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Physicochemical assessment, biodegradation and environmental analysis of perfluoroalkyl phosphonic compounds

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Introduction

Perfluorinated compounds (PFCs) are a class of organofluorine which are used as an inert material due their physical, chemical and biological stability. Due the strong carbonfluorine (C-F) bond associated with PFCs, some of these compounds have high stability and environmental persistence and can be bioaccumulated and biomagnified assessing the presence of these ones in environmental samples , biological samples and food . Because of their persistence and their potential to accumulate they are of toxicological concern . The study of possible metabolites and degradation products should be done in order to establish a direct impact into environment. One group of these compounds are the perfluoroalkyl phosphonic acids (PFAPs) which have been detected in water from Canada and the effect of these compounds has been assessed in rats concluding that the mono-PFPAs and di-PFPAs may also have significant lifetimes in the human body and it was demonstrated that the mono-PFPAs may bind to blood cells underestimating their concentration in plasma and sera samples. The biological fate of the mono-PFPAs and di-PFPAs determined in the study suggested that there was a potential exposure for human and that if exposure does occur, they may be long-lived in the body.

Under the frame of "Environmental ChemOinformatic Marie Curie Initial Training Network", it was proposed the study of: i) to establish different physicochemical properties of of three different PAPS (perfluorohexyl phosphonic acid, perfluorooctyl phosphonic acid, and perfluorodecyl phosphonic acid), ii) to assess the possible biodegradation and released products in effluent water, iii) adapt and validate an analytical method for the analysis of PFPAs and iv) to assess the occurrence of PFPAs in different water samples from Germany. This project was developed in laboratory facilities of Hochschule Fresenius-Institute for Analytical Research (IFAR), in the group of Prof. Dr. Thomas Knepper and in IDAEA-CSIC laboratories.

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order to establish a direct impact into environment. One group of these compounds are the perfluoroalkyl phosphonic acids (PFAPs) which have been detected in water (10) from Canada and the effect of these compounds has been assessed in rats (11) concluding that the mono-PFPAs and di-PFPAs may also have significant lifetimes in the human body and it was demonstrated that the mono-PFPAs may bind to blood cells underestimating their concentration in plasma and sera samples. The biological fate of the mono-PFPAs and di-PFPAs determined in the study suggested that there was a potential exposure for human and that if exposure does occur, they may be long-lived in the body.

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Experimental section

Chemicals and reagents

Perfluoroalkyl phosphonic acids included in this study were: perfluorohexylphosphonic acid (PFHxPA), perlfuorooctylphosphonic acid (PFOPA) and perfluorodecylphosphonic acid (PFDPA) [chemical purity > 98 %; methanol] supplied for Wellington Laboratories Inc. (Canada).

6-Chloroperfluorohexylphosphonic acid (ClPFHxPA) [chemical purity > 98 %; methanol] was used as a surrogate and was purchased from Wellington Laboratories Inc. (Canada).

Ultra pure Milli-Q water was prepared by a Millipore-Q-system (Millipore, Milford, MA, USA). Methanol (MeOH) suprasolv, ammonium acetate salt ($AcNH_4$: MW, 77.08; $\geq$98 %), ammonia, n-hexane and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany).

Physicochemical properties

Partition coefficient water-hexane. In order to assess the fate of PFPAs compounds in environment, the partition coefficient between water and hexane was calculated according OECD guideline 123 (12). 10 ml of a mixture n-hexane: miliQwater (1:1) was prepared in a PP tube with PFPAs at 100 µg/L. The mixture was vortexed 1 min and shaked in an orbital digestor. In order to establish the equilibrium between water-hexane, an aliquot of 0.5 ml of hexane and an aliquot of 0.5 ml of water were taken at different times: 0, 1, 2, 24, 48 and 96 hours.

0.175 ml of water aliquot was diluted with 0.075 ml of MeOH (7:3) and then 2.5 µl of surrogate-internal standard was added in order to obtain a final concentration of 10 µg/L.

0.175 ml of hexane aliquot was dried under N_2 (g), reconstituted in a 0.250 ml of Water:MeOH (7:3) and 2.5 µl of surrogate-internal standard was added in order to obtain a final concentration of 10 µg/L.

