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– a dialysis study

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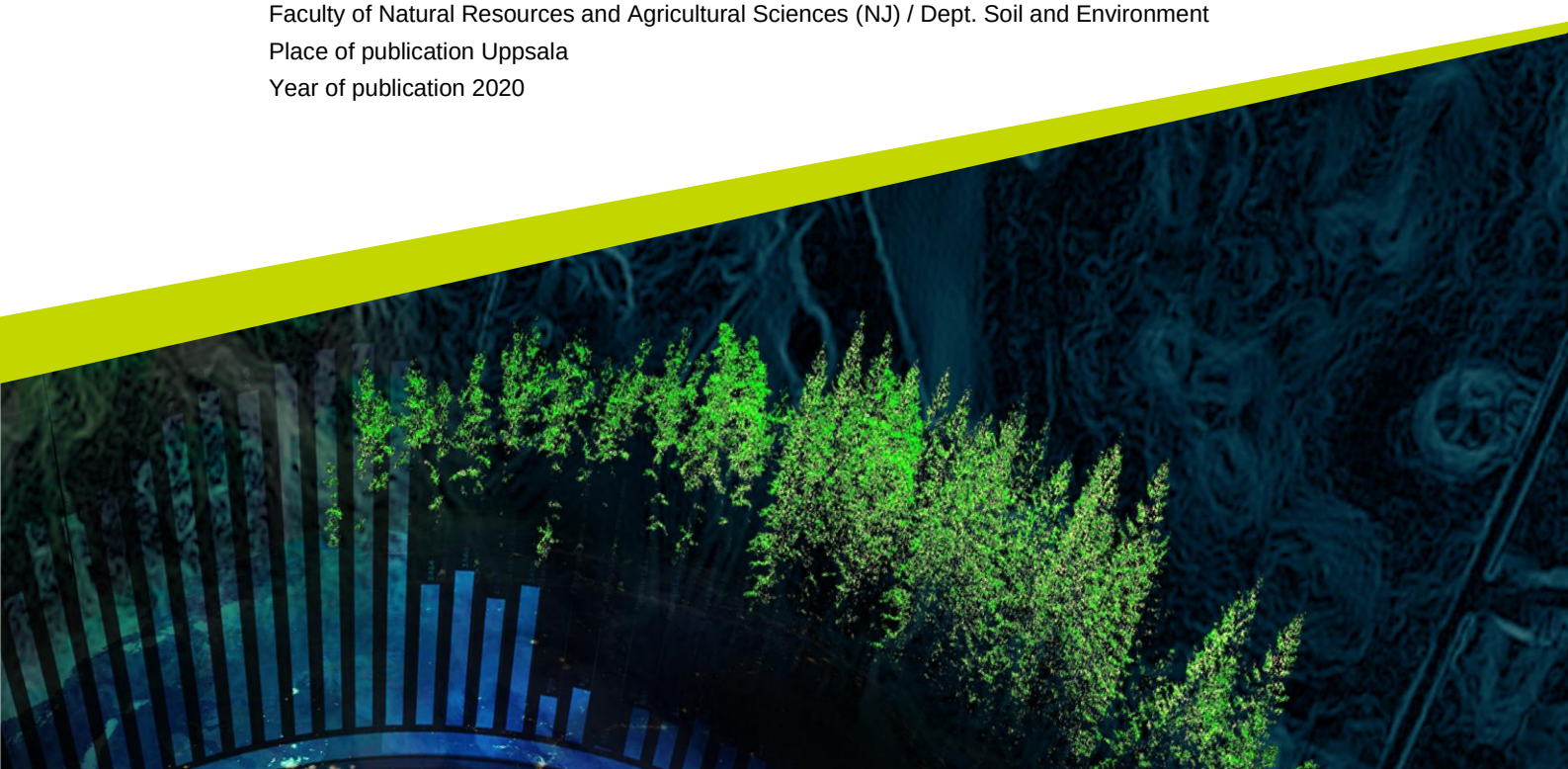
Johannes Kikuchi

BSc project • 15 ECTS credits
Swedish University of Agricultural Sciences, SLU
Faculty of Natural Resources and Agricultural Sciences (NJ) / Dept. Soil and Environment
Place of publication Uppsala
Year of publication 2020



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Bindning av PFAS-ämnen till humus- och fulvosyror – en dialysstudie

Johannes Kikuchi

Supervisor: Hugo de Campos Pereira, Swedish University of Agricultural Sciences (SLU), dept. Soil and Environment

Assistant supervisor:

Examiner: Dan B. Kleja, Swedish University of Agricultural Sciences (SLU), dept. Soil and Environment

Credits: 15 ECTS credits

Level: G2E

Course title: Självständigt arbete i Markvetenskap

Course code: EX0690

Programme/education:

Course coordinating dept: Mark och miljö

Place of publication: Uppsala

Year of publication: 2020

Cover picture:

Title of series:

Part number:

ISSN:

Keywords: PFOS, PFOA, sorption, kinetics, dissolved organic matter, natural organic matter, DOC, DOM, membrane

Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences (NJ)

Dept. Soil and Environment

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Abstract

To make more accurate predictions of the mobility of perfluoroalkyl substances (PFAS) in soil and water, better understanding of PFAS sorption to components of the terrestrial and aquatic systems is needed. This study investigated the possible sorption of a range of PFAS compounds with varying chemistries to humic acid (HA) and fulvic acid (FA) using a dialysis bag experimental set-up.
No sorption of any of the analysed compounds was observed to either fractions of *d*issolved *o*rganic *m*atter (DOM) using this experimental set-up. Our findings suggest that soil water containing fulvic acid does not enhance the solubility of PFAS compounds and is thus not likely to act as a transport vector for these compounds in natural systems. The non-binding of PFAS-compounds to the, in natural systems, solid phase humic acid suggest that PFAS-mobility and retainment in soil due to interaction with organic matter is more likely mediated by the lesser charged and more hydrophobic humin fraction.

Keywords: PFOS, PFOA, sorption, kinetics, dissolved organic matter, natural organic, matter, DOC, DOM, membrane

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Abbreviations

FA	Fulvic acid
HA	Humic acid
NOM	Natural organic matter
DOM	Dissolved organic matter
SOM	Soil organic matter
PFPeA	perfluoropentanoate
PFHxA	perfluorohexanoate
PFHpA	perfluoroheptanoate
PFOA	perfluorooctanoate
PFNA	perfluorononanoate
PFDA	perfluorodecanoate
PFUnDA	perfluoroundecanoate
PFDoDA	perfluorododecanoate
PFTeDA	perfluorotetradecanoate
PFBS	perfluorobutane sulfonate
PFHxS	perfluorohexane sulfonate
PFOS	perfluorooctane sulfonate
6:2 FTSA	6:2 fluorotelomer sulfonate
8:2 FTSA	8:2 fluorotelomer sulfonate
FOSA	perfluorooctanesulfonamide
EtFOSA	ethylperfluorooctanesulfonamide

1. Introduction

Background

Per- and poly fluoroalkyl substances (PFASs) are a large class of organic compounds with surfactant-like properties. PFASs fluorinated carbon tail and charged functional head group allows for both hydrophobic and hydrophilic interactions with various surfaces, compounds and materials (Ahrens, 2011). These unique characteristics makes PFASs very useful in a wide array of applications ranging from lubricants to water repellent fabrics and paints, amongst others (Buck *et al.*, 2011). Historically, the substances have also been widely used in aqueous film-forming foams (AFFFs), which has led to significant contamination at and downstream fire-fighting training sites (e.g. military sites). As a consequence, raw water source contamination with PFASs has been increasingly reported on a global scale over the past decades (Gobelius, Lewis and Ahrens, 2017; Xiao *et al.*, 2017; Høisæter, Pfaff and Breedveld, 2019). In animal- as well as in epidemiological studies, PFASs have been related to several types of cancers, liver damage, decreased birth weights and other adverse health effects (Cordner *et al.*, 2019). Thus, tools to conduct environmental risk assessments of PFAS contaminated sites need to be developed. To make more accurate predictions of the mobility and sorption of PFASs in environmental media such as soil and water, more knowledge is needed on how PFAS transport is mediated and regulated by the specific components that comprise the soil-water system.

PFASs and their structure

The general chemical formula of *per*fluoroalkyl substances is $C_nF_{2n+1}R$, where R represents the functional head group and C_nF_{2n+1} the fully fluorinated aliphatic carbon chain. Common functional groups are, amongst others, carboxylic acids (-CO_2H, perfluoroalkyl carboxylic acids; PFCAs), sulfonic acids (-SO_3H, perfluoroalkane sulfonic acids; PFSAs) and sulfonamides (-SO_2NH_2, perfluoroalkane sulfonamides; FASAs). There are also compounds where not all C atoms are fully fluorinated, these are called *poly*fluoroalkyl substances (Buck *et al.*, 2011).

Chemically and thermally, PFASs are extremely stable compounds that do not easily degrade. This is due to the presence of the perfluoroalkyl moieties (CF_2-moieties) of the carbon chain. Fluorine atoms (F) have the highest electronegativity of all elements and thus a strong tendency to attract electron density. This tendency results in the C-F bond being highly polarized, with negative charge shifted towards the fluorine atom. The strong polarization and thus the electrostatic attraction that arises between the $F^{\delta-}$ and $C^{\delta+}$ is what gives the C-F bond its strength and persistency to resist degradation (O'Hagan, 2008).

PFASs in the environment

PFASs surfactant-like properties render them extremely mobile in the environment as they are soluble in both polar and non-polar media such as water phases and lipid tissues of organisms. Given their high mobility, these compounds are now found spread across the globe from urban areas to the remotest of locations (Giesy and Kannan, 2001). Many studies have investigated the distribution of PFASs between different phases such as the partitioning in the water-solid interface of sediments and soils (Ahrens *et al.*, 2010), the soil-water-plant interface (Gobelius, Lewis and Ahrens, 2017) and between water and aquatic organisms (Xia *et al.*, 2015) amongst others. In natural systems such as waters and soils, natural organic matter (NOM) or soil organic matter (SOM, in the latter case) is considered an important sorbent for PFASs (Du *et al.*, 2014; Milinovic *et al.*, 2015)

Fractions of Soil Organic Matter

Traditionally the organic matter content of soils and sediments have been crudely divided into humic and non-humic substances. The non-humic substances, simply put, comprises biochemicals stemming from the anabolism and metabolism of life. This group of compounds are chemically and structurally identifiable and categorizable into distinct groups such as carbohydrates, lipids, proteins and amino acids etc. Unlike the non-humic substances the humic substances are hard to identify both structurally and chemically. They are the product of the remains after degradation of the non-humic substances in the environment. These compounds are complex and heterogenous with regards to their structure and chemical functionality. However, whether these compounds are supramolecular associations of smaller molecules or actual repolymerizations of the degradation products after biotic and abiotic decomposition is widely debated. (Essington, 2015, pp. 179-190; Paul, 2015, pp. 360-368)

Humic substances

Humic (HA) and fulvic acids (FA) are operationally defined fractions of soil organic matter, characterised by their solubility in acidic and alkaline media. A general method to obtain these fractions of soil organic matter from the soil matrix

is to first extract it (the soil) with 0,5 M NaOH. This is done repeatedly and the non-soluble fraction of organic matter in this step is classified as the humin fraction (Sposito, 2008). The extract containing dissolved organic matter (DOM) is then treated with 6 M HCl which precipitates (below pH 2) the HA fraction while the remaining organic matter in solution is defined as the FA fraction. (Essington, 2015)

Characteristics of HA and FA

The chemical and structural composition of the humic and fulvic acids are reflected in their respective solubilities. Fulvic acids, in general, have a higher oxygen-to-carbon ratio (O/C-ratio) than the humic acids (http://humic-substances.org/elemental-compositions-and-stable-isotopic-ratios-of-ihss-samples/). This, in turn reflect the number of oxygen-containing functional groups, such as carboxyls, hydroxyls, phenols etc. As mentioned earlier, the humic acids precipitates when pH is brought below 2 whilst the fulvic acids remain in solution. This disparity originates in the greater amount of surface charge present in FA compared to HA, which results from the larger number of functional groups present in the former.

Another important parameter differing between the two fractions is the H/C-ratio which represents the degree of aromaticity. A smaller ratio is in general interpreted as the organic matter being more aromatic whilst a larger H/C-ratio is indicative of a greater abundance of aliphatic structures. In general, the fulvic acids have a greater H/C-molar ratio as compared to the humic acids which would indicate a higher degree of aromaticity in the latter compared to the former. (Essington, 2015, pp. 179-190).

Consequently, under field conditions, humic acid is present as solid-phase (i.e. non-dissolved) soil organic matter, whereas the significantly more soluble fulvic acid will be present predominately in the soil water phase.

PFAS and Soil Organic Matter

As stated by Campos Pereira *et al.* (2018), studies of PFAS sorption to pure phases of soil organic matter are scarce. Among the ones that have been made, Zhang et al, (2015) found that the humin fraction accounted for the largest sorption of perfluorooctane sulfonate (PFOS). In addition to PFOS, the humin fraction also contributed the most to sorption of perfluorohexane sulfonate (PFHxS) in another study (Zhao *et al.*, 2014). However, the former study found a rather small contribution to sorption from the humic- and fulvic acid (HA and FA) fractions whilst the latter concluded their contribution to be smaller as compared to humin, but still significant. The length and size (increasing perfluorocarbon chain length)

of the PFASs under study was also determined a contributing factor since PFOS (C_8) displayed greater sorption affinity than PFHxS (C_6) on the same humic substance fraction (Zhao *et al.*, 2014). Similar results of increased sorption affinity with increasing chain lengths of PFASs has been shown in several studies (Higgins and Luthy, 2006; Ahrens *et al.*, 2010; Campos Pereira *et al.*, 2018) and is attributed to the increased hydrophobicity of the PFASs with increasing chain length.

PFAS and Dissolved Organic Matter

A common feature of the previously mentioned studies is that they focused on the PFAS sorption to the solid phases of SOM. If studies on pure phases of solid SOM are scarce, studies on PFAS sorption to pure phases of *dissolved* organic matter (DOM) are even scarcer. Like the humic substances, DOM is an operationally defined fraction. Most commonly it is demarcated as the fraction of organic matter in solution smaller than 0.45 µm (Paul, 2015, pp. 388; Vitale and Di Guardo, 2019). Partitioning and binding of organic contaminants to DOM is believed to be a large contributor to apparent contaminant solubility and thus mobility in terrestrial and aquatic environments (Chiou *et al.*, 1986; Chiou, 2003; Vitale and Di Guardo, 2019). Furthermore, it has been shown that the presence of DOM in the forms of HA and FA (from 1 mg/l) can enhance the bioaccumulation of certain PFASs in the aquatic *Daphnia magna* up until a certain DOM concentration threshold after which the opposite accumulation trend is observed (Xia *et al.*, 2015).

Aim of study

This study aimed to investigate the sorption behaviour of PFASs to the humic fractions of soil organic matter; more specifically, the sorption behaviour onto dissolved HA and FA. The specific objective of the study was, from a series of dialysis experiments, to calculate the organic carbon-normalized HA/FA–water distribution coefficients (K_{OC}) for a range of PFASs of different chemistries. This objective was based on the following hypothesis:

Hypothesis

PFASs bind to humic and fulvic acids. Thus, PFAS concentrations will be higher in dialysis bags containing humic and fulvic acids, as compared to those in the solutions outside of the dialysis bags.

