

Formation of PFOA from 8:2 FTOH in closed-bottle experiments with brackish water

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Abstract The formation of perfluorooctanoate (PFOA) from 1H,1H,2H,2H-perfluorodecanol (8:2 FTOH) was studied for the first time in laboratory experiments with brackish water. The water samples were collected from the Baltic Sea, which is one of the largest brackish water areas in the world and is polluted with PFOA and other perfluorinated compounds. The formation of PFOA was studied in closed-bottle experiments at different water temperatures. As a reference experiment, a modified OECD 310 test was conducted with sludge from a wastewater treatment plant and with brackish water. The PFOA and 8:2 FTOH were concentrated from water samples by solid-phase extraction (SPE) and were analysed using liquid chromatography–mass spectrometry. The effect of oxygen concentration on the formation of PFOA was studied using surface water samples with high and low oxygen contents. Other experiments were performed with oxygen-rich surface water and oxygen-deficient bottom water. The formation of PFOA was observed in all experiments; it was higher in the trial performed with brackish water than in the reference test carried out with sludge. Clear temperature dependence was observed in the formation of PFOA in brackish water tests;

after a 30-day test period, a sixfold increase was observed in the amount of PFOA in surface water between the temperatures of 15 and 20 °C. Microbes were suggested as the major cause of the formation of PFOA, but other environmental characteristics, such as oxygen, could also affect the formation potential of PFOA.

Keywords 8:2 FTOH · PFOA · Environmental fate · Brackish water · Transformation · Precursor

Introduction

Per- and polyfluorinated alkyl substances (PFAS) have been widely used in domestic and industrial applications, e.g. adhesives, paper coatings and pharmaceuticals since the 1950s due to their unique properties (Kissa 2001). Perfluorooctanoate (PFOA) is one of the most studied PFAS, and it has proven to be ubiquitous in the environment. Present studies indicate that PFOA is persistent, bioaccumulative and toxic, and it can migrate via surface water or due to atmospheric transformation of its precursors to even Arctic or Antarctic areas (Prevedouros et al. 2006; Vierke et al. 2012). PFOA is an impurity in fire-fighting foams, and it has been used in aqueous fluoropolymer dispersions. Potential indirect sources of PFOA in the environment are also its precursors such as fluorotelomer alcohols (FTOHs) (Kissa 2001; Taniyasu et al. 2005). Their transportation and the following degradation at the deposition site spread the parent substances far from the main source. The behaviour of FTOHs has been studied, e.g. in air (Stock et al. 2004), surface water and rainwater (Mahmoud et al. 2009).

1H,1H,2H,2H-perfluorodecanol (8:2 FTOH) has been used in the synthesis of surfactants and polymeric materials. It has

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been the dominating poly- and perfluorinated compound in household consumer products such as impregnating agents and lubricants (Fiedler et al. 2010). FTOHs have been applied in the production of paints, adhesives, polishes and caulks due to their unique chemical properties (Kissa 2001; Dinglasan et al. 2004). The calculations indicate that residual 8:2 FTOH contributes up to 80 % of the total fluorotelomer acrylate polymer contribution to PFOA concentrations in global oceans (Van Zelm et al. 2008). The 8:2 FTOH is initially oxidised to telomer acid which is further transformed to unsaturated acid and finally to PFOA (Dinglasan et al. 2004). Several metabolites are formed during the degradation of 8:2 FTOH, and a ca. 45 % loss of those product compounds has been reported (Dinglasan et al. 2004; Wang et al. 2005).

The FTOHs and PFOA end up in the environment through the effluent waters released from the municipal wastewater treatment plants (WWTPs). FTOHs have been measured in WWTP effluents (Mahmoud et al. 2009) and in sewage sludge-treated soils (Ellington et al. 2009). WWTPs and landfills are also atmospheric emission sources of FTOHs (Ahrens et al. 2011; Xiao et al. 2012). Higher levels of PFOA detected in effluent waters than those measured in influent waters refer to the biodegradation of the precursors to PFOA in the wastewater treatment process (Loganathan et al. 2007). Up to a tenfold increase of PFOA concentration due to its formation from precursors was observed in WWTPs by Becker et al. (2008). Increased formation of some perfluorinated compounds has been observed in a WWTP receiving discharges from both municipal and industrial sources when compared to a WWTP that receives only municipal wastewater (Arvaniti et al. 2012). 8:2 FTOH has been observed to biodegrade aerobically in bacterial cultures, activated sludge from municipal WWTPs and soil (Dinglasan et al. 2004; Wang et al. 2005, 2009; Liu et al. 2007). It has also been shown to degrade by photolysis in lake water (Gauthier and Mabury 2005), but its degradation in water has not been intensively studied under laboratory conditions.

The widespread occurrence of PFOA in the aquatic environment has created concern about its indirect sources and the environmental fate of its precursors. In the present study, we carried out preliminary laboratory experiments to see if 8:2 FTOH can transform into PFOA in brackish water. The brackish water from the Baltic Sea was selected as the study medium due to the lack of information concerning the formation of PFOA in this type of water environment. Furthermore, PFOA has earlier been measured as the main PFAS in seawater samples in the vicinity of Helsinki city (Kallenborn et al. 2004). The Baltic Sea is one of the largest brackish water areas in the world, and it is very sensitive to changes due to its unique properties, low depth and low salinity. Eutrophication and hazardous substances have also been identified as major threats for this sea where eutrophication-related oxygen depletion is a wide-spread problem due to

anthropogenic nutrient inputs. The main aim of this study was to investigate whether PFOA is formed from 8:2 FTOH in brackish water and how the different environmental variables affect its formation. Experiments were performed with different test configurations and varying temperature and oxygen conditions. First, the formation of PFOA in brackish water was compared with a reference test where the microbes of the activated sludge from a WWTP caused the formation of this compound. In the other two tests, the effect of oxygen content and the microbiological environment of the water (surface vs. bottom) on the formation of PFOA were studied at different temperatures.

Experimental

Chemicals

The reagents and standards were obtained from Sigma-Aldrich/Fluka. The purity of PFOA was 96 % and that of 8:2 FTOH 97 %. The methanol was HPLC grade and the ammonium acetate pro analysi grade.

