Title
Uptake and Biological Effects of Perfluorooctane Sulfonate Exposure in the Adult Eastern Oyster *Crassostrea virginica*

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Disclaimer
The scientific results and conclusions, as well as any views or opinions expressed herein, are those of the authors and do not necessarily reflect the views of NOAA or the Department of Commerce.

Data availability
Data can be obtained from Allisan Aquilina-Beck on request (allisan.beck@noaa.gov)

Running head
PFOS exposure effects in the adult Eastern oyster

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Title

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Abstract

Perfluorooctane sulfonate (PFOS) is a legacy contaminant that has been detected globally within the environment and throughout numerous species, including humans. Despite an international ban on its use, this unique contaminant continues to persist in organisms and their surroundings due to PFOS's inability to breakdown into nontoxic forms resulting in bioaccumulation. In this study, we analyzed the effects of a technical mixture of PFOS (linear and branched isomers) in the adult Eastern oyster, *Crassostrea virginica,* at two days and seven days exposure. Biomarker analysis (lysosomal destabilization, lipid peroxidation and glutathione assays) in oyster tissue along with chemical analysis (liquid chromatography tandem mass spectrometry) of PFOS in oyster tissue and water samples revealed the oysters' ability to overcome exposures without significant damage to lipid membranes or the glutathione phase II enzyme system; however, significant cellular lysosomal damage was observed. The oysters were able to eliminate up to 96% of PFOS at 0.3 mg/L and 3 mg/L exposures when allowed to depurate for two days in clean seawater. Chemical analysis showed the linear isomer to be the prevailing fraction of the residual PFOS contained in oyster tissue. Results provide insight into possible detrimental cellular effects of PFOS exposure in addition to offering insight into contaminant persistence in oyster tissue.

Keywords

PFOS, Perfluorooctane sulfonate, Eastern oyster, depuration, cellular stress

Introduction

The use of fluorinated organic compounds gained popularity in the 1970's (Kannan et al. 2002; Kissa 2001). Their chemical structure provides thermal stability and resistance to acids, bases, oxidants and reductants making this class of chemical popular for a variety of consumer, industrial and commercial uses (Kissa 2001). Perfluorooctane sulfonate (PFOS), a type of Per- and polyfluoroalkyl substances (PFAS), is an ubiquitous synthetic chemical commonly found in firefighting foams, food packaging, paper products, surface treatments to leather and carpet along with household products, such as, floor polishes and shampoo, to name a few (EPA 2000; Kissa 2001). The United States Environmental Protection Agency (U.S. EPA) guidelines limited the use and production of PFOS and its derivatives in 2000 (EPA 2000). PFOS and PFAS extensive use and manufacturing has caused prevalent environmental pollution in air, soil and water, making PFOS the predominant perfluorinated compound identified in species (Giesy and Kannan 2001; Houde et al. 2011; Jeon et al. 2010b; Martin et al. 2004). The widespread use of PFOS has resulted in contamination identified in remote regions of the Arctic, Atlantic Ocean and Antarctic via ocean currents and atmospheric deposition (Zhao et al. 2012). In 2009 PFOS was listed as a persistent organic pollutant by the United Nations Stockholm Convention (Convention 2009). This classification is named to those chemicals that are "toxic to both humans and wildlife, can persist over long periods in the environment, accumulate in organisms, and can be transported over long distances by air or water" (Convention 2019). These legacy compounds persist in the environment for years due to their strong structural bonds. Perfluoroalkanes and perfluoroalkyl acids (PFAA) -which includes PFOA and PFOS- are organic compounds that contain strong fluorine-carbon bonds instead of the weaker hydrogen-carbon bond (Buck et al 2011). These compounds exist as both branched and linear isomers. The branched isomers are eliminated faster than the linear; however, both are found in the environment, humans and wildlife (Lau 2015; Beskin et al., 2009a; Houde et al. 2008).

The Eastern oyster, *Crassostrea virginica*, is a stationary organism that actively filter feeds to obtain food. When a contaminant, such as PFOS, is present in either the water or through contaminated particulate matter, the oyster can actively bioaccumulate PFOS in its tissues (Jeon et al. 2010b). Bivalves, have long been sentinel organisms for monitoring how chemicals in the aquatic environment are processed within an organism, as well as providing insight into the health of the estuarine environment (Kannan et al. 2002; O'Connor 1996). Since estuaries provide critical habitat for spawning, feeding and protection for a diversity of nascent and juvenile organisms, contamination from air and water pollution could have negative effects on many estuarine species' survival. The oyster, in addition to being a valuable model organism for research, also provides ecosystem services such as water filtration and shoreline stabilization, and furthermore serves as a habitat and food source for other estuarine organisms. The oyster is a significant commercial and recreational fishery, valued at $213.8 million in 2015 (NMFS

2016). Therefore, research focusing on legacy contaminant exposure in oysters has potential for impacts environmentally and economically.

Examination of external stressors such as chemical exposure in invertebrates are commonly employed through bioassays: lysosomal destabilization, lipid peroxidation and glutathione- a phase II detoxification system (DeLorenzo et al. 2006a; Hoguet and Key 2007; Liu et al. 2016; McCarthy et al. 2013). These assays provide health assessment of oxidative stress of an individual and a population. Tools such as these and can identify sublethal stress levels prior to widespread harmful effects on a population (McCarthy et al. 2013; Ringwood et al. 2003). These same assays have been utilized when assessing cellular damage from PFOS exposure (Liu et al. 2007). Lysosomes are cytoplasmic organelles that serve in cellular repair, immune response, and act to contain and concentrate a range of inorganic and organic chemicals (Hwang et al. 2002; Moore 1985; Ringwood et al. 1998). The lysosomal destabilization procedure involves the use of live cells and histological techniques to give an indication of the health of a cell (Khan et al. 2019; Ringwood et al. 1998). External stressors on an organism, such as exposure to contaminants, may lead to accumulation of reactive oxygen species (ROS) in cells, thus overwhelming the cellular system and ultimately causing lipid membrane damage and apoptosis. (Hunter and Moghimi 2010; Hwang et al. 2002; Khan et al. 2019; Ringwood et al. 2003). Studies using cultured hepatocytes of tilapia and the aquatic organism, planarian, have shown an increase in cell apoptosis, oxidative stress and developmental toxicity when exposed to PFOS (Liu et al. 2007; Yuan et al. 2014).

