Bioaccumulation of perfluoroalkyl compounds in marine echinoderms: Results of laboratory-scale experiments with *Holothuria tubulosa* Gmelin, 1791

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Declarations of interest: none

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ABSTRACT Bioaccumulation of six perfluorinated substances (PFASs) was assessed using the marine echinoderm Holothuria tubulosa Gmelin, 1791. Batch experiments were conducted to establish the relationship between concentrations in water, sediment and biota over 197 days. The sample treatment for the determination of compounds involves steps of lyophilization, solvent extraction and clean-up of the extracts with dispersive sorbents. PFASs were then analysed by liquid chromatography–tandem mass spectrometry. During contaminant exposure, detectable levels of compounds were found in all samples collected. Mean concentrations of selected PFASs were higher in sediments than in water samples. This fact is explained by the strong adsorption of these compounds into sediments. Sediment-water distribution coefficients (log Kd) were in the range 0.11 (PFBuA) to 2.46 (PFOA). Beside this, PFASs accumulation was observed in Holothuria tubulosa organisms. The uptake of PFASs was very rapid, reaching the maximum between 22-38 days of assay. Bioaccumulation factors (mean log BAF: 1.16 - 4.39) and biota sediment accumulation factors (mean log BSAF: 1.37 - 2.89) indicated a high bioaccumulation potential for the target compounds. Both parameters increased with perfluorinated carbon chain length ($R2 > 0.93$; $p < 0.05$). In organ-specific distributions of PFASs, greater concentrations were found in intestine than in gonads. Also, male specimens showed higher concentration levels than female (student t test: $t_{cal} = 2.788$, $t_{tab} = 2.262$; $p < 0.05$). These data provide a detailed accounting of PFASs fate and distribution in the marine environment highlighting accumulation at lower trophic levels, a potential source for contamination in higher organisms.

Keywords: Perfluoroalkyl compounds; *Holothuria tubulosa*; Environmental partitioning; Bioaccumulation studies

1. Introduction

Perfluorinated compounds (PFASs) are markedly surface-active agents with unique physicochemical properties due to the combination of a hydrophilic head group and a hydrophobic perfluorinated tail (Martin et al., 2003a). Due to their unique physicochemical properties, including thermal stability and water/oil resistance, PFASs are extensively used in fire-fighting foams, acid plating baths, and in many consumer products such as cleaners, polishes, lubricants, rust inhibitors, shampoos, and cosmetics (Cerveny et al., 2018; Kissa, 2001; Mudumbi et al., 2017). In the last decades, these compounds have been widely investigated due to their persistence, bioaccumulative potential and possible adverse effects on wildlife and humans (Cerveny et al., 2018; Mudumbi et al., 2017; Renzi et al., 2013; Sturm and Ahrens, 2010; Zhao et al., 2012;). Many scientific papers have reported the global distribution and ubiquitous detection in different environmental compartments: water (Campo et al., 2015, 2016; Hu et al., 2016; Flores et al., 2013; Myers et al., 2012; Pan et al., 2014; Pignotti et al., 2017), sediments (Campo et al., 2015, 2016; Gómez et al., 2011), biota (Bertín et al., 2014; Chen et al., 2016; Fujii et al., 2018; Gómez et al., 2011; Hart et al., 2008; Llorca et al., 2012; Pan et al., 2018), and even in human tissues (Kärrman et al., 2008; Martín et al., 2016a, 2016b; Sturm and Ahrens, 2010).

Because of the environmental concern of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the 3M Company voluntarily phased out the production in 2000, and replaced them with shorter-chain chemicals (Ahrens and Bundschuh, 2014; Lindstrom et al., 2011; Renner, 2006). In 2009, PFOS was listed as Persistent Organic Pollutant (Annex B, United Nations Environmental Program) and in 2013 the watch list of priority substances under the European Union Water Framework Directive (2000/60/EC) has been extended to include PFOS and its derivatives (2013/39/EU). The U.S. EPA has also

recently revised the Health Advisory Levels of PFOS and PFOA in drinking water to 70 parts per trillion (Hu et al., 2016; Munoz et al., 2017).

Aquatic sediment PFASs might also be important sources of contamination of aquatic food webs (Armitage et al., 2006; Beyer et al., 2017; Bertin et al., 2014; Higgins and Luthy, 2006; Hong et al., 2015; Houde et al., 2011; Lasier et al., 2011; Moon et al., 2010; Naile et al., 2010; Sedlak et al., 2017; Thompson et al., 2011; Wu et al., 2012). The distribution of water, sediment, and biota as well as the role of the sediment compartment in biota contamination is still poorly understood (Kwadijk et al., 2014). The length of the fluorocarbon chain is an important criterion for PFAS distribution in the environment (Bertin et al., 2014; Ahrens et al., 2009; Higgins and Luthy, 2006). PFASs with eight or more carbon atom backbones are known to be bioaccumulative (Bertin et al., 2014; Ahrens et al., 2011; Loi et al., 2011; Kelly et al., 2009; Kannan et al., 2005). Benthic invertebrates are often exposed to PFAS-contaminated sediments via ingestion of sediment particles. These organisms are key components of aquatic food webs and contribute significantly to fish diets (Jiang et al., 2015; Xing and Chia, 1997). The Holothuria, or sea cucumbers, are a diverse group of the phylum Echinodermata for the most part belonging to the benthic epifauna, living and foraging on the sea floor. They ingest and defecate large amounts of sediment and digest and absorb only the organic and living material. They play an important role in aquatic ecosystems since they stir up and oxygenate the sediment while feeding and mobilizing nutrients, therefore contributing to bioturbation (Kristensen et al., 2012; Uthicke, 2001). Moreover, sea cucumbers are excellent ecotoxicological sentinels because they can bioaccumulate micropollutants (Jiang et al., 2015; Martín et al., 2017; Sugni et al., 2007; Warnau et al., 2006; Xia and Chia, 1997; Xing and Chia, 1997). Additionally, sea cucumbers are considered as a delicacy in many Asian cultures, and are now harvested and traded in

more than 70 countries, which has led to overfishing and rapid worldwide depletion of stocks (Purcell et al., 2012).

Bioaccumulation Factor (BAF) and Biota-Sediment Accumulation Factor (BSAF) are parameters for understanding the partitioning of pollutants from water and sediment into ecological receptors, (Arnot and Gobas, 2006; Conder et al., 2012; Hong et al., 2015; Mackay et al., 2013; Naile et al., 2013; Rocha et al., 2018; Wilkinson et al., 2018; Xu et al., 2014; Zhang and Kelly, 2018). However, field-based BAF and BSAF data for PFASs are still limited, especially for short chain PFASs. Fluorocarbon chain length seems to be related to environmental distribution of PFASs (Ahrens et al., 2009; Bertin et al., 2014; Hong et al., 2015; Kannan et al., 2005; Kelly et al., 2009; Labadie and Chevreuil, 2011; Loi et al., 2011; Myers et al., 2012; Tomy et al., 2004; Zhang and Kelly, 2018; Zhao et al., 2014).

The main purpose of this study was to evaluate the bioaccumulation of six PFASs using the marine echinoderm *Holothuria tubulosa* Gmelin, 1791. Semi-static batch experiments, at three different spiked concentration levels (0.1, 0.5 and 1 mg L^{-1}), were conducted to establish the relationship between contaminant concentrations in water, sediment and biota over a 197 day period. The distribution and partitioning of PFASs between water and sediment (K_d) as well as the BAF and BSAF from intestine and gonads of *Holothuria tubulosa* specimens were calculated and compared with those from other aquatic organisms. To our knowledge, this is the first study on the bioaccumulation behaviour of PFASs in *Holothuria tubulosa*.

2. Materials and methods

2.1. Standards and reagents

All reagents were analytical grade unless otherwise specified. The PFASs, perfluorobutanoic acid (PFBuA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctane sulfonic acid (PFOS), and perfluorooctanoic acid (PFOA), were purchased from Sigma-Aldrich (Steinheim, Germany). The internal standard (IS) perfluorooctanoic acid-$^{13}C_4$ (PFOA-$^{13}C_4$) was supplied by Cambridge Isotope Laboratories (MA, USA). Mixtures of the studied compounds at different concentration levels were freshly prepared by appropriate dilutions of the stock standard in water:ethanol 95:5 (v/v). Water, ethanol, methanol, acetonitrile and acetone (chromatographic analysis grade) were purchased from Romil Ltd. (Barcelona, Spain). Octadecyl functionalized silica (C18) was provided by Sigma-Aldrich (Steinheim, Germany). Ammonium acetate was from Panreac (Barcelona, Spain).

2.2. Holothuria collection and acclimation

The specimens of *Holothuria tubulosa* Gmelin 1791 of a similar size (200 g average) were randomly collected by hand by SCUBA divers at a 10-15 m depth, in May 2016, in the infralittoral zone of San Cristobal Beach (Almuñécar, Granada, Southern Spain; GPS coordinates: 36° 44' 00.7" N; 3° 42' 17.9" W). Specimens were carried to the experimental facility in refrigerated containers at 4 ºC, without any damage. There they acclimated to the experimental conditions during two weeks.

2.3. Experimental conditions and design

Three independent experimental units were arranged at the aquarium facility of the Department of Zoology of the University of Granada (Granada, Spain). Each unit was

composed of nine polypropylene tanks with seawater supply in a closed recirculating system that contains a biological filter, protein skimmer, UV sterilizing unit, aeration system, chiller, heater and water pump. Tanks (42 x 36 x 25 cm) had 25 L capacity and 0.15 m^2 bottom surface. A 5 cm layer of silica sand (0.4-0.8 mm particle size) was placed on the bottom of the tanks. An artificial photoperiod of 12 h light/12 h dark was applied. Figure S1 shows the experimental aquarium.