Adsorption experiments in sludge. PFPAs Adsorption - Desorption experiments were carried out according to the procedure described by the OECD guideline 106 (13), using the indirect method. These experiments were performed using two types of sewage sludge from Beuerbach (Hesse, Germany): activated sludge and final sludge. Very briefly, the procedure

was as follows: the samples were dried under ambient conditions and homogenized. Approximately 2 g of dried sample were introduced into a PP tube and 10 ml of MilliQ-water with $CaCl_2$ (0.01 M), according to ISO 10390-1 (14), in order to minimize the cation exchange. This first part of the experiment was carried out in quadruplicates and two procedural blanks were included consisting in MilliQ-water $CaCl_2$ (0.01 M). The mixture was vortexed 1 min and stirred overnight (~12 h) in an orbital digester. The first aliquot of water (200 µl) was taken as a blank before start the spiked experiments. Then, three of the PP tubes were spiked with PFPAs at optimum level in order to achieve 100 µg/L. The other PP tube with sludge was used as a blank. The prepared tubes were vortexed and stirred in an orbital digester along all experimental process. During the sampling process 200 µl aliquot of water were taken after the following times: 0, 2.5, 4, 7.5, 24 and 48 hours.

Water aliquots were introduced into a PP eppendorf and centrifuged at 4000 rpm, at room temperature for 20 min. 0.175 ml of the supernatant was filtered by 0.45 µm Nylon filter and filled up with 0.075 ml of MeOH. 2.5 µl of surrogate-internal standard was added to obtain a final concentration of 10 µg/L.

Aerobic degradation experiments. Degradation experiments were carried out in order to test if any transformation process can be done by aerobic organisms of WWTPs. The experiments were performed according to OECD guideline 309: Aerobic Mineralization in Surface Water – Simulation Biodegradation Test (15). For experiment purposes, wastewater effluent was taken from Beuerbach WWTP (Hesse, Germany). The wastewater effluent was distributed in seven 250 mL amber glass bottles. 5 bottles were spiked with a mixture of PFPAs to obtain a final concentration of 500 µg/L. Three of the bottles were used to follow the biotransformation processes and the other two ones were spiked with NaN_3 in order to stop the biological activity and to assess other possible physicochemical processes as hydrolysis or glass adsorption. In addition, two non spiked wastewater effluents were used as a blank.

During 3 month the bottles were stirred 24 h/day in an orbital digester and the pH was controlled and maintained constant. Dark conditions were used in order to minimize the algae growth, and aerobic conditions were maintained under aeration.

During first week every two days an aliquot of 0.5 ml was taken from each bottle and once a week during the rest of the experiment. These samples were filtered through 0.45 µm

cellulose filter. Then, 0.35 ml of each one was transferred into a PP vial and filled up with 0.15 ml of MeOH using ClPFHxPA as internal standard.

In order to adapt the organisms to phosphonic degradation one more experiment was carried out in parallel. The effluent blank water was spiked with 10 ng/ml of triphenylphosphinoxid (TPPO). This experiment was performed based on an earlier published work by Knepper et al. (16) were the effluent WWTP water organisms were able to degrade TPPO in 21 days in a biologically active fixed-bed bioreactor (FBBR) experiments simulating a WWTP system. The effluent water was spiked with TPPO and aerobic conditions were maintained shaking experimental bottles in an orbital digester 24 h/day with aeration of the water 20 min, once a day. After 21 days the water was spiked with PFPAs at the same concentration that the previous experiments carried out without TPPO. The same conditions and sampling times were maintained.

Algae adsorption experiments. *Desmodesmus subspicatus* (pond scum, green weed) was used in algae adsorption experiments. In this case, the experiments were carried out adding 6.75, 12.5, 25, 50 and 100 ng/mL of corresponding PFPA into transparent glass bottles. The bottles were exposed to natural light and stirred in an orbital digester along the experiment time. Aliquots of 250 µl were taken after 0, 0.5, 1, 5, 24, 144 and 288 hours of exposure and diluted 1:1 with MeOH in order to stop any biological process. A blank experiment containing the algae medium and algae medium spiked with PFPAs at 100 ng/ml were carried out in order to avoid any possible cross contamination during experiments, analysis process or adsorption of the analytes in the glass. The algae medium was constituted with different nutrients ($NaHCO_3$, $CaCl_2$, NH_4Cl, $MgSO_4$, $MgCl_2$ and KH_2PO_4 at mg/L levels) and trace elements (Na_2EDTA, $FeCl_2$, $MnCl_2$, H_3BO_3, Na_2MoO_4, $ZnCl_2$, $CoCl_2$, $CuCl_2$) at pH of 8. The pH of the samples was continuously controlled.

