

PFAS Levels in White-Tailed Deer Harvested at the JCI/Tyco Fire Technology Center

BACKGROUND

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a large group of human-made chemicals that have been used in industry and consumer products worldwide since the 1950s. PFAS do not occur naturally but are widespread in the environment due to their widespread human use. PFAS don't break down easily and can remain for a long time in the environment where people can be exposed to them. As part of the DNR's response to community concerns regarding PFAS contamination in and around the Johnson Controls, Inc. – Tyco Fire Products (JCI/Tyco) facilities in Marinette, WI, the DNR, in consultation with DHS, investigated whether white-tailed deer from this area were contaminated with PFAS and whether there is any potential risk for consumers of deer harvested near this area.

SAMPLE COLLECTION and ANALYSIS

In February of 2020, under a cooperative agreement with the WDNR, the U.S. Department of Agriculture – Wildlife Services (USDA-WS) harvested 20 deer from the JCI/Tyco Fire Technology Center (FTC). Of these 20 deer, a range of ages were collected (fawn – 4-5 years old) and both sexes were represented (12 females and 8 males). After harvest, the carcasses were transported to a WDNR facility where tissue samples were collected. In addition to muscle tissue (venison), liver and heart were also collected as these tissues are often consumed by hunters and also provide additional indicators of exposure. The following samples were collected from each deer:

- o Muscle tissue –100-200 grams
- o Liver – 100-200 grams
- o Heart – 100-200 grams

In addition, muscle tissue was collected from 12 deer from Price County during Wisconsin's November 2019-gun deer hunt. These deer are considered a control group from an area with no significant sources of PFAS. Of these 12 deer, a range of ages were collected (fawn – 9-12 years old) and both sexes were represented (6 females and 6 males). Muscle samples were collected opportunistically from deer heads submitted to the DNR for Chronic Wasting Disease (CWD) testing. As such, heart and liver tissue were not collected from the Price County deer.

The samples were then transported to the Wisconsin State Laboratory of Hygiene for analysis. The list of PFAS compounds analyzed for in all deer samples can be found in Table 1.

Table 1: PFAS measured in deer tissue harvested at the JCI/Tyco Fire Technology Center and analyzed at the Wisconsin State Laboratory of Hygiene.

| Parameter Name | Abbreviation |
|---|--------------|
| 10:2 Fluorotelomer sulfonic acid | 10:2 FTSA |
| 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid | 11Cl-PF3OUdS |
| 4,8-Dioxa-3H-perfluorononanoic acid | DONA |
| 8:2 Fluorotelomer sulfonic acid | 8:2 FTSA |

| | |
|--|------------|
| 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid | 9Cl-PF3ONS |
| Hexafluoropropylene oxide dimer acid | HFPO-DA |
| N-ethyl perfluorooctanesulfonamidoacetic acid | NEtFOSAA |
| N-methyl perfluorooctanesulfonamidoacetic acid | NMeFOSAA |
| Perfluorododecanesulfonic acid | PFDoS |
| Perfluoro-n-butanesulfonic acid | PFBS |
| Perfluoro-n-decanesulfonic acid | PFDS |
| Perfluoro-n-decanoic acid | PFDA |
| Perfluoro-n-dodecanoic acid | PFDoA |
| Perfluoro-n-heptanesulfonic acid | PFHpS |
| Perfluoro-n-heptanoic acid | PFHpA |
| Perfluoro-n-hexanesulfonic acid | PFHxS |
| Perfluoro-n-hexanoic acid | PFHxA |
| Perfluoro-n-nonanoic acid | PFNA |
| Perfluoro-n-octanesulfonic acid | PFOS |
| Perfluoro-n-octanoic acid | PFOA |
| Perfluorononanesulfonic acid | PFNS |
| Perfluoro-n-pentanoic acid | PFPeA |
| Perfluoro-n-tetradecanoic acid | PFTeA |
| Perfluoro-n-tridecanoic acid | PFTriA |
| Perfluoro-n-undecanoic acid | PFUnA |
| Perfluoropentanesulfonic acid | PFPeS |

RESULTS

Muscle Tissue

Twenty deer were collected in February of 2020 from the JCI/Tyco Fire Technology Center in Marinette, WI. The only PFAS detected in muscle tissue was PFOS and was only detected in 1 deer at 2.67 ng/g (Table 2). A full copy of the analytical report is available at the end of this report.

Table 2: PFAS levels in muscle (in nanograms per gram [ng/g] or parts per billion [ppb]) from deer harvested at the JCI/Tyco Fire Technology Center.

| Abbreviation | # of Muscle Samples with Detections | Range |
|--------------|-------------------------------------|-------|
| 10:2 FTSA | 0/20 | ND* |
| 11Cl-PF3OUdS | 0/20 | ND |
| DONA | 0/20 | ND |
| 8:2 FTSA | 0/20 | ND |
| 9Cl-PF3ONS | 0/20 | ND |
| HFPO-DA | 0/20 | ND |
| NEtFOSAA | 0/20 | ND |
| NMeFOSAA | 0/20 | ND |
| PFDoS | 0/20 | ND |

| | | |
|--------|------|----------------|
| PFBS | 0/20 | ND |
| PFDS | 0/20 | ND |
| PFDA | 0/20 | ND |
| PFDoA | 0/20 | ND |
| PFHpS | 0/20 | ND |
| PFHpA | 0/20 | ND |
| PFHxS | 0/20 | ND |
| PFHxA | 0/20 | ND |
| PFNA | 0/20 | ND |
| PFOS | 1/20 | ND - 2.67 ng/g |
| PFOA | 0/20 | ND |
| PFNS | 0/20 | ND |
| PFPeA | 0/20 | ND |
| PFTeA | 0/20 | ND |
| PFTriA | 0/20 | ND |
| PFUnA | 0/20 | ND |
| PFPeS | 0/20 | ND |

*ND = Not Detected

No PFAS were detected in the muscle samples collected from Price County.

Heart Tissue

The only PFAS detected in heart tissue was PFOS and was detected in 2 deer (range 2.29 – 3.07 ng/g (Table 3). A full copy of the analytical report is available at the end of this report.

Table 3: PFAS levels in heart tissue (in nanograms per gram [ng/g] or parts per billion [ppb]) from deer harvested at the JCI/Tyco Fire Technology Center.

| Abbreviation | # of Heart Samples with Detections | Range |
|--------------|------------------------------------|-------|
| 10:2 FTSA | 0/20 | ND* |
| 11Cl-PF3OUdS | 0/20 | ND |
| DONA | 0/20 | ND |
| 8:2 FTSA | 0/20 | ND |
| 9Cl-PF3ONS | 0/20 | ND |
| HFPO-DA | 0/20 | ND |
| NEtFOSAA | 0/20 | ND |
| NMeFOSAA | 0/20 | ND |
| PFDoS | 0/20 | ND |
| PFBS | 0/20 | ND |
| PFDS | 0/20 | ND |
| PFDA | 0/20 | ND |
| PFDoA | 0/20 | ND |
| PFHpS | 0/20 | ND |

| | | |
|--------|------|----------------|
| PFHpA | 0/20 | ND |
| PFHxS | 0/20 | ND |
| PFHxA | 0/20 | ND |
| PFNA | 0/20 | ND |
| PFOS | 2/20 | ND – 3.07 ng/g |
| PFOA | 0/20 | ND |
| PFNS | 0/20 | ND |
| PFPeA | 0/20 | ND |
| PFTeA | 0/20 | ND |
| PFTriA | 0/20 | ND |
| PFUnA | 0/20 | ND |
| PFPeS | 0/20 | ND |

*ND = Not Detected

Liver Tissue

Six different PFAS compounds (8:2 FTA, PFDA, PFHxS, PFNA, PFOS, and PFUnA) were detected in liver tissue from deer harvested at the JCI/Tyco FTC site (Table 4). One deer, a 2-year-old male, had detectable levels of all 6 of these PFAS in its liver. This same deer had the highest levels of hepatic PFOS (92.0 ng/g). PFOS was detected in all 20 liver samples (range 3.83 – 92.0 ng/g). Considering a primary function of the liver is to filter out contaminants from the bloodstream, it was not unexpected to observe higher levels of PFAS in the liver compared to muscle or heart tissue. A full copy of the analytical report is available at the end of this report.