Experiments

The degradation experiments were performed as closed-bottle tests with brackish water (coastal seawater from the western Gulf of Finland in the Baltic Sea). We first tested the difference between the microbial degradation potential of activated sludge and brackish water by conducting the OECD 310 test (the 28-day headspace test at 20 °C, OECD 2006) using both of these media. A chemical substance that shows positive results in the OECD 310 test can be considered rapidly degradable in the environment and therefore less harmful. A fresh 8:2 FTOH solution was prepared for each experiment in order to avoid any possible degradation and evaporation of 8:2 FTOH in stock solutions during storage. In experiments 1 and 2, the ionic strength was the same (according to OECD 310 test), being higher than in experiments 3 and 4 (without the addition of mineral salts).

Experiment 1 (OECD 310 test with WWTP sludge)

The first experiment was performed according to the OECD 310 guideline (OECD 2006) with inoculum collected from the WWTP of the city of Jyväskylä in March 2010. The WWTP receives wastewaters mainly from domestic sources. The test was conducted in sealed glass bottles with a headspace of air, which provides a reservoir of oxygen for aerobic biodegradation.

Mineral salts medium (volume, 2 L; pH 7.4) which contained 6.8 mL of activated sludge (inoculum collected March 1, 2010 from the aeration tank of the sewage treatment plant) was spiked with 40 µL of the stock solution of 8:2 FTOH (1,265 µg mL⁻¹) in methanol. The mixed solution was divided

into 125 mL glass bottles (100 ml in each) to be used as test and control samples. The spiked 8:2 FTOH concentration was $25.3 \mu\text{g L}^{-1}$ in each bottle ($2.53 \mu\text{g}$ per sample). For blank samples, another 2 L set of mineral salts medium containing activated sludge was prepared. Methanol ($40 \mu\text{l}$) without test substance was added to the medium, and it was divided into glass bottles. The bottles were placed on a rotary shaker and incubated at $22 \text{ }^\circ\text{C}$. In order to prevent the degradation caused by agents other than microbes, the sample bottles were protected from UV and natural sunlight during the test.

The experiment consisted of five sampling days (3, 7, 14, 21 and 28). Three replicate samples were taken each day. Blanks (for each sampling day), abiotic control samples (for days 14, 21 and 28) and 0-day control samples, two replicates for each, were also included in this experiment. Abiotic control samples were sterilized by autoclaving them.

Experiment 2 (modified OECD 310 test with surface brackish water)

The experiment was performed according to the OECD 310 (OECD 2006) test by replacing the activated sludge from the WWTP with brackish water. Surface brackish water was collected from site $59^\circ 50' \text{N}$, $23^\circ 15' \text{E}$ near the shore in March 2010 using a Limnos sampler and stored overnight at $4 \text{ }^\circ\text{C}$ in a 5-L polypropylene (PP) container. The surface brackish water was aerated with an aquarium pump to achieve a highly oxidised initial state. The total organic carbon (TOC) content of the water was 32.7 mg L^{-1} and that of inorganic carbon 16.3 mg L^{-1} (measured with a Shimadzu total organic carbon analyzer TOC-5000A). pH of the water was not measured, but it was assumed to be below 8.0 based on monitoring of water samples in the vicinity of the sampling site (7.2 1 week after our sampling, unpublished data). The same nutrients as in the OECD 310 test were added to 2 L of the brackish water in order to obtain the same mineral salt content as in experiment 1. The initial salinity and concentrations of nutrients in the brackish water were assumed to be considerably lower than those obtained by the addition of mineral salt stock. The medium was then spiked with 8:2 FTOH and divided into exposure bottles as in experiment 1 for test and control samples (abiotic and 0 day). The spiked concentration of 8:2 FTOH was $16.6 \mu\text{g L}^{-1}$ in each bottle ($1.66 \mu\text{g}$ per sample). Blank samples were also included in this test; they were prepared by adding mineral salt and methanol (without 8:2 FTOH) to the brackish water as in experiment 1. Four sampling days (6, 14, 21 and 28 days) were selected for this experiment.

Experiment 3 (oxygen-rich vs. oxygen-deficient surface brackish water)

The formation of PFOA was studied in surface brackish water at different temperatures under different initial oxygen

conditions. The experiment was performed in PP bottles instead of glass bottles required in the OECD test. The use of glass containers was avoided since the PFAS can be adsorbed onto glass (Martin et al. 2004; Ahrens et al. 2011).

The effect of temperature and dissolved oxygen concentration on the formation of PFOA was studied with surface water collected parallel to experiment 2 from the same sampling site. The water was collected in eight 5-L PP containers, four of which were purged with a nitrogen gas flow overnight at room temperature to remove the dissolved oxygen. The oxic ($c(\text{O}_2)=11 \text{ mg L}^{-1}$) and anoxic ($c(\text{O}_2)=1 \text{ mg L}^{-1}$) surface waters were then divided into 0.5-L PP bottles and spiked with $20 \mu\text{L}$ of 8:2 FTOH stock solution in methanol ($2.5 \mu\text{g}$ per sample; the final 8:2 FTOH concentration $5 \mu\text{g L}^{-1}$). We tried to keep the spiked amount of 8:2 FTOH at the same level as in experiments 1 and 2, but the water volume was increased in order to ensure sufficient amount of microbes for adequate formation of PFOA.

The formation of PFOA in brackish water was studied at four different temperatures (5 , 10 , 15 and $20 \text{ }^\circ\text{C}$), which correspond to the water temperatures in this area during spring and summer. The samples were kept in climatic chambers protected from UV and natural sunlight. Three replicate samples were taken from each batch of bottles kept at different temperatures on sampling days, which were 15, 30 and 45-days from the beginning of the experiment. The samples were frozen after sampling. Two replicate samples of both oxic and anoxic surface water were stored frozen immediately after spiking with 8:2 FTOH to be used as control samples. Since the aim of this experiment was to compare the differences in the yield of PFOA at different temperatures and oxygen levels, no abiotic control samples or blanks without 8:2 FTOH spiking were included in this test.

Contrary to experiments 1 and 2, there was no headspace of air left in exposure bottles, and thus no reservoir of oxygen for aerobic biodegradation was available. Salinity in the test bottles resembled the natural conditions more closely than in experiments 1 and 2 because mineral salts were not added to the water. The salinity of the brackish water was assumed to be around 4–5‰ based on monitoring of water samples in the vicinity of the sampling site (4‰ 1 week after our sampling, unpublished data) and on the general conditions suggested by these monitoring data.