The lipid peroxidation assay is an indicator of cellular membrane damage which measures malondialdehyde (MDA), an oxidized product released from a cell when it is unable to protect against ROS-induced damage (Khan et al. 2019). In healthy cells, regulation of ROS is controlled by antioxidants such as glutathione, catalase, superoxide dismutase and glutathione peroxidase (Khan et al. 2019). Glutathione (GSH) is the most abundant low molecular weight thiol in animal cells (Wu et al. 2004). It is a tripeptide antioxidant, which in the presence of ROS, neutralizes it to form the disulfide GSSG, which is then reduced to GSH again by NADPH-dependent glutathione reductase (Wu et al. 2004). This phase II enzyme system indicates whether or not detoxification is occurring in a cell.

In this current study, we exposed the Eastern oyster, *Crassostrea virginica*, to a range of PFOS concentrations and examined the extent of oxidative stress as well as contaminant uptake and depuration during two exposure durations. Results of this study provide insight into possible detrimental cellular effects of acute PFOS exposure as well as duration of chemical persistence in oyster tissue. This information could provide insight into proper depuration rates after PFOS exposure, in addition to improving current understanding of the impacts of PFOS exposure on this important estuarine species.

Materials and Methods

Oyster Collection

A total of 94 adult oysters (70-100 mm in length) were collected in the fall and winter seasons from a reference site commonly used as a control site at the mouth of Leadenwah Creek at North Edisto River on Wadmalaw Island, South Carolina (N 32° 37' 03.9"; W 80° 13' 44.6") (DeLorenzo and De Leon 2010; DeLorenzo et al. 2018; DeLorenzo et al. 2006b). Seawater for testing was collected from Charleston Harbor estuary (N 32° 45ʹ11.52"; W 79° 53ʹ58.31"), filtered (5 µm), UV-sterilized, activated carbon filtered (5 µm), and diluted with deionized water to 20 ppt salinity. The oysters were scrubbed with 20 ppt seawater to remove algae and barnacles then placed in a controlled laboratory aquatic recirculating system to acclimate for 14 d at 25 °C, 20 ppt salinity, and 16-h light: 8-h dark cycle (16L: 8D). Oysters were fed 10 mL of Shellfish Diet® (Reed Mariculture; Campbell, CA) daily until day of exposure. Oyster length and width were measured and recorded before exposures.

Static Non-renewal PFOS 48 Hour Exposure

PFOS (Santa Cruz Biotechnology, CAS 1763-23-1, purity ≥97%) stock solution (10,000 mg/L) was prepared in deionized water. Each adult oyster was exposed to 1 L of PFOS treatment in 20 ppt seawater (0 mg/L, 3 mg/L, 30 mg/L, 300 mg/L) in a glass beaker. Beakers were covered with a clear plastic lid with a hole drilled in it for oxygen exchange and evaporation reduction. After individual oysters were carefully added to the corresponding treatment beaker they were immediately placed into an environmental chamber (Percival Scientific) at 25 °C and a 16L: 8D light cycle and kept statically for 48 h. PFOS concentrations were selected to bracket the effects range reported for aquatic invertebrates, including *C. virginica* (Beach et al. 2006).

After 48 h of exposure, oysters were shucked and whole tissue wet weight (w.w.) was recorded. The hepatopancreas (HP) was dissected, weighed, divided into three sections: two of which were frozen in liquid nitrogen for downstream biomarker analysis and the third section was processed immediately for lysosomal destabilization. Water quality parameters were recorded at the beginning and end of the treatment and were within acceptable test conditions (mean values± standard deviation: Temp °C: 24.9 ± 0.4 °C, D.O. mg/L: 4.3 ± 2.2 mg/L, Salinity ‰: 20 ± 0.2 ppt, and pH 7.5 ± 0.3). PFOS 48 h exposure experiments and biomarker analyses were repeated three times with four or six replicates per treatment group, twice in the fall and once in the winter. A combined total of 16 oysters per treatment group were tested. An additional 2 oysters collected in fall and 4 collected in winter, not subjected to treatment (Baseline) were dissected and analyzed for comparison to controls in bioassay tests.

Static Renewal PFOS Seven Day Exposure

Twenty-four oysters collected in the fall were kept statically for seven days in individual glass beakers containing 1 L volume of PFOS concentrations (0 mg/L, 0.3 mg/L and 3 mg/L). Concentrations for the biouptake exposure were selected to be sublethal to the oysters and at the upper range of environmentally plausible concentrations (Moody et al. 2002).

Eight replicates were made for each treatment and control group. Beakers were covered with a clear plastic lid with a hole drilled in it for oxygen exchange. Each exposure concentration and control seawater was replaced daily for seven days. Mortality was assessed daily and water quality measurements were recorded. Water quality parameters for all treatments during exposures were within acceptable test conditions (mean values± standard deviation: Temp °C: 25.3 ± 0.5; D.O. mg/L: 5.1 ± 1.4; Salinity ‰: 20.0 ± 0.4 and pH: 7.7 ± 0.3). After seven days of exposure, half of the oysters from each treatment group were shucked and whole tissue was flash frozen in liquid nitrogen. The remaining four oysters were placed into clean 20 ppt seawater and allowed to depurate for 48 h. At the end of 48 h depuration, oysters were shucked, flash frozen in liquid nitrogen and stored at -80 °C. All 24 oysters were processed for chemical analysis. Additionally, 1 mL water samples were taken for PFOS analysis from each treatment group at day two of the seven day exposure and 24 h later (day 3), in order to represent a one day dose.

Chemical Analysis

Chemicals

Calibration solutions (0.001 ng/g to 1000 ng/g) were created by using the NIST Reference Material RM 844.

Perfluorinated Sulfonic Acids in Methanol. A $^{13}C_4$-PFOS internal standard (IS) was purchased from Wellington Laboratories (Guelph, Ontario, purity >99%). A technical PFOS mixture containing both linear and branched isomers was purchased from Santa Cruz Biotechnology (CAS 1763-23-1, purity ≥97%). A technical PFOS stock solution (10,000 mg/L in deionized water) was used to spike exposure experiments.

