfluorinated sulfonates including PFOS	90	90	
PFDoS	90	90	
4:2 FTS	90	90	
6:2 FTS	90	90	
8:2 FTS	14	90	Decreasing trend seen from day 7 at 4°C
PFOSA	14	90	Increasing trend seen day 14 onwards at 4°C
N-MeFOSA	7	90	Decreasing trend seen at first stability point 4°C
N-EtFOSA	7	90	Decreasing trend seen at first stability point 4°C
N-MeFOSAA	<7	90	Increase from transformation of MeFOSE
N-EtFOSAA	7	90	Increase from transformation of EtFOSE
N-MeFOSE	<7	90	Loss seen at first stability point 4°C
N-EtFOSE	<7	90	Loss seen at first stability point 4°C

APPENDIX E
SGS Axys Analysis Method MLA-110 Details and Analyte List

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METHOD DETECTION LIMITS AND REPORTING LIMITS

SGS AXYS Method: MLA-110
Instrument Type: UPLC-MS/MS
MDL Protocol: Federal Register 40 CFR Part 136, Appendix B Rev.1 (or * = MDLs determined according to Rev. 2, [2017])
Quantification: Linear regression with 1/x weighting

Matrix	TISSUE			
Units/Sample Size	ng/g based on 2 g sample			
Default Extract Volume	4000 uL			
Analyte	MDL *	US DoD LOD/LOQ	NELAP LOQ	RL based on Low Cal.
Perfluorobutanoate (PFBA)	0.551	1.6	1.6	0.8
Perfluoropentanoate (PFPeA)	0.192	0.8	0.8	0.4
Perfluorohexanoate (PFHxA)	0.203	0.4	0.4	0.2
Perfluoroheptanoate (PFHpA)	0.170	0.4	0.4	0.2
Perfluorooctanoate (PFOA)	0.162	0.4	0.4	0.2
Perfluorononanoate (PFNA)	0.129	0.4	0.4	0.2
Perfluorodecanoate (PFDA)	0.116	0.4	0.4	0.2
Perfluoroundecanoate (PFUnA)	0.151	0.4	0.4	0.2
Perfluorododecanoate (PFDoA)	0.156	0.4	0.4	0.2
Perfluorotridecanoate (PFTrDA)	0.398	0.4	0.4	0.2
Perfluorotetradecanoate (PFTeDA)	0.309	0.4	0.4	0.2
Perfluorobutanesulfonate (PFBS)	0.097	0.4	0.4	0.2
Perfluoropentanesulfonate (PFPeS)	0.129	0.4	0.4	0.2
Perfluorohexanesulfonate (PFHxS)	0.153	0.4	0.4	0.2
Perfluoroheptanesulfonate (PFHpS)	0.154	0.4	0.4	0.2
Perfluorooctanesulfonate (PFOS)	0.354	0.4	0.4	0.2
Perfluorononanesulfonate (PFNS)	0.155	0.4	0.4	0.2
Perfluorodecanesulfonate (PFDS)	0.207	0.4	0.4	0.2
Perfluorododecanesulfonate (PFDoS)	0.291	0.4	0.4	0.2
4:2 fluorotelomersulfonate (4:2 FTS)	0.234	1.6	1.6	0.8
6:2 fluorotelomersulfonate (6:2 FTS)	0.404	1.4	1.4	0.8
8:2 fluorotelomersulfonate (8:2 FTS)	0.670	1.6	1.6	0.8
N-Methylperfluorooctanesulfonamidoacetic acid (N-MeFOSAA)	0.304	0.4	0.4	0.2
N-Methylperfluorooctanesulfonamidoacetic acid (N-EtFOSAA)	0.143	0.4	0.4	0.2
Perfluorooctanesulfonamide (PFOSA), a.k.a FOSA	0.152	0.4	0.4	0.2
N-Methylperfluorooctanesulfonamide (N-MeFOSA)	0.288	0.5	0.5	0.2
N-Ethylperfluorooctanesulfonamide (N-EtFOSA)	0.248	1.0	1.0	0.2
N-Methylperfluorooctanesulfonamidoethanol (N-MeFOSE)	3.357	4.1	4.1	2.0
N-Ethylperfluorooctanesulfonamidoethanol (N-EtFOSE)	1.447	3.1	3.1	2.0
Perfluoro-2-propoxypropanoate (HFPO-DA)	0.460	1.6	1.6	0.8
4-dioxa-3H-perfluorononanoate (ADONA)	0.884	1.6	1.6	0.8
9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)	0.708	1.6	1.6	0.8
11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS)	0.889	1.6	1.6	0.8

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APPENDIX F:
SGS Axys Shipping Chain of Custody

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CHAIN OF CUSTODY

2045 Mills Road West TEL: (250) 655-5800
Sidney, British Columbia, Canada V8L 5X2 FAX: (250) 655-5811

AXYS CLIENT #:

REPORT TO:	***INVOICE TO:***	ANALYSIS REQUESTED
Company	Company	
Address	Address	
Contact	Contact	
Phone	Phone	
FAX	FAX	
E-mail	E-mail	
Project Name/Number:	Sampler's Name: Signature:	

Client Sample Identification	Matrix	Sampling Date	Sampling Time	Container Type/No.	AXYS Lab Sample ID (Lab use only)					

Relinquished by (Signature) Date Time	Received by (Signature) Date Time	Courier	Waybill No.
Relinquished by (Signature) Date Time	Received by (Signature) Date Time	Sample Receipt	
Remarks			Cooler
		Temp °C	
		Custody Seal #	
		Seal Intact Y / N	
		Sample Tags Y / N	