Experiment 4 (surface vs. bottom brackish water)

The formation of PFOA in oxygen-rich surface water and anoxic bottom water (collected from a basin, site $59^\circ 50' \text{N}$, $23^\circ 15' \text{E}$, in July 2010) were compared at the same four temperatures as in experiment 3 (5 , 10 , 15 and $20 \text{ }^\circ\text{C}$). The surface water was collected at the depth of 1 m and the bottom water at the depth of 27 m (1 m above the sea floor). The oxygen content of the surface water was 8 mg L^{-1} and

that of the bottom water 3 mg L^{-1} during sampling. pH of the surface water was 8.1 and that of the bottom water 7.6 in the beginning of the experiment. Measurements were performed with dissolved oxygen metre Palintest Microcomputer 900 and pH metre Mettler Toledo MP220. The salinity of the surface and bottom water were assumed to be similar (5.8 vs. 5.9‰), based on the analyses of water samples at the same sampling site (performed 1 week before our sampling, unpublished data). Again, salinity in test bottles resembled more the natural conditions than in experiments 1 and 2 because mineral salts were not added to the water. Dissolved organic carbon (DOC) was not measured as there was no instrument available at that time.

Aliquots of both waters (in 50-ml PP sterile centrifuge tubes) were spiked with 8:2 FTOH ($2.5 \mu\text{g}$ per sample in $20 \mu\text{L}$ of methanol similarly as in experiment 3) so that the 8:2 FTOH concentration was $50 \mu\text{g L}^{-1}$. As in experiment 3, no headspace of air was left in the test tubes. Anoxic bottom water samples were kept under a nitrogen atmosphere to minimize the accumulation of oxygen into the samples. The lower sample amount was selected because the amount of microbes in the bottom water was assumed to be greater than in the water used for experiment 3 due to summer conditions. The amount of methanol (extra carbon source) per test bottle was the same in both experiments.

In this experiment, sampling was performed more frequently and for a longer time period at 2, 5, 15, 30, 45 and 60 days from the beginning of the experiment with three replicate samples. As control samples, three replicate samples of both surface and bottom brackish waters were analysed immediately after spiking. Since the aim of this test was to compare the difference between surface and bottom water and the effect of temperature in the yield of PFOA, no abiotic control samples or blanks without 8:2 FTOH spiking were included in this experiment. Extra control water samples without FTOH spiking were included in this experiment for pH and oxygen measurement on each sampling day.

Chemical analysis

The samples were extracted using solid-phase extraction (SPE) on the sampling days (excluding experiment 3 samples), and the extracts were analysed with high-performance liquid chromatography–mass spectrometry (HPLC–MS). The samples of experiment 3 were frozen on the sampling day and stored at -20°C . All these samples were extracted later during 1 day. The extracts were run by HPLC–MS as one batch.

The water samples were concentrated with C18 SPE cartridges (Varian, Bond Elut C-18 500 mg/3 mL) conditioned with 3 mL methanol followed by 3 mL UHQ water (ultra high-quality water, internal resistance $\geq 18.2 \text{ M}\Omega \cdot \text{cm}$ at 25°C) using a Vacuum Master sample processing manifold. The water sample was passed through the cartridge, which was

then dried in vacuum at ambient room temperature. The compounds were then eluted with 3 mL methanol. The eluate was either evaporated with nitrogen flow to a smaller volume for adjustment to the final volume of $1,000 \mu\text{L}$ (50/50 methanol/5 mM ammonium acetate), or an aliquot of $300 \mu\text{L}$ (experiment 4) of the eluate was mixed with $300 \mu\text{L}$ ammonium acetate. The sample was filtered with an Acrodisc GHP $0.2\text{-}\mu\text{m}$ syringe filter before analysis.

The chromatographic separation was carried out using an HP Series 1100 binary pump, a vacuum degasser, a thermostated column compartment and an autosampler. The chromatographic column used in this study was Kinetex C18 ($100 \text{ mm} \times 2.1 \mu\text{m}$, $d_p = 2.6 \mu\text{m}$). The binary solvent system consisted of aqueous ammonium acetate buffer (55 mM) and methanol. The methanol proportion was increased from 10 to 100 % during 4 min and kept there for 9 min. After that, the methanol fraction was reduced to 10 % in 3 min for FTOH and in 4 min for PFOA.

Analyses were performed using electrospray ionization MS (HP 1100 MSD, Hewlett-Packard, Palo Alto, CA) because no MS–MS instrument was available during the experiments. FTOHs are typically analysed by gas chromatography–mass spectrometry, but also LC–MS has been reported suitable for analyses of FTOHs (Szostek et al. 2006; Larsen et al. 2006). The MS was operated in the negative selected ion monitoring mode. PFOA was detected as the M^- ion (m/z value, 369; a fragment ion of 413) and 8:2 FTOH as the acetate adduct ion ($\text{M}+59^-$) (m/z value, 523). The operating conditions for the determination of PFOA were as follows: drying gas (N_2) flow rate, 13 L min^{-1} ; temperature, 350°C ; nebulizer pressure, 20 psig; capillary voltage, 3.5 kV and fragmentor, 80 V. The operating conditions for the determination of 8:2 FTOH were as follows: drying gas (N_2) flow rate, 13 L min^{-1} ; temperature, 150°C ; nebulizer pressure, 20 psig; capillary voltage, 3.6 kV and fragmentor, 50 V.

The concentration of PFOA and 8:2 FTOH was calculated using a three-point (experiments 1 and 2) or ten-point (experiments 3 and 4) calibration curve determined with external standards as mass labelled internal standards were not available for the experiments. The analysis method was validated by performing the extraction experiments with standards. Five replicates of 100 ml of water were spiked with PFOA and 8:2 FTOH standards ($0.1\text{--}0.5 \mu\text{g}$ and $0.2\text{--}0.8 \mu\text{g}$, respectively), and the samples were extracted and analysed as described above. The average recoveries of PFOA and 8:2 FTOH in the extraction experiments were 121 (± 16)% and 78 (± 29)%, respectively. The concentrations in the experiments were not corrected for recovery. In experiment 4, the injection losses and matrix effects for PFOA were studied by adding perfluorooctane sulfonate (PFOS) to the extracts. The intensity of PFOS signal was constant in all the samples. The concentrations of PFOA and 8:2 FTOH in samples were corrected by subtracting

corresponding blanks. In experiments 1 and 2, the amount of PFOA/8:2 FTOH in the blanks was reduced from that in the samples before the concentration per volume was calculated. The concentration of PFOA was $<0.03 \mu\text{g L}^{-1}$ in 0-day control samples and $<0.3 \mu\text{g L}^{-1}$ in abiotic control samples. Laboratory contamination observed for PFOA in 0-day controls in experiment 4 was subtracted from other samples of this experiment because there were no blanks without 8:2 FTOH spiking in this test. No blanks were included either in experiment 3, but PFOA was not observed in the control samples of this experiment.