Sample Preparation for Chemical Analysis

Oyster tissue samples were analyzed for PFOS concentrations using methods previously described (Reiner et al. 2012). Briefly, samples were removed from storage and allowed to thaw before all edges were trimmed using a sterilized, methanol rinsed scalpel. Although there has been no indication of PFOS in the storage containers with the oyster tissue samples, trimming the edges ensures any tissue that may have come in contact with the storage container is not extracted. Whole oyster samples were homogenized and internal standard was added to approximately 1 g (mass known) of sample. The samples were extracted using ultrasonication with 0.01 mol/L potassium hydroxide (KOH) in methanol twice and the supernatants were combine and further cleaned using graphitized carbon. Water samples were diluted with deionized water (mass known) and internal standard was added to an aliquot of the diluted sample.

Quality assurance and control methods included blanks and Standard Reference Material (SRM) 1947 Lake Michigan Fish Tissue that were extracted alongside samples. The concentrations of PFOS in SRM 1947 agreed with values reported on the Certificate of Analysis (5.90 ng/g ± 0.39 ng/g).

PFOS Quantification

PFOS was quantified using an established liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Reporting limits (RL) were determined as the lowest calibrant detected, all divided by the mass (in grams) of extracted sample. The method detection limit for the oyster tissues was 0.10 ng/g and 0.001 ng/g for the water samples (Reiner et al. 2012).

Bioaccumulation Factor Calculation

Bioaccumulation factor (BAF) was calculated as the measured average PFOS concentration in oyster tissue (ppb (w.w.)) after seven days exposure, divided by the measured average PFOS concentration in the water (ppb). The oysters were assumed to be in a steady state with the water.

Percent PFOS Elimination Calculation

Percent PFOS elimination from oyster tissue was calculated for the seven day exposure experiment. Average total PFOS concentration in oyster tissue (ppb) for each treatment group was subtracted from the average total PFOS concentration in oyster tissue (ppb) after 48 h depuration. This difference was divided by the average total PFOS concentration in oyster tissue (ppb) for each treatment group and multiplied by 100.

Biomarker Analysis

Lysosomal Destabilization Assay

Oysters were subjected to one of four different treatments: control (0 mg/L), 3 mg/L, 30 mg/L or 300 mg/L, for 48 h. The method was followed as reported in Ringwood et al. (2003). Briefly, oyster HP was dissected out and placed in fresh cold calcium and magnesium free saline (CMFS) (salinity 29 ppt, pH 7.36) where it was agitated for 20 min at 100 rpm on a reciprocating shaker to dissociate the cells. Trypsin, at 0.5 mg/mL concentration (Gibco 25300-054), was added and cells were shaken for an additional 20 min. Samples were transferred to a 1 mL pipet tip containing a 23 micron filter nested in a 1.5 mL microcentrifuge tube and centrifuged at 200 x g for 5 min. Cells were re-suspended in 300 µL CMFS and 300 µL neutral red dye (0.04 mg/mL) was added to filtrate. Cells were incubated at 22 °C in the dark for 1 h. Fifty microliters of cells were pipetted onto a microscope slide and at least 50 individual cells were counted per treatment and visually sorted for dye present in the lysosome (stable) or cytosol (destabilized) at 40x magnification, viewed using a Swift M10T-BTW1-MP Digital Phase Contrast microscope. The percentage of destabilized lysosomes was then calculated.

Lipid Peroxidation Assay

The Lipid Peroxidation (LPx) assay uses a spectrophotometric thiobarbituric acid detection at 532 nm to quantify the amount of (MDA) in a sample. The methods reported in Ringwood et al. (2003) were modified for 96 well plate reading. Oyster HP were frozen in liquid nitrogen and stored at -80 °C. Tissue samples were kept frozen and homogenized at a concentration of 250 mg tissue/mL buffer in cold 50 mM potassium phosphate (K_2PO_4) buffer at pH 7.0. Homogenization was conducted on ice using a Pro Scientific model Pro 200 motor with a 20 mm x 150 mm stainless steel rod for a minimum of 30 sec. Homogenates were centrifuged at 4 °C for 5 min at 13,000 x g. A 10 mM stock solution of MDA was previously heated for 1 h at 50 °C and allowed to cool to room temperature before generating standards. A secondary solution of 3200 µM MDA was used to prepare oyster serial dilutions from 800 µM to 25 µM using K_2PO_4 buffer. Tubes were prepared using 100 µL of either MDA dilution, sample homogenate or only K_2PO_4 buffer (as a blank). The addition of 1400 µL 0.375% thiobarbituric acid/trichloroacetic acid and 14 µL of 2% butylated hydroxytoluene was added to each tube and tubes were heated to 92 °C for 15 min. The tubes were centrifuged at room temp for 5 min at 13,000 x g. Aliquots of 300 µL supernatant were plated in triplicate into a clear Corning 96 well plate and read using a µQuant microplate Spectrophotometer (Bio-Tek Instruments Inc.) in conjunction with KC junior software (Bio-Tek Instruments.). Absorbance readings for each sample and serial dilution were adjusted by subtracting from the blank value and the slope of the standard line was used to determine the amount of MDA in nmol/g (w.w.).

Glutathione Assay

The glutathione assay (GSH) methods followed Ringwood et al. (2003), 5, 5'-dithiobis (2-nitrobenzoic) acid-glutathione (DTNB-GSSG) recycling protocol. Previously frozen HP tissues were weighed and homogenized in 10 times the volume of the weight of the tissue in cold 5% sulfosalicylic acid (SSA). Homogenates were centrifuged at 4 °C for 5 min at 13,000 x g and 100 µL of supernatant was combined with 100 µL 5% SSA. L-glutathione

reduced standards were prepared using 5% SSA at serial dilutions of 200 µM, 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM. A 25 µL aliquot of either sample, standard or 5% SSA (blank) was combined with 0.208 mM NADPH, 10 mM DTNB and deionized water to 1 ml total volume and vortexed. Cuvettes were prepared with 900 µL of the above mixture in addition to adding 50 U/L GSSG reductase enzyme before reading continuously at a wavelength of 405 nm for 90 seconds with 15 second intervals. A spectrophotometer UltroSpec 5300 pro (Amersham Biosciences) with Swift II Reaction Kinetics software (Biochrom Ltd.) was used for obtaining measurements. GSH data were expressed as nmol/g (w.w.).

Statistical Analysis

JMP12 software (Statistical Discovery v.12) was used to run Analysis of Variance (ANOVA) on biomarker assays. A p-value of ≤ 0.05 was used to determine significance. Tukey-Kramer along with Dunnett's tests were used to identify differences among treatment groups.