Table 4: PFAS levels in liver tissue (in nanograms per gram [ng/g] or parts per billion [ppb]) from deer harvested at the JCI/Tyco Fire Technology Center.

| Abbreviation | # of Liver Samples with Detections | Range |
|--------------|------------------------------------|----------------|
| 10:2 FTSA | 0/20 | ND* |
| 11Cl-PF3OUdS | 0/20 | ND |
| DONA | 0/20 | ND |
| 8:2 FTSA | 9/20 | ND - 5.11 ng/g |
| 9Cl-PF3ONS | 0/20 | ND |
| HFPO-DA | 0/20 | ND |
| NEtFOSAA | 0/20 | ND |
| NMeFOSAA | 0/20 | ND |
| PFDoS | 0/20 | ND |
| PFBS | 0/20 | ND |
| PFDS | 0/20 | ND |
| PFDA | 6/20 | ND - 5.72 ng/g |
| PFDoA | 0/20 | ND |
| PFHpS | 0/20 | ND |
| PFHpA | 0/20 | ND |
| PFHxS | 1/20 | ND - 2.05 ng/g |

| | | |
|--------|-------|------------------|
| PFHxA | 0/20 | ND |
| PFNA | 2/20 | ND - 2.79 ng/g |
| PFOS | 20/20 | 3.83 - 92.0 ng/g |
| PFOA | 0/20 | ND |
| PFNS | 0/20 | ND |
| PFPeA | 0/20 | ND |
| PFTeA | 0/20 | ND |
| PFTriA | 0/20 | ND |
| PFUnA | 4/20 | ND - 5.81 ng/g |
| PFPeS | 0/20 | ND |

*ND = Not Detected

DISCUSSION

Each time PFAS are ingested, they can stay inside the body for many years. Repeated exposures to PFAS in the environment can cause PFAS levels in the body to slowly build up to a high level. High levels of PFAS in the body are harmful to human health and especially to the health of pregnant women. High levels of PFAS in the body may increase cholesterol levels, decrease how well the body responds to vaccines, increase the risk of thyroid disease, decrease fertility in women, increase the risk of high blood pressure or pre-eclampsia in pregnant women, and lower infant birth weights¹.

One way to reduce the total amount of PFAS in the body is to avoid consuming food containing high amounts of PFAS. The DNR initiated this study to address local concerns regarding the possibility for PFAS to affect deer harvested from the contaminated area. The DNR collected and tested the muscle, liver, and heart tissue of 20 white-tailed deer and consulted with DHS to determine whether there is any potential health risk for consumers.

In the study, PFOS was detected at low concentrations in the muscle of one deer (2.67 ng/g) and in the heart tissue of two deer (2.29 and 3.07 ng/g). Conversely, PFOS was detected in the liver of all deer (20/20) ranging from 3.83 to 92.0 ng/g (mean = 21.9 ng/g, median = 11.8 ng/g). Due to the lack of PFOS-based consumption guidelines for deer, fish consumption guidelines were used to help estimate those for deer. Consumption of Great Lakes fish is restricted when PFOS levels exceed 10 ng/g in fish tissue². Because no muscle or heart tissues resulted above 10 ng/g, these tissues were not deemed to be a public health concern. However, 11 of 20 liver samples contained PFOS levels above 10 ng/g, warranting further public health recommendation to limit liver consumption.

In general, the liver serves as a filtration organ and is also known to accumulate a variety of other contaminants. Many studies in wild game have shown that contaminants such as PFAS

¹ <https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf>

² <https://www.health.state.mn.us/communities/environment/fish/docs/consortium/bestpracticepfos.pdf>

compounds³, heavy metals⁴, PCBs⁵, dieldrin (insecticide)⁶, and dioxin⁷ are found in higher levels in the liver compared to other tissues. Considered together, the current study results and other available research on liver contaminants suggest that a Do Not Eat advisory for the liver of deer harvested from the contamination area is warranted in order to protect the health of the public. The advisory encompasses a five-mile radius from where the deer were collected and includes what is estimated to be the travel and dispersal range for deer living in this area. The advisory area (Figure 1) can be described as:

From the mouth of the Peshtigo River upstream to Bagley Junction, northeast on a cross country bearing to Nettleton Road, east on Nettleton Rd to N. Nettleton Rd intersecting with the Menominee River.

STUDY LIMITATIONS

The purpose of this study was to examine potential exposure to PFAS of white-tailed deer near the JCI/Tyco FTC. This area was selected to represent high exposure potential of deer to PFAS. Due to the fact that we were not able to analyze liver or other organs from deer from other areas of the state, it is unclear whether the PFAS levels observed in deer liver in the present study are the result of local exposure or whether they are representative of PFAS levels in liver from deer from other parts of the state.

CONCLUSIONS AND RECOMMENDATIONS

Based on our findings, the muscle and heart of white-tailed deer are not likely to result in significant PFAS exposure for consumers of these tissues. As such, no PFOS-based consumption advisories are warranted for the muscle or heart of deer harvested from this location.

Conversely, our findings suggest that the liver of deer harvested from this area is likely to result in significant PFAS exposure for those who consume it. Furthermore, additional research on contaminants in deer support a recommendation for deer consumers to avoid the consumption of deer liver. As such, a Do Not Eat advisory for the liver of deer harvested from this contaminated area is warranted.

³ <https://www.sciencedirect.com/science/article/abs/pii/S0269749112003508>

⁴ <https://www.fws.gov/southwest/es/ArlingtonTexas/pdf/CLNWRDeerStudy2006.pdf>

⁵ <https://www.atsdr.cdc.gov/HAC/pha/Dioxins%20in%20Wild%20Game%20-%20Tittawassee/wildgamedioxinshc042205.pdf>

⁶ <https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.5620180225>

⁷ <https://www.atsdr.cdc.gov/HAC/pha/Dioxins%20in%20Wild%20Game%20-%20Tittawassee/wildgamedioxinshc042205.pdf>

Figure 1: Map of the deer liver consumption advisory area



Deer Tissue Study 2020 – Marinette and Peshtigo

Lab Methods and Data

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ESS ORG METHOD OC14801

Analysis of Perfluorinated Compounds in Fish Tissue by HPLC-MS/MS

ISO/DIS 21675
Matrix: Tissue
Acode: OC14801
Method (,T)

1. Scope and Application

- 1.1. This is a high performance liquid chromatographic triple quadrupole mass spectrometric (HPLC-MS/MS) method applicable to the determination of perfluorinated compounds in fish tissue.
- 1.2. The following compounds and reporting limits are listed below for this method:

| <u>Analyte</u> | <u>MDL</u> <u>(ng/g)</u> | <u>RL</u> <u>(ng/g)</u> |
|---|-----------------------------|----------------------------|
| *Perfluoro-n-butanoic acid (PFBA) | NA | NA |
| Perfluoro-n-pentanoic acid (PFPeA) | 0.911 | 2.00 |
| Perfluoro-1-butanefulfonate (PFBS) | 0.875 | 1.77 |
| *1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTSA) | 1.058 | 1.90 |
| Perfluoro-n-hexanoic acid (PFHxA) | 1.376 | 2.00 |
| Perfluoro-1-pentanesulfonate (PFPeS) | 0.989 | 1.88 |
| 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3-heptafluoropropoxy)-propanoic acid (HFPO-DA) | 1.88 | 2.00 |
| Perfluoro-n-heptanoic acid (PFHpA) | 0.847 | 2.00 |
| Perfluoro-1-hexanesulfonate (PFHxS) | 0.545 | 1.82 |
| Dodecafluoro-3H-4,8-dioxanoanoate (DONA) | 1.065 | 1.89 |
| *1H,1H,2H,2H-Tridecafluorooctane-1-sulfonic acid (6:2 FTSA) | NA | NA |
| Perfluoro-n-octanoic acid (PFOA) | 1.692 | 2.00 |
| Perfluoro-1-heptanesulfonate (PFHpS) | 1.161 | 1.90 |
| Perfluoro-1-octanesulfonate (PFOS) | 1.089 | 1.85 |
| Perfluoro-n-nonanoic acid (PFNA) | 1.326 | 2.00 |
| Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS) | 1.375 | 1.86 |
| 1H,1H,2H,2H-Perfluorodecanesulfonic acid (8:2 FTSA) | 1.088 | 2.00 |
| Perfluoro-n-decanoic acid (PFDA) | 1.631 | 2.00 |
| Perfluoro-1-nonanesulfonate (PFNS) | 1.519 | 2.00 |
| N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA) | 0.804 | 2.00 |
| N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA) | 1.055 | 2.00 |
| *Perfluorooctanesulfonamide (FOSA) | NA | NA |
| Perfluoro-n-undecanoic acid (PFUnA) | 0.760 | 2.00 |
| Perfluoro-1-decanesulfonate (PFDS) | 1.811 | 1.93 |
| Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS) | 1.375 | 1.88 |
| Perfluoro-n-dodecanoic acid (PFDoA) | 1.487 | 2.00 |
| 1H,1H,2H,2H-Perfluorododecane sulfonic acid (10:2 FTSA) | 0.507 | 2.00 |
| Perfluoro-1-dodecanesulfonate (PFDoS) | 1.841 | 1.94 |
| Perfluoro-n-tridecanoic acid (PFTrDA) | 1.210 | 2.00 |
| *N-Methyl Perfluorooctanesulfonamide (N-MeFOSA) | NA | NA |

