

**A QUANTITATIVE ANALYSIS OF PER- AND POLYFLUOROALKYL
SUBSTANCES (PFAS) IN DRINKING AND SURFACE WATERS OF
TEXAS A&M AT GALVESTON**

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This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

A Quantitative Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking and Surface Waters of Texas A&M at Galveston

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Per- and polyfluoroalkyl substances or PFAS are a family of anthropogenic pollutants that consists of approximately 1200 structurally related chemical compounds. PFAS were discovered accidentally over 70 years ago by a DuPont scientist and are found in various applications: cookware, food packaging, metal plating, leather and textile coatings, pesticides, paints, aviation hydraulic fluids, and fire-fighting (aqueous film-forming) foams. PFAS are an area of concern as their structural stability and ability to resist biodegradation enable their bioaccumulation in exposed organisms and biomagnification across food webs. The ability of PFAS to remain stable in the environment coupled with their known toxicity in invertebrate and vertebrate taxa has led to the characterization of some PFAS as persistent organic pollutants (POPs). As a result, there is concern for the health of both wildlife and human populations from direct or inadvertent exposures to PFAS. In this project, I assessed the levels of eleven select PFASs categorized as priority pollutants by the Environmental Protection Agency (EPA), in various drinking water sources across the Texas A&M University at Galveston (TAMUG)

campus and in the final effluent of the campus' wastewater treatment plant (WWTP). Of the PFASs monitored, 73% were detected above the detection limits of the LC-MS/MS used to quantify PFAS levels. The levels of select PFAS (i.e. PFOA and PFOS) obtained in the samples were found to be 172 and 826 times lower than state and federal levels. Therefore, we can conclude that the levels of PFAS detected in the drinking waters at TAMUG and in surrounding surface waters are well below state mandated regulatory levels of concern.

Keywords: PFAS; PFOS; PFOA; contaminant; drinking water

DEDICATION

To Dr. Chen Xu for always encouraging my thirst for knowledge and supporting my crazy research ideas. Thank you for always believing in me and never letting me settle for mediocrity. To Rachel Sellers and Rachel Ball who always provided guidance and a willingness to point me in the right direction to find answers. Lastly, to Dr. Hala and Rayna Nolen, thank you for your courage to take me on as your mentee, supporting my research ideas, and keeping me grounded.

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Finally, without Ms. Tammy Do the collection, and testing would have been a much longer process, thank you for all the aid you were able to provide while I was in the hospital and in various other meetings.

The equipment, collection method, and baseline data for the data curve used for the Examination of Texas A&M's Galveston campus influent and effluent water sources for Per- and Polyfluoroalkyl Substances (PFAS) was provided by PhD student, Rayna Nolen. Ms. Nolen is conducting similar research on a much larger scale and thus the baseline data collection is proprietary and is currently unpublished and will not be in this paper.

NOMENCLATURE

ASEC	Aggie Special Events Center
Blank	Control
C6	Short-chain carbon sequence meant to replace PFAS (<i>Carbons</i> ≤ 6)
C8	Long-chain carbon compound commonly known as PFAS (<i>Carbons</i> ≥ 7)
EPA	Environmental Protection Agency
HFPO	Hexafluoropropylene oxide plus associated dimer salts (C_3F_6O)
ITRC	Interstate Technology Regulatory Council
LC-MS	Liquid Chromatography-Mass Spectrometry (analytical technique)
LC-MS/MS	Liquid Chromatography-Mass Spectrometry/Mass Spectrometer
LIB or Library	Jack K. Williams Library
MARB	Marine Biology
MeOH	Methanol (CH_3OH)
OCSB	Ocean & Coastal Science Building
PFBS	Perfluorobutane sulfonate/sulfonic acid ($C_5HF_9O_3S$)
PFDA	Perfluorodecanoic acid ($C_{10}HF_{19}O_2$)
PFDoA	Perfluorododecanoic acid ($C_{12}HF_{23}O_2$)
PFHpA	Perfluoroheptanoic acid ($C_7HF_{13}O_2$)
PFHxA	Perfluorohexanoic acid ($C_6HF_{11}O_2$)
PFHxS	Perfluorohexane sulfonate/sulfonic acid ($C_6HF_{13}O_3S$)
PFNA	Perfluorononanoic acid ($C_9HF_{17}O_2$)
PFOA	Perfluorooctanoic acid ($C_8HF_{15}O_2$)

PFOS	Perfluorooctane sulfonate/sulfonic acid ($C_8HF_{17}O_3S$)
PFTA	Perfluorotetradecanoic acid ($C_{14}HF_{27}O_2$)
PFTrDA	Perfluorotridecanoic acid ($C_{13}HF_{25}O_2$)
PFUnA	Perfluoroundecanoic acid ($C_{11}HF_{21}O_2$)
PPB	Parts Per Billion
RfD	Reference Dosage
SPE	Solid Phase Extraction
TAMMA(H)	Texas A&M Maritime Academy Hall (cadet dorm building)
WWTP	On campus Waste Water Treatment Plant (effluent collection site)

INTRODUCTION

Understanding the history of per- and polyfluoroalkyl substances (PFAS) from their discovery to their implementation in today's society helps to identify how this family of chemicals made it into our environment and food webs. PFAS are a family of approximately 1200 different chemical compounds, colloquially known as C8 chemicals, due to their structure containing at least eight carbon atoms chained together and bonded with fluorine (DiGiannantonio, 2022). While traditional PFAS are known as C8 due to their structure there are members of this family that contain seven chained carbons such as perfluoroheptanoic acid (PFHpA), perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA). PFAS, specifically perfluorinated compounds, were accidentally discovered in the late thirties by Dr. Roy Plunkett while working on a refrigeration project for the DuPont Company (Seymour & Kirshenbaum, 1987). This accidental discovery was notable in that PFAS appeared to not break down under high temperatures and the other chemicals and compounds were not adhering to this newly discovered substance (Mueller & Yingling, 2020). DuPont recognized the usefulness of PFAS and decided it warranted further study. After more than a decade of testing, DuPont marketed the new chemical as Polytetrafluoroethylene (PTFE) and began selling it commercially under its trademarked name – Teflon™ (DiGiannantonio, 2022).

During the PTFE testing, the scientists discovered many health concerns that they disclosed to the DuPont's; however, the DuPont family decided not to release this information to the public. This decision to keep the dangers secret was soon discovered and made public during the 1960s when over 200 barrels of the substance's waste byproduct were illegally dumped into the Ohio River, contaminating the local communities' water supplies (Lerner, 2015).