Results

48 h PFOS Exposure

Live cells from *C. virginica* were examined for lysosomal integrity. Cells were classified as destabilized if they were unable to retain cellular content, rendering them 'leaking' and thus damaged. Control groups in each repeat experiment were consistently statistically different from PFOS exposed groups by containing a lower percentage of destabilized cells (Figure 1). All PFOS exposed oysters had calculated cellular membrane damage over 60%.

MDA, a measured end product of cellular membrane damage, was used in lipid peroxidation assays to evaluate cellular stress. No significant difference was detected between untreated, control and PFOS exposed groups (Figure 2). Mean observed levels of MDA, for control and all PFOS exposures, were near or below the normal reported level of <150 nmol/g (Ringwood et al. 2002) including a group of 6 oysters (Baseline) not subjected to experimental treatment.

Glutathione was analyzed for all treatment groups including a subset of 6 oysters not subjected to experimental conditions. There was no statistical significant difference among treatment groups and Baseline controls (Figure 3a). However, although the majority of oysters fell within the normal acceptable range of 800-1600 nmol/g (w.w.) (Ringwood et al. (2002)), there were a higher percentage of oysters below the 800 nmol/g (w.w.) in the 3 mg/L and 30 mg/L exposure groups (Figure 3b). Thus, these oysters were considered to be in a 'concerned' state due to decreased levels of GSH.

Seven Day PFOS Exposure

A longer exposure with lower concentrations of PFOS was used to challenge oysters in order to examine PFOS uptake and depuration. Chromatogram analysis of PFOS water concentrations before exposure can be found in supplemental data (Online Resources 1-3). PFOS concentrations were quantified in oyster tissue samples after seven days of exposure and 48 h of depuration. Data revealed the presence of both linear and branched PFOS isomers in oyster tissue after seven days of exposure (Figure 4a). However, following two days of depuration in clean seawater, the linear isomer still remained at high levels whereas the branched isomer had been almost completely eliminated from the tissue (Figure 4b, Table 1). Though, the process of depuration did eliminate a 96% of PFOS from the oyster tissue, concentrations remained over 10 ppm (w.w.) reported in the 3 mg/L group (Table 1). The BAF calculated after the seven day PFOS exposure revealed oysters had incorporated 50 times and 116 times the level of PFOS in the 0.3 mg/L and 3 mg/L aqueous exposures, respectively.

Discussion

C. virginica were subjected to various concentrations of PFOS in seawater. After a 48 h exposure, experimentally tested oysters had significant damage to lysosomal integrity. According to Ringwood et al. (2009), the normal range of lysosomal destabilization occurs in up to 30% of the cells observed. Some individual control oysters displayed a higher percentage of damaged cells. Seasonal effects could be a possible explanation as reported by (Ringwood et al. 2002), however, more experiments are needed to sufficiently conclude seasonality as a factor. A significantly higher percentage of damaged cells were observed in treatment groups regardless of PFOS concentration, which demonstrates the extent of damage cells endure in short exposure times.

MDA analyses indicated that the oysters were able to withstand PFOS exposure up to 300 mg/L without significant harm to their cellular lipid bilayer. Similarly, lipid peroxidation studies with the aquatic invertebrate *Limnodrilus hoffmeisteri*, showed significant differences in MDA levels 24 h after exposure, but no significant difference between treatment groups by 10 days of PFOS exposure (Liu et al. 2016). This indicates that while an immediate response in MDA levels can be observed, some marine organisms can stabilize MDA levels shortly after oxidative stress from PFOS exposure.

The observed trend in decreasing glutathione levels among the 3 mg/L and 30 mg/L treatment groups suggest that the oysters were actively using this phase II enzyme system to metabolize PFOS and protect cellular membranes against oxyradicals. While an increase of glutathione would indicate that the cells are responding to the stressor and actively detoxifying, a decrease indicates the levels are depressed in the cellular system. Once glutathione becomes depleted, the oyster would be vulnerable to cellular oxidative damage (Conners and Ringwood 2000; McCarthy et al. 2013). Similar studies using invertebrates have also shown that oxidative stress, due to PFOS exposure, leads to depletion of GSH (Ding et al. 2015; Liu et al. 2016). As observed in *C. virginica*'s response to 3 mg/L and 30 mg/L dose, *L. hoffmeisteri* showed GSH levels decrease with increasing PFOS concentration (Liu et al. 2016). Though GSH concentration measured at normal levels in the 300 mg/L group in our study, it is possible that the oyster was not actively filtering from the highly toxic environment or that it had actively detoxified the contaminant before harvest rendering GSH levels back to normal. It would be valuable to obtain data 24 h after exposure to observe any acute response. Additionally, it would be interesting to consider the antioxidant defense ability for oysters after a longer PFOS exposure in order to evaluate if damage caused by PFOS exposure in early developmental oyster stages is detrimental to later growth and development.

In order to study PFOS uptake into tissues and effectiveness of oyster depuration, a longer exposure was conducted. Analysis of PFOS in exposure water and oyster tissue revealed the presence of a technical mixture of PFOS isomers, the majority being comprised of linear PFOS at day seven post dose and the minority, containing mono- and di-substituted PFOS. Literature reports the branched isomer is commonly eliminated faster than the linear isomer (Houde et al. 2008; Lau 2015). After the 48 h depuration, a substantial amount (96%) of total PFOS was eliminated from the oyster tissue. Oysters in the present study that actively filtered uncontaminated seawater for two days following exposure to nominal concentrations of PFOS, retained more linear isomer in their tissues. Two days of filtration in clean seawater was insufficient for complete detoxification at the levels of exposure used in this study, thus indicating, that longer depuration times are needed to further reduce PFOS levels in the tissue. Dosing concentrations used in this study are representative of a localized accidental environmental release, such as the one that occurred in Toronto in 2000. The release of aqueous film-forming foam firefighting agents (AFFF), which was composed primarily of PFOS, was estimated to measure 22,000 L (Awad et al. 2011). PFOS surface water concentrations measured shortly after the spill ranged from non-detectable to 2210 µg/L (or 2.21 mg/L) (Moody et al. 2002).