A sample of 0.5 ml was taken from each bottle at experimental time. These samples were filtered through 0.45 µm cellulose filter. Then, 0.35 ml of each were transferred into a PP vial and filled up with 0.15 ml of MeOH using ClPFHxPA as internal standard.

PFPAs in water samples

To assess the presence of PFPAs in different types of water, an analytical method was developed based on solid phase extraction (SPE) followed by LC-MS/MS. SPE procedure was based on the adaption of a previous method published by Llorca et al. (2). In this case, the method was modified in order to improve the efficiency of PFPAs during SPE. The optimized conditions were: i) conditioning: 2 x 2 ml of MeOH, 2 x 2 ml of miliQ water; ii) loading: 150 ml of water at 1 ml/min and dried under N_2; iii) elution: 2 x 2 ml of MeOH (1% NH_3).

The quality assurance include: method limits of detection and quantification (MLOD and MLOQ), the efficiency of the method evaluated by recoveries at three different spiked levels (15, 30 and 60 ng/L) of MiliQ water and effluent water, and the reproducibility intraday of the method at the same spiked levels.

In order to assess the presence of these compounds in water different samples were analyzed, including mineral bottled water, tap water, river surface water and effluent water from Beuerbach WWTP (Hesse, Germany). A total number of 34 samples were analyzed. A blank of miliQ water and all the solvents used during extraction and clean-up procedure were extracted in parallel in order to discriminate possible cross contamination.

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Instrumental analysis

Degradation and algae experiments were followed by analysis using liquid chromatography coupled to a hybrid quadrupole-linear ion trap analyzer (LC-QqLIT). The separation of the analytes was carried out in a Synergi 4u Fusion-RP 80A (50 x 2.0 mm) column in a Perkin Elmer Series 200 HPLC chromatograph (Norwalk, CT, USA). The chromatograph was coupled to a QqLIT mass spectrometer Q Trap 3200 (Applied Biosystems, Foster City, CA, USA) using a Turbo Ionspray interface in electrospray negative mode. The acquisition of the samples was done by selected reaction monitoring mode (SRM). The possible metabolites formation was assessed using the Scan mode in the first quadrupole working under negative and positive ionization conditions. The injection volume was set at 5 µl for the SRM mode, and at 30 µl using the Scan mode.

The analysis of adsorption experiments in sludge, algae experiments and water samples were carried out by liquid chromatography coupled to a triple quadrupole analyzer (LC-QqQ). The separation of the analytes was carried out in a MZ-Aqua Perfect C18 (5µm, 50 x 2.1 mm) column in a HP HPLC chromatograph (Norwalk, CT, USA). The chromatograph was coupled to a QqQ mass spectrometer API 2000 (Applied Biosystems, Foster City, CA, USA) using a Turbo Ionspray interface in electrospray negative mode. The acquisition of the samples was done by selected reaction monitoring mode (SRM) with an injection volume of 5 µl.

In both cases mobile phases were consisting in (A) water:MeOH (95:5) 5 mM $AcNH_4$ and (B) water:MeOH (10:90) 5 mM $AcNH_4$ with the gradient as follows: starting at 70% of A and at 0.5 min decreased to 20% at 2 min. From 2 to 6 min the %A decreased until 10% and was maintained 0.5 min. After this time the %A returned to 70% in 1.5 min and was maintained for 6 min. The total run time was 12 min at flow rate of 400 µl/min.

Main transitions used for quantification and identification, as well as, retention time for the 3 analytes are summarized in table 1.

Table 1: Main transitions of PFPAs

	Retention time (min)	**Transitions**	**Ratio**
PFHxPA	3.9	399 > 399 399 > 79*	1.5
PFOPA	5.1	499 > 499 499 > 79*	1.2
PFDPA	5.8	599 > 599 599 > 79*	1.7

* Quantification transition

Results and discussion

Partition coefficient water-hexane

Table 2 shows the obtained results in the water-hexane partition experiments.