President Nixon established the Environmental Protection Agency (EPA) in December 1970 in response to national environmental protection laws often needing to be addressed due to their confusing and ineffective nature (Britannica, 2023). Their role is to enforce national environmental protection laws; however, the dangers of PFAS remained undisclosed. After decades of investigation, in 2004, the EPA won a \$16 million lawsuit against the DuPont company. During the investigation, the EPA discovered that the DuPont company knowingly withheld the fact that one of the most common compounds of the PFAS family, perfluorooctanoic acid (PFOA), caused congenital disabilities and liver, pancreatic, and testicular cancer in their lab animals. In fact, due to their willful negligence, DuPont was forced to pay out more than a billion dollars in damages and another two hundred thirty-five million dollars for medical monitoring (DiGiannantonio, 2022). Since the EPA's successful lawsuit much of the manufacturing in the United States has been stopped, with the exclusion of firefighting foams and aviation hydraulic fluids (Mørck et al., 2015).

Due to their incredible heat resistance and surfactant-type properties, this chemical family is laborious to break down once in the environment. Once PFAS has entered the water cycle through groundwater or as runoff after a storm, it is effortlessly evaporated and moved into the atmosphere and transported around the globe (D'Eon et al., 2006; Ellis et al., 2003, 2004; Martin et al., 2006; Stock et al., 2004; Yamashita et al., 2005; Young et al., 2007). While some PFASs are easily transported and moved by the atmosphere, others fall into the ocean's global conveyor belt. If the PFAS remains on the surface of the water, snow, or dewy vegetation it is carried into the atmosphere as the sun begins the evaporation process; however, in colder waters the PFAS is carried to the bottom and moved around the globe. On the other hand, as surface waters are transported into the ground so too are the PFAS chemicals. As the groundwater flows into the

sea, it carries along the PFAS and introduces PFAS to the oceanic conveyor belt. Consequently, remote places untouched by modern society are too affected by DuPont's negligence.

Studies conducted in the Netherlands looking at the concentration of PFAS in drinking water and foodstuffs gathered from local grocery stores discovered that the highest concentrations existed in shellfish and lean fish, respectively, and the lowest concentrations subsided in milk, baked goods, chicken, and oils (Noorlander et al., 2011). Another study conducted in Spain on twenty human cadavers (N=20) looked at PFAS concentrations in tissue samples (N=99) gathered from the bones, brains, livers, lungs, and kidneys. The study showed that the lung tissue samples had the highest concentration of PFASs, whereas the bone samples showed no evidence of PFAS. The lungs contained PFBA as their chief concentration with only trace concentrations of GenX chemicals. The brain however, showed a predominance of GenX chemicals with the primarily Perfluorohexanoic acid. Within the kidneys the only homolog that was present with significant concentration levels was that of PFBA. See Appendix A for a more detailed breakdown of the study's findings.

Scientists have conducted multiple studies on the health concerns associated with PFASs over the past quarter of a century that showed substantial bioaccumulation leading to low birth weights, immunocompromised children, cancers, and endocrine and reproductive issues (EWG.org). Other studies have found other maladies, including, but not limited to, enlarged livers, low cholesterol, dose-dependent mortality rates, and slowed bone ossification in lab animals (Goldenthal et al., 1978a,b; Lau et al., 2007; Seacat et al., 2002). In 2015, a study conducted on over 250 women and children from 17 European countries found no distinction in concentration levels regardless of their location; rural or urban. However, the study found a

correlation between mother and child, with the child having higher levels of PFAS in their system (Mørck et al., 2015).

PFAS production has ceased with the exceptions mentioned earlier, but in its place is a new short-chain (6C vs. 8C) chemical family known as Gen X, designed to provide the PFAS benefits without the harmful side effects. Once again, DuPont company created it and began to market Gen X as a less harmful alternative to PFOA (EPA, 2022). Despite the good intentions involved with the C6 manufacturing, DuPont's director of Regulatory Affairs for Haskell Laboratories, Dr. A. Michael Kaplan, in a letter to the EPA, stated that the GenX family of chemicals still possessed the downside of producing cancer in their lab rats (Kaplan, 2010).

As there is no current remediation for PFAS, the EPA has developed reference doses (RfDs) for the Genx, perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorobutanesulfonic acid (PFBS) chemicals. Reference doses are essentially the maximum daily oral dosage, in milligrams, of a toxic substance allowed, without having any serious or adverse effect. These reference doses are based on individual body weight measured in kilograms (mg/kg day). These RfDs are listed based on chronic (lifetime) and subchronic (less than lifetime) consumption (Table B.1.); however, the PFBS, PFOA, and PFOS show only Chronic RfDs for a point of comparison (Table B.2.). Many in the scientific community are working tirelessly to find a solution. In 2020 a study concluded that the non-ionizing radiation from UV light could effectively degrade PFAS, lending itself to the idea that one day there may be a way to destroy these chemicals or safely remove them from our global waters. Despite the success of this experiment, it employed particular parameters that, in a real-world environment, would not be efficient (Olatunde et al., 2020).

Since PFAS is in remote corners of the world, does not degrade, and no remediation exists at present, it would not be difficult to surmise that these chemicals have found their way into our homes and food sources on campus at Texas A&M at Galveston (TAMUG). TAMUG is home to one of the six Maritime Academies in the United States, supporting around 5,000 students, faculty, and staff daily. Therefore, this project sought to quantify the levels of select EPA-priority PFAS in the drinking and surface waters of TAMUG. The project involved collecting various samples from water fountains across campus and collecting downstream surface waters of the effluent from the wastewater treatment plant (WWTP) that services the community at TAMUG. These water samples were run through a special filtration system designed to collect PFAS for creating a concentration to be placed in the LCMS-MS for measurement. In particular, the levels of select PFAS, PFOS, and PFOA were compared with the state regulatory limits to determine whether there was a cause for concern for inadvertent toxicity effects in exposed wildlife and humans.

1. METHODS

Producing a viable product capable of analysis requires several steps and phases. The sample collection phase used one-liter low-density polyethylene (LDPE) Nalgene-type bottles, whereas the quantitative analysis process required a concentrated PFAS sample of only a few milliliters. For this to be possible, the sample water underwent a solid phase extraction (SPE) which moved the PFAS from the sample to a special SPE cartridge filter (sorvent bed) explicitly designed to remove the chemicals from the sample. In preparation for the SPE process, the sorbent beds were conditioned in a precise sequence to ensure the filters did their job and functioned properly. After successfully conditioning the sorbent bed, the sample was introduced to the SPE process after which the extraction method required an elution phase to extract the PFAS from the filters. Finally, the eluate required a concentration phase to remove residual liquids used in the rinsing process and produce a product ready for liquid chromatography-tandem mass spectrometry analysis.

1.1 Sample Collection Phase

The sample collection phase required using one liter LDPE (Nalgene style) bottles to collect duplicate samples from various water bottle-filling water stations from around TAMUG's campus and from a surface water site comprising the final effluent from a wastewater treatment plant (WWTP) servicing the campus (Figure B.1). The drinking water samples were collected primarily from EZH₂O fountains (Figure B.2) located at various buildings and dormitories around the TAMUG campus (Appendix C), these sites included the: Aggie Special Events Center (ASEC), Texas A&M Maritime Academy Hall (TAMMAH), Ocean & Coastal Studies Building (OCSB), Pacific Hall residence hall (PACIFIC), Oceans Hall residence hall (OCEAN),

and the Jack K. Williams Library (LIB), two water samples collected per site (N = 2). In addition, replicate surface water samples were also collected from a stream comprising the final effluent from a WWTP.