A study testing depuration rates on the Pacific oyster, *Crassostrea gigas*, which included a feeding component and differing salinities but considerably lower experimental concentrations of PFOS (10 µg/L), found depurations rates of 28 days were insufficient to completely rid the tissue of PFOS contamination (Jeon et al. 2010b). Bioaccumulation of PFOS in oysters can be magnified through factors such as: ingestion of food, changing salinity concentration, and contaminated sediment (Jeon et al. 2010a; Jeon et al. 2010b; Young et al. 2013). In this current study, oysters were assumed to be in a steady state with the water; they were not fed during experimental trials nor was there any growth due to the short duration of the study. The calculated BAF at PFOS concentrations of 0.3 mg/L and 3 mg/L in the oyster was 50 and 116 times the level of PFOS in the exposure water, respectively. A similar BAF of 127 was calculated for *C. gigas* exposed to 10 ppb PFOS for 28 d (Jeon et al. 2010b). This range of biouptake is similar to that reported for Northern leopard frogs, with bioconcentration factors approximately 30 to 200 (Ankley et al. 2004). As summarized by (Beach et al. 2006), laboratory-derived bioconcentration factors for fish, however, ranged widely depending on species and exposure condition (approximately 500 – 4500). Invertebrate studies that examined PFOS in the field report higher BAF estimates. For example, in zooplankton, *Mysis* (shrimp-like crustacean), and *Diporeia* (amphipod), BAF were estimated as 240, 1200 and 13,000, respectively (Houde et al. 2008). Similarly, oysters and snails sampled in Korean waters were observed to have a mean BAF of 316 and 501, respectively (Hong et al. 2015). Field estimated BAF levels may be higher due to variable environmental conditions, such as food, salinity, substrate and temperature.

After 48 hours in clean seawater, approximately 96% of the total PFOS had been eliminated, indicating the quick and efficient filtering out of PFOS; however, 4% of the contaminant remained in the tissue after depuration. This equates to 0.517 to 10.912 ppm (w.w.) of total PFOS in the oyster tissue, with respect to exposures of either 0.3

mg/L or 3 mg/L. The 3 mg/L exposure resulted in higher PFOS concentrations in tissue than those reported for 'Baseline' levels found in field-collected *C. virginica* (<48- 1,225 ppb (d.w)) (Kannan et al. 2002). Similar to our findings, (Jeon et al. 2010b) reported rapid depuration of PFOS in *C. gigas*, however, residual PFOS still remained in the tissues after a 28 day depuration.

The stability of this contaminant makes it difficult to be completely removed from the body, whether considering invertebrates or higher organisms. Implications of its persistence has been discovered crossing the blood brain barrier along with presence in cord blood and mothers' milk in mammals (Viberg 2015). Additionally in humans, there is more exposure to PFOS apart from ingestion of contaminated meat, fish and shellfish; occupational exposure, indoor dust, household products, and geographical location all contribute to the bioaccumulation of PFOS and its derivatives within the body (DeWitt 2015; EPA 2000; Key et al. 1997). Elimination rates vary greatly from species to species, and is also influenced by sex and age of the animal. The half-life of PFOS in humans is 5.4 years (Lau 2015). Consistent with analysis of oyster tissue in our study, human blood samples analyzed for PFOS, revealed the linear isomer to be the predominant one remaining (Butenhoff 2015; Houde et al. 2011; Lau 2015). In the marine realm, PFOS contamination has been characterized in several taxa of aquatic organisms, including marine mammals. Bottlenose dolphins sampled in the Charleston Harbor, SC estuary, had blood plasma PFOS levels ranging from 316.7–6260 ng/g (w.w.) (Fair et al. 2012). A study in the same estuary measured PFAS in fish tissues at concentrations ranging from 6.4 to 15.5 ng/g (w.w.) (Fair et al. 2019).

This study has shown *C. virginica*'s ability to withstand acute, high concentrations of PFOS exposure. However, PFOS retention remains in tissues after 48 hours of depuration. Though the widespread use of PFOS has been banned, the ongoing presence in the environment and within species still remains. As new chemicals begin to be manufactured as PFOS replacements, careful studies into the fate and biological effects of the novel compounds should be conducted to protect both environmental and human health.

Figure Captions

Fig 1. Percentage of Lysosomal Destabilization of *C. virginica* Exposed to PFOS
Adult *C. virginica* were exposed to different concentrations of PFOS for 48 h after which, lysosomal cells were analyzed for cellular integrity. Asterisks indicate treatments that were significantly different from control (one way ANOVA followed by Dunnett's test). Control 0 mg/L n=15, 3 mg/L n=16, 30mg/L n=15, 300mg/L n=12. Bars represent mean ± standard error.

Fig 2. Lipid peroxidation in Adult *C. virginica* 48 h PFOS exposure at different concentrations
Lipid peroxidation was assessed in hepatopancreas tissue and recorded as MDA nmol/g tissue wet weight. One way ANOVA analysis determined no significant difference between groups. The line represents a normal range of MDA at ≤150 nmol/g. Six wild oysters (Baseline) not subjected to any treatment were examined as a comparison to controls. Control 0 mg/L n=10, 3 mg/L n=9, 30 mg/L n= 10, 300 mg/L n=10. Bars represent mean ± standard error.

Fig 3. Glutathione activity in Adult *C. virginica* 48 h PFOS exposure at different concentrations (A) Glutathione was assessed in hepatopancreas tissue and recorded as GSH nmol/g tissue wet weight. One way ANOVA analysis determined no significant difference between groups. Most oysters fall within in acceptable normal ranges of 800-1600 nmol/g. Baseline oysters were not subjected to treatment conditions. Bars represent mean ± standard error. (B) Oysters that fall within the 'concerned' range of <800 nmol/g expressed as a percent of total number exposed. GSH values are three to four times higher in the 3 mg/L and 30 mg/L treatment groups compared to controls. Baseline n=6, Control 0 mg/L n=16, 3 mg/L n=16, 30 mg/L n= 16, 300 mg/L n=15. One oyster in 300 mg/L group died during experiment.

Fig 4. Extracted ion chromatogram of PFOS (transition 499 > 80) in oyster (*C. virginica*) tissue samples. (A) Tissue sample after seven day exposure to 0.3 mg/L PFOS. (B) Tissue sample after 48 hours depuration in clean seawater. Graphs are of a single oyster representative of their respective groups. L-PFOS is the linear isomer and both the monosubstituted and disubstituted make up the branched isomers.