Statistical analyses

The effects of the treatments on the formation of PFOA in experiments 1 and 2 were tested using one-way ANOVA with the sampling day as the independent variable and PFOA concentration as the dependent variable. In experiments 3 and 4, which also included the effects of temperature and either oxygen content (experiment 3) or water depth (experiment 4; surface vs. bottom brackish water), two-way multivariate analysis of variance (MANOVA) was used. Here, PFOA concentrations on the different sampling days were the dependent variables, whereas temperature and oxygen content (experiment 3) or water depth (experiment 4) were the independent variables. The MANOVA was followed by univariate analyses for each individual sampling day. The general linear model in the statistical package IBM SPSS version 18 was used in the analyses.

Results and discussion

Experiment 1 (OECD 310 test with WWTP sludge)

The formation of PFOA is illustrated in Fig. 1. In the 28-day OECD 310 test with WWTP sludge, an elimination of the 8:2 FTOH was observed as its concentration was $<0.5 \mu\text{g L}^{-1}$ after 3 days ($<0.1 \mu\text{g L}^{-1}$ in other sampling days). The microbial culture used by Dinglasan et al. (2004) degraded 8:2 FTOH in 15 days. In 0-day control samples, the concentration of 8:2 FTOH was $6.4 \pm 0.6 \mu\text{g L}^{-1}$, which was lower than the expected initial concentration. This may be due to losses occurred during analysis or the inadequate dissolution of the 8:2 FTOH in water, as the analysis was performed immediately after spiking. Also, the studied compounds may have been adsorbed onto glassware to some extent. In abiotic controls, the concentration of 8:2 FTOH was also lower than assumed (after 28 days, $0.6 \pm 0.2 \mu\text{g L}^{-1}$). This was possibly due to incomplete sterilization or sorption to sludge or septa and/or evaporation to the gas phase, but it was not possible to investigate gas phase FTOHs. In addition to these factors, there might have been losses during analyses of control

samples. A decrease of 8:2 FTOH concentrations in abiotic controls was also observed in the study of Sáez et al. (2008), but they saw no evidence for the biodegradation of FTOHs in sludge.

There was a highly significant difference between the sampling dates (ANOVA $F=12.985$; $df=4$; $MS=3.164$; $P=0.001$) in the formation of PFOA (Table 1). It is known that the formation of PFOA from 8:2 FTOH occurs through intermediates and the latency period is as expected (Dinglasan et al. 2004). After a 28-day exposure, the concentration of PFOA was 11.2 (± 0.9)% of its expected initial concentration. The concentration of PFOA in abiotic controls was at maximum 0.9 % of the expected initial concentration of 8:2 FTOH.

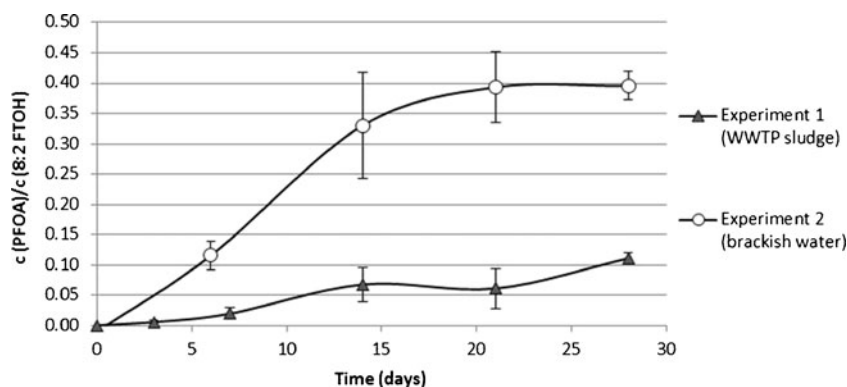
Experiment 2 (modified OECD 310 test with surface brackish water)

As in experiment 1, the concentration of 8:2 FTOH in 0-day control samples ($9.1 \pm 1.7 \mu\text{g L}^{-1}$) was lower than the expected initial concentration. The concentration of 8:2 FTOH was $<0.05 \mu\text{g L}^{-1}$ after 6 days. Levels of 8:2 FTOH in abiotic control samples were similar to those in experiment 1. There was a highly significant difference between the sampling dates (ANOVA $F=17.306$; $df=3$; $MS=13.405$; $P=0.001$) in the formation of PFOA. After 28 days, the concentration of PFOA was 39.5 (± 2.4)% of its expected initial concentration. PFOA was not measurable in abiotic controls, except in one replicate of 28-day abiotic controls ($0.01 \mu\text{g L}^{-1}$).

The formation of PFOA was higher in the brackish water (experiment 2) than in the activated sludge (experiment 1) in the modified OECD 310 test (Fig. 1). This could be due to the presence of microbes that are more capable in forming PFOA from 8:2 FTOH than the microbes in the WWTP sludge. Our aim was not to investigate microbes in water, but sea ice bacterial communities have earlier been studied in this area (Kaartokallio et al. 2008).