Each one-liter Nalgene-type collection bottle received three rinses with source water before collecting the sample. The rinsing process consisted of collecting approximately 250mL of source water then affixing the top and vigorously shaken for five minutes. After five minutes the lid was loosened, and the source water was allowed to run through the threads of the bottle cap. Once collected, the sample bottle was labeled with orange masking tape, and the location from which the sample was collected. The labeled sample was then placed in a Styrofoam cooler with Dr. Hala's name on it to prevent tampering or someone mistakenly grabbing a sample from the wrong study. Finally, the sample-filled cooler was stored in a freezer at twenty degrees Celsius below zero (-20°C) to preserve the integrity of the sample and prevent the possibility of sample degradation. Before starting the extraction and reduction, the first iteration of samples (N = 5) was thawed overnight in a refrigerator at four degrees Celsius (4°C). Once thawed, the solid phase extraction (SPE) device vacuumed the contents through the device, thereby removing the water from the possible PFAS. Performing the SPE required using Agilent SampliQ WAX polymer six milliliter (6mL /150mg) SPE cartridges (Agilent, Cat.# 5982-3667). The sorbent beds needed to be conditioned properly and without error to ensure the SPE filters captured the PFAS as the sample water passed through the beds.

1.2 SPE Conditioning Phase

The lines and stoppers used in the SPE process underwent a thorough methanol rinsing before conditioning the sorbent beds. The SPE conditioning phase began with 15 milliliters (mL) of methanol (CH₃OH) given in increments of five milliliters (5mL) then, followed by an 18mL

rinse of water, ensuring the sorbent beds in each cartridge never ran dry, thus keeping the beds solvated. If a piece of the sorbent bed became dry at any time during this process, the filter would be damaged, and the conditioning phase would need to be conducted again. PFAS possess a greater affinity for methanol than water; thus, the pretreatment allowed the sorbent beds to ensure any PFAS that passed through the SPE cartridge. Water (H₂O) creates hydrogen bonding with any methanol not captured in the filter and carries it away, leaving the beds conditioned and ready for the elution phase.

1.3 The Elution Phase

The SPE device possessed slots for up to 12 cartridges; however, iterations of five proved more manageable as, again, the filters needed to stay solvated. If the filters became dry, the sample would need to be collected again and the sorbent bed reconditioned. Therefore, five samples (one per cartridge) at a time were extracted through the SPE cartridges under vacuum and not exceeding 10 - 15 milliliter per minute (mL/min) flow-through rate (~ 15psi). Once empty, each bottle underwent two 7.5mL (15mL total) ultra-filtered distilled water (Mili-Q water) rinsings. The rinsing consisted of adding the Mili-Q water to the empty bottle, then the top was affixed and then shaken for approximately five minutes. After each rinsing cycle, the water went through a solid phase extraction device to ensure the collection of any residual PFAS. With the PFAS trapped in the filter, eluate collection tubes (one per cartridge) replaced the SPE device. Each SPE cartridge underwent two rinsing cycles with four milliliter (4mL) of methanol to relocate the PFAS to its respective sample tube in a process known as elution.

1.4 The Concentrating Phase

The concentration phase consisted of reducing the elute from four milliliters (4mL) to the required two-tenths of a milliliter (0.2mL) for analysis by the mass spectrometer (MS). The

previous phase left the eluate tubes full of PFAS and residual methanol; removing the methanol would produce the concentration needed for quantitative analysis. Each collected eluate underwent a nitrogen dry-down via a speed vac (90°C for approximately one hour) to acquire the concentrated PFAS needed for liquid chromatography-tandem mass spectrometry (LCMS/MS) analysis. The nitrogen gas and heat helped evaporate the remaining methanol to ensure PFAS sample integrity. Once dried, the concentrated eluate tubes received another one milliliter (1mL) of nitrogen and were placed on a bench mixer. Lastly, the tubes went back through the speed vac for drying and were given an additional two tenths of a milliliter (0.2mL) of methanol in preparation for LCMS/MS analysis.

1.5 Preparing the LCMS/MS

The LCMS/MS uses an acquisition method that determines the desired target ions monitored in the samples. The parameters below, in Table 1, determine the acquisition parameters used to identify and quantify the various PFAS homologs. Each PFAS homolog ran as a 12-point standard curve with the internal standard as the constant. The machine settings are shown below in Table 1.

The mass-to-charge (m/z) ratio of precursor to product ions monitored for the quantification of each PFAS homolog are detailed, along with mass spectrometer fragmentor (Frag) voltage in Volts (V), collision energy (C.E.) voltage in Volts, and chromatographic retention times (R.T.) in minutes (min). The internal standard (I.S.) used for quantifying individual PFAS homologs is also detailed. Also listed are the instrument limit of detection (LOD) for each PFAS. After establishing and programming the parameters into the system, the 27 vials were introduced into the autosampler of the liquid chromatographer or LC (Appendix E).

Table 1. List of Mass Spectrometric Settings used in the Quantification of PFAS.

LCMS Settings							
Compound	Precursor Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)	Frag (V)	C.E. (V)	R.T. (min)	LOD (ng/mL)
13C8-PFOA (I.S.)	421.00	376.10	171.90	75	4	9.8	
PFDA	512.96	469.00	269.00	125	10	11.4	0.19
PFD _o A	612.95	569.00	268.90	120	5	12.5	6.25
PFHpA	362.97	319.00	168.80	85	0	8.8	6.25
PFH _x A	312.97	269.00	237.30	100	8	7.5	1.56
PFH _x S	398.93	98.90	79.90	195	45	8.9	0.78
PFNA	462.96	419.10	218.90	85	4	10.7	6.25
PFOA	412.96	369.00	354.00	75	4	9.8	3.13
PFOS	498.93	98.40	80.00	215	60	10.7	6.25
PFT _r DA	662.95	619.00	269.10	140	9	13.0	3.13
PFUnA	562.95	519.00	269.00	120	10	12.0	0.78
PFTA	712.94	668.90	269.20	140	10	13.4	1.56

1.6 Materials used in the collection and analysis

Two one-liter low-density polyethylene Nalgene style bottles were used per location to collect water samples totaling 14 bottles. Once assembled, the samples required a freezer capable of reaching temperatures of twenty degrees below zero Celsius (-20° C) to prevent potential sample degradation. Six water fountains with bottle-filling capabilities provided the influent samples. Each of the 14 samples needed a separate SPE cartridge. Each cartridge required a 15mL pre-rinse of methanol followed by an 18mL rinse of Mili-Q water whilst attached to the SPE (Figure D.1.). Once empty, the bottle underwent two rinsing cycles of 7.5mL of Mili-Q water and extracted through the SPE after each rinse. Thus, the first extraction stage required 210mL of methanol and 462mL of Mili-Q water. Each SPE cartridge was placed in its protection tube (14) and stored until stage two was ready.