2. Materials and methods

Chemicals and standards

The HA used in the experiments was Pahokee Peat 1S103H and the FA used was Pahokee Peat 2S103F (for information on chemical composition etc, see http://humic-substances.org/elemental-compositions-and-stable-isotopic-ratios-of-ihss-samples/). In total 16 PFASs (purchased from Sigma Aldrich) were analysed including C_4-C_{11} and C_{13} perfluoroalkyl carboxylates (PFCAs) (PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA and PFTeDa), C_4, C_6, and C_8 perfluoroalkane sulfonates (PFSAs) (PFBS, PFHxS, PFOS), perfluorooctane sulfonamide (FOSA), ethyl-perfluorooctane sulfonamide (EtFOSA) and C_6 and C_8 fluorotelomersulfonates (FTSAs) (6:2 FTSA and 8:2 FTSA).

As internal standard, isotopically labelled Wellington laboratories standards $^{13}C_2$ PFHxA, $^{13}C_4$ PFOA, $^{13}C_5$ PFNA, $^{13}C_2$ PFDA, $^{13}C_2$ PFUnDA, $^{13}C_2$ PFDoDA, $^{13}C_2$ PFTeDA, $^{13}C_2$ PFHxDA, $^{18}O_2$ PFHxS, $^{13}C_4$ PFOS, $^{13}C_8$ FOSA, d_5 EtFOSA, $^{13}C_2$ 6:2 FTSA and $^{13}C_2$ 8:2 FTSA were added in MeOH to each sample before analysis.

HA stock solution

A HA stock solution was prepared from the solid Pahokee Peat 1S103H standard by dissolving approximately 300 mg (306 mg) of HA in 10 ml (9.959 g, liquids where weighed rather than volumetrically added) of 0.0001 M NaOH in a glass beaker. MQ-water (filtered through powered activated carbon, LC-PAK, Millipore) was then added until no visible solid particles remained in solution, which amounted to a total MQ addition of 15 ml (14.798 g). This generated a HA stock solution of 12360 mg HA/l. Using the stated carbon content of 56.37 % C for calculations, this stock solution had a total organic carbon (TOC) content of 6967.4 mg C/l.

FA stock solution

A FA stock solution was prepared from the solid Pahokee Peat 2S103F standard by dissolving approximately 100 mg (101 mg) FA in 100 ml (99.37 g) of MQ-water in a glass beaker. This yielded a FA stock solution concentration of 1020 mg FA/l.

With regards to carbon content (51.31 % C for the solid FA standard) the FA stock solution had a TOC-content of 522 mg C/l.

The validity of the stock solution concentrations (HA 6967.4 mg C/l and FA 522 mg C/l) was confirmed by analysing the diluted working solutions (HA 170 mg C/l, dilution factor 40 and FA 25 mg C/l dilution factor 20, described later) for DOC and back calculating with the dilution factor.

For the experiment, working solutions of approximately 170 mg C/l (HA) and 25 mg C/l (FA) were prepared by dilution of the stock solutions in 100 ml volumetric flasks with 1 mM $NaNO_3$ as solvent. Since the HA was dissolved in 0.0001 M NaOH the working solutions had an initial high pH of 9.52 and thus needed pH-adjusting with 1 M HNO_3 (Titrisol®, Supelco) prior to the experimental start to reach the desired pH 4. Test-titrations of HA working solutions were performed and a total addition of 0.11 ml of 1 M HNO_3 was deemed enough to reach and keep the pH of the working solutions around pH 4. The initial FA working solution pH was on average 3.98 and needed no initial pH-adjustments.

Dialysis test set-up

The basic set-up for the dialysis tests (*Figure 1*) consisted of square, 1 l, high density polyethylene (HDPE) bottles, filled with 900 ml of background electrolyte solution (1 mm $NaNO_3$, from here on referred to as *outer solution*). To this solution was added a dialysis bag (Spectra Por 7 regenerated cellulose (RC) membrane, molecular cut-off 1 kD, nominal flat width 45 mm, diameter 29 mm, vol. 6.4 mL cm^{-1}, prewetted in 0.05% sodium azide), pre-filled with a 100 mL solution of dissolved organic matter (HA or FA, from here on referred to as *inner solution*). Dialysis tube clamps (Spectra Por) were used to seal the dialysis bag. All dialysis experiments were performed in triplicate (i.e. $n = 3$).

Addition of PFASs was done by pipetting 100 µl PFAS-stock solution (average PFAS concentration 0,22 mg/ml) directly into the outer solution and carefully rinsing the pipette tip by aspirating and dispensing the outer solution three times before discarding the tip. This, to ensure proper addition and mixing of the stock solution in the outer solution. Thus, the resulting nominal concentrations of individual PFASs in the outer solution were, on average, 25 µg L^{-1}. The 1 l bottles were then sealed and covered to prevent photochemical degradation of the DOM and put on a 1D- horizontal shaker (Gerhardt, model unknown) at a rotational speed of 80 rpm. Subsequent sampling of the inner- and outer solutions began starting at 1 h after the addition of the stock solution to the samples and continued at 24 h, 48 h, 96 h, 192 h and 288 h.

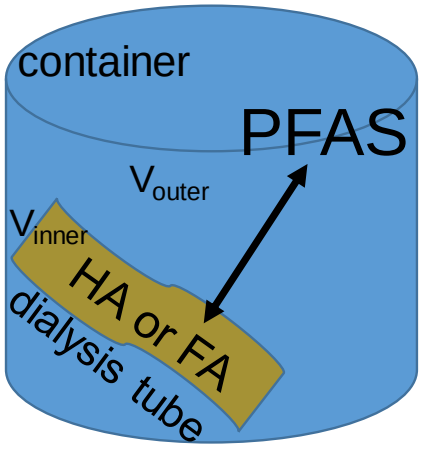

Figure 1. Set-up of dialysis system. V_{Outer} = 900 mL, V_{Inner} = 100 mL. PFASs were initially added to V_{Outer}. The molecular weight cut-off of the dialysis tube was 1 kDa.

Pre-dialysis

Prior to the start-up of the test, the DOM-solutions (inner solutions) were put on pre-dialysis for 48 h to release and discard DOM smaller than 1 kD (Berggren, 1989). Leaching DOM could potentially pose as a sorbent for PFASs in the PFAS-added outer solution surrounding the dialysis tube and thus hamper with observed concentrations at equilibrium. Thus, attempts were made to mitigate the risk of DOM leaching to the outer solution.

The pre-dialysis of the DOM-solutions were performed with the same outer solutions used in the experiments with the exception that no PFASs were added to it. After 48 h the pre-dialysis outer solution was exchanged with fresh outer solution and the testing began as previously described.

To check the pH-stability and also potential leaching of DOM from the dialysis bags the 48 h exchanged outer solutions were analyzed for pH (GK2401C combined pH electrode, Radiometer Analytical), conductivity (mS/m), absorbance at 254 nm ($A_{\lambda 254}$) (AvaSpec-ULS3648 high-resolution spectrometer, Avantes) and DOC (mg/l) (Shimadzu TOC-V_{CPH}). The same measurements were also performed on all replicate inner- and outer solutions at the end of the kinetic study.

Sampling

Sampling of the inner- and outer solutions was performed by removing the 1 l HDPE bottles from the horizontal shaker and transferring 500 µL of either solution to 1.7 ml PP-vials by automatic pipette. On each sampling occasion the inner solutions of all replicates were sampled first followed by sampling of the outer solutions. This procedure was followed to minimize the risk of accidentally

contaminating the inner solutions with high PFAS-content outer solution. The pipette tip was exchanged in between every replicate to further minimize risks of contamination. After sampling, 400 µL of methanol and 100 µL of PFAS internal standard (both in MeOH of liquid chromatography purity grade) was added to each vial and samples were then stored in freezer (-18 °C) until analysis.

Instrumental analysis

Samples were analyzed for PFASs using a DIONEX UltiMate 3000 ultra performance liquid chromatography (UPLC; Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole mass spectrometer (MS/MS) (TSQ Quantiva; Thermo Scientific, Waltham, MA, USA). The injected volume (10 µL) was separated on an Acquity UPLC BEH-C18 analytical column (1.7 µm, 50 mm, Waters, UK) using an eluent gradient of 12 min. Mobile phase was milli-Q water (LC-PAC quality) with 5 mmol L^{-1} ammonium acetate and 2% (v/v) acetonitrile. All integrations were checked manually and concentrations were evaluated using a 9-point calibration curve (0.01–100 ng mL^{-1}, all r^2 values ≥0.99).

Quality control

No fluorinated materials (e.g. tetrafluoroethylene, Teflon™) were used in the experiments to minimize the risk of contamination. Negative blanks, that is tests without addition of the PFAS stock solution, where run in duplicate for the inner and outer solutions in the HA experiment to determine whether PFAS substances were present in any of the materials used in the experimental set-up. If compounds were detected and quantified in at least 3 of the 4 blank solutions, the standard deviations of those average concentrations were used to calculate the limit of quantification (LOQ) in the experiment solutions. This was done by multiplying the standard deviations by a factor 10. In those cases where the standard deviations could not be calculated for the negative blanks, the lowest detected actual concentration of the calibration curve was used to estimate the limit of quantification. More specifically, the average of the lowest detected calibration curve point in the beginning of the instrumental analysis and the after-analysis lowest calibration curve point was used for this estimation. A pre-requisite for determining a valid low calibration curve point was that the observed actual concentration of the point did not deviate > 25 % from the aimed standard concentration of the calibration curve point.

In the FA experiment no negative blanks were run and LOQs were thus only estimated by the lowest observed concentrations of the calibration curve as previously described.

Duplicate positive blanks (that is experimental set ups with addition of the PFAS stock solution but without dialysis bags containing HA or FA) were run to study the behavior of the PFAS transfer through the dialysis membrane without the influence of DOM.

Data handling

Evaluation and statistical testing of the data was performed using Microsoft Excel. Data was checked for normality by calculating the Kolmogorov-Smirnov test statistic (KS test statistic). This test compares the maximum difference between the sample cumulative distribution function and the hypothesized cumulative distribution function. If the calculated KS-statistic (or maximum difference) is smaller than a specific critical value, it cannot be proven that the maximum difference between the functions does not deviate significantly from zero. Thus, indicating that the sample cumulative distribution function behaves no different from the normally distributed hypothesized cumulative distribution function (Miller and Miller, 2010, pp. 63-65).

To determine if or when statistically significant changes of PFAS concentrations in the inner solution no longer occurred, One-Way ANOVA was performed. The least significant difference (LSD) was used as a comparative measure to find after which point in time the difference between the sampling point means became smaller than the difference caused by random variation i.e. smaller than the standard deviation (SD) of the mean (Miller and Miller, 2010 pp. 53-59).

The regular one-sided Student's T-test was used to determine whether the concentration of each PFAS in the inner solution became significantly higher than that of the outer solution, i.e whether sorption and thus higher concentrations of PFAS was observed in the inner solutions containing DOM. This was only done in those cases where the observed average concentration of each triplicate inner solution surpassed the concentration of the outer solution. The two-sided F-test was used to test if sample variances could be pooled or not when calculating the Student's t test-statistic.

3. Results

Evaluated compounds

The following 16 PFAS compounds and precursors were evaluated in the HA experiment: PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS, PFHxS, PFOS, FOSA, EtFOSA, 6:2 FTSA and 8:2 FTSA. In the FA experiment the following 13 PFAS compounds and precursors were evaluated: PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFBS, PFHxS, PFOS, FOSA, EtFOSA, 6:2 FTSA and 8:2 FTSA.

LOQ

The limit of quantification (LOQ) was based either on the lowest detected standard concentration of the calibration curve for each compound, or 10 times the standard deviation of the negative blanks, and are compiled in Table 1 (below).

Table 1.Tabulated values of LOQ for the HA and FA experiments. Limit values are based on either the lowest detected standard concentration of the calibration curve (within 25 % accuracy of aimed standard concentration) or 10 times the standard deviation of the compound in the negative blanks.

Compound	HA LOQ µg/l	FA LOQ µg/l
PFPeA	0.06	-
PFHxA	0.05	0.08
PFHpA	0.05	0.61
PFOA	0.01	0.50
PFNA	0.05	0.11
PFDA	0.03[a]	0.05
PFUnDA	0.03[a]	0.10
PFDoDA	0.02[a]	-
PFTeDA	0.02[a]	-
PFBS	0.01[a]	0.38[b]
PFHxS	0.03[a]	0.19[b]
PFOS	0.09	0.72[c]
FOSA	0.45	0.12
EtFOSA	0.09	0.11
6:2 FTSA	0.01	0.10
8:2 FTSA	0.06	0.06

[a] LOQ determined as 10 times the standard deviation of the blanks.

[b] LOQ estimated by only one calibration curve point.

[c] Difference between calibration and aimed concentration > 25 %.

Distribution of the data

KS-test statistics, critical values and resulting statistical distribution for each compound and DOM-solution are found in Table 2 below. In the HA-experiment only PFDoDA, PFTeDA and the sulfonamide FOSA could be proven to follow a normal distribution. However, after log-transformation EtFOSA was shown to follow a log-normal distribution. In the FA-experiment, data for all PFASs could be proven normally distributed apart from EtFOSA for which neither could be proven.

Table 2. Calculated KS-test statistics and resulting distribution of each compound in the HA and FA experiment (n = 36, α = 0.95).

Compound	HA KS statistic	HA Distribution	FA KS statistic	FA Distribution
PFPeA	0.30	Cannot prove normal distribution	-	-
PFHxA	0.33	Cannot prove normal distribution	0.13	Data normally distributed
PFHpA	0.33	Cannot prove normal distribution	0.19	Data normally distributed
PFOA	0.31	Cannot prove normal distribution	0.21	Data normally distributed
PFNA	0.32	Cannot prove normal distribution	0.12	Data normally distributed
PFDA	0.30	Cannot prove normal distribution	0.12	Data normally distributed
PFUnDA	0.28	Cannot prove normal distribution	0.09	Data normally distributed
PFDoDA	0.14	Data normally distributed	-	-
PFTeDA	0.19	Data normally distributed	-	-
PFBS	0.24	Cannot prove normal distribution	0.14	Data normally distributed
PFHxS	0.30	Cannot prove normal distribution	0.21[a]	Data normally distributed
PFOS	0.28	Cannot prove normal distribution	0.15	Data normally distributed
FOSA	0.13	Data normally distributed	0.14	Data normally distributed
EtFOSA	0.14[b]	Data log-normally distributed	0.08[a]	Cannot prove normal distribution
6:2 FTSA	0.30	Cannot prove normal distribution	0.16	Data normally distributed
8:2 FTSA	0.25	Cannot prove normal distribution	0.18	Data normally distributed

[a] KS test statistic decreased when compound specific data was log-transformed.

[b] KS test statistic decreased and no statistical difference could be proven between the sample cumulative distribution function and the hypothetical cumulative distribution function when data was log-transformed.

[c] KS test statistic decreased but data could not be shown to follow a normal distribution.

Dialysis tests

No increase in PFAS concentration could be observed (statistically shown) in the inner solutions containing HA or FA (HA:Fig. 2-4 ; FA: Fig. 5-6: Figure 11 in Appendix). The results indicate that no (measureable) binding of PFAS occurred to either HA or FA.