Table 1. Mean (± standard error) measured PFOS concentration in exposure water (ppm) and whole oyster tissue (ppb) (w.w.) for the seven day exposure. Measured mean water PFOS concentrations were sampled after 24 h of exposure during the seven day uptake experiment (8 per treatment group). Measured oyster tissue total, linear and branched fractions of PFOS sampled after seven days of exposure (4 per treatment group) and after 48 h depuration: control (n=3, one control oyster died at the end of day seven) and exposed (4 per treatment group). PFOS percent elimination was calculated and reported from oyster tissue after depuration. Mean BAF calculated for oyster tissue after seven day exposure.

Online Resource 1-3.
Extracted ion chromatogram of PFOS (transition 499 > 80) in water samples 24 hours post dose (1) control water, (2) 0.3 ppm dosed water, and (3) 3.0 ppm dosed water.

Figures/Legends –Made in Excel

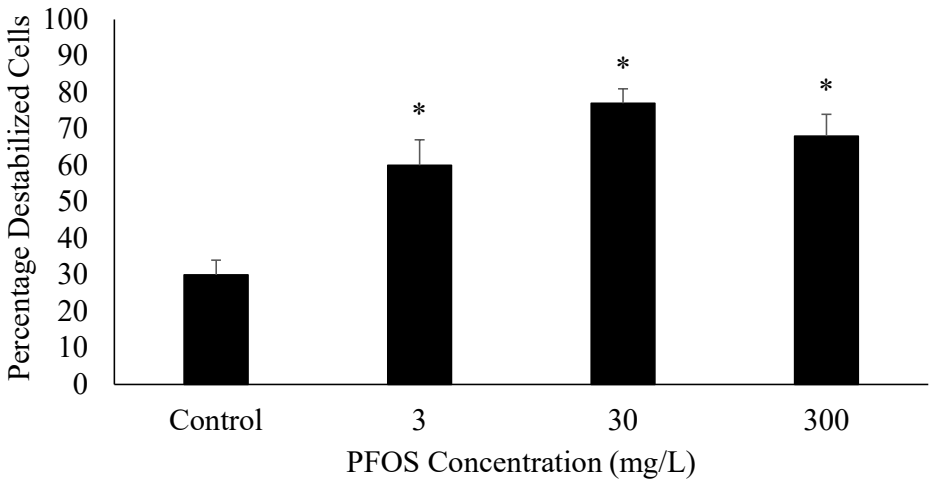


Fig1

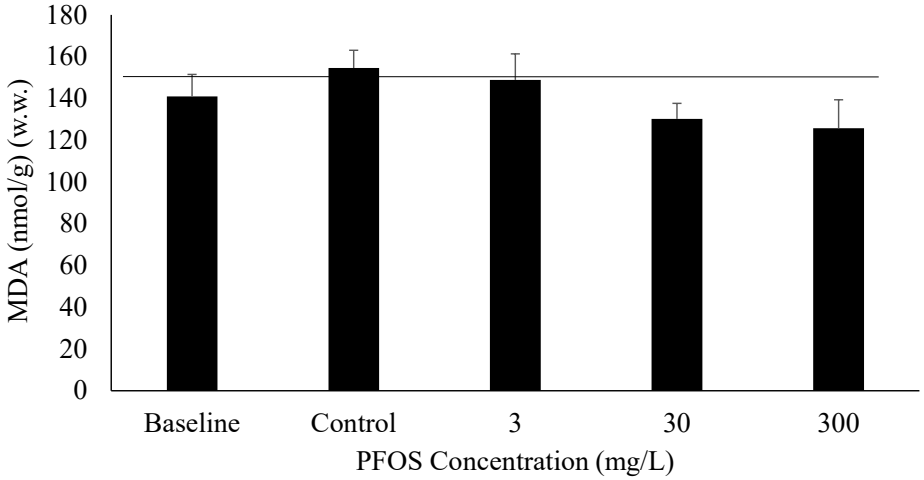


Fig2

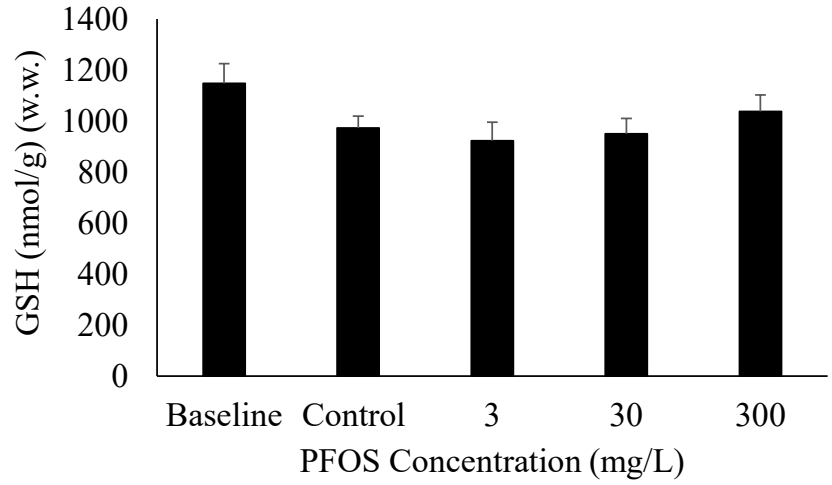

1400
1200
1000
800
600
400
200
0
GSH (nmol/g) (w.w.)
Baseline
Control
3
30
300
PFOS Concentration (mg/L)

Fig3a

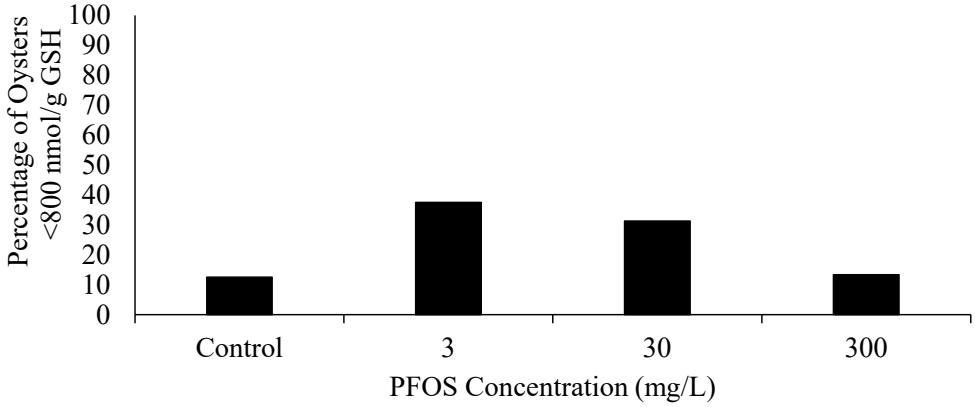

100
90
80
70
60
50
40
30
20
10
0
Percentage of Oysters <800 nmol/g GSH
Control
3
30
300
PFOS Concentration (mg/L)