In stage two, the SPE tubes were subjected to a four milliliter (4mL) methanol rinses twice to move the PFAS from the SPE filters to the elute tubes, requiring an additional 112mL of

methanol and 14 elute tubes. Finally, using a nitrogen dry-down system (Figure D.2.) allowed for the evaporation of the residual methanol from the elute tubes. Once the drying was complete, one milliliter (1mL) of methanol, 14mL in total, was added and placed on a bench mixer, thus ensuring no residual PFAS remained on the sides of the elute tubes. Again, the elute tube underwent nitrogen dry-down to remove the methanol. After which, the dried-out elute tubes were given two tenths of a milliliter (0.2mL) of methanol for the LCMS/MS.

In total, this project required 27 five microliter vials; 14 one-liter (1L) LDPE Nalgene bottles, SPE tubes with filters, elute tubes, and falcon tubes; 5 lines and beakers; 3 mechanical pipettes; 1 LCMS/MS, SPE, speed vac nitrogen dry-down system, and water catchment system; 462mL of Mili-Q water and approximately 339mL of methanol (CH₃OH).

2. RESULTS

The LCMS/MS analysis showed no detection of perfluorononanoic acid (PFNA) or perfluorodecanoic acid (PFDA) across any of the samples, thereby excluding these homologs from the results. The highest concentration of PFAS across all samples came from Perfluorohexanoic acid (PFHxA) with a mean of 2.4 parts per billion (ppb) and a standard deviation of 0.7ppb. Perfluorododecanoic acid (PFDoA) and Perfluorotridecanoic acid (PFTrDA), despite being present in the OCSB and ASEC these samples had the lowest mean and standard deviation of 0 (zero) ppb. Table 2 below shows the mean and standard error of the PFAS collected per site. It is important to note that the means and standard error for the PFAS concentrations are in parts per trillion (ppt) for ease of reading and comparison with EPA regulations. If one wishes to see the ppb results, divide by one thousand. The normalized PFAS homolog levels per collection site (Figure 1) illustrate that, except for the ASEC building, PFHxA concentrations were much higher than the other homologs.

Table 2. List of mean and standard error per PFAS homolog per collection site.

PFAS Detected Per Site									
Sample Site	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFUnA	PFDoA	PFTTrDA	PFTA
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
ASEC	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.7 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.3
TAMMA H	1.9 ± 0.2	0.9 ± 0.5	0.8 ± 0.1	1.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
OCSB	2.9 ± 1.1	1.0 ± 0.4	1.3 ± 0.4	2.3 ± 0.1	0.5 ± 0.5	0.6 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
PACIFIC	2.6 ± 1.1	1.0 ± 0.4	1.1 ± 0.1	2.2 ± 0.1	0.7 ± 0.7	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
OCEAN	3.5 ± 0.9	1.2 ± 1.1	1.1 ± 0.2	1.7 ± 0.5	0.4 ± 0.4	0.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LIB	2.8 ± 0.6	0.5 ± 0.2	0.0 ± 0.0	0.9 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
WWTP	2.8 ± 0.6	0.8 ± 0.0	0.8 ± 0.1	2.1 ± 0.0	0.4 ± 0.4	0.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

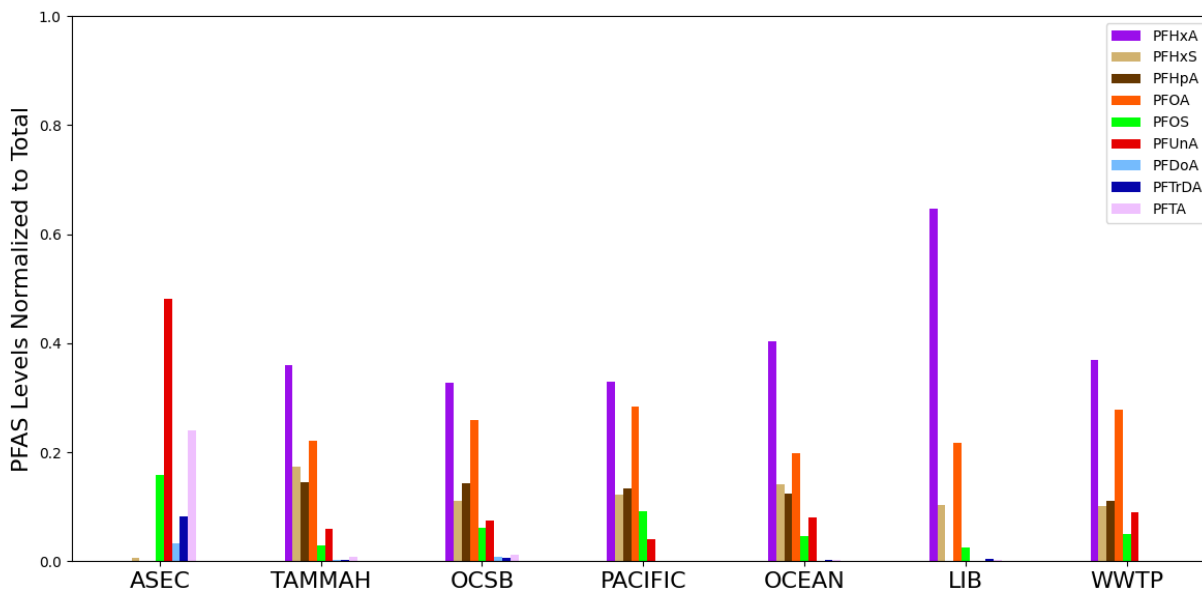


Figure 1. Normalized homologous PFAS concentrations per collection site.

On the other hand, when one analyzes the non-normalized data (Table 3), it becomes apparent that there were several significant outliers. An example is shown for the homolog PFOS between samples A and B for the TAMMA Hall (T-A and T-B, respectively). This trend continued for all PFOS between the A and B samples but was not exclusive to PFOS but other homologs as well. These outliers were concerning as they pushed the concentration means well above federal and state regulations.

One possible explanation for these outliers could be the redundant use of some of the equipment. The same equipment was used between samples, specifically the lines from the 1L bottle to the SPE but was subjected to a rinsing between sample extractions. The simple rinse of the lines may have been inadequate or improperly performed. Another possibility is that water bubbled out of the tubes during the nitrogen dry-down because the gas turned too high.