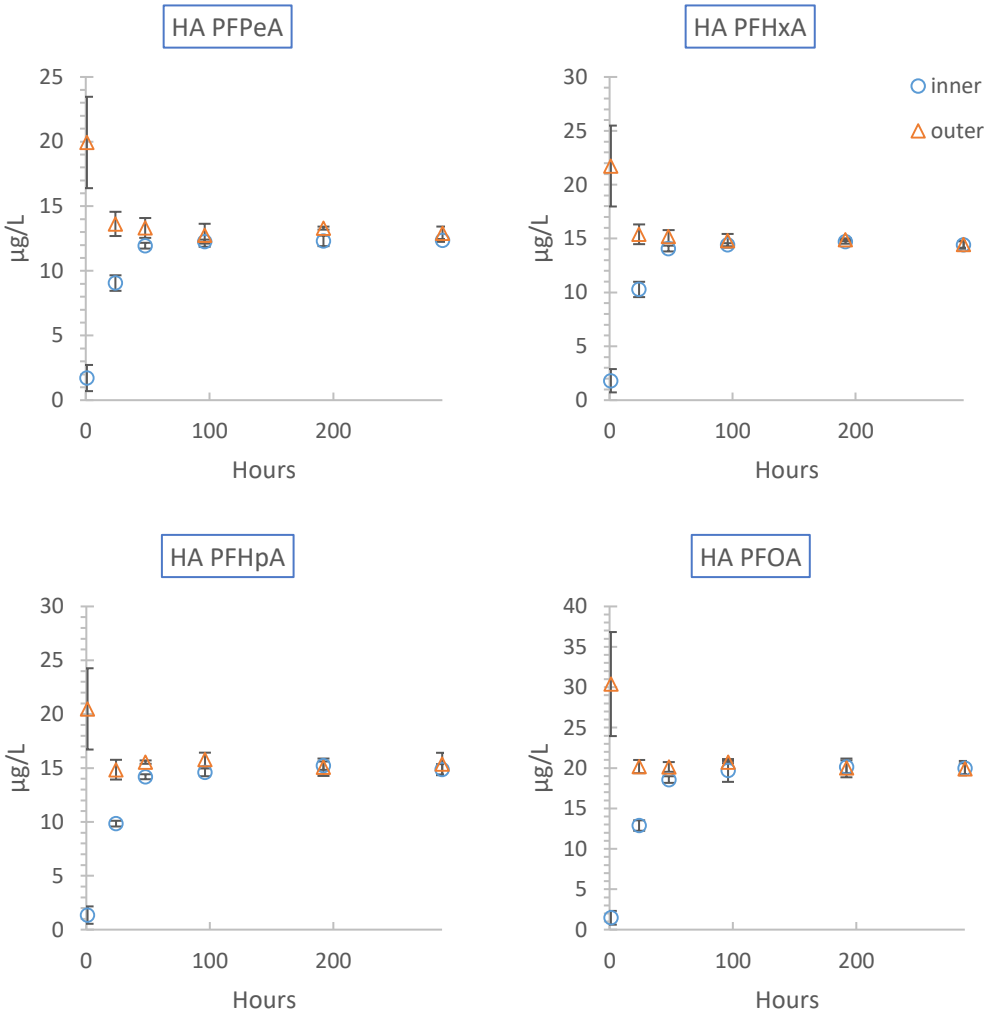

Figure 2. Distribution over time of the C_5-C_8 perfluorocarboxylates PFPeA, PFHxA, PFHpA and PFOA with HA as inner solution. pH = 3.2. Whiskers represent replicate standard deviations *(n = 3).*

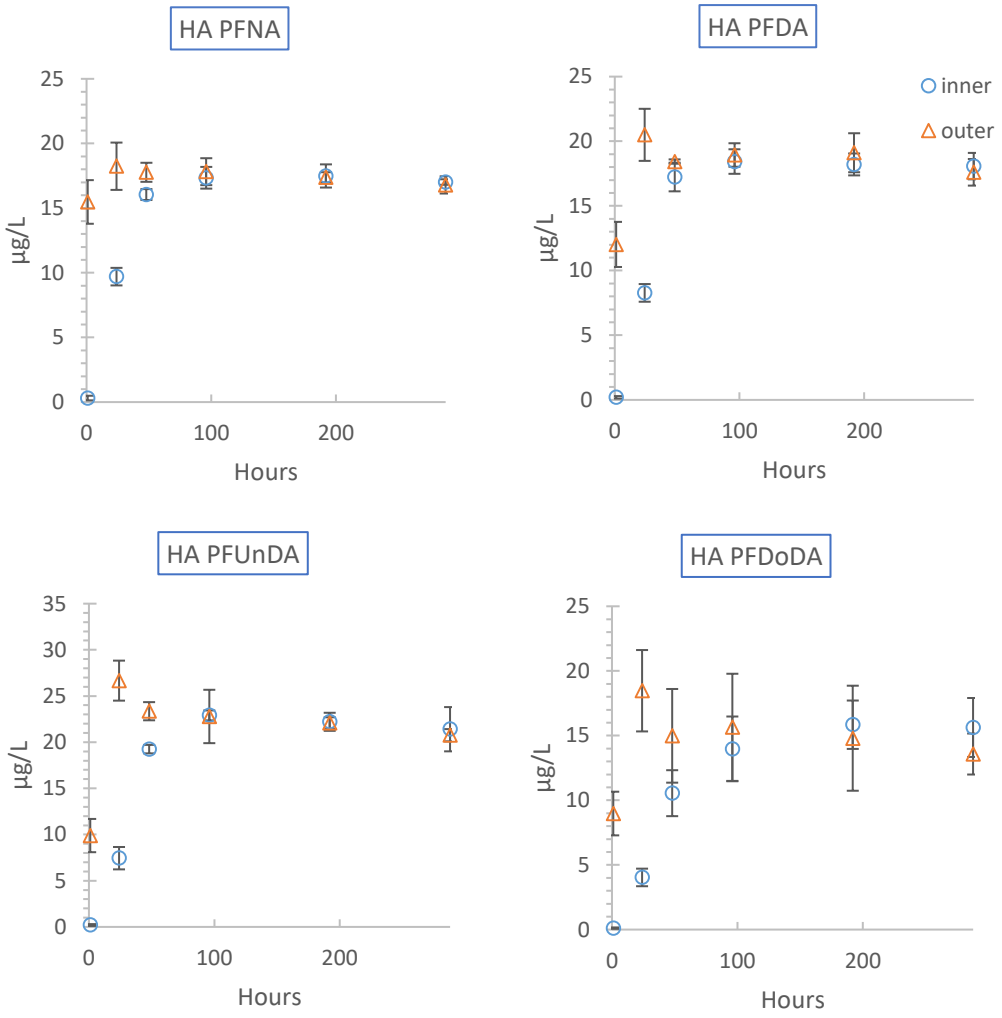

Figure 3. Distribution over time of the C_9-C_{11} carboxylates PFNA, PFDA, PFUnDA and PFDoDA with HA as inner solution. pH = 3.2. Whiskers represent replicate standard deviations (*n = 3*).

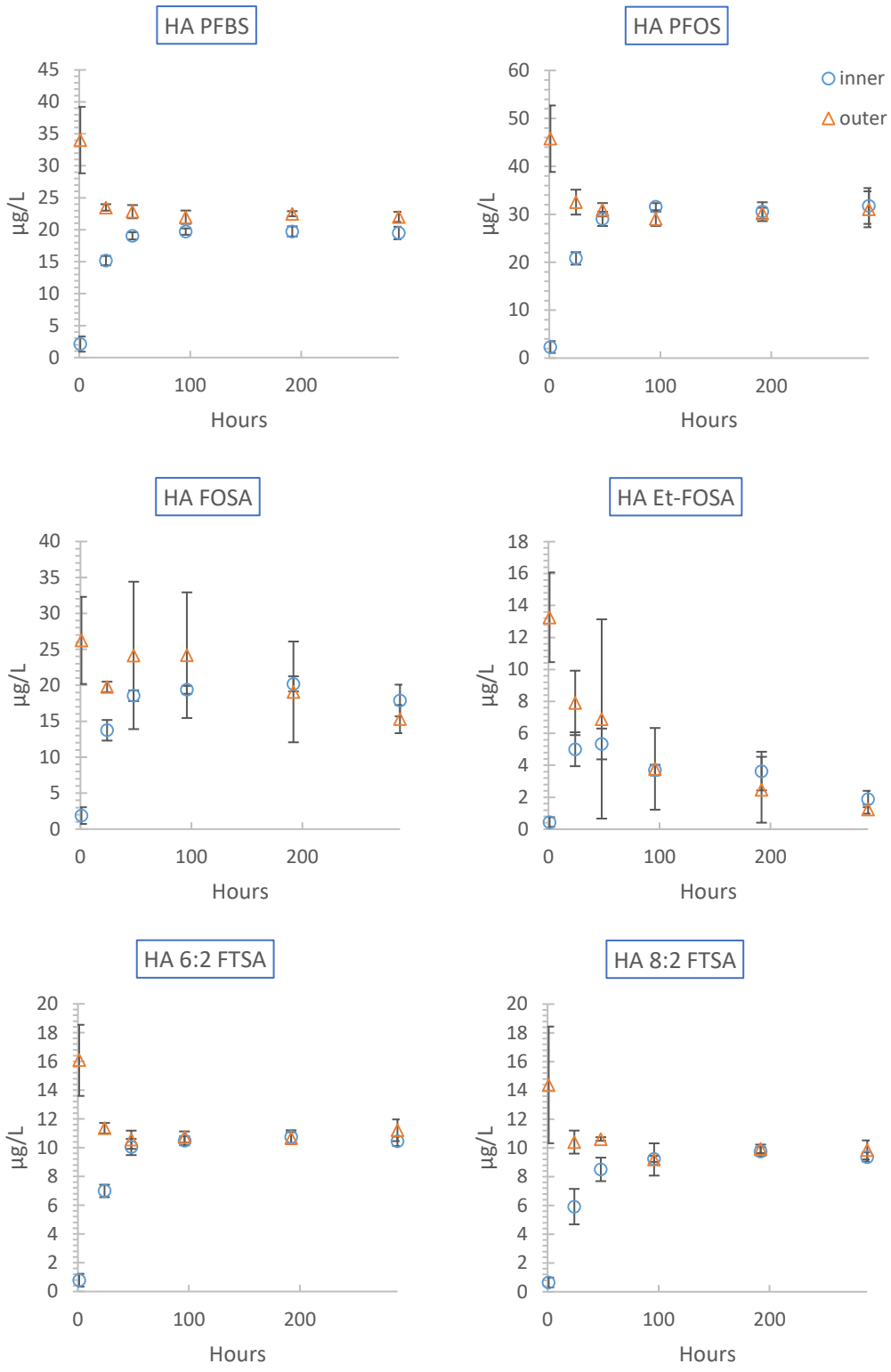

Figure 4. Distribution over time of PFBS, PFOS, FOSA, EtFOSA, 6:2 FTSA and 8:2 FTSA with HA as inner solution. pH = 3.2. Whiskers represent replicate standard deviations (n = 3).

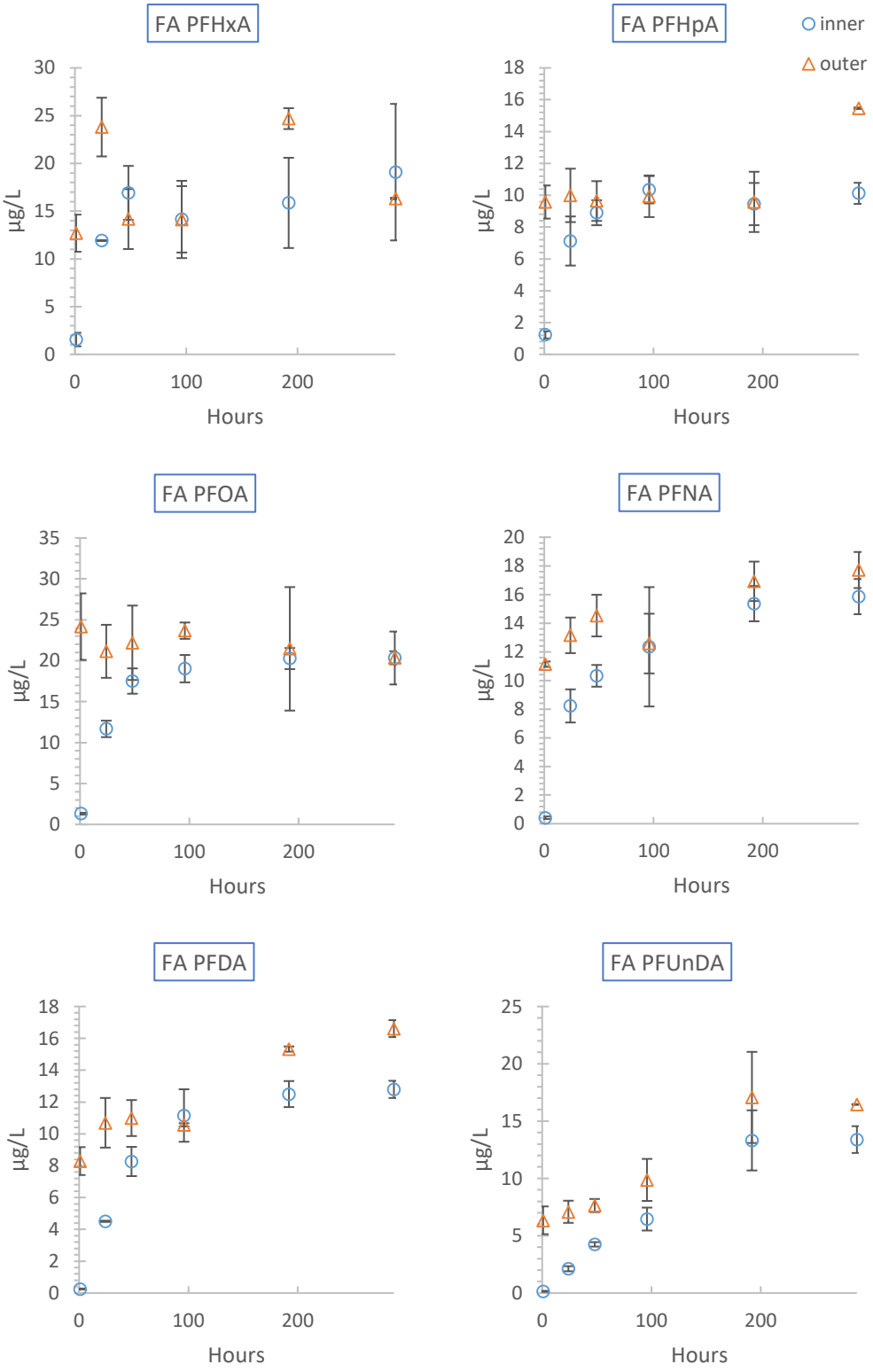

Figure 5. Distribution over time of the perfluorocarboxylates C_5-C_{10} PFHxA, PFHpA, PFOA, PFNA, PFDA and PFUnDA with FA as inner solution. pH = 4.0. Whiskers represent replicate standard deviations (n = 3).