The different chemical environment might also have affected the formation of PFOA. The difference in the formation of PFOA was most likely not caused by different salinity because the amount of mineral salts was the same per test bottle in both experiments. There were also no significant differences in pH in experiments 1 and 2 (7.4 vs. 7.2). Instead, DOC and nitrate were assumed to have been at a higher level in water in experiment 2 than in experiment 1. Based on nutrient analyses of a water sample taken in the vicinity of our sampling site 1 week after our sampling, the level of nitrate was high ($270 \mu\text{g L}^{-1}$, unpublished data) in the brackish water, whereas there was no added nitrate in the medium of experiment 1. DOC was not added in experiment 1 either, but in brackish water in the vicinity of our sampling site, it has been measured at a level of about $400 \mu\text{mol C L}^{-1}$ (Hoikkala et al. 2012). It has been suggested that in aqueous photolysis of 8:2 FTOH, in

Fig. 1 The ratio of PFOA concentrations to initial 8:2 FTOH concentration in modified OECD 310 experiments



which the hydroxyl radical is the main degradation agent, the higher production of PFOA in natural water compared to synthetic field water was caused by factors such as DOC which are capable of producing hydroxyl radicals in natural waters (Gauthier and Mabury 2005). Higher nitrate concentration resulted in more efficient formation of PFOA in synthetic field water systems, while DOC inhibited it.

Experiment 3 (oxygen-rich vs. oxygen-deficient surface brackish water)

The 8:2 FTOH concentration was 0.16 ± 0.06 and $0.34 \pm 0.13 \mu\text{g L}^{-1}$ after 15 days in oxygen-rich and oxygen-

deficient surface water, respectively. The concentrations of 8:2 FTOH in 0-day controls (3.1 ± 1.9 and $4.1 \pm 0.4 \mu\text{g L}^{-1}$, respectively) were closer to the expected initial concentration than in experiments 1 and 2.

The formation of PFOA increased with the longer exposure time period and also when the temperature was higher (Fig. 2). This was valid for both oxygen concentrations. Clear temperature dependence was observed for the formation of PFOA; after a 30-day period, a sixfold increase was observed in the amount of PFOA in oxygen-rich surface water between the temperatures of 15 and 20 °C. At the lowest temperature (5 °C), the formation of PFOA was low even after 45 days of exposure, and there was no difference

Table 1 MANOVA (upper panel) and ANOVA (lower panel) for effects of temperature and oxygen on PFOA concentrations in experiment 3*

Effect	Wilk's λ	<i>F</i> value	<i>df</i>	<i>P</i>		
Temperature	0.000	106.097	9, 34.223	0.000		
Oxygen	0.050	88.370	3, 14	0.000		
Temperature \times oxygen	0.002	50.767	9, 34.223	0.000		
Source	Dependent	SS	<i>df</i>	MS	<i>F</i>	<i>P</i>
Temperature	PFOA15	0.09	3	0.03	10.513	0.000
	PFOA30	3.433	3	1.144	171.333	0.000
	PFOA45	16.585	3	5.528	1,233.963	0.000
Oxygen	PFOA15	0.018	1	0.018	6.387	0.022
	PFOA30	1.178	1	1.178	176.366	0.000
	PFOA45	0.913	1	0.913	203.717	0.000
Temperature \times oxygen	PFOA15	0.023	3	0.008	2.735	0.078
	PFOA30	2.575	3	0.858	128.502	0.000
	PFOA45	1.535	3	0.512	114.228	0.000
Error	PFOA15	0.046	16	0.003		
	PFOA30	0.107	16	0.007		
	PFOA45	0.072	16	0.004		
Total (corrected)	PFOA15	0.177	23			
	PFOA30	7.293	23			
	PFOA45	19.105	23			

*The MANOVA results are the overall results using the concentrations on all sampling days as dependent variables. The ANOVA results report the treatment effects on each day individually and can be interpreted when effects in the MANOVA are significant. The numbers 15, 30 and 45 in the "Dependent" column of the ANOVA table indicate the PFOA sampling days

in its formation between different oxygen levels during the test periods studied. At the other temperatures, a difference in the formation of PFOA was detected between different oxygen levels, the formation of PFOA being faster in oxygen-rich water. Substantial formation of PFOA after 45 days of exposure was observed already at 10 °C in oxygen-rich water. At 20 °C, the PFOA level in water with an initially low oxygen concentration reached that in water with an initially high oxygen concentration during 45 days. After 30 days, the concentration of PFOA in oxygen-rich water was 37.6 (±4.2)% of the expected initial concentration of 8:2 FTOH.

Statistically, the effects of both temperature and oxygen concentration on PFOA formation were manifested as a significant interaction in the MANOVA that included all sampling days (Table 1, upper panel). The main effects of both variables were also statistically significant. This allowed us also to interpret the ANOVA results of the individual sampling dates (Table 1, lower panel), for which the interaction approached significance on sampling day 15 and was thereafter statistically significant for the remaining sampling days. The main effects of temperature and oxygen were statistically significant on all sampling days.

The higher formation of PFOA in oxygen-rich water is most likely explained by more favourable conditions for microbes in this type of environment. FTOH has been reported to biodegrade aerobically in bacterial cultures (Dinglasan et al. 2004). It has been proposed that 8:2 FTOH is initially oxidised to 8:2 aldehyde catalysed by alcohol dehydrogenase. Our aim was not to investigate other metabolites

than PFOA, but in other studies, fluorotelomer acids (2H,2H-perfluorodecanoic acid, 8:2 FTCA and its unsaturated form, 8:2 FTUCA) have been measured as metabolites of 8:2 FTOH in a microbial enrichment culture (Dinglasan et al. 2004), the unsaturated telomer acid being the predominant metabolite.

Experiment 4 (surface vs. bottom brackish water)

The concentration of 8:2 FTOH decreased faster in the bottom water than in the surface water (Fig. 3). Temperature dependence was observed for both bottom and surface water. The concentrations of 8:2 FTOH in 0-day controls (23.0 ± 6.4 and $21.5 \pm 5.3 \mu\text{g L}^{-1}$ in oxygen-rich and oxygen-deficient water, respectively) were closer to the expected initial concentrations than in experiments 1 and 2. This better agreement of the measured initial concentrations with the assumed nominal concentration in experiments 3 and 4 compared to experiments 1 and 2 might be explained by different experimental set ups that enhanced the solubility of 8:2 FTOH. In experiments 1 and 2, 8:2 FTOH was spiked to a solution which was mixed and divided into sample bottles. On the other hand, in experiments 3 and 4, 8:2 FTOH was spiked directly to each sample bottle. Furthermore, there was a 2-month delay before frozen samples of experiment 3 were analysed. This might explain the better recovery of 8:2 FTOH compared to that in experiment 4.