Fig3b

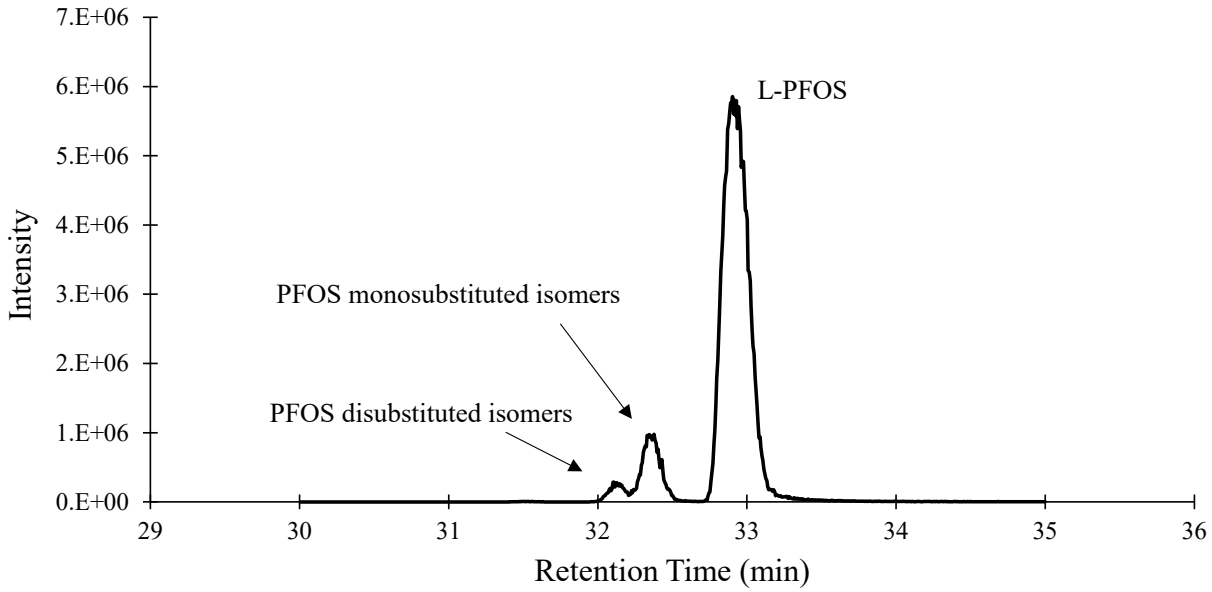

7.E+06
6.E+06
5.E+06
4.E+06
3.E+06
2.E+06
1.E+06
0.E+00
Intensity
L-PFOS
PFOS monosubstituted isomers
PFOS disubstituted isomers
29
30
31
32
33
34
35
36
Retention Time (min)

Fig4a

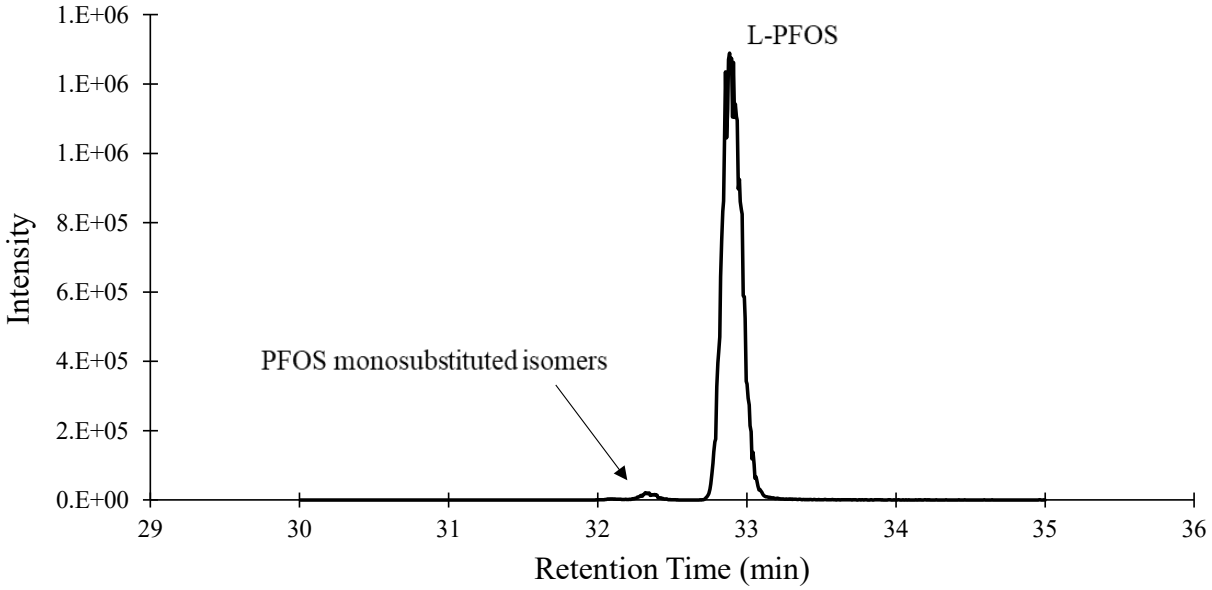
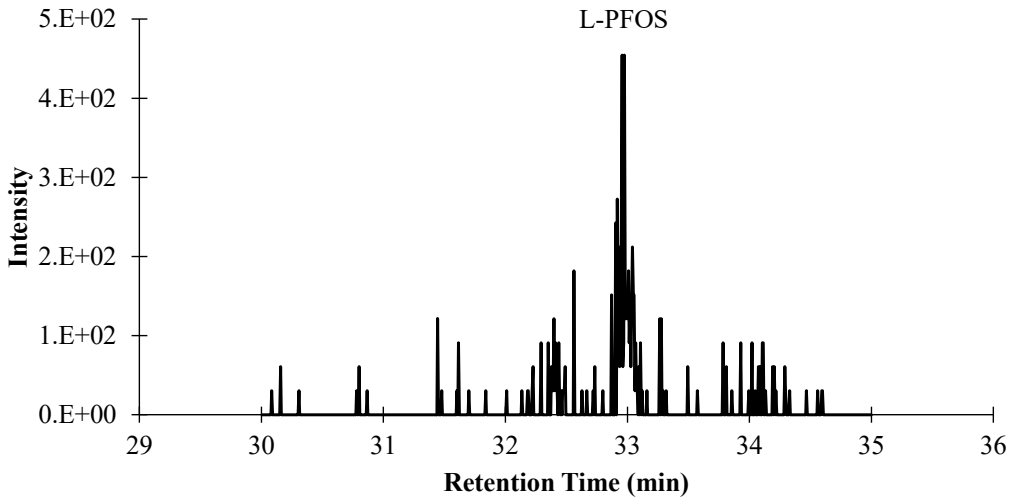