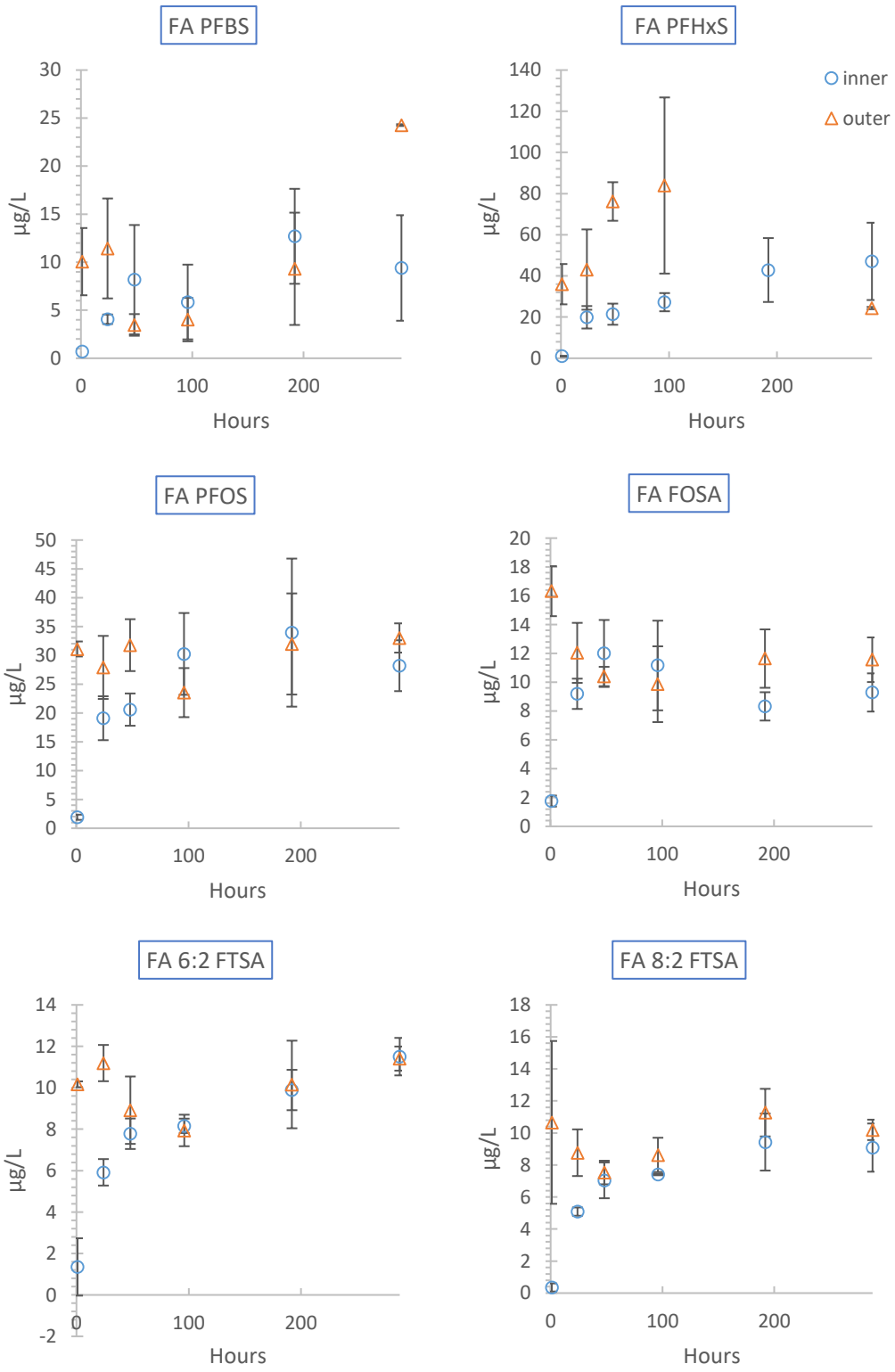

Figure 6. Distribution over time of PFBS, PFHxS, PFOS, FOSA, 6:2 FTSA and 8:2 FTSA with FA as inner solution. pH = 4.0. Whiskers represent replicate standard deviations (n = 3).

HA experiment

In the HA-experiment PFCAs, PFSAs and FTSAs with CF_2-moiety chain lengths C_4-C_7, (PFPeA, PFHxA, PFHpA, and PFOA), C_3-C_8 (PFBS, PFHxS and PFOS) and C_6-C_8 (6:2 FTSA and 8:2 FTSA) exhibited very similar equilibration patterns across the dialysis membrane with equivalent concentrations on both sides of the membrane within 2-3 days. As can be seen in Fig. 2 and 4, the decrease in concentrations for these compounds in the outer solution was consistently countered by a corresponding increase in concentration of the inner solution. For the PFCAs with CF_2-moiety chain lengths C_9-C_{11} and C_{13} (i.e. PFNA, PFDA, PFUnDA, PFDoDA and PFTeDA) the inner solution exhibited a similar pattern as the shorter-chained PFCAs. However, the first sampling occasion of the outer solution (1 h) had consistently lower concentration than the sampling at 24 h (which had the highest concentration of all sampling points of the outer solutions) for these compounds as can be seen in Fig. 2 and Figure 11, Appendix (PFTeDA).

PFCAs with CF_2-moiety chain lengths C_{11} and C_{13} (PFDoDA and PFTeDA) showed larger deviations within replicates (Fig. 2 and Figure 11, Appendix) as compared to shorter-chained PFCAs. Amongst these two PFCAs, equivalent concentrations between the inner- and outer solutions was observed only for PFTeDA (C_{13}) after 288 h (12 days). At this point the inner solution concentration of PFTeDA just slightly surpassed the concentration of the outer solution, though testing with the Student's t-test could not prove the difference to be statistically significant at the 95 % significance level. The results for the one-sided Student's T-test to test for higher concentrations in the inner solutions are shown in Table 3 below.

The FOSAs (FOSA and EtFOSA) exhibited a deviating behavior (Fig. 3), with larger replicate standard deviations (much like PFCAs with CF_2-moiety chain lengths C_{11} and C_{13}) and a somewhat decreasing trend in total concentration over time on both sides of the dialysis membrane. For these compounds the inner solution concentrations surpassed the outer solution concentration at 192 h (8 days). EtFOSA increased the most in its inner solution concentration but, as with the longer chained PFCAs, no statistical significance could be proven for either of these observed increases.

Table 3. Student's t-test to test for significant increases in solution concentration for compounds where the observed average inner solution concentration surpassed that of the outer (n = 3, $\alpha = 0.05$).

Compound	h	DOM	Inner $\bar{x}$	Inner SD	Outer $\bar{x}$	Outer SD	t_{calc}	t_{crit} $\alpha = 0.95$	Significant Y/N[b]
PFDoDA	192	HA	15.8	1.9	14.8	4.1	0.40	2.13	N
PFDoDA	288	HA	15.6	2.3	13.6	1.6	1.28	2.13	N
PFTeDA[a]	192	HA	1.5	0.3	2.8	2.4	0.94	2.92	N
PFTeDA	288	HA	1.6	0.3	1.3	0.6	0.97	2.13	N
FOSA[a]	192	HA	20.2	1.0	19.1	7.0	0.27	2.92	N
FOSA	288	HA	17.9	2.2	15.3	2.0	1.52	2.13	N
EtFOSA	192	HA	3.6	1.2	2.5	2.1	0.85	2.13	N
EtFOSA	288	HA	1.9	0.5	1.3	0.3	1.88	2.13	N
PFDA[a]	96	FA	11.2	1.6	10.6	0.1	0.61	2.92	N
PFBS	48	FA	8.2	5.7	3.5	1.1	1.41	2.13	N
PFBS	96	FA	5.9	3.9	4.0	2.3	0.70	2.13	N
PFBS	192	FA	12.7	4.9	9.3	5.8	0.77	2.13	N
PFOS	96	FA	30.2	7.1	23.5	4.3	1.41	2.13	N
PFOS	192	FA	33.9	12.8	32.0	8.8	0.22	2.13	N
FOSA	48	FA	12.0	2.3	10.4	0.7	1.14	2.13	N
FOSA	96	FA	11.2	3.1	9.9	2.6	0.55	2.13	N
6:2 FTSA	96	FA	8.2	0.3	7.9	0.8	0.46	2.13	N

[a] Samples of the inner and outer solutions could not be proven to come from populations with the same variance and standard deviations of the mean could not be pooled.
[b] Y/N = Yes/No

FA experiment

The dialysis experiment with fulvic acid showed much larger standard deviations within replicates as compared to those in the experiment with humic acid. As can be seen in Fig. 5 and 6, kinetic trends are not very clear. For PFCAs of chain length C_8 and longer there seemed to be a consistent increase in concentrations over time in both inner- and outer solutions (Figure 5). A somewhat similar equilibration trend could be seen for PFOS and FOSA but not for PFBS and PFHxS (Fig. 6). Among the two FTSAs the 6:2 FTSA seemed to consistently increase in overall concentration over time, much like the PFCAs C_8-C_{10}. The 8:2 FTSA behaved similarly though not as pronounced (Fig. 6).

In the FA-experiment higher concentrations in the inner solutions during the testing period was observed for PFDA (96 h), PFBS (48, 96 and 192 h), PFOS (96 and 192 h), FOSA (48 and 96 h), EtFOSA (48 and 96 h) and 6:2 FTSA (96 h). However, for these compounds the standard deviations of the mean inner- and outer-solution concentrations were overlapping and the Student's T-test could not prove any statistically significant increases in concentrations of the inner solutions (Table 3)

As already stated, in general, the standard deviations within replicates for each PFAS were much larger in the FA-experiment than in the HA-experiment.

One-Way ANOVA and the Least Significant Difference (LSD)

By evaluating the differences in sampling point mean concentration over time in the inner solution for each compound with One-Way ANOVA and comparing these differences or (presumed differences) with the *L*east *S*ignificant *D*ifference (LSD), the time in which statistically significant changes seized to occur could be determined. These results are compiled in Table 4 along with the number of degrees of freedom for rows and replicates, the average difference in concentration in the determined time interval and the LSD of each compound.

pH, conductivity, DOC and SUVA

To monitor the potential leaching of the inner solutions into the outer solutions the following supporting parameters were analyzed in the pre-dialysis outer solution (regarded as t = 0) and the inner and outer solution at 288 h (day 12) for all replicates; pH, conductivity, DOC and absorbance at 254 nm. These results are presented below in Table 5. Furthermore, the working solutions of HA (170 mg C/l) and FA (25 mg C/l) were analyzed for DOC and absorbance at 254 nm to

quantify the actual DOC concentrations of the solutions and to get a reference point for the starting concentrations of the inner solutions in the experiment.

The specific UV-absorbance (SUVA) is a parameter commonly used to characterize the degree of aromaticity of the DOM. It is calculated as the ratio of absorbance at 254 nm and DOC (mg/l) and has the units of l/mg C m. The higher the ratio, the more aromatic the DOM (Weishaar *et al.*, 2003). Resulting SUVA-values of respective solutions are also presented in Table 5.

Table 4. Determined equilibration times with the One-Way ANOVA and least significant difference (LSD) analysis. For compounds with several determined equilibration times the concentration differences fluctuated between being significant and non-significant throughout the duration of the test. ($\alpha = 0.95$)

Compound	HA $t_{equilibrium}$ (h)	FA $t_{equilibrium}$ (h)	HA d.f (h, n) / FA d.f (*h*, *n*)	Concentration difference at $t_{equilibrium}$ HA / FA (µg/l)	HA LSD / FA LSD (µg/l)
PFPeA	48-96	-	5, 10/-	0.317 / -	0.930 / -
PFHxA	48-96	24-48	5, 10/5, 10	0.353 / 2.772	1.010 / 7.042
PFHpA	48-96	24-48	5, 10/5, 10	0.407 / 1.777	0.823 / 1.778
PFOA	48-96	48-96	5, 10/5, 10	1.127 / 1.509	1.573 / 3.109
PFNA	96-192	24-48	5, 10/5, 10	0.139 / 2.101	1.084 / 3.432
PFDA	48-96	96-192	5, 10/5, 10	1.197 / 1.348	1.518 /1.546
PFUnDA	96-192	1-96 & 192-288	5, 10/5, 10	0.693 / 1.973 & 2.126 & 2.209 & 0.072	2.140 / 2.222
PFDoDA	96-192	-	5, 10/-	1.867 / -	3.132 / -
PFTeDA	24-48 & 192-288	-	5, 10/-	0.217 & 0.159 / -	0.337 / -
PFBS	48-96	Never significant	5, 10/4, 8	0.673 / -	1.470 / -
PFHxS	-	24-48	5, 10/5, 10	- / 1.510	- / 18.795
PFOS	48-96	24-48	5, 10/5, 10	2.550 / 1.499	3.544 / 11.656
FOSA	48-96	24-48	5, 10/5, 10	0.824 / 2.800	2.324 /3.172
EtFOSA	24-48 & 96-192	1-96 & 192-288	5, 10/5, 10	0.331 & 0.062 / 1.175 & 0.603 & 0.715 & 0.023	1.450 / 2.120
6:2 FTSA	48-96	only at 48-96	5, 10/5, 10	0.431 / 0.382	0.771 / 1.584
8:2 FTSA	48-96	48-96 & 192-288	5, 10/5, 10	0.733 / 0.369 & 0.345	1.160 / 1.898

Table 5. Measured pH, conductivty (S/m), DOC (mg/l), absorbance at 254 nm and calculated SUVA-values (l/mg C m) of the pre-dialysis solution (Outer t_0), inner- and outer solutions at 288h (12 d) and the working solutions of HA (170 mg C/l) and FA (25 mg C/l). For all parameters with a calculated standard deviation (SD) *n = 3*.

Sample	pH	pH SD	Conductivity S/m	Conductivity SD	DOC (mg/l)	DOC SD	Abs 254	Abs SD	SUVA (l/mg C m)	SUVA SD
HA Outer t_0	3.0	0.000	0.61	0.01	4	1.8	0.1	0.02	2.2	1.5
HA Outer 12d	3.2	0.002	0.56	0.01	16	0.4	0.1	0.01	0.7	0.1
HA Inner 12d	3.1	0.002	1.21	0.05	168*	4.7	10.2*	0.1	6.1	0.1
HA 170 mg C/l	-	-	-	-	172*	-	11.6*	-	6.7	-
FA Outer t_0	3.9	0.004	0.21	0.004	6	5.8	0.02	0.01	1.3	1.7
FA Outer 12d	4.0	0.01	0.21	0.001	16	0.9	0.03	0.01	0.2	0.1
FA Inner 12d	3.9	0.006	0.56	0.02	34	1.3	1.5	0.05	4.5	0.1
FA 25 mg C/l	-	-	-	-	29	-	2.0	-	6.7	-

* Results were calculated from analysis of diluted samples due to limited calibrated/linear range of the instruments.

4. Discussion

Non-Gaussian distributions

The data could not be proven to be normally distributed for every compound despite log-transformation (see Table 2). Examples of causes for non-normal distributions like heavy tailings or asymmetrical appearances are (amongst others); analytical errors, inadequate experimental set up or sampling and measurements performed by several different people (Miller and Miller, 2010, pp. 154-155). Regardless, the usual statistical tests (like Student's T-test and ANOVA) are only applicable when the data follows a normal distribution. In cases where data is not normally distributed one is referred to using non-parametric statistical tests. However, the use of these types of tests were not investigated further since it was deemed outside the scope of this study. Despite not being fully valid (from a statistical point of view), the more common parametric statistical tests were used to evaluate data that could not be shown to follow a Gaussian distribution.

PFAS-behavior during dialysis tests

As already stated, no sorption for any of the analyzed PFASs to either HA or FA could be proven using this experimental set up. The kinetic behavior observed seems to only reflect the equilibration of PFASs across the dialysis membrane (Fig. 1-6, Fig. 10, Appendix) but no more than this. These results are in stark contrast to studies showing, compared to humin, the lesser, though still measurable PFAS sorption to HA and FA (Zhao *et al.*, 2014; Zhang *et al*., 2015). Xia *et al.* (2015) performed kinetic dialysis bag tests (Spectra Por 6, molecular cut-off 7000 D, equilibration time of 7 days) as a part of their study of PFAS bioaccumulation in Daphnia magna and could calculate log partition coefficients ($\log K_{HA}$ (l/kg)) for humic acid in the range of 4.21 – 4.98 for PFOS, PFOA, PFNA, PFDA, PFUnDA and PFDoDA. The main difference in their study, compared to this one, being that the HA solution in the dialysis bag was fortified with PFAS, instead of the outer solution of artificial fresh water.

The resulting Student's T-tests in this (Table 3) showed no significant increases in concentration of the inner solutions as compared to the outer. Thus, the

hypothesis that PFAS concentrations should increase in solutions containing DOM due to sorption must be rejected.