| | | |
|---|-------|------|
| *2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (N-MeFOSE) | NA | NA |
| *N-Ethyl Perfluorooctanesulfonamide (N-EtFOSA) | NA | NA |
| *2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (N-EtFOSE) | NA | NA |
| Perfluoro-n-tetradecanoic acid (PFTeDA) | 0.647 | 2.00 |
| *Perfluoro-n-hexadecanoic acid (PFHxDA) | NA | NA |
| *Perfluoro-n-octadecanoic acid (PFODA) | NA | NA |

*Laboratory is not reporting results for these Analyte

2. Summary of Method:

- 2.1. Perfluorinated compounds (PFCs) or Per and polyfluoroalkyl substances (PFAS) present in fish tissue samples are detected by an alkaline digestion/solid phase extraction, eluted with 3% NH₄OH in MeOH, and evaporated to dryness under nitrogen gas. The contents of the tube are reconstituted in 1ml MeOH, and stored at room temperature. Prior to analysis by HPLC-MS/MS the contents are transferred to an autosampler vial via syringe filter (0.2 um). Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are analyzed using a turbo ion spray triple quadruple mass spectrometer in the negative ionization mode.
- 2.2. List Regulatory Deviations: None.

3. Safety and Waste Management:

- 3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences;
O:\SOP\Safety\Final\AD_SAFETY_GENOP_102_Chemical_Hygiene_Plan.doc.
- 3.1.1. All laboratory waste, excess reagents, and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines for Wisconsin State Laboratory of Hygiene is located at the O:\SOP\EHD\Division_Wide\Final\EHD_GENOP_038_SOP_Waste_Management.doc.
- 3.1.2. The University of Wisconsin chemical safety and disposal guideline can also be found on <http://ehs.wisc.edu/disposal-services/>.

4. Sampling Handling and Preservation:

- 4.1. Samples must be frozen prior to transportation and frozen and shielded from light as soon as possible upon arrival at the lab. Perfluorinated compounds have been shown to be stable for several months under these conditions.
- 4.2. Sample should be stored in high density polyethylene (HDPE) or polypropylene (PPE) containers prior to analysis and long term storage, containers or any contact with PTFE surfaces must be avoided. Samples final extracts should be stored frozen in the laboratory.
- 4.3. Samples need to be extracted and analyzed within one year of collection. After samples are extracted, they need to be analyzed within 30 days.

5. Interferences: Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference is unknown until further sample analysis is completed.

6. Reagents and Standards:

6.1. Reagents

- 6.1.1. Methanol, Reagent grade
- 6.1.2. Ammonium Acetate, Reagent grade
- 6.1.3. Sodium acetate, Reagent grade
- 6.1.4. Sodium Hydroxide (NaOH) pellets, Reagent grade
- 6.1.5. Ammonium hydroxide, Concentrated
- 6.1.6. 18 Mohm water

6.2. Reagent Preparation

- 6.2.1. 2mM Ammonium Acetate in Water (5% MeOH): Add 0.154 g of ammonium acetate to 1 liter of 18 Mohm water/Methanol mix (950mL:50mL)
- 6.2.2. 3% ammonium hydroxide in methanol: Add 6 mL ammonium hydroxide to 200 mL methanol.
- 6.2.3. 10 mM NaOH in methanol: Add 0.40 g NaOH to 1 liter methanol.
- 6.2.4. 25 mM sodium acetate buffer (pH 4): Add 2.05 g sodium acetate to 1 liter of 18 Mohm water. Adjust to pH 4 using glacial acetic acid.

6.3. Standards

- 6.3.1. Prepare stock standard solutions by obtaining a known weight of pure material or pre-made mix of target compounds. Over time some of the analytes can adsorb onto glass surfaces, therefore glass containers should be avoided whenever possible. Standards will be diluted and stored in polypropylene vials at 4°F and brought to room temperature before use.
 - 6.3.1.1. Final working standard concentration for this method is at 100ng/ml, (0.1ul/ml) PFASTiWrk1 in Horizon. Actual concentration for each compound can be found in section 16, Table 2.
- 6.3.2. Current Target compounds are composed of premade mix of 18 compounds at 2ug/ml (EPA-537PDS-R1 Wellington laboratories) that have both the branched and linear forms and additional 18 individual compounds purchased at 50 ug/ml. Standards should be re-evaluated or replaced after 12 month or if signs of degradation are observed.
- 6.3.3. Stock internal standard solution contains 19 individual compounds with ¹³C isotopically labeled compounds at a nominal concentration of 50 ng/ml and 9 isotopically labelled mix at 2ug/ml.
- 6.3.4. Second Source Standard - Second source standard from a different vendor or different lot number from the same vendor will be run to verify the calibration curve.

Revision: 6

Effective Date: 4/10/20 - present

Replaces: ESS ORG METHOD14801, Revision 5

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7. Apparatus:

- 7.1. Sample tubes: Avoid use of Teflon septa/cap liners
- 7.2. Analytical Balance capable of accurately weighing to the nearest 0.0001 g.
- 7.3. 50 ml conical screw capped polypropylene centrifuge tubes, graduated
- 7.4. 15-ml conical screw capped polypropylene centrifuge tubes, graduated
- 7.5. Vortex mixer
- 7.6. Disposable polypropylene Luer tip syringes
- 7.7. 13-mm syringe filters, and 0.2 micron nylon filter
- 7.8. 2-ml polypropylene auto-injector vials with polypropylene septa
- 7.9. SPE extraction manifold apparatus
- 7.10. TurboVap
- 7.11. SPE cartridge, SPE columns OASIS WAX 3 cc
- 7.12. Disposable polypropylene pipettes
- 7.13. Waters I class Acquity UPLC
- 7.14. SCIEX Q-Trap 5500 Triple Quad mass spectrometer
- 7.15. HPLC/MS/MS Instrument Conditions
 - 7.15.1. The HPLC-MS/MS method is performed on an Applied Biosystems/SCIEX API 5500 triple quadrupole mass spectrometer which is interfaced to Waters Acquity UPLC system equipped with a degasser, autosampler and column heating compartment.
- 7.16. General Method Parameters
 - 7.16.1. Synchronization Mode: LC Sync
 - 7.16.2. Auto-Equilibration: Off
 - 7.16.3. Acquisition Duration: 13 minutes 30 seconds
 - 7.16.4. Number of Scans: 810
 - 7.16.5. Period In File: 1
 - 7.16.6. Acquisition Module: Acquisition Method
 - 7.16.7. Software version: Analyst 1.63
- 7.17. Source height setting-3, Source L/R setting-5
- 7.18. Waters Acquity Pump Method
 - 7.18.1. Pump Model: Waters Acquity UPLC
 - 7.18.2. Column: Acquity UPLC BEH C18 1.7MM 2.1x50mm Column, (waters Part# 186002350)