The faster decrease of the concentration of 8:2 FTOH in the bottom water than in the surface water (Fig. 3) might

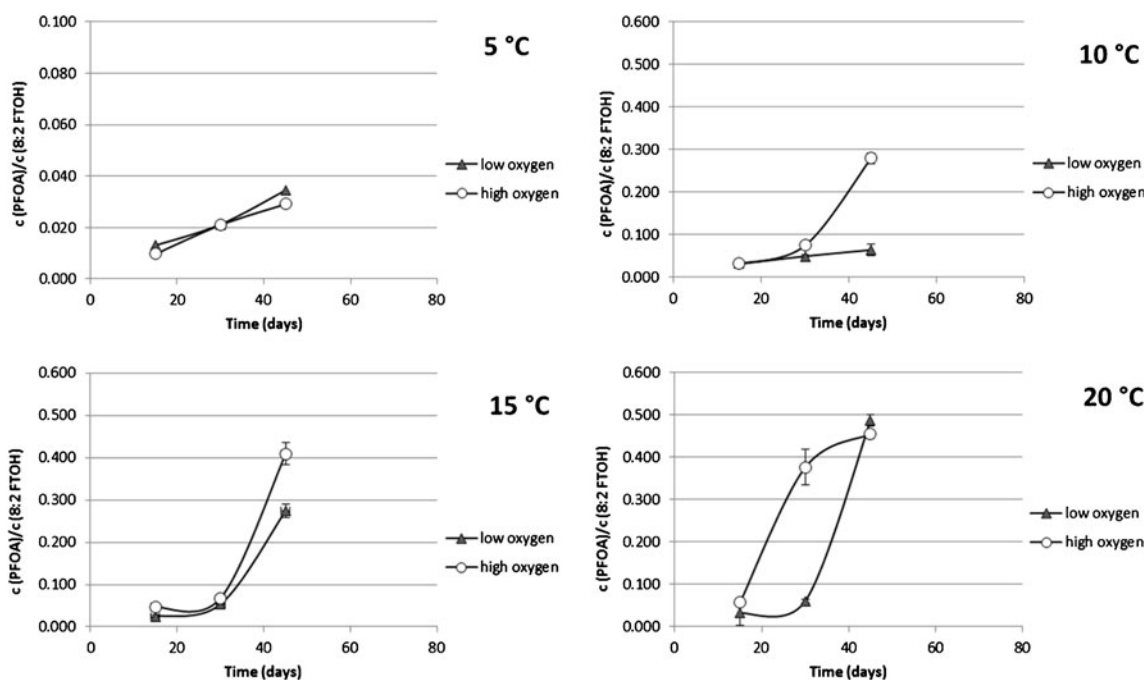


Fig. 2 The ratio of PFOA concentrations to initial 8:2 FTOH concentration in experiment 3 (high-oxygen vs. low-oxygen surface water) at different temperatures

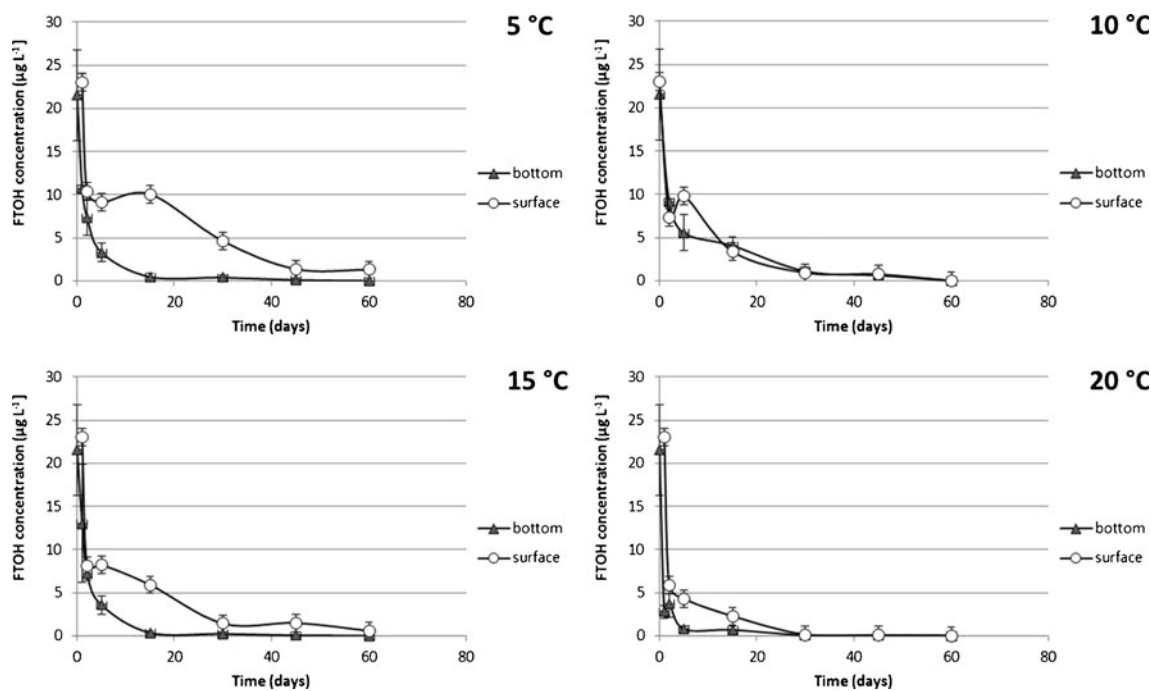


Fig. 3 8:2 FTOH concentrations in experiment 4 (surface vs. bottom water) at different temperatures

indicate that there were more microbes capable for degrading 8:2 FTOH in the bottom water or that there were more nutrients and carbon sources for the microbes in bottom water compared to surface water. There are no data on DOC in the water from the sampling site of experiment 4, but on the other hand, no great differences in DOC between

surface and bottom water for other sites near our sampling sites have been observed (Hoikkala et al. 2012).