The physicochemical characteristics of the water were monitored daily throughout the experimental period and remained approximately stable with mean values of temperature = 20 °C, salinity = 37 PSU (conductivity = 50.3 mS cm^{-1}), pH = 8.3, dissolved oxygen concentration = 6 mg L^{-1} (90 % saturation level), nitrates = 50 mg L^{-1}, nitrites = 0.1 mg L^{-1}, ammonium = 0.25 mg L^{-1}. The following instruments were used for the measurements: Hanna Instruments® digital thermometer (model HI Checktemp 1), HACH® sensION™ + EC5 portable conductivity/TDS meter with a conductivity probe (model 50 60), HACH® HQ40d portable multi meter with a pH probe (model pHC101) and a luminescent dissolved oxygen probe (model LDO101). Nitrate, nitrite and ammonium analyses were performed using Hanna instruments® Test Kits (HI 3874, 3873 and 3826, respectively). Water temperature was measured with an AKO® 14716 and a RENA® TE-2000 thermostats connected to HAILEA® HC130A water chillers and generic aquarium electric heaters.

Feeding. Holothuria specimens were fed once a week with 10 g minced dry *Laminaria* algae (ALGAMAR® Kombu).

Experimental design. Specimens (108) were randomly distributed in 3 batches of 36 specimens each one putting 4 animals per tank into the experimental units. After an acclimation period of two week, stock solutions of PFASs were added to each experimental unit to achieve contaminant doses of 1.0 mg L^{-1} (Batch-1), 0.5 mg L^{-1} (Batch-2) and 0.1 mg L^{-1} (Batch-3), with spiked water concentration in the three batches differing by a factor of 2 to

10. The concentration levels used were selected in base on the concentration levels measured previously in these organisms up to 3.97 ng/g for PFOA (Martín et al., 2017) and in order to observe clear tendency and differences between the spiked concentrations. The experiment lasted six months and sampling was conducted before contaminant administration and then on days 1, 8, 15, 22, 27, 38, 53, 86 and 197.

Sample collection. At each sampling, three Holothuria specimens and corresponding 10 mL aliquots of water and 1 g of sediment were collected, by hand, from each experimental unit using polypropylene bottles pre-cleaned with acetone and methanol. The digestive tracts as well as the gonads were dissected out from sea cucumbers (Figure S2) and the outer body wall was discarded. Dissected material was placed in sterile polythene bags and stored in the laboratory deep freezer at -40 ºC until analysis.

2.4. Quantification of PFASs

PFASs were extracted and analysed from water, sediment and holothuria samples using the method previously published by Martín et al. (2017) and Martín et al. (2014) briefly modified.

Holothuria (gonads and intestine) and sediment samples were freeze-dried, homogenized and grounded into powder. Aliquots of the samples (0.5 g) were weighed into 12 mL glass vials, containing 100 µL of a methanol solution (250 ng mL^{-1}) of $PFOA^{13}C_4$. The samples were vortexed twice for homogenization in 7 mL of acetonitrile for 2 min and centrifuged for 10 min at 4050 × g. For clean-up, disperse solid phase extraction (d-SPE) was applied. The supernatants obtained from the two extractions of each sample were combined in a 50 mL polypropylene conical tube containing 800 mg of C18 sorbent. The mixture was hand-shaken for 2 min and centrifuged for 5 min at 4050 × g. The solvent was evaporated to dryness at room temperature under a nitrogen stream and the extract was reconstituted in 0.25 mL of

methanol:water (50:50, v/v) and filtered through a 0.22 µm nylon filter. A 20 µL aliquot of the extract was injected into the LC instrument. Water samples were collected in brown bottles pre-cleaned with acetone and methanol. Prior to analysis, water samples were filtered through a 0.45 µm membrane filter. Subsequently 2 mL of sample was evaporated to dryness at room temperature under a nitrogen stream and the extract was reconstituted in 0.25 mL of methanol:water (50:50, v/v) and filtered through a 0.22 µm nylon filter. A 20 µL aliquot of the extract was injected into the LC instrument.

Water samples were filtered through a 0.45 µm membrane filter. Sample pH was adjusted to 2 by addition of sulfuric acid 40 % (v/v) and then PFOA$^{13}C_4$ was added to achieve a concentration of 100 ng mL^{-1}. Oasis HLB cartridges were conditioned using 3 mL of methanol, 3 mL of 0.5 N hydrochloric acid, and 3 mL of deionized water. The acidified sample (5 mL) was percolated through the cartridge at a flow rate of 10 mL min^{-1}. Then, the cartridges were washed with 3 mL of deionized water, dried for 10 min, and eluted with four aliquots of 1 mL of methanol at a flow rate of 1 mL min^{-1}. The eluates were collected in 10-mL collection tubes and evaporated to dryness at room temperature by a gentle nitrogen stream. Finally, the extracts were reconstituted in 0.25 mL of methanol:water (50:50, v/v) and filtered through a 0.22 µm nylon filter. A 20 µL aliquot of the extract was injected into the LC instrument.

Liquid chromatography-tandem mass spectrometry analysis was performed using a HALO C-18 Rapid Resolution (50 × 4.6 mm i.d., 2.7 µm particle size) column. The compounds were separated using a gradient mobile phase consisting of an aqueous buffer solution of acetic acid/ammonium acetate (pH 4.4) (solvent A) and methanol buffered with the same composition (solvent B). The gradient program was as follows: 0-14 min, linear gradient from 28 to 70 % of solvent B, from 70 % to 80 % of solvent B in 5 min, and then increased to 100 % in 6 min and held for 2 min. Flow rate was 0.6 mL min^{-1}; column

temperature was maintained at 30 ºC; injection volume was 20 µL. Two multiple reaction monitoring (MRM) transitions were selected for each analyte for quantification and confirmation of compounds. The mass spectrometer was operated in negative ESI mode. MS/MS features are shown in Table 1.

Table 1

The validation characteristics of the method (linearity and range, sensitivity, trueness and precision) are described in the Supplementary material and summarized in Table 2.

Table 2

4. Results and discussion

4.1. PFASs distribution in water and sediment

Water samples used in the experiment batches collected before spiking revealed trace amounts of PFASs (< 2 ng mL^{-1}), indicative of negligible transfer of contaminants to the water during the spiking process. During contaminant exposure, detectable levels of the six compounds were found in all samples. The mean (n=3) of the measured water concentrations of target compounds ranged between 0.03 ng mL^{-1} (PFOS) to 32.7 ng mL^{-1} (PFOA) in the low exposure batch (Batch-3), between 0.10 ng mL^{-1} (PFOS) to 139 ng mL^{-1} (PFBuA) in the medium exposure batch (Batch-2) and between 0.62 (PFOS) to 276 ng mL^{-1} (PFBuA) in the high exposure batch (Batch-1). PFAS concentration profiles were relatively similar throughout the exposure phase in the three batches. In the water compartment, PFBuA was the most abundant compound in all the experiments, while PFOS was the least abundant. The

mean and standard deviation of the concentration levels measured in water and sediment samples during the trials in the three batches are shown in the Supplementary materials Table S1. Figure 1 shows the fate profile of PFASs in Batch-1 over the 197 day experiments.

Figure 1

The compounds were detected at higher concentration in sediment samples than in water samples. These results suggest that sediments may be an important source of PFASs for benthonic biota. The mean (n=3) of the measured concentrations of individual target compounds ranged between 0.42 ng g^{-1} dry matter (d.m.) (PFOS) to 87.9 ng g^{-1} (d.m.) (PFBuA) in Batch-3, between 7.08 ng g^{-1} (d.m.) (PFHxS) to 152 ng g^{-1} (d.m.) (PFBuA) in Batch-2, and between 17.6 ng g^{-1} (d.m.) (PFHxA) to 695 ng g^{-1} (d.m.) (PFOS) in Batch-1. The kinetics of disappearing of C6-C8 and PFOS significantly fitted to an asymptotic model. A similar profile was also found between the spiking amount and the PFASs concentration in the aqueous phase for all target compounds, which suggests that spiking levels had no influence on the partitioning in the sediment.

A similar distribution of PFASs in water, sediment and biota were found in a study conducted by Campos et al. (2015) in the Llobregat River ecosystem (Mediterranean area, NE Spain). All samples were positive with at least one PFAS, being the most frequently found PFBuA, PFOA and PFOS. Mean PFAS concentrations measured in sediments (0.01–3.67 ng g^{-1}) and biota (0.79– 431 ng g^{-1}) samples were higher than those found in water (0.01–233 ng L^{-1}).

4.2. Partition between water and marine sediment

The field estimated partition factor between marine sediment and water was determined as $K_d=C_{sed}/C_w$; where C_{sed} is the concentration of PFASs measured on sediment in ng g^{-1} (d.w.) and C_w is PFASs concentration in the aqueous phase in ng mL^{-1} (Ahrens et al., 2011). Data on PFASs partitioning are shown in Table 3.

Table 3

The average log K_d of PFASs in Batch-1 was between 0.11 (PFBuA) and 2.46 (PFOS). No mayor differences were observed between the three spiked batches. Overall, the partition coefficient increased with PFAS length chain ($R^2>0.884$; $p<0.05$). The experimental data suggest that in the marine environment, PFASs are mainly adsorbed to suspended solids rather than to dissolved phase, with adsorption increasing with chain length.