7.18.3. Acquity UPLC Pump Method Properties (Binary Solvent Manager)

| | |
|--|-------------------------------|
| Minimum Pressure (psi) | 0.0 |
| Maximum Pressure (psi) | 17000 |
| Dead Volume (µl) | NA |
| Maximum Flow Ramp (ml/min ²) | NA |
| Maximum Pressure Ramp (psi/sec) | NA |
| Left Compressibility | NA |
| Right Compressibility | NA |
| Left Dead Volume (µl) | NA |
| Right Dead Volume (µl) | NA |
| Left Stroke Volume (µl) | NA |
| Right Stroke Volume (µl) | NA |
| Solvent A1 | 2mM Ammonium acetate (5%MeOH) |
| Solvent A2 | |
| Solvent B1 | Methanol |
| Solvent B2 | |

7.18.4. Step Table:

| Step | Total Time (min) | Flow Rate (µl/min) | A1(%) | B1(%) |
|------|------------------|--------------------|-------|-------|
| 0 | 0.00 | 300 | 95 | 5 |
| 1 | 0.50 | 300 | 95 | 5 |
| 2 | 1 | 300 | 40 | 60 |
| 3 | 8.5 | 300 | 10 | 90 |
| 4 | 9.0 | 300 | 0 | 100 |
| 5 | 10.5 | 300 | 0 | 100 |
| 6 | 11.5 | 300 | 95 | 5 |
| 7 | 13.5 | 300 | 95 | 5 |

7.19. Sample Manager Properties

| | |
|-----------------------------|--------------------|
| Autosampler Model | Sample Manager FTN |
| Syringe Size (µl) | NA |
| Injection Volume (µl) | 10.00 |
| Target Column Temperature | 40.0 C |
| Column temp Alarm Band | Disabled |
| Target Sample Temperature | 5.0 C |
| Syringe Draw Rate | Automatic |
| Needle Placement | Automatic |
| Load Ahead | Disabled |
| Loop Offline | Automatic (Min) |
| Wash Solvent Name | Acetonitrile |
| Pre-inject Wash Time | 0 (sec) |
| Post-Inject Wash Time | 6 (sec) |
| Purge Solvent Name | Methanol |
| Pre/Post- Aspirate Air Gap | Automatic |
| Auto add Mix Stroke Cyc/Vol | Automatic |
| Temperature Control | Not used |

7.20. MS/MS Method Properties:

7.20.1. Period 1:

7.20.1.1. Scans in Period: 810

7.20.1.2. Relative Start Time: 0.00 msec

7.20.1.3. Experiments in Period: 1

7.20.2. Period 1 Experiment 1:

| | |
|-------------------|-------------|
| Scan Type: | MRM (MRM) |
| Scheduled MRM | Yes |
| Polarity: | Negative |
| Scan Mode: | N/A |
| Ion Source: | Turbo Spray |
| Resolution Q1: | Unit |
| Resolution Q3: | Unit |
| Intensity Thres.: | 0.00 cps |
| Settling Time: | 0.0000 msec |
| MR Pause: | 5.0070 msec |
| MCA: | No |
| Step Size: | 0.00 Da |

7.20.3. Parameters:

| Analyte | Internal Standard Used to quantify target compound | Q1 Mass (Da) | Q3 Mass (Da) | Dwell (msec) | DP | CE | CXP |
|---------------|--|--------------|--------------|--------------|------|-----|-----|
| PFBA-1 | 13C4-PFBA | 212.962 | 168.906 | 30 | -25 | -14 | -17 |
| 13C4-PFBA-1 | | 216.9 | 172 | 30 | -50 | -14 | -15 |
| PFPeA-1 | 13C3-PFPeA | 262.908 | 218.904 | 30 | -35 | -12 | -21 |
| 13C3-PFPeA-1 | | 266.006 | 221.9 | 30 | -15 | -14 | -9 |
| PFBS-1 | 13C3-PFBS | 298.839 | 80 | 30 | -25 | -70 | -9 |
| PFBS-2 | 13C3-PFBS | 298.839 | 99 | 30 | -25 | -36 | -9 |
| 13C3-PFBS-1 | | 301.849 | 79.978 | 30 | -100 | -78 | -5 |
| 4:2FTSA-1 | 13C2-4:2FTSA | 326.853 | 306.926 | 30 | -85 | -28 | -19 |
| 4:2FTSA-2 | 13C2-4:2FTSA | 326.853 | 80.974 | 30 | -95 | -50 | -7 |
| PFPeS-1 | 18O2-PFHxS | 348.95 | 79.945 | 30 | -25 | -86 | -9 |
| PFPeS-2 | 18O2-PFHxS | 348.95 | 98.933 | 30 | -25 | -40 | -9 |
| PFHxA-1 | 13C2-PFHxA | 312.898 | 269 | 30 | -45 | -14 | -13 |
| PFHxA-2 | 13C2-PFHxA | 312.898 | 119 | 30 | -45 | -30 | -7 |
| 13C2-PFHxA-1 | | 314.907 | 270 | 30 | -55 | -14 | -13 |
| HFPO-DA | 13C3HFPO-DA-1 | 328.968 | 285 | 30 | -5 | -8 | -5 |
| 13C3HFPO-DA-1 | | 332.045 | 287 | 30 | -20 | -8 | -41 |
| PFHpA-1 | 13C4-PFHpA | 362.858 | 318.9 | 30 | -40 | -14 | -13 |
| PFHpA-2 | 13C4-PFHpA | 362.858 | 169 | 30 | -40 | -24 | -15 |

| | | | | | | | |
|----------------|--------------|---------|---------|----|------|------|-----|
| 13C4-PFHpA-1 | | 367.007 | 168.947 | 30 | -65 | -22 | -13 |
| PFHxS-1 | 18O2-PFHxS | 398.841 | 79.9 | 30 | -5 | -86 | -7 |
| PFHxS-2 | 18O2-PFHxS | 398.841 | 98.9 | 30 | -5 | -42 | -9 |
| 18O2-PFHxS-1 | | 402.878 | 84 | 30 | -20 | -86 | -7 |
| ADONA | 13C4-PFOS | 376.77 | 251 | 30 | -60 | -18 | -11 |
| 6:2FTSA-1 | 13C2-6:2FTSA | 426.897 | 406.968 | 30 | -75 | -32 | -39 |
| 6:2FTSA-2 | 13C2-6:2FTSA | 426.897 | 80.936 | 30 | -75 | -82 | -15 |
| 13C2-6:2FTSA-1 | | 428.808 | 80.985 | 30 | -100 | -80 | -7 |
| PFOA-1 | 13C4-PFOA | 412.877 | 368.9 | 30 | -55 | -14 | -17 |
| PFOA-2 | 13C4-PFOA | 412.877 | 168.9 | 30 | -55 | -24 | -13 |
| 13C4-PFOA-1 | | 416.89 | 372 | 30 | -75 | -16 | -17 |
| PFHpS-1 | 13C4-PFOS | 448.837 | 79.917 | 30 | -145 | -94 | -5 |
| PFHpS-2 | 13C4-PFOS | 448.837 | 98.909 | 30 | -145 | -96 | -9 |
| PFOS-1 | 13C4-PFOS | 498.802 | 79.9 | 30 | -40 | -70 | -7 |
| PFOS-2 | 13C4-PFOS | 498.802 | 98.9 | 30 | -40 | -57 | -11 |
| 13C4-PFOS-1 | | 502.8 | 80 | 30 | -40 | -102 | -9 |
| PFNA-1 | 13C5-PFNA | 462.817 | 419 | 30 | -55 | -16 | -17 |
| PFNA-2 | 13C5-PFNA | 462.817 | 219 | 30 | -55 | -24 | -19 |
| 13C5-PFNA-1 | | 467.855 | 423 | 30 | -55 | -16 | -13 |
| 9Cl-PF3ONS-1 | 13C4-PFOS | 530.725 | 351 | 30 | -105 | -36 | -27 |
| 8:2FTSA-1 | 13C2-8:2FTSA | 526.91 | 506.922 | 30 | -15 | -38 | -9 |
| 8:2FTSA-2 | 13C2-8:2FTSA | 526.91 | 80.92 | 30 | -15 | -78 | -7 |
| 13C2-8:2FTSA-1 | | 528.878 | 79.975 | 30 | -5 | -104 | -7 |
| PFDA-1 | 13C2-PFDA | 512.83 | 469 | 30 | -40 | -16 | -13 |
| PFDA-2 | 13C2-PFDA | 512.83 | 218.9 | 30 | -40 | -26 | -17 |
| 13C2-PFDA-1 | | 514.848 | 470 | 30 | -55 | -16 | -15 |
| PFNS-1 | 13C4-PFOS | 548.893 | 79.966 | 30 | -90 | -112 | -9 |
| PFNS-2 | 13C4-PFOS | 548.893 | 98.942 | 30 | -90 | -106 | -7 |
| N-MeFOSAA-1 | d3-N-MeFOSAA | 569.863 | 419.1 | 30 | -80 | -30 | -7 |
| N-MeFOSAA-2 | d3-N-MeFOSAA | 569.863 | 512 | 30 | -80 | -36 | -13 |
| d3-N-MeFOSAA-1 | | 572.777 | 514.984 | 30 | -25 | -32 | -13 |
| PFDS-1 | 13C4-PFOS | 598.811 | 79.916 | 30 | -25 | -120 | -9 |
| PFDS-2 | 13C4-PFOS | 598.811 | 98.957 | 30 | -25 | -108 | -15 |
| PFUdA-1 | 13C2-PFUdA | 562.846 | 519 | 30 | -60 | -18 | -13 |
| PFUdA-2 | 13C2-PFUdA | 562.846 | 268.9 | 30 | -60 | -28 | -13 |
| 13C2-PFUdA-1 | | 564.855 | 520 | 30 | -90 | -18 | -15 |
| N-EtFOSAA-1 | d5-N-EtFOSAA | 583.962 | 419 | 30 | -120 | -37 | -10 |
| N-EtFOSAA-2 | d5-N-EtFOSAA | 583.962 | 525.8 | 30 | -120 | -37 | -10 |
| d5-N-EtFOSAA-1 | | 588.841 | 531.041 | 30 | -20 | -32 | -31 |