The results of the One-Way ANOVA and LSD analysis (Table 4) showed that most PFASs reached equilibrium in the inner solution in the time interval 48 – 96 h during the HA experiment (CF_2-moiety chain length PFCAs C_4-C_7, C_9, PFSAs C_3 and C_8, FOSAs C_8 and FTSAs C_6 and C_8). During the FA experiment equilibrium seems to have been reached at one time interval earlier, between 24 – 48 h (CF_2-moiety chain length PFCAs C_5-C_7 and C_9, PFSAs C_6 and C_8, FOSAs C_8 and FTSAs C_8. One might speculate that the equilibration time would correlate positively with increasing chain length of the compounds. However, the data set is inconsistent in this regard since for example PFNA (C_8) reached equilibrium at 96-192 h while the longer chained PFDA (C_9) did so one interval step earlier in the HA experiment.

There were also compounds that seems to have drifted in and out of equilibrium; PFTeDA (C_{13}) and EtFOSA (C_8) in the HA experiment and PFUnDA (C_{10}) and 8:2 FTSA (C_8) of the FA experiment. These variations could potentially be linked to the apparent over all loss and or increase of these compounds in solution (both inner- and outer solution) over time. The HA PFTeDA (Figure 11, Appendix) and EtFOSA (Fig. 4) both decreased over time while the FA PFUnDA (Fig. 5) and 8:2 FTSA (Fig. 6) increased. Furthermore, the FA 6:2 FTSA only reached equilibrium at one time interval and exhibited the continual increase in overall concentration throughout the experiment (Fig. 6 and Table 4). A concentration decrease in both solutions could indicate sorption losses, perhaps to the walls of the vessels or to the dialysis membrane itself. An increase in both solutions could, following the same logic, indicate that desorption was taking place. If, when first adding the PFAS-stock solution to the outer solution, a fast, initial sorption to the vessel walls occurred, starting concentrations in both solutions would be low but as desorption increased, over all concentrations in the system would also increase.

It should be mentioned that the FA PFNA and PFDA (Fig. 5) also exhibited an increasing over all concentration trend. However, this trend was not pronounced enough to result in the inner solution reaching equilibrium several times in the LSD-comparison as previously described for HA PFTeDA, PFUnDA and the FA 6:2 FTSA.

Comparison of absolute amount PFAS (ng) of inner- and outer solutions and blank samples

To investigate what the addition of PFAS-stock solution to the outer solutions yielded in starting concentrations, MeOH blanks (addition of PFAS-stock solution in pure MeOH) and positive blank samples (addition of PFAS-stock solution to sample containers with dialysis bags without HA or FA inside) were made. Using the MeOH-blanks as a measure of total added amounts of PFAS to the systems, mass balances of the last sampling point for the analyzed compounds were calculated (Table 6). The absolute amounts (ng) from the MeOH blanks showed rather good agreement with the sum of absolute amounts of the inner and outer solutions (ng) for PFCAs with CF_2-moiety chain lengths C_4 – C_7 in the HA experiment, Figure 7. Calculated mass balances did not deviate more than 14 % from the considered total amount of the systems (Table 6) This, however, was not the case for the mass balance and sum of the inner- and outer solution absolute amounts of PFCAs C_8 – C_{13} (Fig. 7 and Fig. 12, Appendix, Table 6). For these compounds the mass balance ranged from 194 – 283 %. For the PFSAs, mass balances and the MeOH blanks and sums of inner- and outer solutions all seemed to be in rather good agreement with each other (Fig. 8 and Table 6). The same appears to be true for FOSA and the FTSAs (6:2 FTSA and 8:2 FTSA)Fig. 8, Fig. 12 (Appendix) and Table 6. However, the EtFOSA mass balance and inner and outer absolute amounts diverged a lot from the quantified total amounts of the MeOH blanks (Fig. 8 and Table 6).

Table 6. Mass balances for PFAS at time t = 288 hours in the dialysis experiment.

Compound	HA 288 h (ng absolute)	FA 288 h (ng absolute)	MeOH-blank (ng absolute)	HA Mass Balance [%] *	FA Mass Balance [%] *
PFPeA	12799		11190	114	-
PFHxA	14374	16513	-	-	-
PFHpA	15250	14865	16692	91	89
PFOA	19825	20252	20825	95	97
PFNA	16732	17440	8642	194	202
PFDA	17559	16155	6687	263	242
PFUnDA	20714	16069	7309	283	220
PFDoDA	13708	6879	5315	258	129
PFTeDA	1301		2051	-	-
PFBS	21653	22700	22674	95	100
PFHxS	23035	26475	24588	94	108
PFOS	30947	32378	29491	105	110
FOSA	15474	11282	20439	76	55
Et-FOSA	1311	1070	23289	6	5
6:2 FTSA	11079	11361	10550	105	108
8:2 FTSA	9766	10035	11508	85	87

*$\sum_{\text{inner + outer}} t_{288\ h}$ / MeOH blank * 100

Results for the mass balances and absolute amounts of PFCAs $C_5 – C_7$ in the FA experiment (Fig. 9 and Table 6) was not as uniform as the PFCA $C_4 – C_7$ of the HA experiment. In particular, the FA PFHxA inner- and outer solution absolute sum varied extensively between good agreement with the quantified amounts of the MeOH blanks and the calculated theoretical amount (that is the aimed weight of each compound when preparing the PFAS-stock solution) of the PFAS-stock solution (Fig. 9). Moving up in CF_2-moiety chain lengths, the sum of inner- and outer-solution PFCAs $C_8 – C_{10}$ showed better agreement with the MeOH blank levels initially. After 96 h, however, the sums of the total amount started to increase, diverging more and more from that of the MeOH blanks. This increasing trend is clearly seen in the mass balance (range 202-220 %) of the last sampling point of

these compounds (Table 6) and is similar to the mass balances of the HA tests. The sulfonates (PFHxS and PFOS) of the FA experiment differed from each other, with the sums of inner- and outer solution amount of PFOS converging with the absolute amount of the MeOH blanks whilst the PFHxS amount at 24 h and 48 h massively increased to far above the quantified amounts of the MeOH blanks (Fig. 10). However, at the end of the test the mass balances for both PFHxS and PFOS was 108 % and 110 % respectively. The FOSA mass balances (HA 76 % and FA 55 %) sum of inner- and outer solution amounts was consistently considerably lower than what would be expected as compared to the MeOH blank results. Mass balances of FTSAs (6:2 FTSA and 8:2 FTSA) was in the range of 85 – 108 % (Table 6) and varied to some extent for the absolute amounts of inner- and outer solutions but were overall in good agreement with the results from the MeOH blanks (Fig. 10).

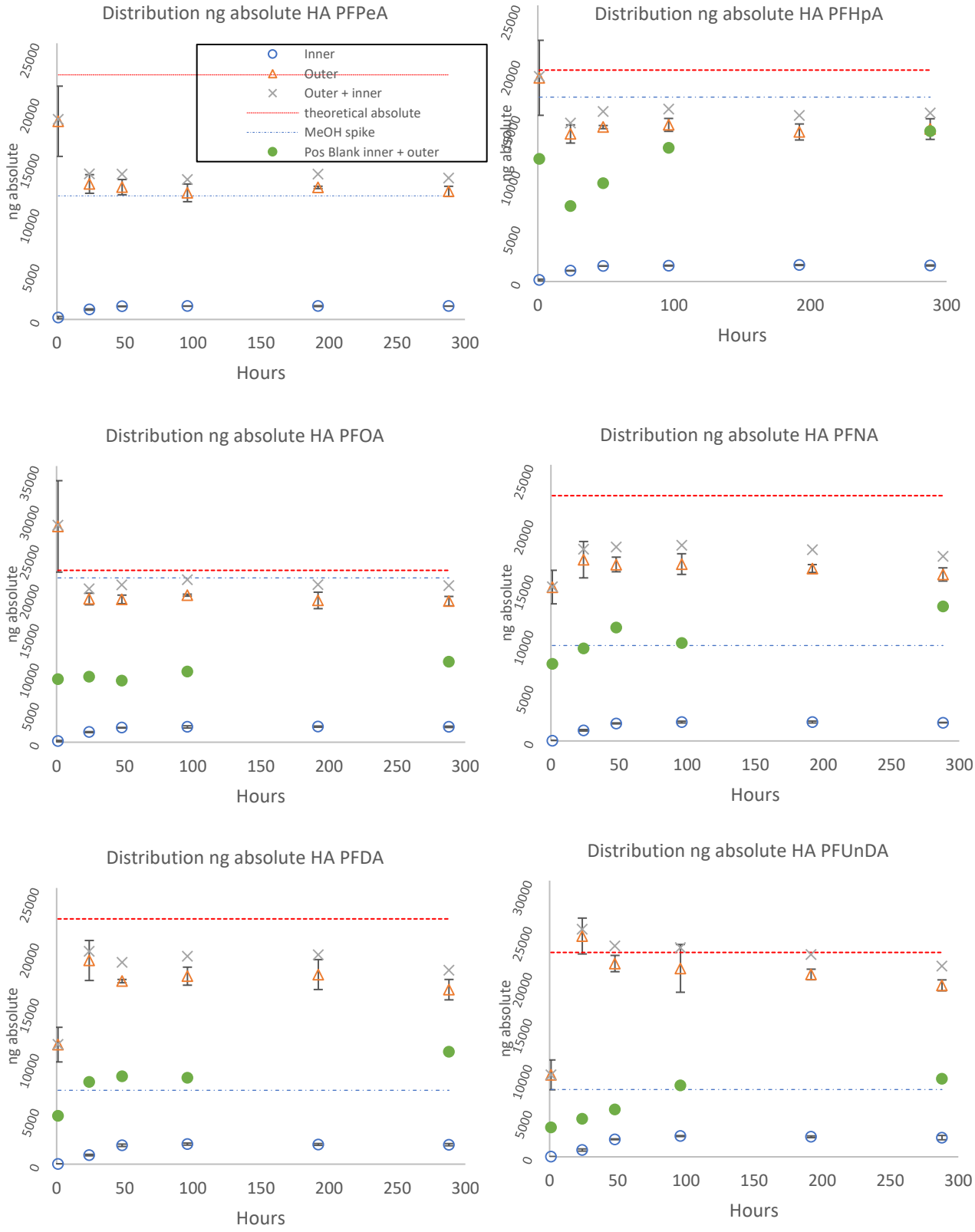

Figure 7. Absolute amounts (ng) of PFPeA, PFHpA, PFOA, PFNA, PFDA and PFUnDA of the MeOH blanks, inner-, outer solutions and the sum of inner- and outer solutions in the positive blanks and in the HA experiment. Whiskers represent standard deviations (*n = 3*). *pH 3.2.*

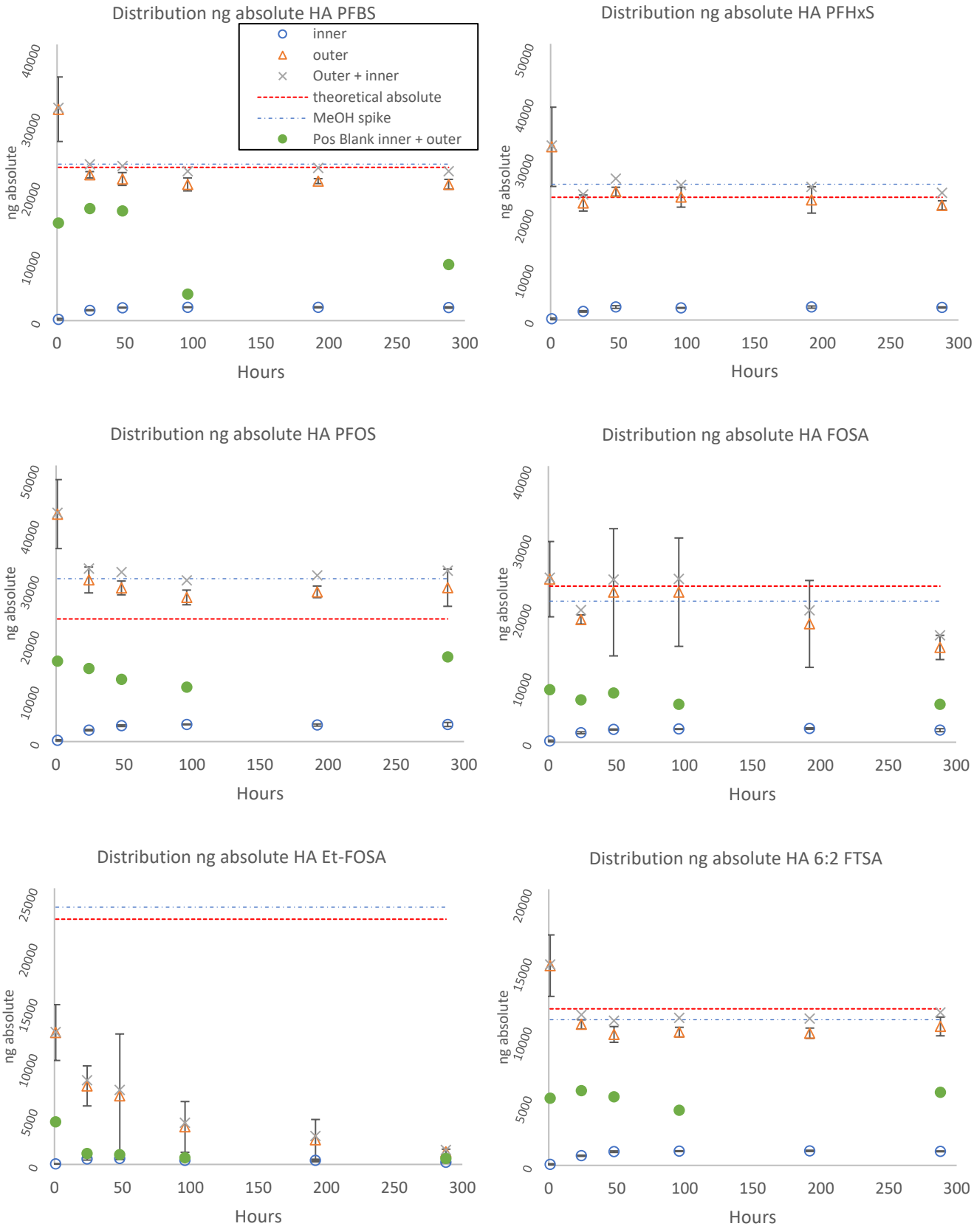

Figure 8. Absolute amounts of PFBS, PFHxS, PFOS, FOSA, EtFOSA and 6:2 FTSA of the MeOH blanks, inner-, outer solutions and the sum of inner- and outer solutions in the positive blanks and in the HA experiment. Whiskers represent standard deviations ($n = 3$). *pH 3.2.*