Table 2: partition coefficient (D) hexane-water

	Concentration (µg/L)								
	PFHxPA			PFOPA			PFDPA		
	Hexane	Water	D	Hexane	Water	D	Hexane	Water	D
24h	< 0	94.1		3.12	73.7	0.042	1.61	22.8	0.071
48h	< 0	106		0.691	89.5	0.008	1.24	24.8	0.050
96h	1.29	112	0.012	0.97	85.1	0.011	3.39	18.6	0.182

D = [Concentration hexane / Concentration water]

As it can be seen, these compounds were more hydrophilic due the phosphonic group than hydrophobic due the perfluoroalkyl chain.

This experiment concluded that these compounds can be found in environmental waters.

Adsorption experiments in sludge

Figure 1 summarizes adsorption/desorption experiments. As can be seen, long chain compounds were adsorbed to sludge, whereas for the shortest one the compound can be found in both phases. With these experiments were established the percentages distributed in both phases after the equilibrium achieved after 10 h of contact. In the case of PFDPA this percentage of adsorption in sludge was around 80% of exposure and 70% for the activated sludge. In the case of PFOPA the percentage of adsorption was similar to PFDPA adsorption in sludge but in activated sludge the percentage of adsorption was lower (45%).

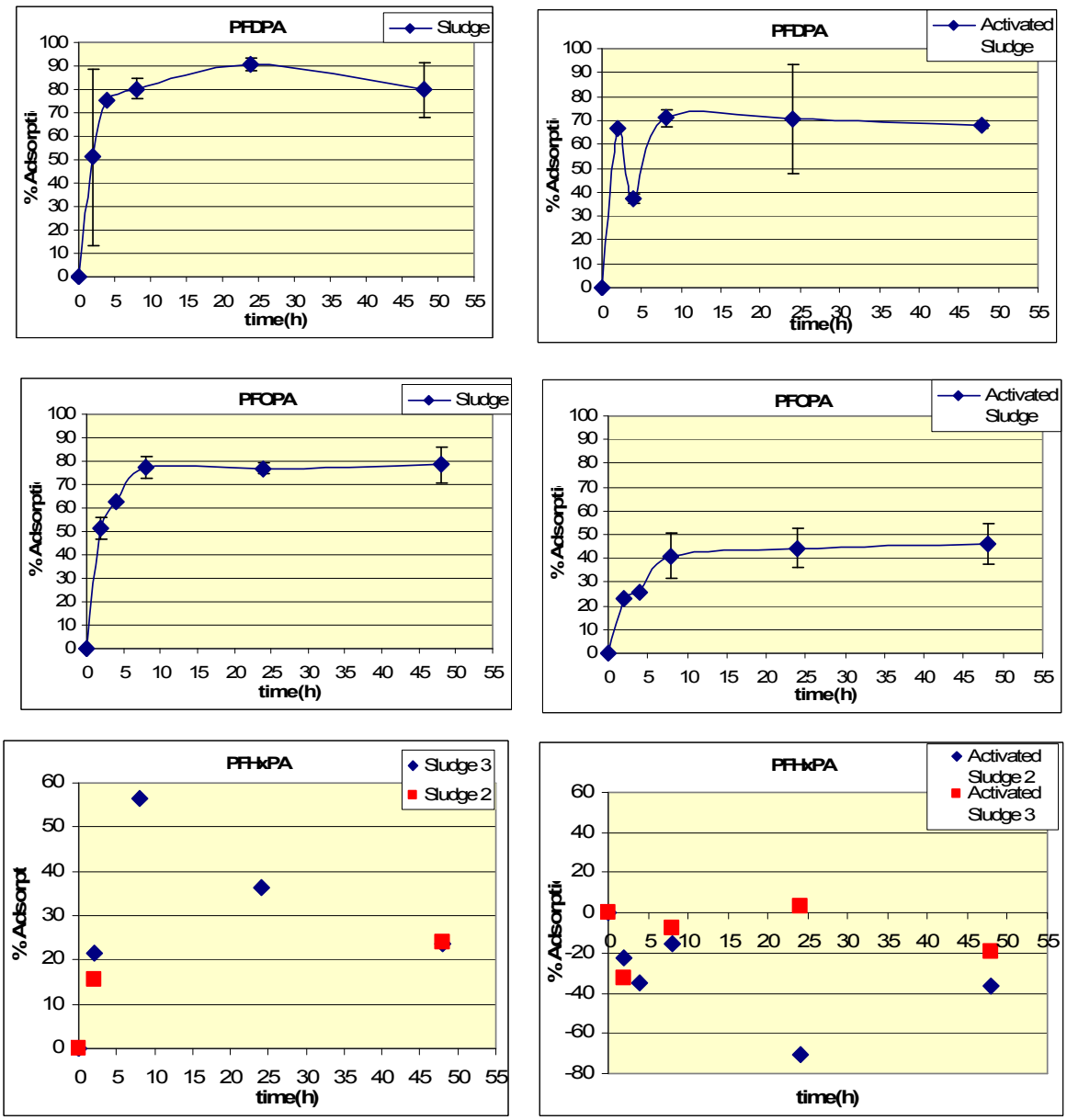

Figure 1: percentage of adsorption vs time of exposure.

The distribution coefficient (K_d) based on equation of figure 2 was calculated, where A_{eq} is the percentage of adsorption at equilibrium (%), V_0 is the initial volume of aqueous phase and m_{soil} is the mass of the soil used in the experiment.

$$K_d = \frac{A_{eq}}{100 - A_{eq}} \cdot \frac{V_0}{m_{soil}} (cm^3 g^{-1})$$

Figure 2: distribution coefficient equation according OECD guideline 106 (13).