| | | | | | | | |
|---------------|---------------|---------|---------|----|------|------|-----|
| FOSA-1 | 13C8-FOSA | 497.854 | 77.977 | 30 | -80 | -86 | -9 |
| FOSA-2 | 13C8-FOSA | 497.854 | 63.952 | 30 | -80 | -124 | -7 |
| 13C8-FOSA-1 | | 505.827 | 63.969 | 30 | -115 | -130 | -9 |
| 11Cl-PF3OUdS | 13C4-PFOS | 630.757 | 451 | 30 | -95 | -38 | -19 |
| 10:2FTSA-1 | 13C2-10:2FTSA | 626.742 | 606.963 | 30 | -100 | -44 | -27 |
| 10:2FTSA-2 | 13C2-10:2FTSA | 626.742 | 587.007 | 30 | -100 | -48 | -17 |
| PFDoA-1 | 13C2-PFDoA | 612.828 | 569 | 30 | -65 | -20 | -17 |
| PFDoA-2 | 13C2-PFDoA | 612.828 | 168.9 | 30 | -65 | -36 | -19 |
| 13C2-PFDoA-1 | | 614.834 | 570 | 30 | -105 | -18 | -15 |
| PFTTrDA-1 | 13C2-PFDoA | 662.782 | 619 | 30 | -75 | -18 | -17 |
| PFTTrDA-2 | 13C2-PFDoA | 662.782 | 169 | 30 | -75 | -36 | -11 |
| PFDoS-1 | 13C4-PFOS | 698.73 | 79.951 | 30 | -150 | -130 | -11 |
| PFDoS-2 | 13C4-PFOS | 698.73 | 98.867 | 30 | -150 | -80 | -9 |
| N-MeFOSA-1 | d3-N-MeFOSA | 511.973 | 168.923 | 30 | -65 | -36 | -15 |
| N-MeFOSA-2 | d3-N-MeFOSA | 511.973 | 218.986 | 30 | -65 | -32 | -17 |
| d3-N-MeFOSA-1 | | 514.931 | 157.121 | 30 | -20 | -24 | -15 |
| N-MeFOSE | d7-N-MeFOSE | 615.821 | 59 | 30 | -50 | -80 | -7 |
| d7-N-MeFOSE | | 622.956 | 59 | 30 | -60 | -78 | -7 |
| PFTeDA-1 | 13C2-PFTeDA | 712.763 | 668.9 | 30 | -35 | -20 | -25 |
| PFTeDA-2 | 13C2-PFTeDA | 712.763 | 168.9 | 30 | -35 | -36 | -11 |
| 13C2-PFTeDA-1 | | 714.788 | 218.964 | 30 | -80 | -34 | -1 |
| N-EtFOSE | d9-N-EtFOSE | 629.837 | 59 | 30 | -15 | -74 | -7 |
| d9-N-EtFOSE | | 638.957 | 59 | 30 | -55 | -76 | -7 |
| N-EtFOSA-1 | d5-N-EtFOSA | 525.856 | 168.98 | 30 | -55 | -40 | -11 |
| N-EtFOSA-2 | d5-N-EtFOSA | 525.856 | 218.97 | 30 | -55 | -38 | -5 |
| d5-N-EtFOSA-1 | | 530.896 | 168.979 | 30 | -70 | -38 | -11 |
| PFHxDA-1 | 13C2-PFDoA | 812.818 | 768.981 | 30 | -40 | -20 | -21 |
| PFHxDA-2 | 13C2-PFDoA | 812.818 | 168.964 | 30 | -40 | -40 | -15 |
| 13C2-PFHxDA-1 | | 814.776 | 218.953 | 30 | -60 | -40 | -13 |
| PFODA-1 | 13C2-PFHxDA | 912.752 | 868.961 | 30 | -5 | -22 | -27 |
| PFODA-2 | 13C2-PFHxDA | 912.752 | 168.935 | 30 | -5 | -44 | -15 |

7.20.4. Parameter Table (Period 1 Experiment 1):

| | |
|------|----------|
| CUR: | 30.00 |
| GS1: | 30.00 |
| GS2: | 30.00 |
| IS: | -4500.00 |
| TEM: | 650.00 |
| ihe: | ON |
| CAD: | Medium |
| EP | -10.00 |

7.20.5. Electron Multiplier Settings

| Detector Parameters (Negative): | |
|---------------------------------|--------|
| CEM | 2200.0 |
| DF | 200.0 |

7.21. Tune Check

7.21.1. Before any analysis is done, the MS/MS detector should pass a polypropylene glycol (PPG) tune check. A standard containing 300 µM of SCIEX Mixed PPG solution is run. The spectra should meet the recommended SCIEX operating criteria before samples are run. A tune check should be performed periodically (ex. Quarterly and after PM or other service) See example SCIEX tuning criteria in 9.2.2 below:

7.21.2. SCIEX tuning criteria for PPGs in negative Turbo Ion Spray mode (NOTE: cps = counts per second and FWHM = field width at half mass.

| Mass | Q1 cps | Mass criteria | Q3 cps | FWHM |
|------|----------|----------------|----------|----------------|
| 934 | >= 2.0e7 | 0.6 to 0.8 amu | >= 2.0e7 | 0.6 to 0.8 amu |
| 2036 | >= 3.0e6 | 0.6 to 0.8 amu | N/A | N/A |

7.22. Instrument manuals can be found at [M:\EHD\ESS\(4900\)\ESS Org\(4940\)\Method Related Documents\Instrument Support Documents](M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\Instrument Support Documents)

8. Quality Control

8.1. For general quality control, procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure "ESS ORG QA0001 QAWRKSHT" located at [O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\Quality Assurance \(QA\)\ESS ORG QA 0001_Horizon and QA.docx](O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\Quality Assurance (QA)\ESS ORG QA 0001_Horizon and QA.docx)

8.2. Laboratory Reagent Blanks and Apparatus Check - The analyst must demonstrate that all sample apparatus and reagent interferences are under control. Before a new set of samples is extracted, a laboratory reagent blank (LRB) must be analyzed. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

8.2.1. Results for sample apparatus and sample centrifuge tubes will be kept at this location: [M:\EHD\ESS\(4900\)\ESS Org\(4940\)\Method Related Documents\PFAS\PFAS in Tissue\Bottle QC Checks](M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\PFAS in Tissue\Bottle QC Checks)

8.3. Initial demonstration of capability (IDC)

8.3.1. Initial demonstration of capability (IDC) needs to be performed before any samples can be analyzed by new personnel.