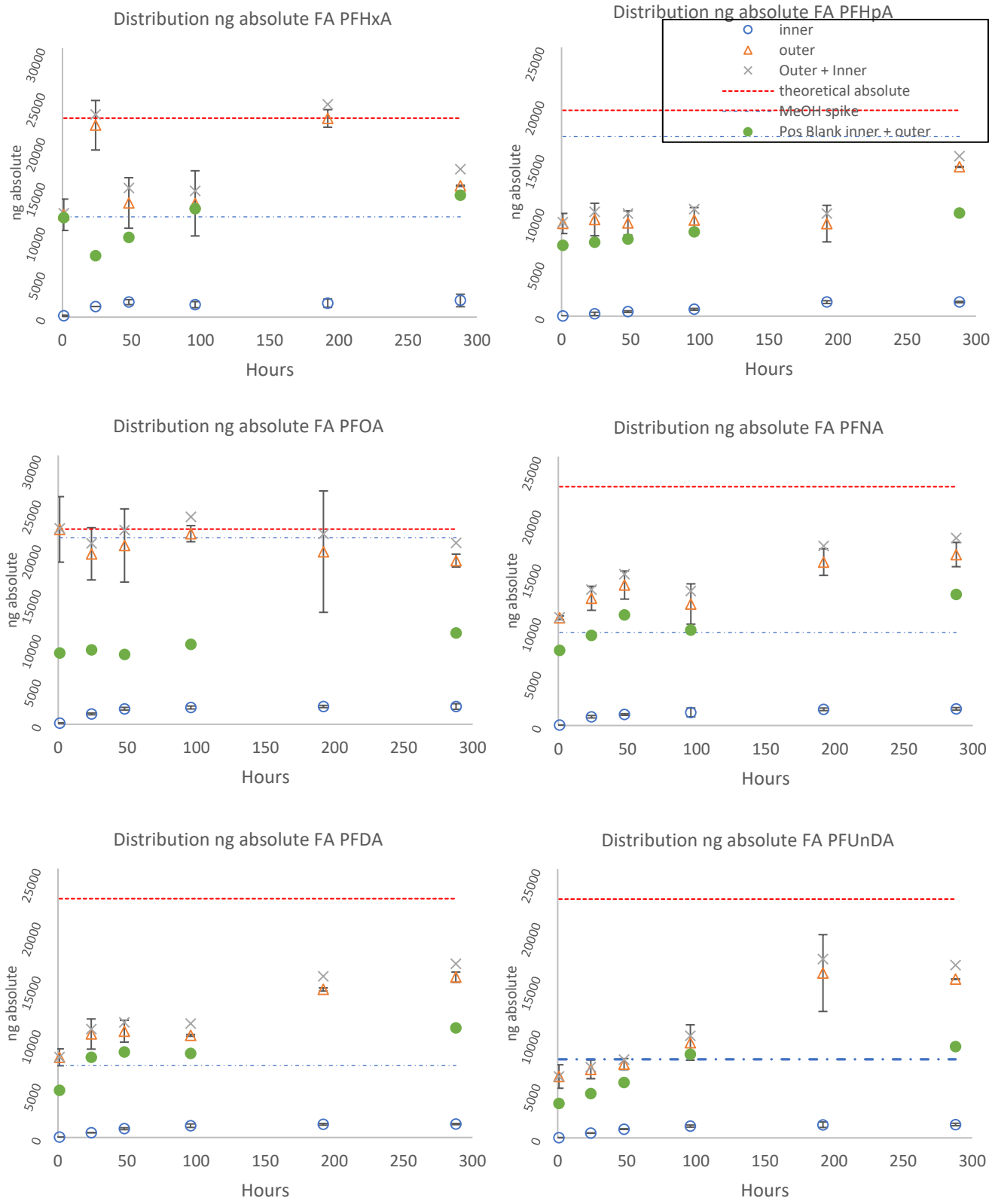

Figure 9. Absolute amounts of PFHxA, PFHpA, PFOA, PFNA, PFDA and PFUnDA of the MeOH blanks, inner-, outer solutions and the sum of inner- and outer solutions in the positive blanks and in the FA experiment. Whiskers represent standard deviations (*n = 3*). *pH = 4.0.*

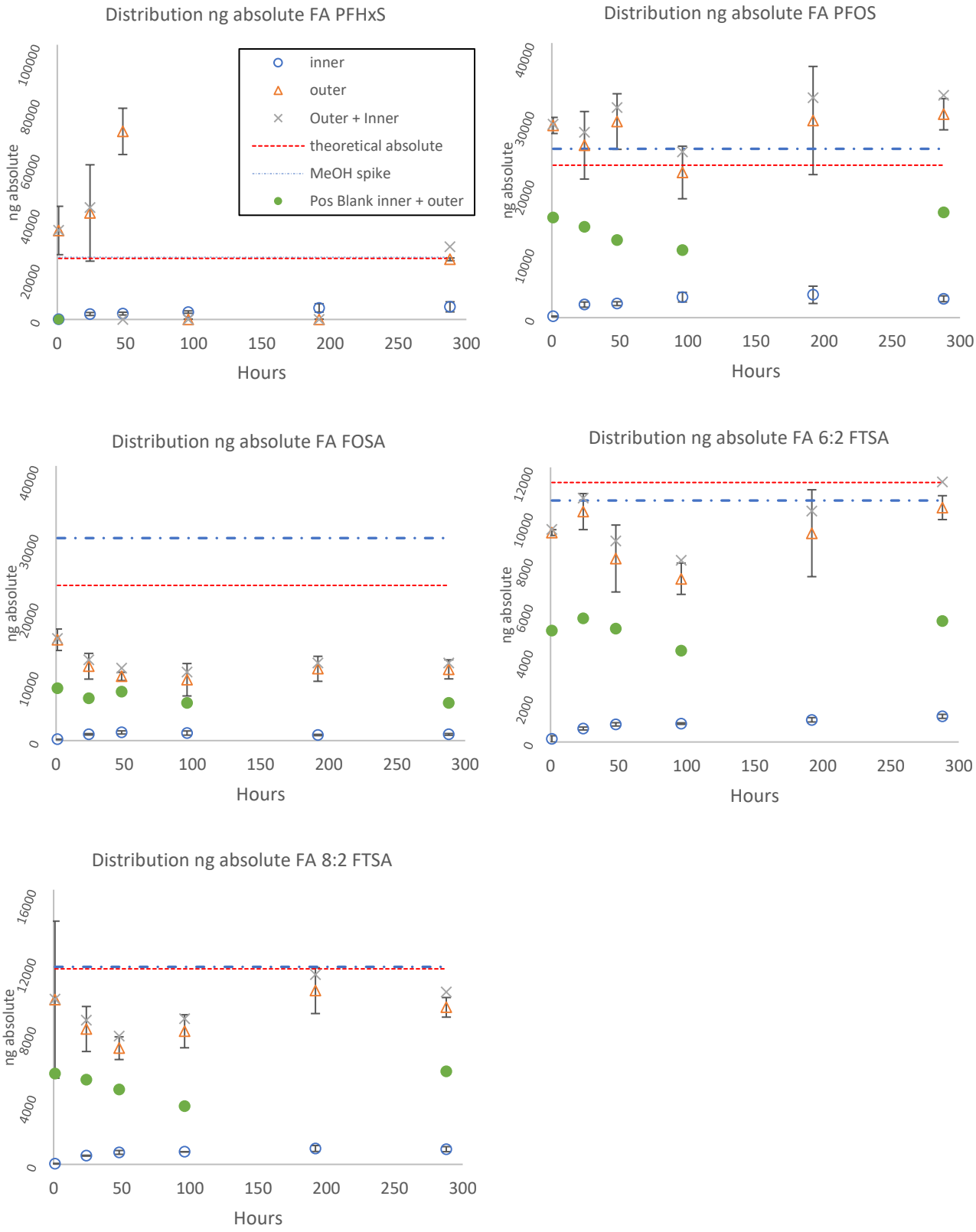

Figure 10. Absolute amounts of PFHxS, PFOS, FOSA, 6:2 FTSA and 8:2 FTSA of the MeOH blanks, inner-, outer solutions and the sum of inner- and outer solutions in the positive blanks in the FA experiment. Whiskers represent standard deviations (*n = 3*). *pH = 4.0.*

Sorption to DOM

In this dialysis experiment no PFAS sorption to the DOM fractions studied could be shown. A possible explanation for this can be found in Chiou *et al.* (1986) and their discussion about solubility enhancement due to DOM. Solubility enhancement would in this case mean apparent increases in solubility due to sorption of organic compounds to DOM. They showed that for DOM concentrations below 100 mg/l no solubility enhancement (or sorption) could be found for relatively water-soluble solutes, which is somewhat in line with what was shown in this study. Admittedly, the HA concentration used was 170 mg C/l but still no sorption was observed. Moreover, the effects of DOM solubility enhancement also only seem to be valid for highly water insoluble solutes which is not the case for any of the PFASs included in this study. Drawing on Chiou *et al.* (1986), lack of PFAS-sorption in this study might be related to that the concentrations of DOM simply was too low in order to significantly enhance the apparent solubility (which would be the same as sorption to the dissolved phase and true at least for the FA concentration). Another possibility could be that PFASs are too water-soluble to exhibit any of the partition-like behavior needed for sorption to the dissolved organic phases (Chiou *et al.*, 1986). Yet another line of thought could be that the polarity of the DOM and the charged functional head group of most PFASs results in such strong electrostatic repulsion that little sorption is observed. Though HA is less polar than FA both types of DOM might be too charged for PFASs sorption.

Spread in the results between HA and FA

As mentioned previously one of the main difference of the two data sets is the spread of the results in the equilibration trend and in each triplicate analysis for each compound (as exemplified in Fig. 1 and 5). It is in no way clear as to why this discrepancy occurs. The most obvious path would be to try to explain this difference from the inherently different characteristics of the DOM fractions; HA being more aromatic with less surface charge and FA having more surface charge but less aromaticity (Essington, 2015, pp. 179-190). Examining the supporting parameters (Table 5); the HA experiment was carried out at pH 3,2 ± 0,002 ($\overline{x}$ ± sd, $n = 3$) and the FA experiment at 4,0 ± 0,01 ($\overline{x}$ ± sd, $n = 3$). This low pH would most likely neutralize some of the variable charges on both DOM fractions. A reduction in surface charge would favor hydrophobic interactions between the aliphatic CF_2-chains of the PFASs and reduce the electrostatic repulsion that the negatively charged functional head groups gives rise to. However, since no sorption could be shown this does not explain the differences in the variability.

The data for DOC (mg/l) and SUVA (l/mg C m) during pre-dialysis shows that the DOM leached was less aromatic (SUVA 2,2 ± 1,5 for HA and 1,3 ± 1,7

($\bar{x}$ ± sd, *n = 3)* for FA, (Table 5). During the 12 day test the leached DOM became even less aromatic with an average SUVA of 0,7 ± 0,1 ($\bar{x}$ ± sd, *n = 3)* in the HA outer solution and 0,2 ± 0,1 ($\bar{x}$ ± sd, *n = 3)* in the FA outer solution. The inner solutions SUVA values were 6,1 ± 0,1 and 4,5 ± 0,1 ($\bar{x}$ ± sd, *n = 3)* for HA and FA respectively after the end of the experiment. The higher SUVA-value for HA was to be expected since HA is more aromatic than FA. It is hard to draw any conclusions from the characteristics of the leaching DOM that would explain the larger spread of the PFAS data observed between the HA- and FA-trials.

Speculative discussion on reasons for spread in the data

Since the separation of HA from SOM comes from first separating away the humin fraction and then precipitating it (the HA) out of solution leaving the FA fraction soluble, one might speculate that the molecular size range of the HA is limited to the confines of, on the upper end that which is still soluble after treatment with base and on the lower end that which is precipitated out of solution while adding acid. Perhaps one could argue that this in-betweenness of the HA fraction put firm limits on what size range the included molecules might have. If the FA contains molecules that cover a much larger size range, especially in the smaller domains, perhaps it is possible that some of these, probably smaller compounds might interact in such a way that analysis variance could increase.

5. Conclusion, implications and outlook

Conclusion

This kinetic dialysis study attempted to elucidate the PFAS sorption behavior to two different fractions of DOM, humic acid and fulvic acid. However, no sorption to either humic nor fulvic acid could be observed using this experimental set-up. Thus the hypothesis that PFAS-compounds binds to humic- or fulvic acid had to be rejected.

Environmental implications

Our results suggest that in soil water, which often contains smaller or larger concentrations of fulvic acid, dissolved PFASs are speciated as freely dissolved ions (or freely dissolved molecules in the case of non-dissociated PFASs such as FOSA). This implies that fulvic acid is not likely to act as a solubility enhancing component in the soil solution, or for that matter, not as a "transport vector" when PFASs are transported in the environment. The observed non-binding to humic acid in our experiments indicate that when PFASs are sorbed to solid-phase soil organic matter, other organic fractions, such as for example the lesser-charged humin-like components, are more likely to contribute to the binding of PFASs to the soil.

Improvements to the experimental scheme and future tests

There are many questions to be answered and the role of and interaction with both SOM and DOM for PFAS compounds are just only beginning to unravel.

If studies like this are to be performed again some suggestions for improvement might be to have triplicates that could be ended at each sampling occasion. This would mean that one experiment would comprise of 18 bottles (3 bottles * 6 sampling occasions = 18 bottles / experiment). In this way possible errors occurring from sampling would be limited to simply that sampling occasion and not propagated within the same solutions throughout the experiment. Another benefit of having triplicate set ups for every sampling occasion would be that it enables

measuring all supporting parameters as the trial is proceeding. Of course, the large downside would be the cost and time of preparing the set up for such an experiment

An outlook for continual work on dialysis experiments with DOM and PFAS might be to look at differing concentrations of DOM and see if perhaps variance of the PFAS analysis is DOM-concentration-dependent. Other interesting variants of dialysis studies could be using different pH values, ionic strengths and perhaps also to study more PFAS compounds.

6. References

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Acknowledgements

I would like to thank my supervisor Hugo de Campos Pereira for giving me the opportunity to work with him on this project and for having the patience with me during this very extended time of writing. I would also like to thank Vera Franke and Rickard Tröger for valuable conceptual inputs and ideas regarding potential problems with the experimental set up.

Appendix

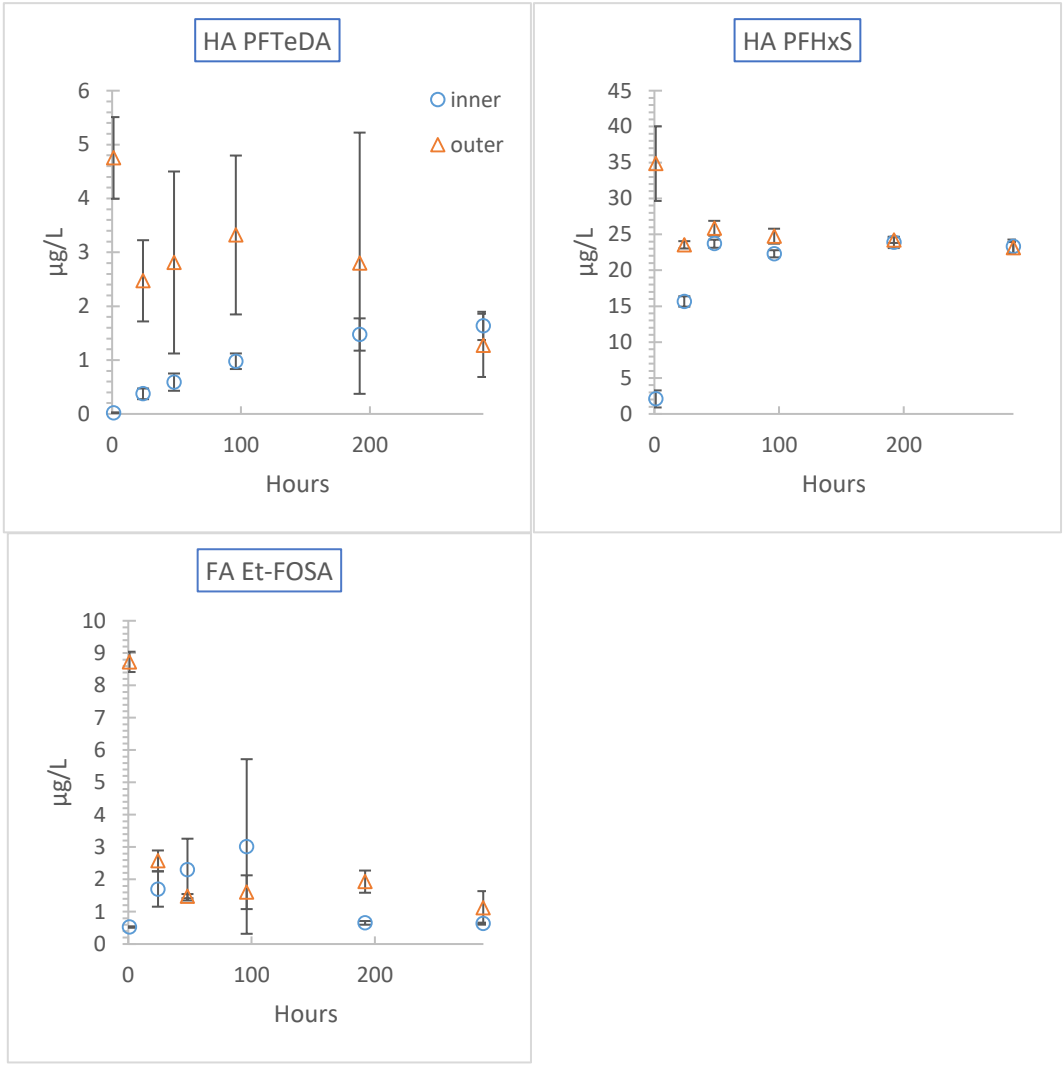

Figure 11. Distribution over time of PFTeDA and PFHxS with HA as inner solution and EtFOSA with FA as inner solution. Whiskers represent replicate standard deviations (n = 3), pH 3,2 (HA) and pH 4 (FA).