K_d data are essential for modelling transport and environmental fate of contaminants (Ahrens et al., 2009, 2011; Higgins et al., 2007), but data in short chain PFASs are scarce (Labadie and Chevreuil, 2011). Yang et al. (2011) evaluated the occurrence and partitioning of PFASs in water and sediment from Liao River and Taihu Lake in China. The concentrations of PFOS and long chain perfluorocarboxylic acids in sediments were much higher than in water samples, indicating a preferential partition of these PFASs into sediment. The average log K_d of PFASs found was between 2.16 and 2.88. These values are similar to those reported by Higgins and Luthy (2006), who studied PFAS sorption to sediments and found log K_{oc} of PFOS and PFOA of 2.68 ± 0.09 and 2.11 cm^3 g^{-1}, respectively. They also reported that perfluorocarbon chain length was the key structural feature influencing sorption, with each CF_2 moiety contributing 0.50–0.60 log units to log K_{oc}, and the sulfonate moiety contributing 0.23 log units compared to carboxylate analogs. Field-based K_d and organic carbon-normalized sediment-water distribution coefficients (K_{oc}) were also determined for

PFASs in a coastal environment by Ahrens et al. (2011) and their results suggested that sediment characteristics have a crucial influence on PFAS sorption capacity.

4.3. PFAS bioacumulation in Holothuria tubulosa specimens

Before spiking, PFASs were detected in *Holothuria tubulosa* samples at concentration levels between 26 and 386 ng g^{-1} (d.w.). These concentrations were 100-fold lower than those found after eight days of exposure in Batch-1. All target PFASs were detected in the biota at higher levels than in water and sediment, which highlights the need for special attention to the potential effects of PFASs on these species. As with PFAS distribution in sediment, PFOS and PFOA were the two most abundant in *Holothuria* samples. Benthonic organisms are frequently exposed to sediment-associated PFASs by ingestion of sediment particles (Martín et al., 2017). PFAS uptake was rapid and after only 8 days of exposure to 1 mg L^{-1}, the intestine and gonads concentrations of PFOA were 196 and 37.1 mg kg^{-1} (d.w.), respectively. The mean and standard deviation of the concentration levels measured in intestine and gonad samples during the trials in the three batches are shown in the Supplementary materials Table S2. For most of the PFASs, concentrations in *Holothuria tubulosa* increased over time until day 38, as shown in Figure 1. At day 38 mean concentrations (n=3) of PFOA were up to 216 and 271 mg kg^{-1} (d.w.) in Batches 1 and 2, respectively. At day 197, PFOA concentration in *Holothuria tubulosa* organisms decreased to 1.6 and 6.6 mg kg^{-1} (d.w.) in Batches 1 and 2, respectively. Moreover, no significant differences were found between the spiked level in Batches 2 and 3. These results suggest that *Holothuria tubulosa* do not have an active mechanism for excretion of PFASs, which has also been reported for rainbow trout exposed to PFASs (Martin et al., 2003a). Higher concentrations of compounds were detected in the intestine of *Holothuria tubulosa* compared to gonads. As reported previously, PFASs

accumulate primarily in the intestine, blood or liver, but not in adipose tissues. This may be partially attributed to the inherent lipophobic properties of the fluorinated chain (Martin et al., 2003a). Similar results were reported by Hong et al. (2015) who found higher concentrations of PFASs in fish intestine compared to other organs and tissues such as liver or gills. The long half-life of compounds can be attributed to the fact that they are metabolically inert and that they enter enterohepatic recirculation, a process that is more effective as the fluorinated chain length increases (Martin et al., 2003b). Concentrations of the target compounds were significantly higher in male gonads than in female gonads (Student's *t* test: $t_{cal} = 2.788$, $t_{tab} = 2.262$; $p < 0.05$). This could be explained by the physiological differences between males and females that arise during the processes of maturation as well as by the different feeding behaviour (Cerveny et al., 2018; Shubert et al., 2016; Wang et al., 2013).

4.4. Bioaccumulation factors (BAFs), biota-sediment accumulation factors (BSAFs)

Differences have been found between the empirical BAF data available for individual PFASs in different organisms, probably due to the nature of field data. Thus, more field-based biological data should provide a better understanding of species-specific bioaccumulation characteristics (Hong et al., 2015; Zhao et al., 2014). In our work, BAF was calculated as C_{hol}/C_w (L kg^{-1}); where C_{hol} is the concentration in intestine or gonads of *Holothuria tubulosa* specimens on dry weight basis (ng g^{-1} d.w.), and C_w is freely dissolved concentration in water (ng mL^{-1}) (Table 3). Chemical bioacumulation is considered for BAF values > 1000, 2000 or 5000 L kg^{-1} by various regulatory authorities (Arnot and Gobas, 2006).

Our results show a clear correlation between BAF increases and higher K_{ow} and therefore also with perfluoroalkyl chain length. LogBAF values were positively correlated with the chain length in all organs ($R^2 > 0.93$; $p < 0.05$) (Figure 2). These findings are in agreement

with those reported by Kwadijk et al. (2010) for C7-C9 acids and C4/C8 sulfonates and with findings reported by Labadie and Chevreuil (2011) who calculated a correlation of $R^2 > 0.86$ for fish liver and muscle.

Figure 2

In some cases, the estimated log BAF for a given compound was dependent on the corresponding PFAS concentrations in spiked water. In particular, BAF values in Batch-1 were sometimes significantly lower than those found in Batch-3. This was the case for short-chain compounds, PFBuA, in intestine and gonads. However, as reported by Hong et al. (2015), BAF in Batch-3 might be underestimated because of the possible overestimation of freely dissolved PFAS in water.

As shown in Table 4, log BAFs for PFASs varied slightly between organisms. This might be due to the different capacities to accumulate and/or metabolize compounds of the different species. For example, according to the results by Hong et al. (2015), gastropods seem to accumulate shorter-chain PFASs (C6 to C9 perfluorocarboxylic acids and C4 to C6 perfluorosulphonic acids), but fish or shrimp tend to accumulate longer-chain compounds (C10 to C11 perfluorocarboxylic acids and C8 to C10 perfluorosulphonic acids). Kwadijk et al. (2010) determined field-based BAFs for PFAS in European eel (*Anguilla anguilla*) and suggested that PFASs with <7 fluorinated carbons are not bioaccumulative, in agreement with Xu et al. (2014), Lasier et al. (2011) and Conder et al. (2008). Wilkinson et al. (2018) assessed the spatial bioaccumulation of PFASs, pharmaceuticals, illicit drugs, plasticisers and metabolites in river sediment, aquatic plants and benthic organisms (amphipod crustaceans [*Gammarus pulex*] and aquatic snails [*Bithynia tentaculata*]) with similar results to those found in our work. While several studies have reported field-based BAFs for PFOS and

PFOA, there is less available literature on BAF data for short-chain PFASs, detected mostly in water. Nevertheless our results show that, short chain PFASs are also bioaccumulative.

Previous studies have also demonstrated that trophic dilution can occur and that lower-level organisms tend to have the highest BAFs when organisms at higher trophic levels are able to better metabolize a given substance (Arnot and Gobas, 2006). Therefore, the lower BAFs values of PFASs in higher trophic organisms (shrimp and fish) compared with benthic organisms or zooplankton might reflect the higher metabolic capacity of shrimp and fish. Similar behaviour has been reported for other families of emerging pollutants such as pharmaceutical active compounds (Xie et al., 2017). However, it should be noted that there are inherent difficulties in interpreting and comparing field BAFs. The concept of BAF assumes that the sampled organisms exist in a steady state with the surrounding water, but PFAS concentrations in water may vary significantly with time and location. In addition, mobile organisms such as fish are exposed to a wide range of PFAS concentrations in the water.

BSAF is other relevant parameter used to describe and predict bioaccumulation of organic pollutants in aquatic organisms through sediments (Zhao et al., 2014; Labadie and Chevreuil, 2011). BSAF was calculated as BSAF = C_{hol}/C_{sed}; where C_{hol} is the concentration in intestine or gonads of *Holothuria* specimens in ng g^{-1} (d.m.), and C_{sed} is the concentration measured in sediment in ng g^{-1} (d.m.) (Table 3).

As shown in Table 3, log BSAF values of PFASs vary considerably, from 1.80 (PFBuA) to 2.89 (PFOA) and from 1.37 (PFOA) to 1.71 (PFBuA) in Batch-1 for intestine and gonads, respectively. Relatively higher log BSAF values were found for PFOS and PFOA in intestine, while higher values were found for PFBuA in gonads. PFHxA showed the lowest accumulation. BSAF also increased with perfluorinated chain length (Figure 2, $R^2 > 0.92$), although this correlation became less significant at lower PFAS concentrations ($R^2 > 0.67$).

373 The relationship between bioaccumulation, fluorinated carbon chain length, and end
374 functional groups has also been reported in other aquatic organisms such as mussels (Liu et
375 al., 2011), rainbow trout (Martin et al., 2003a,b), common carp (Inoue et al., 2012), and
376 *Lumbriculus variegates* (Lasier et al., 2011). The general trend is observed as that BSAF
377 increased along with the increment of log K_{ow}, as in the relationship between log BAF and
378 log K_{ow}, BSAF increases were also observed with increasing log K_{ow}.

379 The comprehensive evaluation of bioaccumulation in freshwater and marine environment
380 of organic contaminants using BAFs and BSAFs is still limited (Zhang and Kelly, 2018).
381 Table 4 shows BAF and BSAF values reported in the literature for different organisms.