8.3.2. Assessing precision and accuracy

- 8.3.2.1. Four laboratory fortified blanks will be prepared near the midpoint of the calibration curve. The relative standard deviation (RSD) of the results of the replicates must be within 30% and average recovery must be between 65-135% or IDC study must be repeated.
- 8.3.2.2. If extracted internal standard recoveries do not fall between 50-150%, IDC must be repeated, the only exception for this is *FOSA and other neutral compounds like *NMeFOSA, *NEtFOSA, *NMeFOSE and *NEtFOSE, the allowable EIS recovery for this compound is between 20-150%.
- 8.3.3. Currently the laboratory is using water as a matrix for IDC, Laboratory Control Samples (LCS) and Method Blanks (MB).
 - 8.3.3.1.1.
- 8.4. MDL Study - Seven LFB's are spiked at a level one to five times the estimated LOD. The LFB's (as well as seven lab blanks) are to be extracted using the established method. The MDL is calculated by following the standard operating procedure O:\SOP\EHD\Division Wide\Final\EHD_QA_116_LOD_Procedures.doc
- 8.5. **Method Blank:** The result are expected to be less than the highest of the following
 - ½ the MRL or 1/10 of the sample concentration
 - Blank results are not subtracted from samples
- 8.6. **Laboratory Control Samples (LCS)**
 - 8.6.1. Precision can be assessed with sample duplicates or laboratory control sample duplicate (LCSD) and matrix spike duplicate (MSD). If sufficient sample is available, with each batch, the laboratory should analyze one tissue sample in a duplicate per batch or for every 10 samples. Matrix spikes and matrix spike duplicate are not required for this method, however recommended when possible.
 - 8.6.2. Laboratory control samples are processed in similar manner and conditions as samples, including all sample preparation steps such as centrifuging and filtering prior to injection.
 - 8.6.3. Laboratory control samples for tissues are spiked at a concentration that is midrange of the standard curve. Recoveries for PFDS, PFDoS, and 4:2 FTS are expected to be within 40-135%, For PFHxDA, PFODA, and NMeFOSA, the recoveries are expected to be within 50-135%; for the rest of the compounds recoveries are expected to be within 60-135%.
 - 8.6.3.1. If the above recoveries are not met the data needs to be appropriately flagged. The following flags should be included for samples and analyte impacted.
 - 8.6.3.1.1. LCSL: The Laboratory Control Spike (LCS) does not meet the lower QC limit.
 - 8.6.3.1.2. LCSU: The Laboratory Control Spike (LCS) does not meet the upper QC limit.

8.7. **Extracted internal standards (EIS)**

- 8.7.1. All samples and quality control samples need to be spiked with internal standards. Isotopically labeled internal standards are added at the beginning of the extraction process.
- 8.7.2. A minimum signal to noise ratio of 10:1 is expected in order to report quantitative results for the target analytes. If this minimum is not achieved result will not be reported.
- 8.7.3. For FOSA, *NMeFOSA, *NEtFOSA, *NMeFOSE, and *NEtFOSE, the EIS recoveries are expected to be within 10-150% (of average recovery of calibration standards) in samples. For the rest, the EIS recoveries are expected to be within 25-150%.
- 8.7.4. It is expected that the exact isotopically labeled analogs for the EIS are used as long as they are commercially available. For those compounds that do not have one, alternate EIS is used. Alternate internal standard must be isotopic and either from the same functional group as the target analyte, elute close to the target analyte, or have the same chain length as the target analyte.
- 8.7.5. If EIS is out of the above range for CCV and ICV for any of the compounds reinject, if reinjection fails a new calibration curve needs to be generated.
- 8.7.6. If EIS is out of range for LCS and samples; if recovery is low reinject and if reinjection fails flag data. If EIS recovery is high and there is no detection reinjection isn't necessary however data needs to be flagged.
- 8.7.7. Current concentration of EIS for each compound can be found in section 16.

8.8. Identification of Analytes

- 8.8.1. The retention time window for all compounds is monitored. In addition, each compound that is detected is confirmed by a confirmation ion pair.
- 8.8.2. Retention times of the target analytes and the EIS are expected to fall within 0.4minutes of the established absolute retention times.
- 8.8.3. When an initial calibration is performed, it is expected that the midrange standard is used to establish absolute retention times. On non-calibration day, the first CCV is used to establish absolute retention times.
- 8.8.4. Lab wide policy regarding procedure of initial identification of retention time can be found at [O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\General \(GENOP\)\ESS ORG GENOP 0072 RT Window Procedure.docx](O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\General (GENOP)\ESS ORG GENOP 0072 RT Window Procedure.docx)

9. **Method Calibration**

9.1. Working Standard Preparation Procedure

- 9.1.1. Standard must be brought to room temperature before use. A minimum of 5 standard curve levels for linear regression and minimum of 6 calibration points for quadratic regressions are required. Suggested levels are as follows: 2.0, 5.0 10.0, 25.0 50.0 and 100 ng/g. These standard curves are a volumetric dilutions of stock methanol standard that reflects extraction procedure for 0.5g of tissue

- sample. Refer to Table 4, section 16, for calibration standard concentration and spiking concentration of working standard.
- 9.1.2. The Applied Biosystems Analyst data system is used to prepare an internal standard linear calibration curve for each analyte. An R value of greater than or equal to 0.990 will be used as guidance to verify the acceptability of the curve.
 - 9.1.3.
 - 9.1.4. Branched and linear isomers must be used as quantitative calibration standards where commercially available.
 - 9.1.5.
 - 9.1.6. Absolute retention times are set using a midpoint standard from the initial calibration on calibration days. If using a previously generated curve, an initial CCV can be used to update retention time.
 - 9.1.7. Second Source (ICV) - needs to be analyzed with each new initial calibration and before analysis of sample. Recovery must be 70-130% of the spiked amount. If the second source fails, samples may not be analyzed.
 - 9.1.8. PFOA branch qualitative Standard
 - 9.1.8.1. Analyze Technical grade PFOA standard that includes branch isomers to confirm retention time with a new calibration curve, (this isn't necessary if retention time has not shifted).
 - 9.1.9. Standard log and completed started forms can be found at this location:
[M:\EHD\ESS\(4900\)\ESS Org\(4940\)\Standards Log](M:\EHD\ESS(4900)\ESS Org(4940)\Standards Log)
 - 9.2. The working calibration curve must be verified on each working day by the injection of one or more calibration standards at the beginning and end of each analytical run, or after the analysis of 10 samples if 10 or more samples are analyzed in an analysis day.
 - 9.3. Continuing Calibration Check and verification (CCV)
 - 9.3.1. The instrument's sensitivity should be verified by analyzing a CCV at or below the MRL on non-calibration days at the start of each run and each analyte should have recovery of $\pm 50\%$. CCVs that are above MRL must be between 70-130%. If CCV fails, make a new standard (CCV) and reinject. , then a
 - 9.3.2. A new calibration curve should be generated if CCV and ICV continue to fail or retention times have shifted significantly. Do not run samples if CCV or ICV fails.
 - 9.3.3. .
 - 9.3.4. Internal standard responses for each batch are recorded and kept in excel file at this location: [M:\EHD\ESS\(4900\)\ESS Org\(4940\)\Method Related Documents\PFAS\PFAS in Tissue\Internal Standard Criteria](M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\PFAS in Tissue\Internal Standard Criteria)
 - 9.4. (Initial Calibration Blank (ICB))
 - 9.4.1. It is expected that the ICB is analyzed immediately after the highest standard in the initial calibration and before the ICV to demonstrate the instrument is free from levels of contaminants.

9.5. Continuous Calibration Blank (CCB)

9.5.1. It is expected that the CCB is analyzed immediately after each CCV to demonstrate the instrument is free from levels of contaminants that would bias results.

9.5.2. Results for CCB and ICB are expected to be less than one-half the MRL.

9.6. It is expected that the same EIS as those used in samples are added to the initial calibration standards, ICV, CCVs, and ICBs at the same concentration used in samples. The recoveries of the EIS are expected to be within 50-150%.