Table 3. LCMS-MS results of the homolog concentrations for every sample given in parts per trillion or ng/L

Per and Polyfluoroalkyl Substances (PFAS) Homologs											
Site	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA	PFUnA	PFDoA	PFTrDA	PFTA
A-A	0.00	0.00	0.00	0.00	-0.11	0.00	0.00	0.68	0.08	0.22	0.63
A-B	0.00	0.02	0.00	0.00	0.43	0.00	0.00	0.63	0.01	-0.01	0.02
T-A	2.10	0.44	0.90	1.27	0.31	0.00	0.00	0.64	0.02	0.01	0.05
T-B	1.76	1.44	0.65	1.12	3725.22	0.00	0.00	0.00	0.01	0.02	0.03
OB-A	3.99	1.35	1.61	2.41	5.35	0.00	0.00	0.66	0.10	0.10	0.19
OB-B	1.74	0.59	0.89	2.12	1.10	0.00	0.00	0.63	0.03	0.01	0.01
PAC-A	1.45	0.60	0.94	2.11	1.44	0.00	0.00	0.63	0.01	0.01	-0.01
PAC-B	3.73	1.33	1.16	2.36	93.03	0.00	0.00	0.00	0.00	-0.01	-0.02
OS-A	4.38	2.31	1.26	2.22	329.85	0.00	0.00	0.75	0.01	0.04	0.04
OS-B	2.55	0.11	0.86	1.20	0.81	0.00	0.00	0.63	0.00	-0.01	0.00
LIB - A	2.21	0.26	0.00	0.84	6.02	0.00	0.00	0.00	0.00	-0.01	0.00
LIB- B	3.42	0.64	0.00	1.05	0.23	0.00	0.00	0.00	0.01	0.04	0.02
W-A	2.20	0.76	0.76	2.09	3.67	0.00	0.00	0.72	0.00	0.00	-0.01
W-B	3.48	0.80	0.94	2.18	0.76	0.00	0.00	0.67	0.00	0.02	-0.02

Note: 1. Sample sites are abbreviated to fit within the margins of this paper and are abbreviated as A is ASEC, T is TAMMA, OB is OCSB, PAC is Pacific dormitory, OS is Oceans dormitory, LIB is Library, W is the WWTP. As noted earlier, two samples (A and B) were collected from each site.

However, comparing the two most hazardous homologous of PFAS (PFOA and PFOS) against the federal and regulatory guidelines issued by the Texas Commission on Environmental Quality (TCEQ) and the EPA respectively as shown in Figure 2 we see the results were well below their standards.

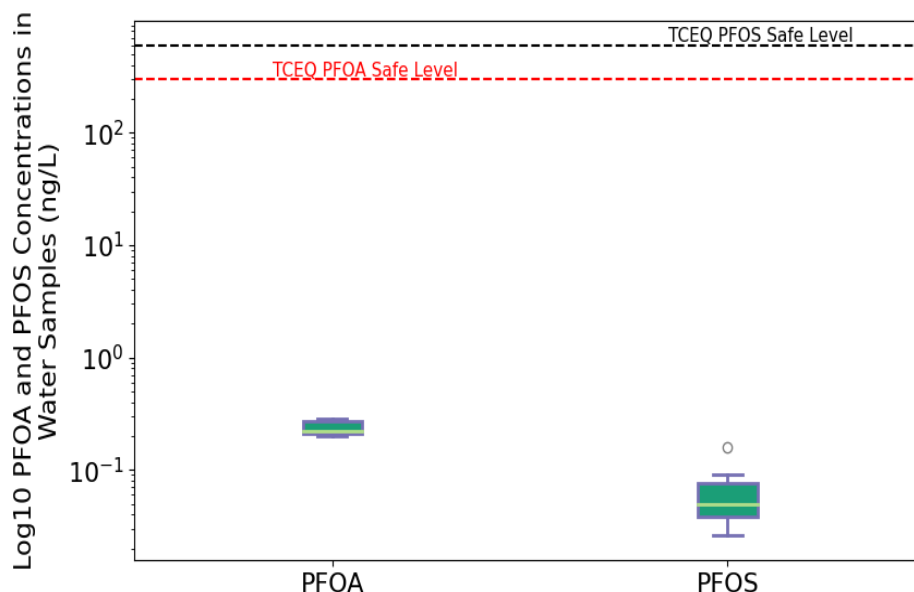


Figure 2. PFOS and PFOA comparison with state regulatory limits. Only shown is the TCEQ limits as state regulations cannot supersede the more stringent federal limits.

The results, when compared with the federal regulatory guidelines for reference dosages (mg/kg-day) for chronic (daily) and subchronic (non-daily) consumption set forth by the Environmental Protection Agency (Table 4), showed the campus’ PFAS levels were well below the federal limits (Environmental Protection Agency, 2021). Table 5 shows the Chronic RfDs for the new GenX and the more dangerous PFAS homologs.

Reference dosages for PFAS chemicals in which excess of these would lead to bioaccumulation to the point of developing various health concerns. Chronic RfD is defined as how much is safe for daily (for life) consumption, whereas the classification of subchronic is anything less than daily. Each amount is also expressed in scientific notation for ease of reading.

Table 4: Final chemical Reference Dosages (RfD) measured in mg/kg-day

PFAS chemical	Subchronic RfD (Scientific notation)	Chronic RfD (Scientific notation)
GenX	0.00003 (3×10^{-5})	0.000003 (3×10^{-6})

In the table below, the sub-chronic RfDs were removed as some of the PFAS chemicals have not been tested and therefore would lack a point of comparison.

Table 5: Chronic RfD comparisons among the leading PFAS chemicals measured in mg/kg-day

PFAS chemical	Chronic RfD (Scientific notation)
GenX	0.000003 (3×10^{-6})
PFBS	0.0003 (3×10^{-4})
PFOA	0.00002 (2×10^{-5})
PFOS	0.00002 (2×10^{-5})

Note: PFOA and PFOS RfDs were recorded in 2016 and the EPA is currently in the process of reevaluating their toxicity.

When calculating the safe RfDs, one must consider the average weight for 20-year-old males and females, which according to the Center for Disease Control, are approximately 200 and 171 pounds (lbs), respectively. However, one must convert pounds into kilograms (kg) for easy calculations; thus, the pounds listed above for males and females must be divided by roughly 2.2, making the average body weights approximately 91 and 77 kilograms, respectively. Under the general guidelines that one liter of water weighs about one kilogram and that the average water consumption should be around four liters (roughly four kilograms) for males and three liters (around three kilograms) for females (Behney, 2004), the computations of RfD equate to 8.51×10^{-6} milligrams (8.51 nanograms) for males and 6.21×10^{-6} milligrams (6.21 nanograms) respectively. Comparing the safe chronic RfDs for PFOA and PFOS against the concentrations found on campus (1.813×10^{-3} mg) shows that campus homologs are significantly lower than EPA regulations by approximately 213 percent.

3. CONCLUSION

As illustrated throughout this paper, PFAS are a dangerous anthropogenic chemical family that poses serious health concerns. These emerging chemicals have found their way into the global water cycle, transporting them around the United States and to remote corners of the world. Cities such as Baltimore have reportedly joined other cities such as Philadelphia in suing 3M, Chemours, DuPont, and hundreds of other manufacturers of aqueous film-forming foams (Mayor Brandon M. Scott, 2022) to rectify and recover from the costs of reverse osmosis and charged carbon filtration systems. In one report, areas of New Mexico remain arid throughout the year except for snowmelt and summer floods which churn their way to the Rio Grande, along with 16 other counties, are now contaminated with PFAS. Furthermore, testing conducted by New Mexico's Environmental Department and the United States Geological Survey has revealed that military bases throughout the region have found PFAS in private and public wells which supply groundwater for neighboring communities as well as those that supply agricultural lands and livestock (Goldberg, 2021).