382

383 **Table 4**

384

385 Higgins et al. (2007) investigated the BSAF of PFASs for the first time using
386 *Lumbriculus variegatus* (oligochaete) to predict bioaccumulated compounds under controlled
387 conditions. Our results were in most cases slightly higher than those in oligochaete (-0.08 to
388 0.49) (Higgins et al., 2007; Lasier et al., 2011), oyster (-1.4 to 0) (Zhao et al., 2014), mussel (-
389 1.2 to -0.2) (Zhao et al., 2014), or fish (0.4 to 3.0) (Hong et al., 2015; Naile et al., 2013;
390 Pignotti et al., 2017). This finding suggests that benthic invertebrates may have a stronger
391 capacity to bioaccumulate PFASs than other organisms. Contaminants appeared to be
392 differentially bioaccumulative in different biota, indicating that there may be a need for a
393 species-specific BAF/BSAF classification system. This seems to be due to differences related
394 to food sources, feeding guild, uptake and excretion rates, and metabolism (Naile et al., 2010,
395 2013). This finding has been recently published (e.g., Hong et al., 2015; Lagesson et al.,
396 2016; Wilkinson et al., 2018) which suggests further research is warranted.

397 In the light of the increasing need to propose animal models different from vertebrate

models, the use of echinoderms represents a promising alternative for future bioaccumulation and ecotoxicological studies of emerging pollutants. It is unfortunate that the European Water Framework Directive (WFD) only uses fish species to assess compliance with Environmental Quality Standards (EQSs) stablished in fish, which are not adapted to other organisms.

4. Conclusions

The present work provides the first description of the partitioning and bioaccumulation behavior of six PFASs in marine environment using field estimated partition factor and various bioaccumulation metrics in *Holothuria tubulosa* specimens. Semi-static batch experiments spiked with compounds were conducted over a period of 197 days. Results suggest that sediments may be an important source of PFASs for benthic biota. Absorption and bioaccumulation of PFASs were strongly influenced by compounds structure, as demonstrated by the strong correlation between log K_d or log BAF and log BSAF and perfluoroalkyl chain length. Higher levels were observed in *Holothuria tubulosa* intestine and gonads than in water or sediments, which highlights the need for special attention to the potential effects on these species. PFASs concentrations were generally higher in the intestine than in gonads. Both the log BAFs and log BSAFs (>1.1) indicate potential bioaccumulation of PFASs in benthic aquatic organisms, even of short chain compounds. The highest mean log BAF in this work was for PFOS in the intestine of *Holothuria tubulosa,* which indicates that this is a highly bioaccumulative contaminant. BSAF values were generally slightly higher than BAF values, which may indicate that accumulation in the intestine occurs primarily via sediment rather than via water, while the opposite was observed in gonads. Furthermore, PFASs uptake by *Holothuria tubulosa* is concentration dependent, with higher sediment concentrations resulted in higher BSAFs.

We hope that these findings could provide essential information to support the risk assessment and management of these chemicals. There are still remaining knowledge gaps calling for further research and clarification, including the study of compounds whose uptake and accumulation mechanisms deviate from the standard partitioning model and the factors affecting the trophic transfer of pollutants in terms of metabolism and uptake routes.

Acknowledgements

This study was supported by the Spanish Ministry of Economy and Competitiveness (Project No. CTM2017-82778-R).

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701

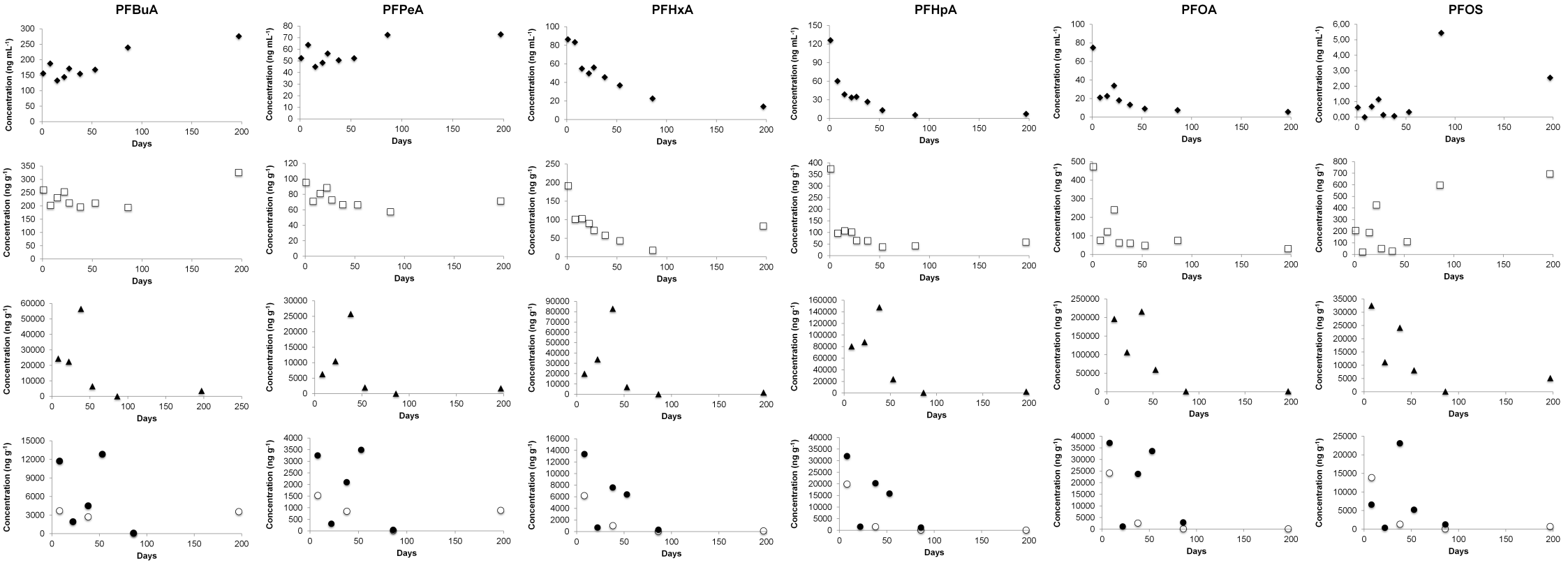
702 Figure Captions

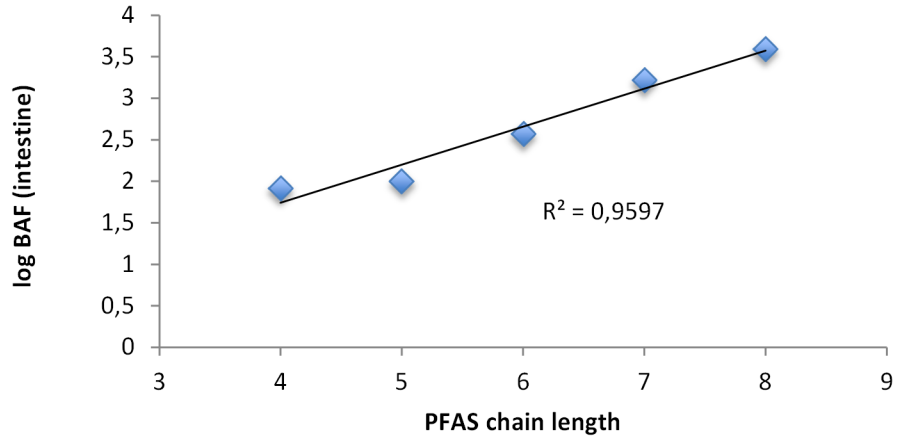
703

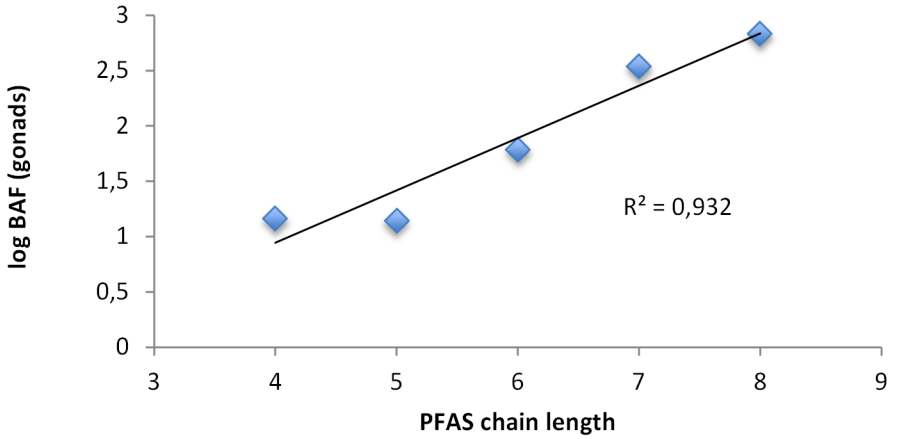
704 **Fig. 1.** Fate profile of PFASs in the high exposure tank along 197 days trial. ◆: water
705 samples; □ : sediment samples; ▲: intestine biota samples; ○: female gonad biota
706 samples; ● : male gonad biota samples.