9.7. Transition Ion Ratio

9.7.1. The primary and secondary ion transition ratios response must be assessed and be within 50-150% of the midpoint standard in calibration days or from the beginning CCV on non-ICAL days. Flag the data if transition ion ratio is out of range.

$$\text{Transition Ion Ratio} = \frac{\text{Quantitation Ion Abundance}}{\text{Confirmation Ion Abundance}}$$

9.7.2. Transition ion ratio for each batch can be found at this location:

[M:\EHD\ESS\(4900\)\ESS Org\(4940\)\Method Related Documents\PFAS\PFAS in Tissue\ION Transition ratio](M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\PFAS in Tissue\ION Transition ratio)

10. Sample Preparation Procedure

10.1. Samples should be blended to hominization using dry ice prior to extraction. (Sample can also be blended to visual homogeneity (up to 60 sec.) using a Tissue Terror probe-style hand-held blender.) Bring the sample to room temperature and transfer 0.5g (± 0.5) of tissue into a 50 ml polypropylene centrifuge tube. Record the actual weight of the sample.

10.2. Spike the sample with mass labeled internal standard solution at the same concentration as the standards. Allow ISTD to equilibrate for at least 10min before continuing with the extraction procedure.

10.3. Add one-half (0.5) mL of 18 Mohm water..

10.4. Vortex sample and add nine (9) mL of 10 mM NaOH in methanol via serological pipette.

10.5. Securely cap the 50 mL polypropylene tube(s) and place it on a shaker (orbital or rocker) for at least 16 hours at room temperature.

10.6. Following ≥ 16 hour alkaline digestion, centrifuge samples at 2000 rpm for 5 minutes.

10.7. Transfer 1.0 mL of the clear, upper layer to a 15-mL polypropylene conical tube, and add 9 mL 18 Mohm water.

10.8. Prepare a 12 port manifold by carefully cleaning all surfaces with methanol, and affix SPE cartridges (OASIS WAX, 3 cc, 60 mg).

10.9. Condition cartridges with the following sequence of reagents, 4 mL per reagent, flow at approximately 1 mL/minute;

10.9.1. 3.0% NH_4OH in methanol

10.9.2. Methanol

10.9.3. 18 Mohm water

- 10.10. Load samples onto SPE cartridges and adjust flow to about 0.5 mL per minute.
 - 10.11. After entire sample has passed, wash cartridges with 25 mM sodium acetate buffer at pH 4, flow approximately 1 mL/minute (note that this is a wash process and eluents are not be collected);
 - 10.12. Allow vacuum air to pass through emptied SPE cartridges for up to 5 minutes as a drying step.
 - 10.13. Elute SPE cartridges into properly-labeled collection tubes (15-mL polypropylene) using 8 mL of 3% NH₄OH in methanol. Adjust flow at about 1 mL/minute. (Use 3 mL of 3% NH₄OH in Methanol elution solvent to rinse the 15-mL polypropylene tube prior to elution).
 - 10.14. Evaporate eluent just to dryness under nitrogen gas (5 – 15 psi) using a TurboVap at 65°C.
 - 10.15. Reconstitute in 1 mL methanol, cap tightly, and store at 4°F until time of analysis.
 - 10.16. Prior to analysis, bring extract to room temperature and filter sample reconstitutes using 0.22-um Glass Fiber Filter and transfer to autosampler vial.
 - 10.17. Cap autosampler vials using caps that are absent of Teflon.
- 11. Calculations:** Calculations are performed using the Applied Biosystems Analyst software, performing a multilevel calibration, and using a linear fit.
- 11.1. Sample results for analytes with isotopically labeled analogs will be calculated using isotope dilution.
 - 11.2. Target analytes and their extract labeled analog must elute within 0.1 min.
 - 11.3. Sample and quality control blanks results are reported to the MDL. Results between MDL and MRL shall be qualified as estimated concentrations.
 - 11.3.1. Non-detections are reported as <MDL.
 - 11.3.2. The laboratory will be using the lowest point of on ICal as MRL (reporting limit).
 - 11.4. The primary and secondary ion transition ratios must be assessed and be within 50-150% of the value calculated from the midpoint standard on calibration day or from beginning CCV on non-calibration days.
 - 11.4.1. Transition Ratio = Quantitation Ion abundance/ Confirmation Ion abundance
 - 11.5. The signal to noise ratio must be greater than or equal to 3:1 for quantitation ions and confirmation ions.
- 12. Data Management:** Data is collected and calculations are made on a PC-based system running SCIEX Analyst Software by the analyst. Perfluorinated analyte data is transcribed onto the sample worksheet, reviewed, and transferred to the Laboratory's LIMS system manually by the analyst (or designee). It is then reviewed by peers or the section supervisor before being released.
- 12.1. Horizon/HDX

- 12.1.1. Data is collected using SCIEX Analyst Software. Sample and QC results may be manually entered in Horizon system or uploaded via HDX to the Horizon system and peer reviewed against the raw data before electronically releasing to the client.
- 12.1.2. Upload via HDX:
 - 12.1.2.1. With the results table open in Analyst right click anywhere on the table to bring up a drop-down menu. Select "full" to get all compounds and all injections into one table.
 - 12.1.2.2. Highlight the entire file and copy the data into an excel file and save it as a .CSV file.
 - 12.1.2.3. Make sure that the Sample Name is in row B, Calculated Concentration column is in row D, the Acquisition Date column is in row H, and the Analyte Peak Name column is in row J on the .CSV file.
 - 12.1.2.4. The Sample Name (row B) must contain the Horizon number of any sample or QC otherwise the upload won't work correctly.
 - 12.1.2.5. Open HDX. Make sure you have the correct parser loaded (ORG_LCMS). Click on the "Files to Parse" button and find the file that you want to upload into Horizon. Select the file you want to upload and click "Open."
 - 12.1.2.6. On the HDX screen make sure you have typed in the correct analytical batch number and the correct operator. Click "Start Parsing" at the bottom to complete the process.
 - 12.1.2.7. Current horizon identification of PFAS in tissue working standard is PFASiWrk1.

13. Definitions: General definitions of terms that may be used in this method can be found in the following documents.

- 13.1. Check the reference methods, see Section 16.
- 13.2. TNI Standard, EL-V1M2-ISO-2009, Section 3.0, Terms and Definitions, The NELAC Institute, 2009 and is located at O:\Teams\EHD QC Team\Accreditation\NELAC\2009 NELAC standards with ISO.pdf.
- 13.3. Chapter NR 149, Laboratory Certification and Registration, Wisconsin Department of Natural Resources, Wisconsin State Legislative Reference Bureau, Register November 2018 No. 755. See Section NR149.03 Definitions.

14. Method Performance:

- 14.1. Where applicable the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure.
 - 14.1.1. MDL procedure is located at <O:\SOP\EHD\Division Wide\Final\EHD QA 116 LOD Procedures.doc>.
 - 14.1.2. IDC procedure is located at <O:\SOP\EHD\Division Wide\Final\EHD QA 115 rev 0 DOCs.docx>.