Just as the remote counties of New Mexico, which experience little to no rainfall, have shown levels of PFAS, making it easy to understand how PFAS has spread to isolated regions of the globe. Due to this global spread of PFAS, coupled with the inoperable water fountain filtration lights, there was concern that these chemicals may have infiltrated our campus water sources. Before this study, no other tests have ever examined the campus surface waters for harmful and dangerous contaminants, thereby exacerbating the concerns of at least one young scientist. As such, this project aimed to quantify PFAS levels found in the campus' influent and

effluent surface waters and the drinking water provided via various water fountains.

Additionally, should the levels found have exceeded state and federal regulations, this project would have provided support for briefing campus administrators.

Mass spectrometry made it possible to quantify the concentration levels of select priority PFAS found in the surface and drinking water of TAMUG. The analysis aimed at comparing the levels found on campus against applicable state and federal regulatory guidelines. Overall, the analysis showed low levels in effluent and water fountains. To note, the concentration levels for this comparison were measured in parts per trillion (PPT, ng/L).

The analysis of specific PFAS homologs revealed a predominance of mostly short carbon chain PFAS, such as the new GenX (C6) chemicals PFHxS and PFHxA along with the long carbon chained (C8) chemicals PFOA, PFOS and PFHpA (C7). This predominance of short chain PFAS is not uncommon as it is consistent with the homologs found in the environment (Aly et al., 2020; Nolen et al., 2022). This is consistent with the previously mentioned reports that GenX was meant as a direct replacement for the more dangerous PFOS and PFOA. Having their homologous production ceased in accordance with the federal government (except for aircraft hydraulic oils and firefighting foams) one would expect to see lower concentrations of PFOS and PFOA and a prevalence of short-chain GenX chemicals.

According to the United Nations, of the PFAS homologs, PFOS is considered the highest priority for the toxicological effects in exposed humans and wildlife (Ankley et al., 2020; UNEP, 2017). In this study, PFOS comprised less than 20% of all measured PFAS homologs and exhibited the highest concentration of 0.7 ppt (ng/L) in drinking water. A comprehensive toxicity survey conducted by Beach et al. (2006) identified PFOS water concentrations less than 1.2 parts per billion (ppb, $\mu\text{g/L}$) or 1200 ppt to be protective against adverse toxicity effects in exposed

shellfish, fish, and amphibians (such as effects on survival, growth, embryo-larval hatching success). Therefore, the protective level for wildlife health effects is ~1,700x higher than the levels detected in surface waters from around the TAMUG campus. Furthermore, the comparison of PFOA and PFOS levels against TCEQ-established levels showed state regulatory levels to be greater than 170 times higher than those detected during this project.

This project concludes that while there are various concentrations of PFAS in the influent and effluent surface and drinking waters at TAMUG, the levels are well below state and federally mandated regulatory levels of concern. Regardless of the observations, the continued exposure and accumulation of PFAS in biota and their associated toxicity effects warrants continued monitoring efforts and regulatory vigilance. As PFAS are an emerging family of chemical contaminants, there are still knowledge gaps within the scientific community. New information may dictate the frequency at which these sources are tested. However, as in the Baltimore example above, the PFAS concentrations in the drinking water were exceptionally low and within regulatory requirements, yet the state calls for quarterly testing as “the fact that they are present is reason enough to proactively prepare for future protections” (Baltimore, 2022).

As Texas A&M University at Galveston draws life from the Gulf of Mexico and Galveston Bay, we must continually monitor the surrounding states and their watersheds' activities that contribute to these bodies of water. The watersheds that lead into the Gulf extend from Montana in the west to Pennsylvania in the east, making monitoring more difficult. Due to the complexity of trying to monitor all the mid-US states, keeping track of these contaminants in Louisiana, Arkansas, Oklahoma, and Texas would be more efficient. In recent news, the EPA announced on 6 March that under President Biden's Bipartisan Infrastructure Law five billion dollars will be allocated over the next five years to address these emerging contaminants in

several states, such as Texas, Louisiana, Arkansas, Oklahoma, and New Mexico (Robledo & Durant, 2023).

In 2021 however, the United States Department of Defense (U.S. DoD) issued a goal of conducting a preliminary PFAS assessments on 700 of their sites and military installations by 2024. To date, approximately 30 percent of the DoD sites remain untested and anticipate an extra 200 million to complete their assessment. Additionally, the U.S. Defense Department is estimating another two billion dollars to address the issues they find (Department of Defense, 2022). As this money is different from the two billion allocated by the Bipartisan Infrastructure Law making it a total of four billion dollars to assess and address PFAS in a handful of states and multiple DoD sites.

In Louisiana, a study conducted by the Water Collaborative of Greater New Orleans found that out of the 31 sites tested along the Mississippi River, five tested positive, of which two sites contained perfluorobutanoic acid (PFBA), one with PFBA and PFOS (Dandridge et al., 2022). In June 2022, the U.S. Army tested their installation's drinking water and found that all the surveyed sites tested positive for PFAS concentration levels greater than federal regulations (Walter et al., 2022). The State Representative, Congressman Troy A. Carter, responded by pushing for a law allowing over 26 million dollars to address emerging contaminants in the state's drinking water (Rep. Carter, 2023).

According to a study by Physicians for Social Responsibility in Texas, PFAS was often used to reduce the friction between the drill and the ground when drilling oil and gas wells in at least 73 counties over the past decade. The Executive Director of Liveable Arlington estimates that over five million state residents live within a half mile of a fracking site (Javidan, 2023). In response to this study, Penny Morales Shaw (Texas State Representative) has filed a bill to study

the use of PFAS in fracking and its potential exposure to the public to determine if these chemicals should be banned from future fracking (H.B. 4577, 2023). In addition to this bill, Texas will receive roughly 115 million dollars from the Bipartisan Infrastructure Law to aid in the infrastructure and treatment of emerging contaminants and water testing (Robledo & Durant, 2023).

The Defense Department, as part of their ongoing preliminary assessment of military installations, also examined Pine Bluff Arsenal's fire station, training area, and storage area and found PFAS in the soil at the fire station and in the groundwater of the training area (Schmidt et al., 2021). The PFAS levels found in the soil and groundwater likely are due to the fire station and training areas using aqueous firefighting foams. Under the Bipartisan Infrastructure Law, Arkansas is due to receive close to 19 million dollars (Robledo & Durant, 2023), which they plan to spend on 24 new water sampling stations (Central Arkansas Water, 2021). In response to the high costs associated with these studies and remediation attempts, Arkansas' legislative branch passed House Bill 1351 (ACT 315) in addition to the U.S. House of Representatives initiating bill H.R. 2467. Together, these bills will ban the use of Class B firefighting foams containing PFAS, with a phase-out date no later than 2024 (H.B.1351, 2023 and H.R. 2467, 2021).