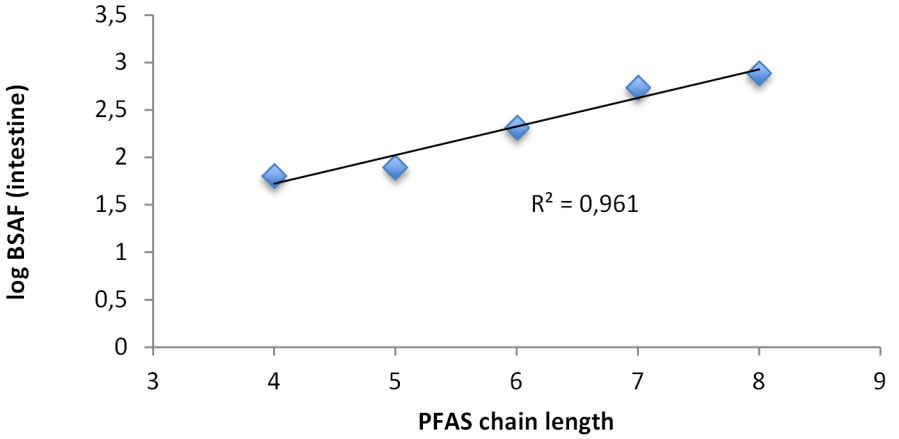
707

708 **Fig. 2.** Relationships between log BAF (top) / log BSAF (bottom) and perfluorinated carbon
709 chain length in intestine and gonad samples.


PFBuA
PFPeA
PFHxA
PFHpA
PFOA
PFOS
Concentration (ng mL-1)
Concentration (ng g-1)
Days


log BAF (intestine)
PFAS chain length
R² = 0,9597


log BAF (gonads)
PFAS chain length
R² = 0,932


log BSAF (intestine)
PFAS chain length
R² = 0,961

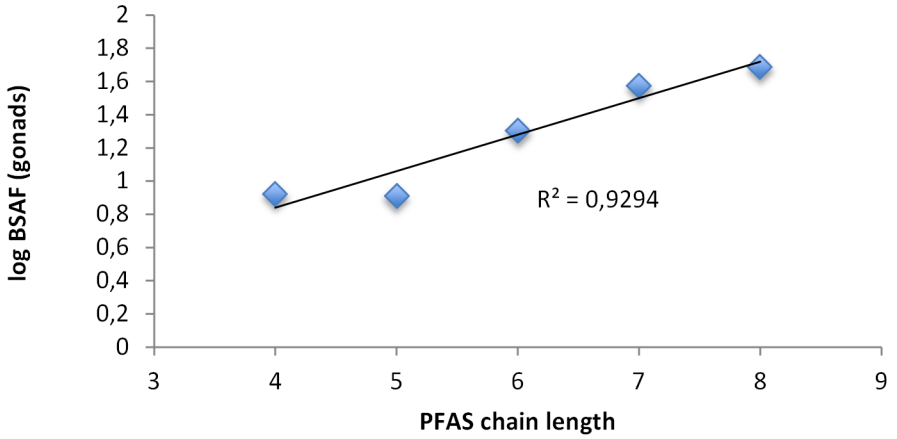

log BSAF (gonads)
PFAS chain length
R² = 0,9294

Table 1
MRM conditions and ESI mode used for LC-MS/MS of PFASs.

PFAS	Internal standard	MRM 1	MRM 2	Fragmentor (V)	Collision energy (eV)
PFBuA	PFOA-$^{13}C_4$	213> 169	213> 51	55	0
PFPeA	PFOA-$^{13}C_4$	263 > 219	263 > 69	68	0
PFHxA	PFOA-$^{13}C_4$	313 > 269	313 > 119	60	0
PFHpA	PFOA-$^{13}C_4$	363 > 319	363 > 169	68	0
PFOA	PFOA-$^{13}C_4$	413 > 369	413 > 169	68	4
PFOS	PFOA-$^{13}C_4$	499 > 80	499 > 51	145	40

MRM 1: transition used for quantification; MRM 2: transition used for confirmation

Table 2
Limit of detection and limit of quantification, precision and recovery of target compounds in *Holothuria tubulosa* specimens, sediment and water samples.

PFASs	*Holothuria* specimens				Sediment samples				Water samples			
	LOD (ng g^{-1} d.w.)	LOQ	RSD (%)	Rec (%)	LOD (ng g^{-1} d.w.)	LOQ	RSD (%)	Rec (%)	LOD (ng mL^{-1})	LOQ	RSD (%)	Rec (%)
PFBuA	0.008	0.03	12	97	0.008	0.03	0.2	101	0.004	0.02	6.7	89
PFPeA	0.008	0.03	3.2	103	0.008	0.03	8.0	99	0.004	0.02	13	100
PFHxA	0.008	0.03	2.2	92	0.008	0.03	8.5	94	0.004	0.02	2.3	102
PFHpA	0.008	0.03	15	88	0.008	0.03	5.7	92	0.004	0.02	6.3	90
PFOA	0.008	0.03	5.5	96	0.008	0.03	1.8	84	0.004	0.02	6.3	93
PFOS	0.008	0.03	1.3	88	0.008	0.03	9.4	74	0.004	0.02	9.0	93

LOD: Limit of detection; LOQ: Limit of quantification; RSD: Relative Standard Deviation; Rec: Recovery

Table 3
K_d (n=27), BAF (n=15) and BSAF (n=15) values of PFASs measured in *Holothuria tubulosa* at three exposure concentrations.

Batch	**log Kd**		**log BAF (intestin)**		**log BAF (gonads)**		**log BSAF (intestin)**		**log BSAF (gonads)**	
Batch-1 (1 mg L^{-1})	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
PFBuA	-0.09–0.22	0.11	1.11–2.56	1.91	-0.45–1.88	1.16	1.04–2.08	1.80	1.28–2.25	1.71
PFPeA	-0.10–0.26	0.12	1.37–2.71	2.00	-0.23–1.82	1.14	1.38–2.59	1.90	1.21–2.14	1.67
PFHxA	-0.11–0.35	0.21	2.09–3.26	2.57	1.13–2.24	1.79	1.32–3.16	2.31	1.29–2.07	1.70
PFHpA	0.21–0.90	0.50	2.55–3.74	3.22	1.66–3.08	2.54	1.65–3.36	2.74	1.34–1.73	1.53
PFOA	0.54–1.00	0.73	2.57–4.21	3.59	1.51–3.57	2.83	1.73–3.56	2.89	1.10–1.48	1.37
PFOS	2.04–2.57	2.46	3.31-5.54	4.39	2.48–5.52	3.72	0.87–2.93	2.06	1.26–2.28	1.63
Batch-2 (0.5 mg L^{-1})	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
PFBuA	-0.58–0.24	-0.09	1.50–2.79	1.82	1.27–1.67	1.54	1.33–2.75	1.95	1.73–2.07	1.87
PFPeA	-0.61–0.27	-0.10	1.52–2.93	1.99	1.38–1.53	1.49	1.74–2.89	2.14	1.40–2.48	1.81
PFHxA	-0.09–0.35	0.13	2.25–3.73	2.86	1.61–2.37	1.89	2.11–3.49	2.72	1.15–2.22	1.78
PFHpA	0.44–1.00	0.74	2.84–4.31	3.34	2.01–2.73	2.34	2.19–3.48	2.59	0.79–1.85	1.58
PFOA	0.63–1.43	0.90	2.52–4.52	3.39	1.68–2.51	2.18	1.74–3.49	2.59	0.93–1.81	1.51
PFOS	0.79–2.54	1.91	3.28–4.91	4.08	2.77–4.06	3.53	1.79–2.85	2.35	1.32–2.13	1.58
Batch-3 (0.1 mg L^{-1})	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
PFBuA	-0.17–0.36	0.12	1.59–2.97	2.23	1.70–2.69	2.08	1.35–2.96	2.08	-0.56–1.78	0.92
PFPeA	-0.30–0.33	0.04	1.63–3.10	2.32	1.54–2.44	1.95	1.62–3.21	2.25	-0.63–1.66	0.91
PFHxA	0.02–0.53	0.33	2.06–3.34	2.86	1.45–2.67	2.14	1.64–3.18	2.49	0.02–2.12	1.30
PFHpA	0.27–0.91	0.75	2.33–4.22	3.35	1.61–3.09	2.51	1.62–3.41	2.55	0.10–2.63	1.57
PFOA	0.37–1.19	0.80	2.41–4.38	3.37	1.72–3.07	2.40	1.21–3.55	2.48	0.13–2.84	1.69
PFOS	1.08–1.98	2.00	3.74–4.67	4.23	3.46–4.65	4.11	1.89–3.39	2.09	-0.09–2.83	1.48

Table 4
Literature log K_d, log BAF and log BSAF values for PFASs measured in aquatic organisms.