- 14.2. Data generated within the last three years will be located in filing cabinet across from cubical SC213 or stored in the basement. Any data older than three years is archived, stored at Wisconsin State Record faculties and then destroyed after meeting its required retention time

15. References:

- 15.1. Ye, X., Strynar, M., Nakayama, S., Varns, J., Helfant, L., Lazorchak, J., Lindstrom, A. "Perfluorinated Compounds in Whole Fish Homogenates from the Ohio, Missouri, and Upper Mississippi Rivers, USA", *Environmental Pollution*, 156:3, 1227-1232.
 - 15.2. "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry". 3M Environmental Laboratory Method Number ETS-8-4.1 (03/01/99).
 - 15.3. Kurunthachalam, K., Franson, J.C., Bowerman, W.W., Hansen, K.J., Jones, P.D., and Giesy, J.P. "Perfluorooctane Sulfonate in Fish-Eating Water Birds Including Bald Eagles and Albatrosses", *Environ. Sci. Technol.* 2001, 35, 3065-3070.
 - 15.4. Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O. "Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices", *Environ. Sci. Technol.* 2001, 35, 766-770.
 - 15.5. "Test Methods For Evaluating Solid Waste Physical/Chemical Methods (SW-846) Third Edition," 1996.
 - 15.6. "Methods for the Determination of Organic Compounds in Drinking Water," US EPA/600/4-88/039, 1995.
 - 15.7. "Quality Assurance Manual", NELAC QA Manual.
 - 15.8. TNI Standard, EL-V1M2-ISO-2009, The NELAC Institute, 2009
 - 15.9. Determination of selected perfluorinated alkyl acids in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS). Method 537.1 EPA/600/R-08/092.
 - 15.10. Chapter NR 149, Laboratory Certification and Registration, Wisconsin Department of Natural Resources, Wisconsin State Legislative Reference Bureau, Register November 2018 No. 755.
 - 15.11. "PFC Analysis Kit for ACQUITY UPLC System Guide" 71500183002/Revision A, (2009).
 - 15.12. Water quality- Determination of polyfluorinated alkyl substances (PFAS) in water- Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS). ICS:13.030.50 ISO/DIS 21675 (Nov,2018)
 - 15.13. Wisconsin PFAS Aqueous (Non-potable Water) and Non-Aqueous Matrices Method Expectations. Wisconsin Department of Natural Resources Notice of Final Guidance & Certification, Doc ID EA-19-0001, 2019
16. Tables, figures, diagrams, charts, checklists, appendices: PFC in Fish Extraction Log on page 16.

16.1. **Table 1 :Current Internal Standard Concentration**

| Compound | Conc (ng/mL) |
|---|--------------|
| Sodium perfluoro-1-hexane[18O2] Sulfonate | 75.6 |
| Sodium perfluoro-1-[13C4]Octanesulfonate | 76.4 |
| Perfluoro-n-[13C4]butanoic acid | 80 |
| Perfluoro-n-[13C2]hexanoic acid | 80 |
| Perfluoro-n-[13C4]octanoic acid | 80 |
| Perfluoro-[13C5]nonanoic acid | 80 |
| perfluoro-[13C2]decanoic acid | 80 |
| perfluoro-[13C2]undecanoic acid | 80 |
| perfluoro-[13C2]dodecanoic acid | 80 |
| d3-N-MeFOSA | 40 |
| d5-N-EtFOSA | 40 |
| 13C2-6:2 FTSA | 80 |
| 13C2-8:2 FTSA | 80 |
| 13C2-FOUEA | 40 |
| 13C4-8:2diPAP | 40 |
| 13C3-PFBS | 40 |
| 13C3-PFPeA | 40 |
| 13-C4PFHpA | 80 |
| 13C2-PFTeDA | 200 |
| 13C2-PFHxDA | 200 |
| 13C8-FOSA | 160 |
| d3-N-MeFOSAA | 80 |
| d5-N-EtFOSAA | 80 |
| d7-N-MeFOSE | 40 |
| d9-N-EtFOSE | 40 |
| 13C3-HFPO-DA | 2000 |

| | |
|---------------|----|
| 13C2 4:2 FTS | 80 |
| 13C2 10:2 FTS | 80 |

16.2. **Table 2: Current Working Standard (Target compound mix) at 0.1 ug/ml**

| Compound | Conc (ng/mL) |
|-----------------|---------------------|
| PFHxA | 100 |
| PFHpA | 100 |
| PFOA | 100 |
| PFNA | 100 |
| PFDA | 100 |
| PFUnA | 100 |
| PFDoA | 100 |
| PFTTrDA | 100 |
| PFTeDA | 100 |
| PFBS | 88.5 |
| PFHxS | 91.2 |
| PFOS | 92.6 |
| N-MeFOSAA | 100 |
| N-EtFOSAA | 100 |
| HFPO-DA | 100 |
| DONA | 94.5 |
| 9Cl-PF3ONS | 93 |
| 11Cl-PF3OUdS | 94 |
| PFBA | 100 |
| PFPeA | 100 |
| PFHxDA | 100 |
| PFODA | 100 |

| | |
|-----------|------|
| PFPeS | 93.8 |
| PFHpS | 95.2 |
| PFNS | 96 |
| PFDS | 96.4 |
| PFDoS | 96.8 |
| 4:2 FTSA | 95 |
| 6:2 FTSA | 99 |
| 8:2 FTSA | 100 |
| 10:2 FTSA | 100 |
| FOSA | 100 |
| N-MeFOSA | 99 |
| N-EtFOSA | 96 |
| N-MeFOSE | 100 |
| N-EtFOSE | 100 |

16.3. Table 3: Standard For Direct Injections (Calibration Curve)

| Target Concentration | Aliquot of 0.1 ug/mL Mix | Aliquot of IS | MEOH |
|----------------------|--------------------------|---------------|----------|
| STD 2.0ng /ng | 1.0 uL | 5uL | 994uL |
| STD 5.0 ng/g | 2.5 uL | 5uL | 992.5uL |
| STD 10 ng/g | 5 uL | 5uL | 990uL |
| STD 25 ng/g | 12.5 uL | 5uL | 982.5 uL |
| STD 50 ng/g | 25uL | 5uL | 970.uL |
| STD 100ng/g | 50uL | 5uL | 945 uL |

16.4. Table 4: Standards for LCS and Matrix Spike (Calculated to reflect 0.5g of Sample)

| LCS Concentration | Aliquot of 0.1 ug/mL Mix | Aliquot of IS |
|--------------------------|---------------------------------|----------------------|
| 2.0 ng/ng | 10 uL | 50uL |
| 5.0 ng/g | 25 uL | 50uL |
| *10 ng/g | 50 uL | 50uL |
| 25 ng/g | 125 uL | 50uL |
| 50 ng/g | 250uL | 50uL |

*Current target concentration for LCS is at 10ng/g

PFC in Fish Extraction Log

Queue/Batch Numbers: _____ **Method** _____
#: _____

Start Date: _____ **Analyst initials:** _____

Date Finished: _____ **Analyst Initials:** _____

Spiking Standard Information:

Matrix-Spike Standard ID: _____

Internal Standard ID: _____

| Sample # | Matrix-Spike | Matrix Duplicate | |
|----------|--------------------------|--------------------------|------------------|
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | Balance ID: |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | Water bath Temp: |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | Thermometer : |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |
| _____ | <input type="checkbox"/> | <input type="checkbox"/> | |

Solvent/Reagent Lot #'s

Methanol Manufacture: _____ Lot: _____
 Ammonium Acetate Manufacture: _____ Lot: _____
 Sodium Acetate Manufacture: _____ Lot: _____
 NaOH Manufacture: _____ Lot: _____

Issuing Authority: Wisconsin State Lab of Hygiene
Analysis of Perfluorinated Compounds in Fish Tissue by HPLC-MS/MS
ESS ORG METHOD OC14801

Revision: 6

Effective Date: 4/10/20 - present

Replaces: ESS ORG METHOD14801, Revision 5

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NH₄OH

Manufacture: _____ Lot: _____

Issuing Authority: Wisconsin State Lab of Hygiene
Analysis of Perfluorinated Compounds in Fish Tissue by HPLC-MS/MS
ESS ORG METHOD OC14801
Revision: 6
Effective Date: 4/10/20 - present
Replaces: ESS ORG METHOD14801, Revision 5
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17. Signatory Page:

17.1. Written by:

17.1.1. Instrumental: Curtis Hedman
Title: Microbiologist Advanced
Unit: ESS Organic Chemistry

Date: Revision 1, 5/17/2010
Rev. 2, 11/15/2010

17.1.2. Sample Prep: Jim Tortorelli
Title: Senior Chemist
Unit: ESS Organic Chemistry

Date: Revision 1, 5/17/2010

17.2.

Revised by: Robel Kebede
Title: Senior Chemist
Unit: ESS Organic Chemistry

Date: Revision 6, 4/2/2020

17.3.

Reviewed by: Brandon Shelton
Title: Advanced Chemist
Unit: ESS Organic Chemistry

Date: Revision 6, 3/27/2020

17.4.

Approved by: Erin Mani
Title: ESS Organic Supervisor
Unit: ESS Organic Chemistry

Date: Revision 6, 4/10/2020