Oklahoma's Red River, one of the significant tributaries of the Mississippi River, winds its way through New Mexico, Texas, Oklahoma, Arkansas, and Louisiana, eventually dumping into the Atchafalaya River. Louisiana's Atchafalaya River is a depository for the Mississippi River (Kleiner, 2019), the largest body of water that feeds the Gulf of Mexico and flows into Galveston Bay under certain weather conditions. For this reason, monitoring the PFAS activities of these rivers are significant for the predictive analysis of what may happen to the Gulf of Mexico and subsequently the influent waters of Texas A&M's Galveston Campus.

The Tar Creek Superfund Site, the most toxic body of water in Oklahoma, does not flow into the Red or Canadian Rivers and thereby avoids the Mississippi; however, Cache Creek meanders through Fort Sill and flows into the Red River. However, in May 2021, when the Army tested the waters of the military installation for PFOA and PFOS, the concentrations were undetectable by their labs (Pihulic, 2021). In contrast to Fort Sill, the McAlester Army ammunition plant had concentration levels of around three parts per trillion in drinking water (Rogers, 2021). The main issue is the PFOS and PFOA levels found on Altus, Vance, and Tinker Air Force Bases (AFB), which according to the Environmental Working Group, were over one million ppt in 2017 (EWG, 2022). These bases have astronomically high concentrations of PFOS and PFOA due to their required use of firefighting foam. The Altus AFB results are significant because the Air Force Base is less than ten miles from the Red River, which borders Oklahoma and Texas. Under President Biden's Bipartisan Infrastructure Law, Oklahoma will receive approximately 21 million dollars in 2023 (Robledo & Durant, 2023). Oklahoma will likely begin its fight against PFAS by addressing the clean-up at Altus, Vance, Tinker Air Force Bases, and Tulsa and Will Rogers World Airport, which have PFAS levels above one thousand ppt.

Recently, the EPA has issued a proposal to establish and legally enforce a maximum national standard for the concentration levels of six PFAS homologs (PFOA, PFOS, PFNA, PFHxS, PFBS, and the new GenX family of chemicals) with a future goal of achieving a contamination level of zero. If approved, under the new guidelines, the two most hazardous homologs, PFOS and PFOA, will be restricted to four parts per trillion (4 ng/L) and the others to a Hazard Index of 1 (Environmental Protection Agency, 2023a). The method for determining the Hazard Index is shown below in Appendix F. While the PFAS concentrations found in the effluent and influent sources around campus did not exceed the new EPA proposed standards,

with the PFOS outliers, more testing is needed to determine if the water bottle filler stations are truly within the new legal limits. Furthermore, when repeating this project in the future, one must consider testing the influent source before and after the water treatment facility on campus and between the stages of the campus' water filtration system. Additionally, more than two samples should be collected from each site to enable the researcher to assess which are genuine outliers accurately.

Moreover, samples should be taken directly from the filters of the water fountains and the campus filtration system. Sampling this way may determine how much PFAS, if any, are captured by the campus filtration system. Similarly, this will allow for the quantification of PFAS held within the filters, thereby eliminating any possible lurking variables which may or may not have skewed the results found during this project.

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APPENDIX A: TISSUE DISTRIBUTION

Based on PFAS accumulation in the cadavers, one can surmise that PFBA has a solid propensity to accumulate in the lungs and kidneys. In contrast, PFHxA tends to gather in the brain and liver, and the two most dangerous homologs, PFOA and PFOS, gather primarily in the liver.

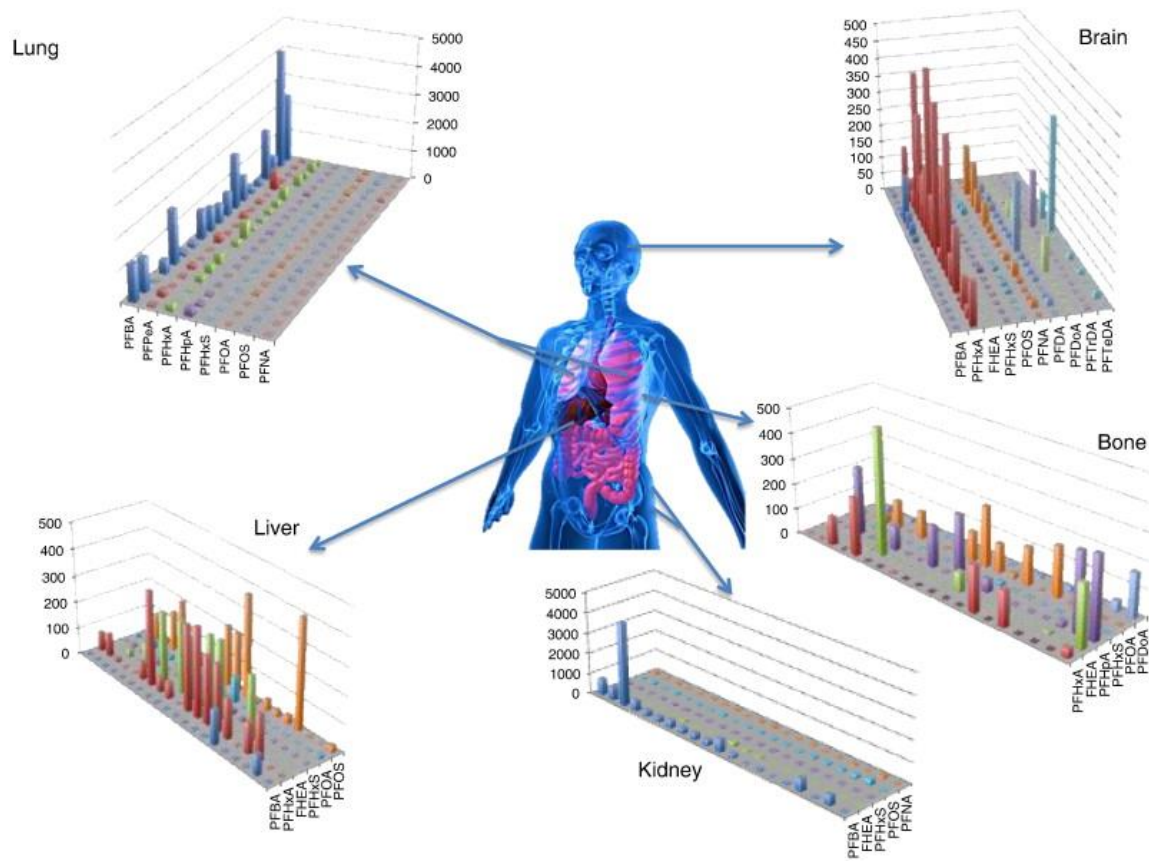


Figure A.1. Concentrations of PFAS homologs (in ng/g) found in various tissue per cadaver from Catalonia, Spain (Mørck et al., 2015)

APPENDIX B: SAMPLE COLLECTION PHASE

Sample collection from various water sources across campus. The one liter polypropylene collection bottles were rinsed with source no less that three times with each rinse being captured and shaken for at least five minutes. After the 15 minutes the source water was collected and frozen for future analysis.