Temperature dependence was observed for the formation of PFOA in both bottom and surface water (Fig. 4), resembling that observed in experiment 3. As in experiment 3, there was no significant difference in the formation of PFOA

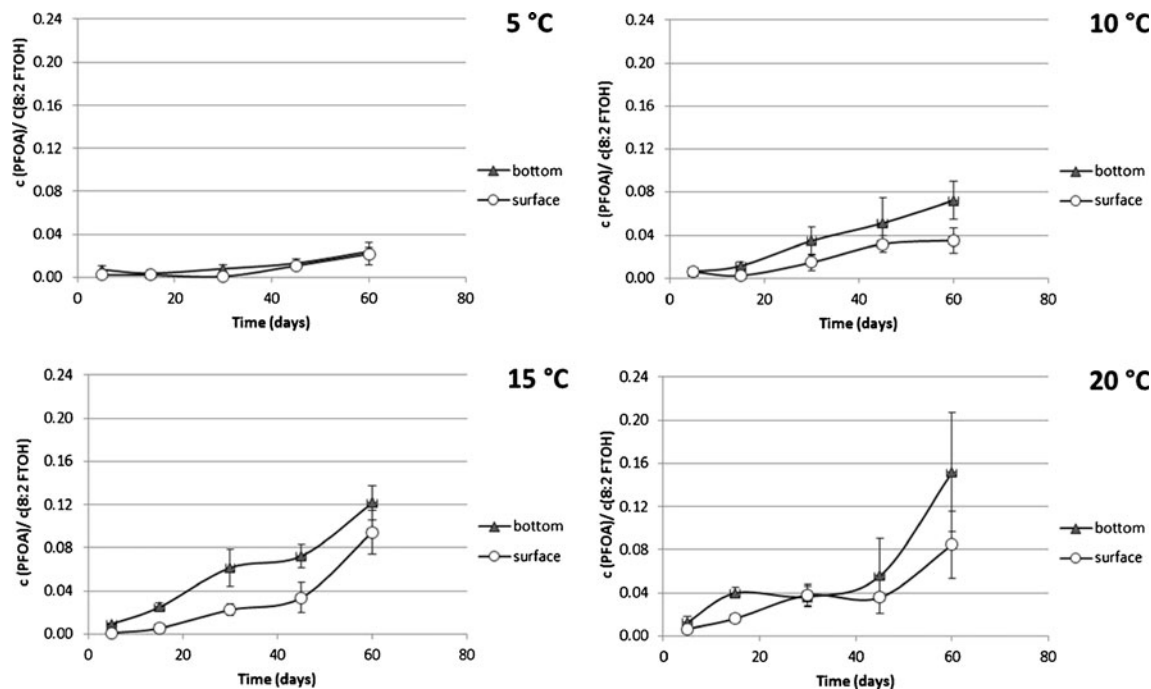


Fig. 4 The ratio of PFOA concentrations to initial 8:2 FTOH concentration in experiment 4 (surface vs. bottom water) at different temperatures

Table 2 MANOVA (upper panel) and ANOVA (lower panel) testing effects of temperature and water depth (oxygen, pH, nutrients etc.) (surface or bottom water) on PFOA concentrations in experiment 4*

Effect	Wilk's λ	<i>F</i>	<i>df</i>	<i>P</i>		
Temperature	0.005	10.009	18, 31.59	0.000		
Water depth	0.107	15.365	6, 11	0.000		
Temperature × water depth	0.082	2.504	18, 31.59	0.012		
Source	Dependent	SS	<i>df</i>	MS	<i>F</i>	<i>P</i>
Temperature	PFOA5	0.265	3	0.088	2.726	0.079
	PFOA15	2.004	3	0.668	62.147	0.000
	PFOA30	4.167	3	1.389	20.879	0.000
	PFOA45	4.412	3	1.471	6.392	0.005
	PFOA60	15.619	3	5.206	6.71	0.004
Water depth	PFOA5	0.295	1	0.295	9.122	0.008
	PFOA15	0.761	1	0.761	70.832	0.000
	PFOA30	1.232	1	1.232	18.519	0.001
	PFOA45	1.654	1	1.654	7.187	0.016
	PFOA60	0.774	1	0.774	0.997	0.333
Temperature × water depth	PFOA5	0.027	3	0.009	0.277	0.841
	PFOA15	0.196	3	0.065	6.092	0.006
	PFOA30	1.166	3	0.389	5.84	0.007
	PFOA45	0.749	3	0.25	1.085	0.384
	PFOA60	2.601	3	0.867	1.117	0.371
Error	PFOA2	0.169	16	0.011		
	PFOA5	0.518	16	0.032		
	PFOA15	0.172	16	0.011		
	PFOA30	1.064	16	0.067		
	PFOA45	3.682	16	0.23		
Total (corrected)	PFOA60	12.414	16	0.776		
	PFOA2	0.909	23			
	PFOA5	1.104	23			
	PFOA15	3.134	23			
	PFOA30	7.629	23			
	PFOA45	10.497	23			
	PFOA60	31.408	23			

*The MANOVA results are for the overall effects using the concentrations on all sampling days as dependent variables. The ANOVA results report the treatment effects on each day individually and can be interpreted when effects in the MANOVA are significant. The numbers 5, 15, 3, 45 and 60 in the “Dependent” column of the ANOVA table indicate the PFOA sampling days

between different waters at a low temperature (5 °C). The PFOA results of sampling day 2 were not included due to suspected laboratory contamination of these samples (most likely during the extraction). At the highest temperature (20 °C), the formation rate of PFOA was higher in the bottom water than in the surface water, although a latency period was observed in the formation of PFOA in the bottom water between 15 and 30 days. At 20 °C, the concentration of PFOA in bottom water was 3.6 (±1.0)% of the expected initial concentration of 8:2 FTOH after 30 days of exposure. The higher formation of PFOA at temperatures of 10, 15 and 20 °C during 45 and 60 days in the bottom water, compared

to the surface water, could indicate that microbial activity was higher in the bottom water. For these microbes, the low oxygen content was presumably high enough to form more PFOA than in the surface water with a higher oxygen level. Differences in the formation of PFOA between the surface and bottom waters could also be due to differences in the DOC, nutrients and pH of the water. Similarly to oxygen levels, the pH was lower in the bottom water in the beginning of the experiment (pH 7.6 vs. 8.1 and oxygen 4.5 vs. 7.5 mg L⁻¹). Based on analyses of water samples (performed 1 week before our sampling), the phosphate concentrations were higher in the bottom water than in the surface water, but the