1.E+06
1.E+06
1.E+06
8.E+05
6.E+05
4.E+05
2.E+05
0.E+00
Intensity
L-PFOS
PFOS monosubstituted isomers
29
30
31
32
33
34
35
36
Retention Time (min)

Fig4b

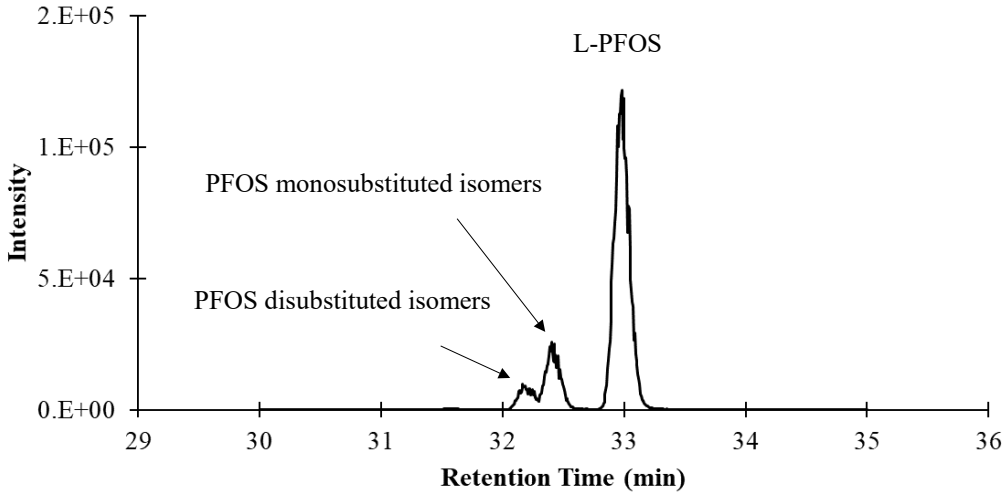
PFOS Fraction	Nominal Water PFOS Concentration (ppm)	Measured Water PFOS Concentration (ppm) Immediately After Dosing	Measured Water PFOS Concentration (ppm) After 24 h of Exposure	Measured Tissue PFOS Concentration (ppm) After Seven Day Exposure	Measured Tissue PFOS Concentration (ppm) After 48 h Depuration	Percent Elimination of PFOS from Oyster Tissue from Day 7 to Day 9	BAF
Total PFOS Linear PFOS Branched PFOS	0	0.00019 ± 0.00006	0.00018 ± 0.00003	0.0104 ± 0.003 0.0099 ± 0.0029 0.0004 ± 0.0001	0.0044 ± 0.0008 0.0042 ± 0.0008 0.0002 ± 0.0000		
Total PFOS Linear PFOS Branched PFOS	0.3	0.258 ± 0.015	0.246 ± 0.021	12.821 ± 2.051 10.939 ± 1.706 1.882 ± 0.353	0.517 ± 0.066 0.510 ± 0.066 0.006 ± 0.001	-96% -95% -100%	50
Total PFOS Linear PFOS Branched PFOS	3	0.2165 ± 0.342	1.908 ± 0.091	252.152 ± 52.259 174.909 ± 36.549 77.242 ± 15.935	10.912 ± 4.776 10.398 ± 4.533 0.522 ± 0.244	-96% -94% -99%	116

Table1

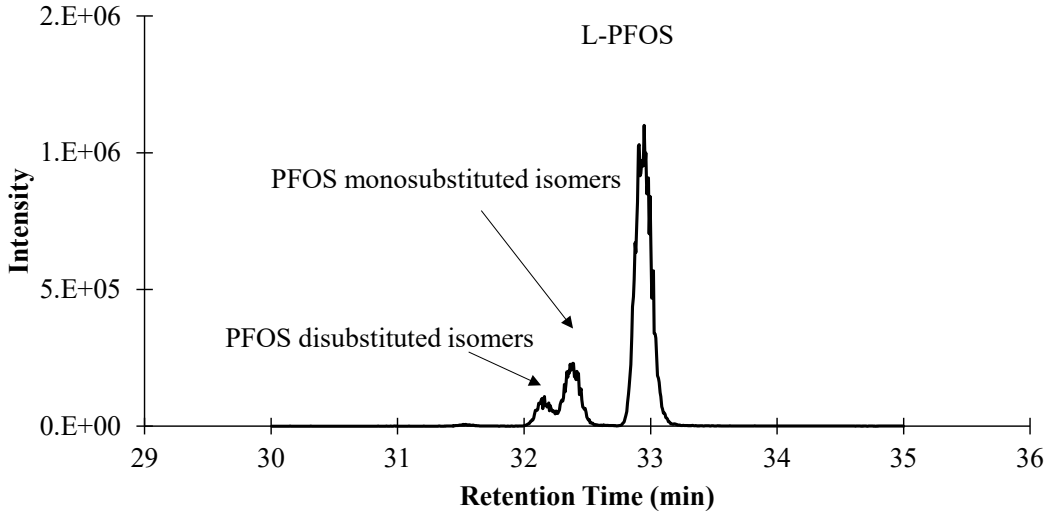
Online Resource: 1-3



1


2.E+05
1.E+05
5.E+04
0.E+00
Intensity
L-PFOS
PFOS monosubstituted isomers
PFOS disubstituted isomers
29
30
31
32
33
34
35
36
Retention Time (min)

2


2.E+06
1.E+06
5.E+05
0.E+00
Intensity
L-PFOS
PFOS monosubstituted isomers
PFOS disubstituted isomers
29
30
31
32
33
34
35
36
Retention Time (min)

3

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