PFCs	Log K_d	Log BAF	Log BSAF	Biota	Reference
PFBuA	-0.09–0.22	1.11–2.56	1.04–2.08	Echinoderm (*Holuthuria tubulosa* intestine)	This work
		-0.45–1.88	1.28–2.25	Echinoderm (*Holuthuria tubulosa* gonads)	This work
	0.70–3.23				Campo et al., 2016
	0.99–3.37	0.95-3.58		Fish (*Barbus graellsii, Ccyprinus carpio, Micropterus salmoides*)	Campo et al., 2015
			-1.4–-0.7	Oyster	Zhao et al., 2014
			-1.2–-0.3	Mussel	Zhao et al., 2014
PFPeA	-0.10–0.26	1.37–2.71		Echinoderm (*Holuthuria tubulosa* intestine)	This work
		-0.23–1.82	1.21–2.14	Echinoderm (*Holuthuria tubulosa* gonads)	This work
	1.11				Pico et al., 2012
	2.44–4.82	6.38		Fish and eel (*Salmo trutta, Gobio lonzanoi, Pseudochrondrostoma polylepis, Mycroptero salmoides, Barbus guiraonis, Lepomis gibbosus, Alburnus alburnus, Esox lucius, Anguila anguila*)	Campo et al., 2016
	2.37-2.43	3.53–3.94		Fish (*Barbus graellsii, Ccyprinus carpio, Micropterus salmoides*)	Campo et al., 2015
PFHxA	-0.11–0.35	2.09–3.26	1.32–3.16	Echinoderm (*Holuthuria tubulosa* intestine)	This work
		1.13–2.24	1.29–2.07	Echinoderm (*Holuthuria tubulosa* gonads)	This work
	1.18				Pico et al., 2012
	0.80				Labadie and Chevreuil, 2011
		1.4		Fish (*Cyprinus carpio*)	Pignotti et al., 2017
		1.3		Fish (*Liza sp*.)	Pignotti et al., 2017
				Fish (*R. rutilus*)	Pignotti et al., 2017
		0.42		Fish (*S. erythropthalmus*)	Pignotti et al., 2017
		1.1		Fish (S. glanis)	Pignotti et al., 2017
		0.90		Fish (*Squalius laietanus*)	Pignotti et al., 2017
		1.8		Fish (*A. alburnus*)	Pignotti et al., 2017
		1.8		Fish	Hong et al., 2015
		2.4		Bivalve	Hong et al., 2015
		1.6		Crab	Hong et al., 2015
		2.2		Gastropod	Hong et al., 2015
		1.8		Shrimp	Hong et al., 2015
PFHpA	0.21–0.90	2.57–4.21	1.65–3.36	Echinoderm (*Holuthuria tubulosa* intestine)	This work
		1.66–3.08	1.34–1.73	Echinoderm (*Holuthuria tubulosa* gonads)	This work
	1.97				Campo et al., 2016
	1.26				Pico et al., 2012
	0.8	1.60	-0.50	Fish (*Leuciscus cephalus*)	Labadie and Chevreuil, 2011
		1.9		Fish	Hong et al., 2015
		1.8		Bivalve	Hong et al., 2015
		1.5		Crab	Hong et al., 2015
		1.9		Gastropod	Hong et al., 2015
		1.8		Shrimp	Hong et al., 2015
		0.75–2.26		Fish (*Hemigrapsus sanguineus, Sesarma pictum, Hemigrapsus penicillatus, Helice tridens tridens,*	Naile et al., 2013

				Philyra pisum)	
		1.69		Bivalve (*Mytilus edulis, Mactra veneriformis, Nuttallia olivacea, Sinonovacula constricta*)	Naile et al., 2013
		1.14–1.61		Crab (*Acanthogobius flavimanus, Sebastes schlegeli, Tridentiger obscurus, Hexagrammos otakii, Mugil cephalus*)	Naile et al., 2013
		1.81		Gastropod (*Littorina brevicula, Monodonta labio, Umbonium thomasi, Glossaulax didyma, Monodonta labio*)	Naile et al., 2013
			0.18	Oligochaete (*Lumbriculus variegatus*)	Lasier et al., 2011
PFOA	0.54–1.00	2.57–4.21	1.73–3.56	Echinoderm (*Holuthuria tubulosa* intestine)	This work
		1.51–3.57	1.10–1.48	Echinoderm (*Holuthuria tubulosa* gonads)	This work
	1.71–4.56				Campo et al., 2016
	1.27–2.91	2.91		Fish (*Barbus graellsii, Cyprinus carpio, Micropterus salmoides*)	Campo et al., 2015
	0–2.13				Pan et al., 2014
	1.55				Pico et al., 2012
	2.28				Yang et al., 2011
	2.17–2.54				Ahrens et al., 2010
	2.18–2.48				Zhou et al., 2010
	1.83				Kwadijk et al., 2010
	2.11				Higgins and Luthy, 2006
		2.21	2.76	Aquatic snails (*Bithynia tentaculata*)	Wilkinson et al., 2018
		1.75	2.76	Amphipod crustaceans (*Gammarus pulex*)	Wilkinson et al., 2018
		2.2		Fish (*Cyprinus carpio*)	Pignotti et al., 2017
		2.0		Fish (*Liza sp.*)	Pignotti et al., 2017
		2.1		Fish (*R. rutilus*)	Pignotti et al., 2017
		1.9		Fish (*S. erythropthalmus*)	Pignotti et al., 2017
		2.1		Fish (*S. glanis*)	Pignotti et al., 2017
		2.0		Fish (*Squalius laietanus*)	Pignotti et al., 2017
		2.3		Fish (*A. alburnus*)	Pignotti et al., 2017
		1.9		Fish	Hong et al., 2015
		2.1		Bivalve	Hong et al., 2015
		2.6		Crab	Hong et al., 2015
		2.5		Gastropod	Hong et al., 2015
		1.8		Shrimp	Hong et al., 2015
		0.99–1.82		Fish	Xu et al., 2014
		1.47		Zooplankton	Xu et al., 2014
		1.94		Phytoplankton	Xu et al., 2014
			-0.90	Oyster	Zhao et al., 2014
		1.95		Crustacean (*Daphnia magna*)	Dai et al., 2013
		1.05-2.24		Fish (*Hemigrapsus sanguineus, Sesarma pictum, Hemigrapsus penicillatus, Helice tridens tridens, Philyra pisum*)	Naile et al., 2013
		1.65		Bivalve (*Mytilus edulis, Mactra veneriformis, Nuttallia olivacea, Sinonovacula constricta*)	Naile et al., 2013
		1.47–1.89		Crab (*Acanthogobius flavimanus, Sebastes schlegeli, Tridentiger obscurus, Hexagrammos otakii, Mugil cephalus*)	Naile et al., 2013
		1.70		Gastropod (*Littorina brevicula, Monodonta labio, Umbonium thomasi, Glossaulax didyma, Monodonta labio*)	Naile et al., 2013

		2.08		Fish (Silver carp)	Wang et al., 2013
		1.93		Prawn	Wang et al., 2013
		1.62		Snakehead	Wang et al., 2013
		1.77		Tire track eel	Wang et al., 2013
		1.32		Fish (Crucian carp)	Wang et al., 2013
		0.70–0.97		Common Carp (*Cyprinus carpio L.*)	Inoue et al., 2012
		1.9–3.7		Floating plants	Shi et al., 2012
		1.0–2.1		Fish (*Leuciscus cephalus*)	Labadie and Chevreuil,~~et al.~~, 2011
			0.07	Oligochaete (*Lumbriculus variegatus*)	Lasier et al., 2011
		1.1–1.2		Mussel (*Perna viridis*)	Liu et al., 2011
		2.43		Zooplankton	Loi et al., 2011
		2.26		Fish	Fujii et al., 2007
			-0.28–-0.02	Echinoderm (*L. Variegatus*)	Higgins et al., 2007
		1.91		Zooplankton	Houde et al., 2006
		-1.42		Rainbow trout (*Oncorhynchus mykiss*)	Martin et al., 2003a
PFOS	2.04–2.57	3.31-5.54	0.87–2.93	Echinoderm (*Holuthuria tubulosa* intestine)	This work
		2.48–5.52	1.26–2.28	Echinoderm (*Holuthuria tubulosa* gonads)	This work
	1.07–3.70	5.37		Fish and eel (*Salmo trutta, Gobio lonzanoi, Pseudochrondrostoma polylepis, Mycroptero salmoides, Barbus guiraonis, Lepomis gibbosus, Alburnus alburnus, Esox lucius, Anguila anguila*)	Campo et al., 2016
	0.47–2.03				Campo et al., 2015
	1.78–2.12	2.3–3.80	0.97–1.81	Eel, Perch, Pike	Kwadijk et al., 2014
	2.32–3.32				Pan et al., 2014
	2.4	3.5–5.2	-0.3–1.5	Fish (*Leuciscus cephalus*)	Labadie and Chevreuil, 2011
	2.15				Pico et al., 2012
	2.88				Yang et al., 2011
	2.1				Ahrens et al., 2010
	2.35				Kwadijk et al., 2010
	2.30–3.60				Zhou et al., 2010
	2.68				Higgins and Luthy, 2006
		2.64	3.46	Aquatic snails (*Bithynia tentaculata*)	Wilkinson et al., 2018
		2.77	3.46	Amphipod crustaceans (*Gammarus pulex*)	Wilkinson et al., 2018
		3.0		Fish (*Cyprinus carpio*)	Pignotti et al., 2017
		0.68		Fish (*Liza sp.*)	Pignotti et al., 2017
		2.3		Fish (*R. rutilus*)	Pignotti et al., 2017
		1.9		Fish (*S. erythropthalmus*)	Pignotti et al., 2017
		2.0		Fish (*S. glanis*)	Pignotti et al., 2017
		2.0		Fish (*Squalius laietanus*)	Pignotti et al., 2017
		2.4		Fish (*A. alburnus*)	Pignotti et al., 2017
		2.9		Fish	Hong et al., 2015
		2.5		Bivalve	Hong et al., 2015
		2.5		Crab	Hong et al., 2015
		2.7		Gastropod	Hong et al., 2015
		2.9		Shrimp	Hong et al., 2015
		2.95–3.71		Fish	Xu et al., 2014
			-0.5–0.0	Oyster	Zhao et al., 2014
			-0.8–-0.2	Mussel	Zhao et al., 2014
			-1.64	Larvae (*Chironomus riparius*)	Bertin et al., 2014
		2.25		Crustacean (*Daphnia magna*)	Dai et al., 2013
		2.92–4.49		Fish (*Hemigrapsus sanguineus, Sesarma pictum, Hemigrapsus penicillatus, Helice tridens tridens,*	Naile et al., 2013