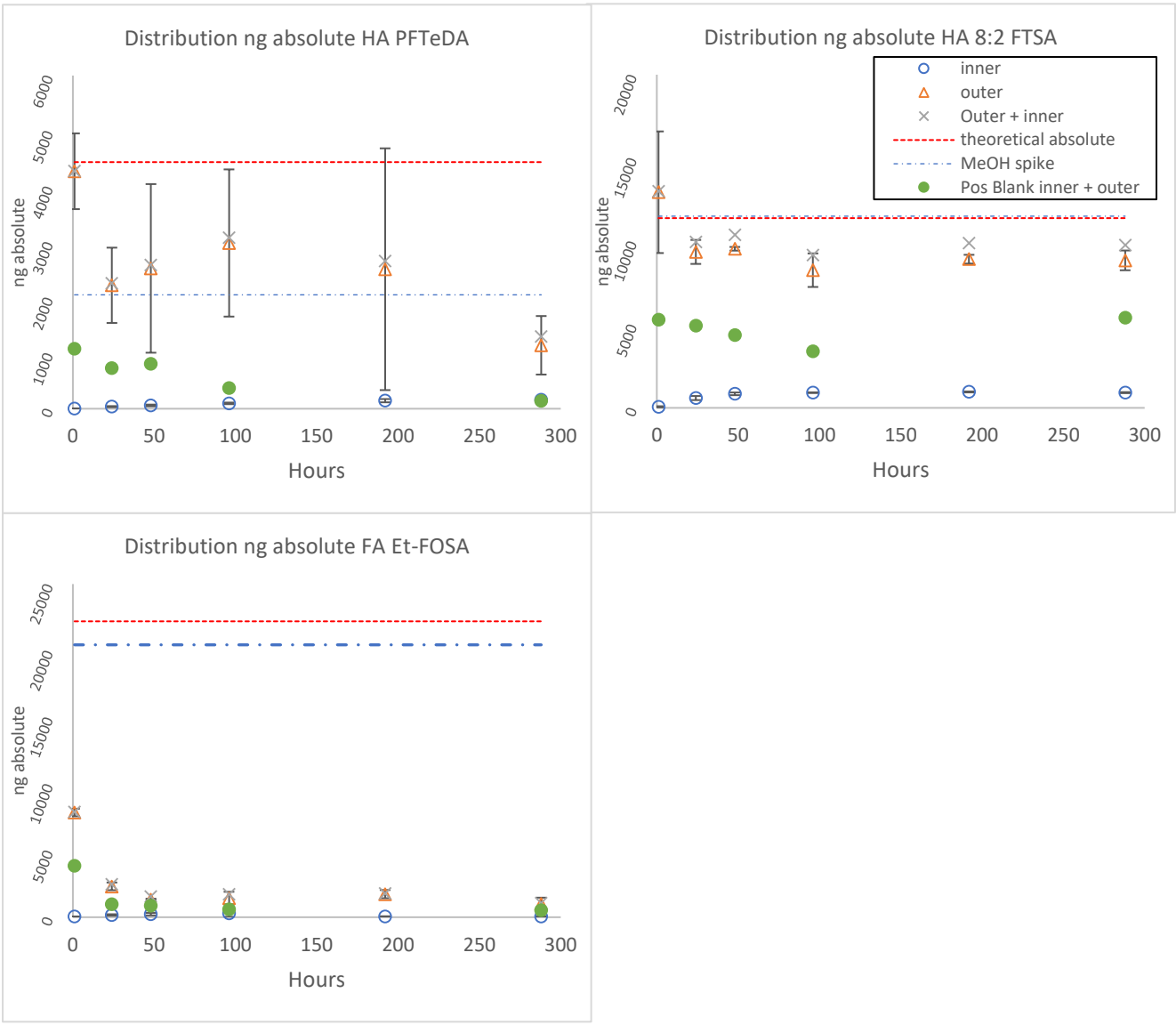

Figure 12. Absolute amounts of PFTeDA (HA), 8:2 FTSA (HA), EtFOSA (FA) and the MeOH spike, inner-, outer solutions and the sum of inner- and outer solutions of the positive blanks . Whiskers represent standard deviations (*n* = *3*), pH 3,2 (HA) and pH 4 (FA).

Sample	HA/FA	Replicate	Compartment	Hour	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTeDA
					ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}
Pos B inner 1 h	POS	B	Inner	1	0.025	0.637	0.605	0.339	0.124	0.06	0.064	0.037	0.006
Pos A inner 24 h	POS	A	Inner	24	0.404	7.829	4.215	3.019	2.891	2.195	0.719	0.351	0.035
Pos B inner 24 h	POS	B	Inner	24		4.403	3.165	3.565	3.675	1.736	0.669	0.626	0.029
Pos A inner 48 h	POS	A	Inner	48		7.494	4.906	5.145	5.833	4.444	1.919	0.847	0.072
Pos B inner 48 h	POS	B	Inner	48		6.912	6.013	5.508	5.959	3.783	1.977	0.674	0.059
Pos A inner 96 h	POS	A	Inner	96	0.025	7.207	8.4	9.159	7.08	6.397	4.067	1.104	0.101
Pos B inner 96 h	POS	B	Inner	96		12.463	6.212	7.86	7.76	6.18	4.6	1.247	0.1
Pos A inner 12 d	POS	A	Inner	288		6.28	7.847	11.717	9.187	8.562	8.702	3.244	0.054
Pos B inner 12 d	POS	B	Inner	288		15.698	9.451	12.39	9.998	6.712	8.835	2.895	0.071
Pos A outer 1 h	POS	A	Outer	1	0.017	10.028	6.517	7.777	7.081	4.971	2.972	2.344	1.216
Pos B outer 1h	POS	B	Outer	1	0.324	14.481	7.956	9.856	8.414	4.738	4.143	2.314	1.18
Pos A outer 24 h	POS	A	Outer	24		5.842	6.898	9.311	8.609	8.234	4.322	2.764	0.883
Pos B outer 24 h	POS	B	Outer	24	0.256	8.012	7.571	8.422	9.303	7.905	4.704	2.537	0.731
Pos A outer 48 h	POS	A	Outer	48		13.187	7.436	8.436	9.674	8.272	5.715	3.009	1.074
Pos B outer 48 h	POS	B	Outer	48	0.12	5.017	7.299	7.747	11.899	8.507	5.315	2.477	0.706
Pos A outer 96 h	POS	A	Outer	96		10.936	8.841	8.893	8.373	8.337	8.546	2.784	0.359
Pos B outer 96 h	POS	B	Outer	96	0.397	13.832	6.998	9.127	9.75	7.682	7.804	2.616	0.442
Pos A outer 12 d	POS	A	Outer	288	0.04	10.705	11.013	11.293	15.106	10.247	8.749	4.179	0.151
Pos B outer 12 d	POS	B	Outer	288	0.185	17.195	8.419	8.806	9.961	10.759	8.247	4.364	0.144
HA_A_inner_1h	HA	A	inner	1	1.007	1.027	0.718	0.806	0.189	0.116	0.129	0.056	0.015
HA_B_inner_1h	HA	B	inner	1	1.269	1.37	1.054	1.118	0.225	0.136	0.133	0.082	0.008

HA_C_inner_1h	HA	C	inner	1	0.291	0.31	0.251	0.277	0.056	0.045	0.045	0.028	0.004
HA_A_inner_24h	HA	A	inner	24	4.877	5.521	4.788	6.243	4.836	3.879	3.034	1.623	0.132
HA_B_inner_24h	HA	B	inner	24	4.349	4.819	4.916	6.266	4.52	4.007	3.949	2.211	0.198
HA_C_inner_24h	HA	C	inner	24	4.362	5.077	5.053	6.814	5.198	4.525	4.179	2.212	0.229
HA_A_inner_48h	HA	A	inner	48	6.1	7.021	7.22	9.185	7.784	8.58	9.379	4.687	0.279
HA_B_inner_48h	HA	B	inner	48	5.883	7.179	6.992	9.152	8.116	9.189	9.833	6.296	0.382
HA_C_inner_48h	HA	C	inner	48	5.941	6.911	7.075	9.521	8.176	8.084	9.661	4.838	0.224
HA_A_inner_96h	HA	A	inner	96	6.063	7.138	7.32	9.245	8.412	9.077	11.755	7.683	0.569
HA_B_inner_96h	HA	B	inner	96	6.216	7.222	7.109	9.68	9.158	9.744	11.351	7.736	0.47
HA_C_inner_96h	HA	C	inner	96	6.121	7.281	7.468	10.624	8.452	8.827	11.245	5.542	0.428
HA_A_inner_8d	HA	A	inner	192	6.252	7.418	7.817	9.927	8.847	8.785	10.96	7.282	0.714
HA_B_inner_8d	HA	B	inner	192	5.928	7.396	7.574	9.793	9.131	8.944	11.648	8.994	0.897
HA_C_inner_8d	HA	C	inner	192	6.301	7.248	7.436	10.524	8.253	9.586	10.703	7.486	0.6
HA_A_inner_12d	HA	A	inner	288	6.117	7.046	7.401	9.633	8.631	8.55	9.557	6.72	0.74
HA_B_inner_12d	HA	B	inner	288	6.19	7.404	7.699	10.489	8.462	8.998	10.603	7.729	0.742
HA_C_inner_12d	HA	C	inner	288	6.225	7.18	7.225	9.861	8.433	9.57	11.947	8.994	0.968
HA_A_outer_1h	HA	A	outer	1	9.626	10.395	9.613	13.663	7.024	5.639	4.453	4.131	2.219
HA_B_outer_1h	HA	B	outer	1	11.879	12.934	12.365	18.895	8.669	7.007	5.985	5.45	2.808
HA_C_outer_1h	HA	C	outer	1	8.394	9.269	8.755	13.029	7.524	5.387	4.399	3.879	2.1
HA_A_outer_24h	HA	A	outer	24	7.006	7.771	7.829	10.559	9.108	10.066	12.115	7.98	1.139
HA_B_outer_24h	HA	B	outer	24	7.162	8.116	7.515	9.804	10.041	11.334	13.695	11	1.651
HA_C_outer_24h	HA	C	outer	24	6.284	7.209	6.927	9.91	8.211	9.345	14.187	8.726	0.916
HA_A_outer_48h	HA	A	outer	48	6.854	7.728	7.859	9.971	8.488	9.137	11.534	6.41	0.848
HA_B_outer_48h	HA	B	outer	48	6.91	7.809	7.761	9.826	9.215	9.208	12.225	9.583	2.377

HA_C_outer_48h	HA	C	outer	48	6.219	7.271	7.704	10.405	8.954	9.303	11.273	6.482	0.991
HA_A_outer_96h	HA	A	outer	96	6.866	7.712	8.205	10.44	8.9	9.65	10.508	6.977	1.529
HA_B_outer_96h	HA	B	outer	96	5.996	7.079	7.905	10.302	9.435	9.806	13.059	10.18	2.455
HA_C_outer_96h	HA	C	outer	96	6.268	7.403	7.557	10.37	8.394	8.953	10.609	6.303	0.998
HA_A_outer_8d	HA	A	outer	192	6.662	7.429	7.58	9.916	8.856	9.059	10.669	6.545	0.754
HA_B_outer_8d	HA	B	outer	192	6.708	7.518	7.917	10.627	8.469	10.424	11.274	9.717	2.797
HA_C_outer_8d	HA	C	outer	192	6.591	7.39	7.113	9.476	8.745	9.186	11.147	5.934	0.645
HA_A_outer_12d	HA	A	outer	288	6.655	7.254	8.178	10.286	8.474	8.846	10.052	6.965	0.551
HA_B_outer_12d	HA	B	outer	288	6.17	7.064	7.743	9.971	8.685	9.29	10.714	7.477	0.963
HA_C_outer_12d	HA	C	outer	288	6.553	7.356	7.142	9.62	8.033	8.264	10.366	5.921	0.395
FA_A_inner_1h	FA	A	inner	1		0.467	0.56	0.59	0.21	0.111	0.057	10.802	0.026
FA_B_inner_1h	FA	B	inner	1		0.699	0.743	0.698	0.162	0.139	0.063	1.142	0.012
FA_C_inner_1h	FA	C	inner	1		1.164	0.537	0.677	0.251	0.132	0.097		0.011
FA_A_inner_24h	FA	A	inner	24		5.98	4.361	6.224	4.617	2.273	1.124	1.14	0.04
FA_B_inner_24h	FA	B	inner	24		5.933	3.503	6.016	4.236	2.251	1.124	16.552	0.046
FA_C_inner_24h	FA	C	inner	24	-2.602	5.963	2.821	5.268	3.48	2.233	0.929	4.383	0.011
FA_A_inner_48h	FA	A	inner	48		10.082	4.641	9.207	4.733	3.642	2.013	14.751	0.043
FA_B_inner_48h	FA	B	inner	48		7.746	4.71	9.205	5.454	4.553	2.152	0.716	0.099
FA_C_inner_48h	FA	C	inner	48		7.544	3.999	7.867	5.298	4.2	2.201		0.099
FA_A_inner_96h	FA	A	inner	96		6.626	5.661	10.387	8.58	5.919	3.185	17.176	0.112
FA_B_inner_96h	FA	B	inner	96		8.988	4.995	8.722	4.951	6.172	3.746		0.196
FA_C_inner_96h	FA	C	inner	96		5.6	4.86	9.434	4.994	4.635	2.748	1.396	0.091
FA_A_inner_8d	FA	A	inner	192		7.391	4.981	9.603	7.052	5.783	5.466	3.16	0.071
FA_B_inner_8d	FA	B	inner	192		5.887	5.217	9.946	7.704	6.417	8.065		0.055