Figure B.1. This is the collection site for the Effluent stream of TAMUG's WWTP. This stream head is comprised mostly of final effluent at low tide and drains directly into Galveston Bay. Water samples collected from this site are therefore used in the analysis



Figure B.2.. This is a example of the EZH2O water bottle filler stations from which the drinking water samples were collected. These were the primary source of collection as the filter lights (as seen in this figure) are inoperable.

APPENDIX C: COLLECTION SITE OVERVIEW



Figure C.1. Google Earth view of the sampling locations showing bay proximity and diverse site collection.

APPENDIX D: SPE, ELUTION, AND CONCENTRATION PHASES

In stage one of PFAS extraction the 1L samples gathered in polypropylene bottles underwent solid phase extraction (SPE). SPE allowed the movement of PFAS from the sample to the filter of the SPE cartridge.

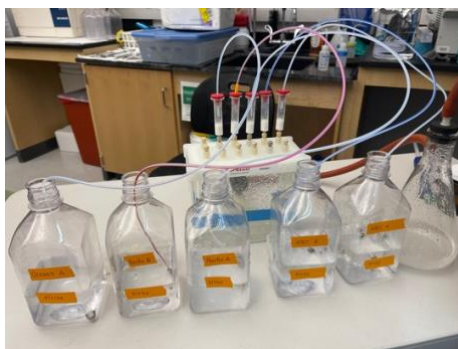


Figure D.1. Samples being extracted from 1L bottles via SPE to catch PFAS in the filters of their respective SPE tube. Weighted lines were placed inside the bottles and an air tight seal was created on the SPE tubes. The SPE had a vacuum line which lead to the 1L erlenmeyer catchment flask which was attached to a vacuum system.

Once the SPE completed the elution process the SPE tubes were treated with methanol to move the PFAS from the filters into catchment tubes which were subjected to the nitrogen drydown process.



Figure D.2. Nitrogen drydown process. Elute tubes containing elute and residual methanol were placed in beakers of water and set upon a heater. To quickly evaporate the methanol, gently blowing nitrogen was introduced.

APPENDIX E: PREPARING LCMS/MS FOR ANALYSIS

Once the five microliter vials were ready they were placed in the LCMS/MS oven for analysis.

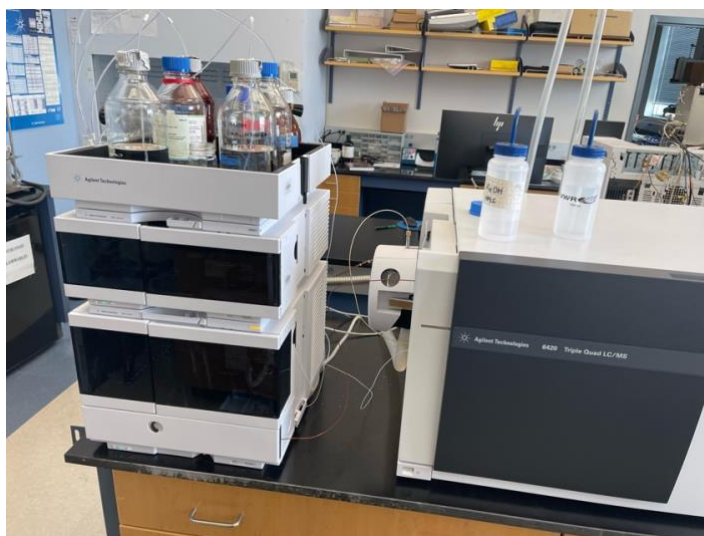


Figure E.1. The LCMS/MS used on this project containing the oven for Liquid Chromatography (left) prior to the fragmented ions and molecules being sent over to the Mass Spectrometer (right) for analysis.

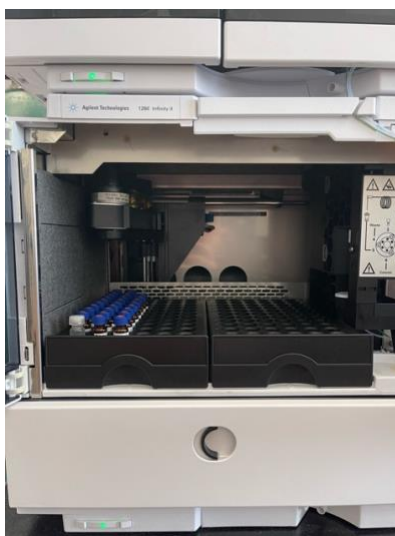


Figure E.2. The 5 μ L (microliter) vials and internal standard placed inside the oven of the LCMS/MS ready for analysis.

APPENDIX F: EPA HAZARD INDEX MEASUREMENT

Under the new EPA proposal several PFAS homologs will be measured using a Hazard Index and anything above one will face legal repercussions. To understand how the EPA will calculate the Hazard Index is provided below.

How do I calculate the Hazard Index?

The Hazard Index (HI) is made up of a sum of fractions. Each fraction compares the level of each PFAS measured in the water to the highest level determined not to have risk of health effects.

Step 1. Divide the measured concentration of Gen X by the health-based value of 10 ppt

Step 2. Divide the measured concentration of PFBS by the health-based value of 2000 ppt

Step 3. Divide the measured concentration of PFNA by the health-based value of 10 ppt

Step 4. Divide the measured concentration of PFHxS by the health-based value of 9 ppt

Step 5. Add the ratios from steps 1, 2, 3 and 4 together

Equation

$$\text{Hazard Index} = \left(\frac{[\text{GenX}_{\text{water}}]}{[10 \text{ ppt}]} \right) + \left(\frac{[\text{PFBS}_{\text{water}}]}{[2000 \text{ ppt}]} \right) + \left(\frac{[\text{PFNA}_{\text{water}}]}{[10 \text{ ppt}]} \right) + \left(\frac{[\text{PFHxS}_{\text{water}}]}{[9.0 \text{ ppt}]} \right)$$

Step 6. To determine HI compliance, repeat steps 1-5 for each sample collected in the past year and calculate the average HI for all the samples taken in the past year.

Step 7. If the running annual average HI greater than 1.0, it is a violation of the proposed HI MCL.

Figure F.1. EPA guidelines for calculating Hazard Index. The Hazard Index will not be used for PFOA or PFOS. It is important to note that the brackets above indicate concentration levels and HFPO-DA (hexafluoropropylene oxide dimer acid) are the GenX chemicals listed above (Environmental Protection Agency, 2023b)