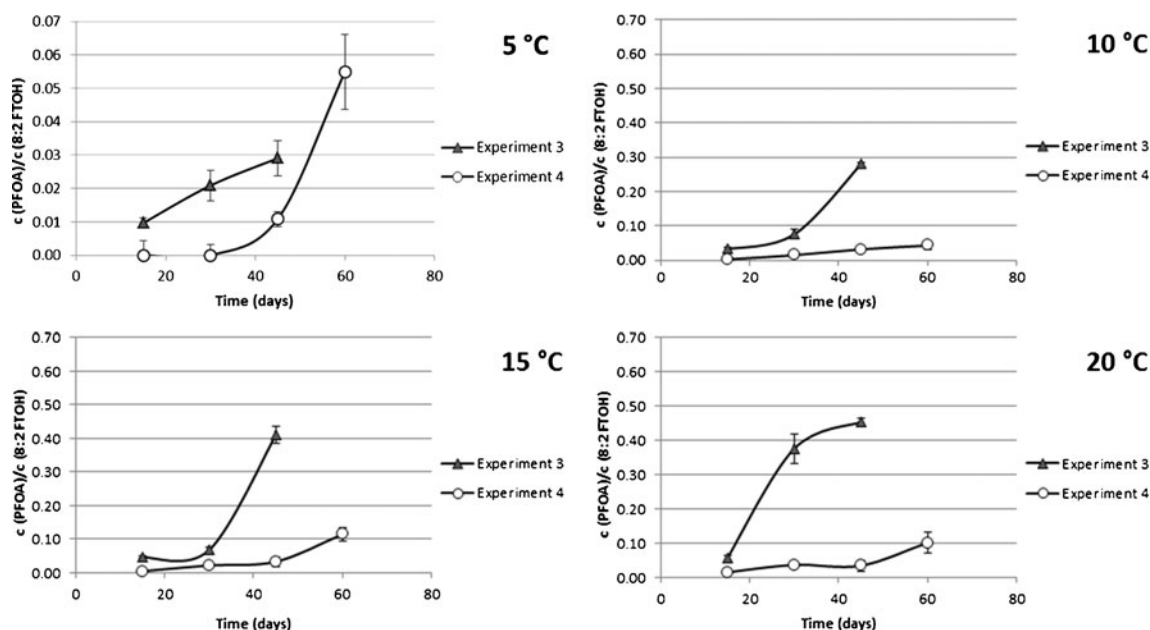


Fig. 5 The ratio of PFOA concentrations to initial 8:2 FTOH concentration in surface water in experiment 3 vs. experiment 4

nitrate concentrations were low in both waters (unpublished data).

There were also differences in pH and oxygen conditions in the control samples between sampling days. In the surface water, pH increased during first days but then decreased. Microbial activity could possibly explain this increase. Oxygen levels decreased in the control samples during the experiment, the decrease being most marked in the surface water at the highest temperature. However, the oxygen levels increased again later, except in the surface water at 5 °C. The decrease of oxygen concentration during first 5 days could be due to the consumption of oxygen in microbial activity. The oxygen level in the bottom water reached that in the surface water in 60 days at all temperatures tested.

Statistically (in parallel to experiment 3), the differences between the bottom and surface waters manifested themselves as a significant interaction between temperature and water depth (microbes/ oxygen/pH/nutrients), as well as significant main effects of these factors, when all sampling dates were included in the MANOVA (Table 2, upper panel). In the ANOVA (Table 2, lower panel), the different rates at which PFOA was formed in surface and bottom waters, and at different temperatures, showed up as a statistically significant interaction on sampling dates 15 and 30. The main effect of water depth was significant on all sampling days except day 60, whereas, as the main effect, temperature approached significance on day 5 and was thereafter significant throughout the experiment.

Differences in the conditions could explain why the formation of PFOA from 8:2 FTOH in the surface water in

experiment 4 was lower than in experiment 3 (Fig. 5). For example, the amount of PFOA was ten times higher in experiment 3 after 30 days of exposure at 20 °C compared to experiment 4. This might be explained by the ten times greater amount of water in experiment 3 compared to experiment 4, so that the amount of microbes, nutrients and DOC would have been lower per test bottle in experiment 4 compared to experiment 3. There could also possibly have been differences in the activity/composition of microbes due to the different sampling sites and probably differences in salinity and pH, too. In water samples taken in the vicinity of our sampling sites (performed before or 1 week after our sampling), the salinity and pH were at a lower level (4.1 vs. 5.8‰ and 7.2 vs. 8.3‰, respectively) and nutrients, such as nitrate and silicate (270 vs. 1 $\mu\text{g L}^{-1}$ and 1,300 vs. 220 $\mu\text{g L}^{-1}$, respectively), at a higher level in water collected near the sampling site of experiment 3 water compared to water collected in the sampling site of experiment 4 water.

The presence of FTOHs in WWTP effluents and sewage sludge-applied soils, and the formation of highly persistent PFOA in an aquatic environment (Ellington et al. 2009; Mahmoud et al. 2009; Yoo et al. 2010), can be a long-lasting concern regarding surface waters. The release of FTOHs from commercial or industrial products to the aquatic environment might be more significant in the future. The release may increase through landfills as, e.g. acrylate-linked fluoropolymer can degrade in soil to form 8:2 FTOH and PFOA (Russell et al. 2008). As FTOH biotransformation products are toxic to aquatic species (Phillips et al. 2007), more knowledge about the biotransformation of FTOHs in the aquatic environment is

needed for the estimation of the environmental risks of PFAS. Although the transformation of 8:2 FTOH to other more stable and harmful substances is not complete, it can have significant effects on the environment due to the possible cocktail effect of pollutants (Celander 2011).

Conclusions

The formation of PFOA from 8:2 FTOH observed under laboratory conditions in brackish water indicates that FTOHs can be transformed into PFOA in natural waters. Microbes are most likely responsible for this process. From our data, it is apparent that environmental conditions can clearly affect the formation of PFOA from its precursor 8:2 FTOH. Clear temperature dependence was observed in the formation of PFOA in brackish water tests; after a 30-day period, a sixfold increase was observed in the amount of PFOA in surface water between the temperatures of 15 and 20 °C. This shows that the formation is more intense during summertime, but oxygen also affects the production of PFOA in the aquatic environment. pH, salinity, DOC and nutrients may also contribute to the formation of PFOA. Further studies are needed in order to obtain more information about the effects of environmental characteristics, including, e.g. microbial communities, TOC and ionic strength, on the formation of PFOA from its precursors in the aquatic environment.

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