		Philyra pisum)	
1.89		Bivalve (*Mytilus edulis, Mactra veneriformis, Nuttallia olivacea, Sinonovacula constricta*)	Naile et al., 2013
1.72–2.41		Crab (*Acanthogobius flavimanus, Sebastes schlegeli, Tridentiger obscurus, Hexagrammos otakii, Mugil cephalus*)	Naile et al., 2013
2.33		Gastropod (*Littorina brevicula, Monodonta labio, Umbonium thomasi, Glossaulax didyma, Monodonta labio*)	Naile et al., 2013
3.58		Fish (Silver carp)	Wang et al., 2013
2.65		Prawn	Wang et al., 2013
3.48		Snakehead	Wang et al., 2013
2.26		Fish (Common carp)	Wang et al., 2013
3.04		Tire track eel	Wang et al., 2013
2.38		Fish (Crucian carp)	Wang et al., 2013
2.85–3.11		Common Carp (*Cyprinus carpio L.*)	Inoue et al., 2012
3.0–4.1		Floating plants	Shi et al., 2012
	0.49	Oligochaete (*Lumbriculus variegatus*)	Lasier et al., 2011
2.4–2.6		Mussel (*Perna viridis*)	Liu et al., 2011
3.82–4.66		Fish	Fujii et al., 2007
	-0.08–0.09	Oligochaete (*Lumbriculus variegatus*)	Higgins et al., 2007
-0.49		Rainbow trout (*Oncorhynchus mykiss*)	Martin et al., 2003a

Fig. S1. Photo of an open specimen of *Holoturia tubulosa*.

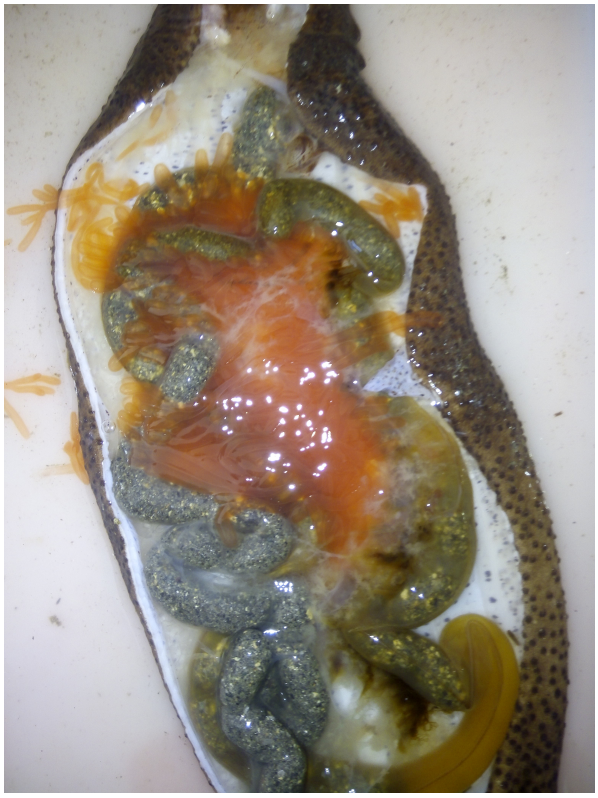
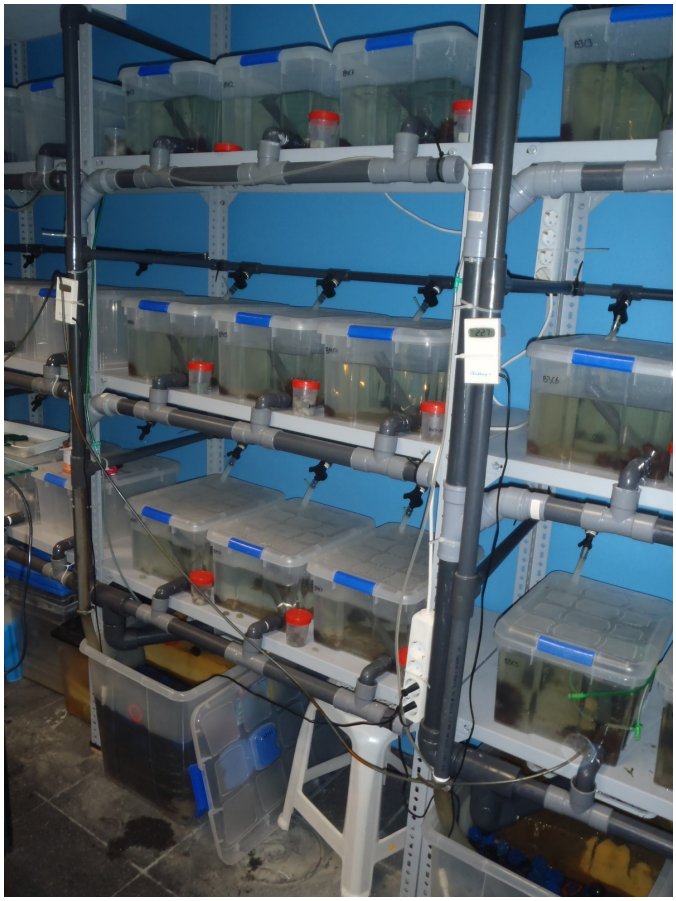


Fig. S2. Image of the experimental aquarium.



Instrumentation and software

Chromatographic analyses were performed on an Agilent 1200 series LC system (Agilent, USA) equipped with a vacuum degasser, a binary pump, an autosampler, and a thermostated column compartment. The LC system was coupled to a 6410 triple quadrupole mass spectrometer (MS/MS) equipped with an electrospray ionization (ESI) source (Agilent). Ionization of analytes was carried out using the following settings: MS capillary voltage 3000 V, drying-gas flow rate 9 L min^{-1}, drying-gas temperature 350 ºC, and nebulizer pressure 0.28 MPa. A vortex-mixer (IKA, Staufen, Germany), an ultrasound-HD bath (Selecta, Barcelona, Spain), a Spectrafuge™ 24D centrifuge (Labnet International, Inc., Edison, NJ, USA), and a sample concentrator (Stuart, Staffordshire, UK) were also used.

Validation requirements

The method was validated according to the International Conference on Harmonization (ICH) guidelines for analytical method validation (ICH Quality Guidelines).

Matrix-matched calibration standards were prepared at eight different analyte concentration levels. The mixtures were vortexed for 2 min and then left to stand for 24 h at 4 ºC in the dark before analysis.

Limit of detection (LOD) and limit of quantification (LOQ) are fundamental aspects that need to be examined in the validation of the analytical method in order to determine whether the analyte is present in the sample. The LOD is the minimum amount of analyte detectable in the sample while the LOQ is the minimum amount that

can be quantified. In this work, these parameters were calculated from the signal-to-noise ratio (LODs = 3, LOQ = 10) obtained from injecting several blank samples fortified with decreasing amounts of the compounds of interest.

A recovery assay was carried out to validate the accuracy of the method in terms of trueness and precision. Fortified blank samples for each compound at three concentrations levels were analyzed. Precision, expressed as relative standard deviation (%, RSD) was determined from triplicate spiked samples during the same day and in six different days, and the trueness was evaluated using the recovery data.

The selectivity of the method was determined by comparing the chromatograms of blank with the corresponding spiked sample.

Validity of the analytical results was verified by some simple quality assurance and quality control (QA/QC) measures. Procedural blanks (samples from a tank without analyte contamination) were injected to monitor background contamination. Blanks were processed in the same way as the samples and injected into the LC-MS/MS system. No quantifiable amounts (<LODs reported in Table 2) of target compounds were detected. Additionally, in order to evaluate possible contaminations and the variability of the instrumental analysis, standards (spiked blank samples at 50, 200 and 500 ng g-1 d.w.) and a standard in the initial mobile phase (100 ng mL-1) were injected by triplicate every 20 samples. Matrix-matched calibration standards were prepared at eight different analyte concentration levels. Taking into account the substance specific responses and the concentration levels in natural samples, different concentration ranges were used for each matrix (from 0.1 to 500 μg g^{-1} d.w. for holothurian and sediment samples and from 0.01 to 300 ng mL^{-1} for water samples). The mixtures were vortexed for 2 min and then left to stand for 24 h at 4 ºC in the dark before analysis. This allows the analytes to come into full contact with the sample.

Table S1. Concentration values (mean and standard deviation) of PFASs measured in water and sediment samples at three exposure batches.