FA_C_inner_8d	FA	C	inner	192	-6.526	10.516	3.968	10.846	8.284	6.548	6.447	0.942	0.037
FA_A_inner_12d	FA	A	inner	288		12.807	4.883	8.95	8.271	6.102	6.598	0.468	0.103
FA_B_inner_12d	FA	B	inner	288		5.725	5.442	12	8.29	6.454	6.166		0.062
FA_C_inner_12d	FA	C	inner	288		10.093	4.852	9.549	7.217	6.633	7.322	0.254	0.067
FA_A_outer_1h	FA	A	outer	1	-2.175	7.462	4.214	10.507	5.468	4.146	2.868	1.345	1.159
FA_B_outer_1h	FA	B	outer	1	2.07	5.689	4.912	11.367	5.655	4.581	2.776	0.12	1.108
FA_C_outer_1h	FA	C	outer	1	-7.19	5.885	5.232	14.373	5.578	3.705	3.87	1.529	1.121
FA_A_outer_24h	FA	A	outer	24		12.55	4.257	8.98	6.155	5.955	3.888	5.578	0.65
FA_B_outer_24h	FA	B	outer	24		13.008	5.908	12.22	7.288	4.468	3.752	0.42	1.16
FA_C_outer_24h	FA	C	outer	24		10.147	4.821	10.525	6.281	5.619	2.991	0.365	0.812
FA_A_outer_48h	FA	A	outer	48	0.854	7.15	4.101	9.018	7.079	5.532	3.811	-0.026	0.639
FA_B_outer_48h	FA	B	outer	48	-0.99	8.619	5.243	10.748	6.647	4.914	4.105	0.15	0.714
FA_C_outer_48h	FA	C	outer	48		5.485	5.111	13.523	8.065	6.042	3.532	3.694	0.659
FA_A_outer_96h	FA	A	outer	96		4.963	5.603	12.412	5.127	5.304	4.31	7.77	0.505
FA_B_outer_96h	FA	B	outer	96		8.999	5	11.507	7.143	5.32	5.987	0.082	0.544
FA_C_outer_96h	FA	C	outer	96		7.23	4.297	11.591	6.593	5.228	4.506	5.031	0.487
FA_A_outer_8d	FA	A	outer	192		11.802	5.785	14.407	8.836	7.664	8.055	10.469	0.289
FA_B_outer_8d	FA	B	outer	192	3.214	12.894	3.9	6.87	8.879	7.744	10.717	8.651	0.247
FA_C_outer_8d	FA	C	outer	192	10.682	12.336	4.688	10.905	7.665	7.581	6.828	10.373	0.216
FA_A_outer_12d	FA	A	outer	288									
FA_B_outer_12d	FA	B	outer	288	7.099	8.135	7.749	9.895	9.3	8.493	8.237	3.752	0.167
FA_C_outer_12d	FA	C	outer	288	7.273	8.191	7.715	10.461	8.41	8.117	8.213	3.834	0.151

Sample	HA/FA	Replicate	Compartment	Hour	PFBS	PFHxS	PFOS	FOSA	EtFOSA	6:2 FTSA	8:2 FTSA
					ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}	ng mL^{-1}
Pos B inner 1 h	POS	B	Inner	1	2.471		0.129	0.803	0.276	0.255	0.032
Pos A inner 24 h	POS	A	Inner	24	5.642	18.092	5.909	5.629	0.68	1.752	0.588
Pos B inner 24 h	POS	B	Inner	24	8.165	1.955	4.28	3.994	0.599	2.083	1.814
Pos A inner 48 h	POS	A	Inner	48	2.327	17.209	8.629	5.423	0.496	5.508	2.657
Pos B inner 48 h	POS	B	Inner	48	7.494	5.873	6.982	5.453	0.654	4.076	3.657
Pos A inner 96 h	POS	A	Inner	96	12.959	11.503	14.839	4.278	0.331	4.752	3.308
Pos B inner 96 h	POS	B	Inner	96	10.997		11.686	5.63	0.922	3.58	2.547
Pos A inner 12 d	POS	A	Inner	288	7.822	28.466	13.647	5.257	0.359	5.086	6.729
Pos B inner 12 d	POS	B	Inner	288	8.039	95.427	9.889	4.514	0.388	5.807	6.833
Pos A outer 1 h	POS	A	Outer	1	7.885	16.328	13.893	8.432	4.209	5.321	6.188
Pos B outer 1h	POS	B	Outer	1	22.997	7.116	18.433	8.301	4.299	5.433	5.547
Pos A outer 24 h	POS	A	Outer	24	10.89		14.778	6.73	0.973	6.709	5.532
Pos B outer 24 h	POS	B	Outer	24	23.675	4.26	13.523	5.861	1.064	4.881	5.167
Pos A outer 48 h	POS	A	Outer	48	22.849		10.806	7.51	1.141	6.326	4.599
Pos B outer 48 h	POS	B	Outer	48	11.466	3.602	12.573	7.128	0.688	3.649	4.421
Pos A outer 96 h	POS	A	Outer	96	1.561		10.118	5.6	0.61	4.493	3.759
Pos B outer 96 h	POS	B	Outer	96	4.424	1.858	8.87	5.475	0.601	3.469	3.136
Pos A outer 12 d	POS	A	Outer	288	2.526	9.746	18.061	5.486	0.634	5.835	5.538
Pos B outer 12 d	POS	B	Outer	288	13.831	-	13.467	5.633	0.491	4.757	5.044
HA_A_inner_1h	HA	A	inner	1	1.185	1.01	1.325	1.039	0.256	0.396	0.487
HA_B_inner_1h	HA	B	inner	1	1.57	1.797	1.674	1.468	0.36	0.612	0.333
HA_C_inner_1h	HA	C	inner	1	0.405	0.323	0.475	0.318	0.06	0.166	0.145
HA_A_inner_24h	HA	A	inner	24	7.779	7.512	10.761	7.551	2.369	3.589	3.23
HA_B_inner_24h	HA	B	inner	24	7.168	7.358	9.649	6.122	3.088	3.248	2.253
HA_C_inner_24h	HA	C	inner	24	7.805	8.612	10.789	6.944	2.052	3.65	3.395
HA_A_inner_48h	HA	A	inner	48	9.195	11.567	14.624	8.931	2.53	5.322	4.14
HA_B_inner_48h	HA	B	inner	48	9.685	10.777	13.731	9.216	3.204	4.76	3.914

HA_C_inner_48h	HA	C	inner	48	9.678	13.193	15.2	9.673	2.272	4.992	4.707
HA_A_inner_96h	HA	A	inner	96	9.572	10.879	16.214	9.934	2.004	5.145	4.647
HA_B_inner_96h	HA	B	inner	96	10.044	11.536	15.506	9.389	1.674	5.421	4.504
HA_C_inner_96h	HA	C	inner	96	9.951	11.022	15.66	9.733	1.875	5.155	4.709
HA_A_inner_8d	HA	A	inner	192	9.651	12.063	16.097	10.069	1.274	5.598	5.046
HA_B_inner_8d	HA	B	inner	192	9.622	12.886	15.533	10.637	2.47	5.385	4.752
HA_C_inner_8d	HA	C	inner	192	10.322	10.866	14.179	9.59	1.716	5.132	4.842
HA_A_inner_12d	HA	A	inner	288	9.217	11.666	14.51	8.24	0.775	5.371	4.68
HA_B_inner_12d	HA	B	inner	288	9.821	11.114	15.088	8.373	0.822	5.048	4.53
HA_C_inner_12d	HA	C	inner	288	10.192	12.155	17.993	10.212	1.241	5.243	4.85
HA_A_outer_1h	HA	A	outer	1	16.105	15.532	20.132	11.745	6.185	7.109	5.409
HA_B_outer_1h	HA	B	outer	1	19.941	22.001	26.781	16.594	8.205	9.44	9.398
HA_C_outer_1h	HA	C	outer	1	14.99	14.733	21.736	11.023	5.507	7.559	6.767
HA_A_outer_24h	HA	A	outer	24	11.671	10.937	17.712	9.596	3.112	5.779	4.803
HA_B_outer_24h	HA	B	outer	24	12.027	11.826	15.189	10.297	5.071	5.786	5.203
HA_C_outer_24h	HA	C	outer	24	11.533	12.55	15.897	9.75	3.676	5.464	5.604
HA_A_outer_48h	HA	A	outer	48	10.955	12.519	15.342	7.737	1.058	5.175	5.246
HA_B_outer_48h	HA	B	outer	48	11.973	12.85	16.226	17.732	6.976	5.02	5.319
HA_C_outer_48h	HA	C	outer	48	11.311	13.399	14.851	10.767	2.318	5.63	5.368
HA_A_outer_96h	HA	A	outer	96	11.319	11.248	14.105	7.76	0.589	5.159	5.145
HA_B_outer_96h	HA	B	outer	96	10.38	12.702	14.088	16.495	3.145	5.541	4.628
HA_C_outer_96h	HA	C	outer	96	11.239	13.164	15.379	12.027	1.935	5.412	4.025
HA_A_outer_8d	HA	A	outer	192	11.021	11.28	14.435	7.223	0.564	5.356	5.098
HA_B_outer_8d	HA	B	outer	192	11.357	11.393	15.298	13.571	2.422	5.522	4.809
HA_C_outer_8d	HA	C	outer	192	11.378	13.631	15.551	7.827	0.721	5.106	5
HA_A_outer_12d	HA	A	outer	288	10.948	11.623	17.679	8.454	0.783	6.041	4.919
HA_B_outer_12d	HA	B	outer	288	10.639	11.994	14.391	7.933	0.506	5.399	5.268
HA_C_outer_12d	HA	C	outer	288	11.426	11.086	14.482	6.56	0.593	5.375	4.608
FA_A_inner_1h	FA	A	inner	1	0.349	0.439	0.79	0.698	0.254	0.337	0.125
FA_B_inner_1h	FA	B	inner	1		0.58	0.861	1.077	0.259	1.472	0.083
FA_C_inner_1h	FA	C	inner	1	-0.064	0.501	1.212	0.855	0.275	0.218	0.319

FA_A_inner_24h	FA	A	inner	24	1.787	12.11	11.032	5.178	1.156	2.707	2.598
FA_B_inner_24h	FA	B	inner	24	2.28	10.863	10.199	4.152	0.761	3.318	2.639
FA_C_inner_24h	FA	C	inner	24	2.026	6.885	7.398	4.466	0.633	2.853	2.397
FA_A_inner_48h	FA	A	inner	48	7.373	9.471	11.61	4.85	0.632	3.517	3.329
FA_B_inner_48h	FA	B	inner	48	2.557	13.658	10.445	7.174	1.25	3.9	4.151
FA_C_inner_48h	FA	C	inner	48	2.34	8.994	8.823	5.972	1.572	4.253	3.083
FA_A_inner_96h	FA	A	inner	96	4.21	11.18	19.109	4.878	0.948	4.123	3.733
FA_B_inner_96h	FA	B	inner	96	0.69	15.483	12.315	7.364	3.049	4.23	3.702
FA_C_inner_96h	FA	C	inner	96	3.885	14.2	13.947	4.501	0.529	3.89	3.681
FA_A_inner_8d	FA	A	inner	192	6.341	13.317	14.447	4.69	0.331	4.609	3.705
FA_B_inner_8d	FA	B	inner	192	3.88	22.202	24.271	4.08	0.297	5.504	5.062
FA_C_inner_8d	FA	C	inner	192	8.823	28.775	12.193	3.718	0.354	4.724	5.382
FA_A_inner_12d	FA	A	inner	288	7.762	16.73	15.042	4.987	0.306	5.696	3.929
FA_B_inner_12d	FA	B	inner	288	2.454	19.621	15.676	5.068	0.309	5.328	4.319
FA_C_inner_12d	FA	C	inner	288	3.883	34.243	11.578	3.882	0.333	6.228	5.383
FA_A_outer_1h	FA	A	outer	1	5.143	12.346	14.803	7.345	4.261	5.003	8.265
FA_B_outer_1h	FA	B	outer	1	6.719	20.977	15.862	9.072	4.287	5.121	3.857
FA_C_outer_1h	FA	C	outer	1	3.224	20.66	15.988	8.064	4.545	5.127	3.868
FA_A_outer_24h	FA	A	outer	24	2.774	14.522	15.967	5.06	1.103	5.139	4.212
FA_B_outer_24h	FA	B	outer	24	6.667	17.432	10.837	7.126	1.364	5.636	5.183
FA_C_outer_24h	FA	C	outer	24	7.708	32.678	15.051	5.873	1.394	6.011	3.756
FA_A_outer_48h	FA	A	outer	48	1.345	41.397	13.552	4.891	0.706	5.388	3.883
FA_B_outer_48h	FA	B	outer	48	1.481	32.719	16.056	5.557	0.769	4.109	3.352
FA_C_outer_48h	FA	C	outer	48	2.382	40.116	18.044	5.153	0.748	3.878	4.06
FA_A_outer_96h	FA	A	outer	96	0.926	22.061	14.21	4.231	0.915	4.13	3.739
FA_B_outer_96h	FA	B	outer	96	1.93	64.585	10.767	6.448	0.987	4.243	4.798
FA_C_outer_96h	FA	C	outer	96	3.184	39.242	10.319	4.115	0.503	3.535	4.409
FA_A_outer_8d	FA	A	outer	192	2.06	7.75	10.963	5.263	0.886	4.778	4.928
FA_B_outer_8d	FA	B	outer	192	4.088	30.324	19.062	6.991	1.162	4.205	6.41
FA_C_outer_8d	FA	C	outer	192	7.821	1034.108	17.929	5.209	0.847	6.254	5.568
FA_A_outer_12d	FA	A	outer	288							

FA_B_outer_12d	FA	B	outer	288	12.103	11.997	17.402	6.328	0.743	5.91	4.872
FA_C_outer_12d	FA	C	outer	288	12.168	12.389	15.61	5.233	0.381	5.499	5.322

Sample	HA/FA	Replicate	Compartment	Hour	pH	Cond	DOC	Abs 254	SUVA
Pos A inner 12 d	POS	A	Inner	288	5.643	165.8	16.38	0.015664	0.095632
Pos B inner 12 d	POS	B	Inner	288	5.676	192.4	14.89	0.006124	0.04113
Pos A outer 1 h	POS	A	Outer	1	5.726	173.2	4.382	0.001283	0.029282
Pos B outer 1h	POS	B	Outer	1	5.706	166.7	0.3331	0.000235	0.070447
Pos A outer 12 d	POS	A	Outer	288	5.574	166.3	16.59	0.007898	0.047607
Pos B outer 12 d	POS	B	Outer	288	5.655	162.9	15.49	0.007433	0.047984
HA_A_inner_12d	HA	A	inner	288	3.134	1252	164.62	10.26049	6.232834
HA_B_inner_12d	HA	B	inner	288	3.134	1210	165.28	10.10635	6.114683
HA_C_inner_12d	HA	C	inner	288	3.13	1160	173	10.38017	6.000098
HA_A_outer_1h	HA	A	outer	1	3	607.7	5.907	0.073795	1.249273
HA_B_outer_1h	HA	B	outer	1	3	611.7	2.427	0.096033	3.956869
HA_C_outer_1h	HA	C	outer	1	3	600	4.785	0.066614	1.392152
HA_A_outer_24h	HA	A	outer	24	3.37				
HA_B_outer_24h	HA	B	outer	24	3.37				
HA_C_outer_24h	HA	C	outer	24	3.37				
HA_A_outer_12d	HA	A	outer	288	3.165	550.4	16.13	0.121251	0.751712
HA_B_outer_12d	HA	B	outer	288	3.169	567.4	15.73	0.127238	0.808887
HA_C_outer_12d	HA	C	outer	288	3.166	569.6	16.46	0.104299	0.633651
FA_A_inner_12d	FA	A	inner	288	3.947	580	32.4	1.463591	4.517256
FA_B_inner_12d	FA	B	inner	288	3.937	552	33.17	1.533719	4.623815
FA_C_inner_12d	FA	C	inner	288	3.936	545	34.96	1.554802	4.447375
FA_A_outer_1h	FA	A	outer	1	3.952	201.8	1.006	0.031736	3.154677
FA_B_outer_1h	FA	B	outer	1	3.947	208.3	4.325	0.023005	0.531903
FA_C_outer_1h	FA	C	outer	1	3.945	209.5	12.29	0.012778	0.103967
FA_A_outer_12d	FA	A	outer	288	3.967	213.4	15.14	0.036746	0.24271
FA_B_outer_12d	FA	B	outer	288	3.984	213.4	16.88	0.041737	0.247258
FA_C_outer_12d	FA	C	outer	288	3.966	211.9	16.38	0.016852	0.102881
HA_stam							172.08	11.57472	6.726359
FA_stam_25 mg/l DOC							29.29	1.959497	6.689985