		FBPuA		PFPeA		PFHxA		PFHpA		PFOA		PFOS	
						Water samples							
Batch	**Day**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blank	0	1.2	0.4	--	--	0.25	0.03	2.1	0.1	2.1	0.6	0.06	0.01
Batch-1	1	156	1	52.4	0.7	86	2	126	1	75.8	0.2	0.60	0.01
	8	188	0.3	63.8	0.2	83	1	60.2	0.3	21.0	0.2	--	--
	15	133	2	45.0	0.2	54.9	0.2	38.6	0.2	22.7	0.2	0.70	0.01
	22	144	1	48.4	0.1	49.8	0.6	33.7	0.6	33.9	0.3	1.20	0.01
	27	172	0.3	56.4	0.2	56.1	0.3	34.8	0.4	18.2	0.3	0.14	0.01
	38	155	1	50.6	0.1	45.5	0.4	26.8	0.4	13.3	0.1	0.07	0.03
	53	168	1	52.3	0.3	36.8	0.2	13.1	0.3	9.1	0.1	0.33	0.02
	86	240	2	72.4	0.2	22.7	0.4	5.4	0.1	7.5	0.2	5.44	0.04
	197	276	4	72.9	0.3	14.0	0.3	7.2	0.1	5.7	0.2	2.55	0.01
Batch-2	1	99.3	0.1	32.1	0.1	36.5	0.3	18.9	0.30	10.8	0.1	--	--
	8	87.8	0.3	28.3	0.1	23.8	0.2	16.9	0.21	84	1	2.72	0.01
	15	84.6	0.3	27.2	0.1	18.5	0.2	7.5	0.1	7.2	0.1	0.75	0.04
	22	112	0.2	36.6	0.2	19.1	0.1	7.3	0.1	7.8	0.1	0.23	0.07
	27	81.0	0.9	25.4	0.3	12.1	0.1	5.2	0.1	7.6	0.3	0.47	0.05
	38	92.7	0.3	28.6	0.1	14.5	0.1	9.9	0.3	8.2	0.2	--	--
	53	151	1	44.4	0.4	17.6	0.3	8.2	0.3	9.2	0.3	0.53	0.01
	86	98.1	0.6	26.8	0.2	4.45	0.03	2.8	0.1	3.1	0.1	0.10	0.02
	197	139	1	34.3	0.2	5.66	0.03	5.6	0.1	4.4	0.5	0.37	0.03
Batch-3	1	33	2	11.1	0.2	17.5	0.4	20.3	0.2	12.8	0.3	0.03	0.02
	8	21.8	0.3	7.7	0.1	10.8	0.2	9.2	0.2	12.8	0.2	0.19	0.01
	15	18.3	0.3	6.5	0.1	7.8	0.2	6.0	0.7	6.5	0.1	0.39	0.03
	22	19.9	0.4	7.1	0.1	6.7	0.1	4.2	0.5	4.8	0.1	--	--
	27	18.8	0.3	6.6	0.1	4.9	0.1	4.9	0.1	11.7	0.4	0.26	0.01
	38	25.7	0.1	8.9	0.1	5.9	0.1	4.9	0.1	6.6	0.4	0.05	--
	53	25.3	0.1	8.2	0.1	5.0	0.2	4.6	0.3	4.7	0.6	0.04	--
	86	26.6	0.3	8.4	0.1	2.9	0.3	3.3	0.1	4.6	0.1	0.07	0.01
	197	27.3	0.2	7.3	0.1	2.4	0.1	5.2	0.2	5.2	0.4	0.17	0.02

Table S1 cont. Concentration values (mean and standard deviation) of PFASs measured in water and sediment samples at three exposure batches.

Batch	Day	FBPuA		PFPeA		PFHxA		PFHpA		PFOA		PFOS	
						Sediment samples							
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blank	0	1.8	0.3	--	--	2.6	0.1	11.2	0.3	25.7	0.5	--	--
Batch-1	1	260	4	95.4	0.3	191	1	374	2	472	5	206	3
	8	202	2	71.0	0.8	101	1	96	1	77	3	20.4	0.9
	15	231	1	81.0	0.1	103	2	107	3	122	6	189	3
	22	253	2	88.6	0.3	90	2	101	1	240	5	425	10
	27	211	3	72.8	0.4	71	1	65	2	63	1	51	1
	38	196	2	66.6	0.7	58	1	64	2	60	1	28	1
	53	211	4	66.4	0.2	43	2	37	1	48	1	110	3
	86	194	4	57.3	0.2	18	1	42	1	75	2	597	13
	197	326	2	71.4	0.9	83	1	58	1	31	1	695	15
Batch-2	1	122	2	40	1	48	2	52	1	46	2	1.8	0.3
	8	152	2	53	2	54	3	97	1	--	--	--	--
	15	85	5	26	1	20	1	33	2	34	3	4.6	0.6
	22	89	2	27	3	18	1	34	1	46	2	33.7	0.7
	27	66	1	20	1	18	2	51	1	79	1	161	5
	38	101	3	31	1	25	1	67	2	89	3	68	1
	53	87	2	25	1	14.1	0.2	36	1	49	2	72	1
	86	26	1	6.6	0.1	8.8	0.1	27.6	0.2	24	1	6.0	0.2
	197	98	3	21	1	7.1	0.3	24.6	0.7	119	3	42.9	0.1
Batch-3	1	29	1	8.7	0.2	18.4	0.5	38.0	0.9	30.4	0.7	0.40	0.02
	8	50	1	16.6	0.3	36.8	0.7	--	--	--	--	--	--
	15	23	2	6.8	0.2	14.6	0.1	37.0	0.3	42.6	0.7	8.7	0.5
	22	21	1	5.5	0.1	9.7	0.9	27.8	0.3	33	1	8.95	0.3
	27	26	2	7.7	0.2	12.9	0.3	31.9	0.5	57	0.9	18.1	0.6
	38	29	2	8.2	0.2	12.0	0.2	32.3	0.4	41.3	0.4	17.8	0.1
	53	34	1	10.1	0.1	11.5	0.1	32.4	0.3	36.4	0.3	20.6	0.4
	86	88	2	23.3	0.3	8.8	0.1	30.0	0.4	29.3	0.3	21.8	0.7
	197	19	1	3.7	0.1	6.3	0.4	26.4	0.9	80.8	2	16.4	0.2

SD: Standard Deviation

Table S2. Concentration values (mean and standard deviation) of PFASs measured in intestine and gonads of *Holothuria tubulosa* during trial at three exposure batches.

Batch	**Day**	**PFBuA**		**PFPeA**		**PFHxA**		**PFHpA**		**PFOA**		**PFOS**	
Intestine samples													
Blank	0	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blank	0	50.1	6.7	26	3	137	15	281	9	386	15	95	4
Batch-1	8	24437	2970	6333	688	19974	1530	80543	5722	196268	23442	32530	2925
	22	22413	1749	10534	991	33918	2831	87877	4897	106478	10842	11164	1133
	38	56507	6377	25771	2987	82982	9365	147956	9179	216068	23408	24158	1602
	53	6502	843	2023	77	7061	116	24049	2554	59672	5334	8107	622
	86	29.7	5.1	22.6	2.8	191	16.9	737	133	1282	78	128	15
	197	3559	451	1723	88	1722	101	2569	110	1676	128	5179	434
Batch-2	8	3223	165	1265	273	6968	481	17457	583	27403	1533	5148	298
	22	4521	208	2239	205	8745	464	8110	541	18166	1738	2890	331
	38	56612	6023	24360	2265	78154	8026	201936	24767	271577	19784	38963	1718
	53	8824	637	6496	359	25407	1670	21530	3646	42396	1907	4433	197
	86	3100	174	2160	119	3677	202	7573	439	3904	217	1657	221
	197	6898	521	1138	125	1011	87	3836	153	6604	781	30125	3351
Batch-3	8	4145	247	1129	67	5995	258	14562	878	25193	1330	3743	231
	22	18640	1826	8899	143	14602	1260	70863	6714	115143	11661	18339	1409
	38	10753	1039	3385	189	5904	664	35773	1047	32361	1627	2518	238
	53	2955	139	2062	114	5734	567	7181	494	16410	927	1526	83
	86	1967	328	959	35	2685	371	6727	414	3631	236	366	45
	197	1058	83	309	15	278	19	1095	198	1322	181	1265	171
Male gonad samples													
Blank	0	64	2	12	1	26	2	62	5	69	6	32	4
Batch-1	8	11712	105	3248	75	13344	355	31933	987	37105	888	6590	100
	22	1922	99	305	21	685	8	1524	20	1104	51	346	41
	38	4481	101	2095	130	7593	540	20260	1195	23761	2209	23088	94
	53	12825	1371	3489	302	6391	692	15806	1502	33599	3979	5203	491
	86	85.1	5.7	43.1	1.0	304	5	1186	79	2856	19	1235	13

Batch-2	8	2901	265	869	11	1037	23	2104	77	4014	103	1586	93
	38	3355	330	963	11	1073	18	2241	173	2661	184	1247	27
	86	4564	388	905	10	1038	14	1495	26	296	41	1137	62
Batch-3	8	2404	31	522	29	912	84	2542	86	2249	242	541	75
	22	1326	16	249	27	231	10	517	26	571	12	338	14
	38	1296	10	282	12	169	12	201	14	351	12	371	21
	53	3997	71	1462	10	914	38	2248	169	3091	84	1232	81
	86	1836	97	580	33	1326	31	2691	191	1264	59	540	11
Female gonad samples													
Blank-Female	0	117	18	47	6	136	11	323	7	298	6	56.1	1.6
Batch-1	8	3690	195	1524	41	6186	343	19901	1257	24110	1854	13847	1529
	38	2702	59	842	16	1011	26	1534	38.7	2576	68	1299	22
	86	53.3	1.1	13.6	1.8	18.5	0.2	54.8	0.35	102	8	31.4	1.6
	197	3543	56	883	56	121	17	72.2	0.59	113	11	696	10
Batch-2	22	5204	255	1227	127	781	44	746	67	1058	80	1158	186
	38	3744	13	864	12	743	36	1537	13	2126	210	2132	162
	53	2791	34	1055	13	1147	64	2064	140	2311	129	894	15
	86	2902	35	890	18	708	53	1162	95	396	35	771	73
Batch-3	8	4813	91	1569	29	3357	74	11375	740	14997	328	5719	187
	22	1805	14	543	15	323	1.6	321	2	416	40	318	31
	38	12497	109	2475	49	1969	82	2467	15	2530	38	2391	112
	53	1831	104	470	19	875	60	2292	168	2358	143	745	74
	86	5791	58	1240	59	1351	10	3080	28	1322	22	455	40

SD: Standard Deviation

References

ICH Quality Guidelines, Topic Q2 (R1): Validation of Analytical Procedures: Text and Methodology, (2005).