The K_d calculated for sludge were: [11.9 ± 1.7] cm^3/g for PFOPA and [14.7 ± 5.6] cm^3/g for PFDPA. For activated sludge the results of the K_d were: [12.4 ± 5.2] cm^3/g for PFOPA and [42.7 ± 5.9] cm^3/g for PFDPA. As can be seen in both cases, the K_d of PFDPA was higher than PFOPA values. This result was expected since PFDPA has a higher C chain than PFOPA and, consequently, the molecule is likely to be more lipophilic.

In the case of PFHxPA experiments the equilibrium was not established.

Aerobic degradation experiments

Aerobic degradation experiments performed for PFHxPA and PFOPA concluded that no degradation processes occurred along all the experiment exposure.

Figure 3 shows the results obtained during the analysis of effluent water experiments for PFDPA.

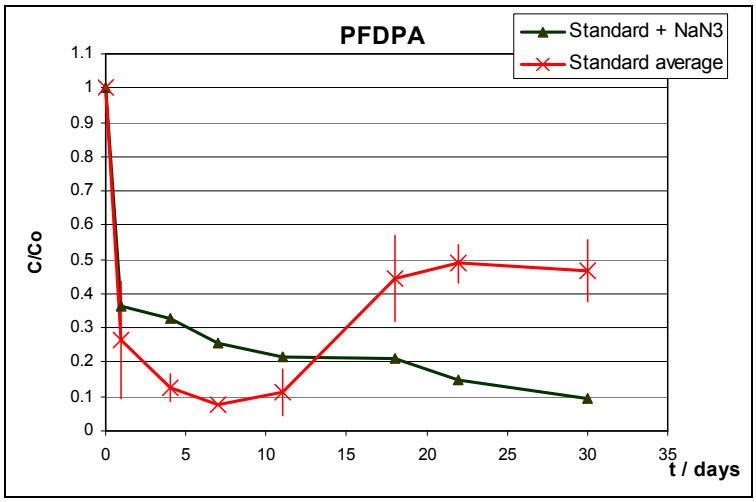

Figure 3: relative level of PFDPA in effluent water vs. time of exposure. Error bars of triplicates.

As it can be seen, between the 2nd and the 10th day of experiment, the levels of PFDPA (red colour line) were lower than the level of this compound adsorbed in the glass (experiment with standards and NaN_3, green line). It could indicate that this compound was degraded, metabolized or adsorbed by the organisms. The possible degradation or metabolization was ruled out because there was not found any degradation product during the Scan analysis.

Along this experiment it was observed the generation of a membrane or biofilm inside the water experiment, which began the 2nd experimental day and the filtration of the aliquot by 0.45 µm cellulose filter was difficult. It could be that the decrease of the PFDPA was related to the generation of this membrane.

In order to discriminate the fate of PFDPA, four aliquots of 0.5 ml were extracted without filtration using dilution [1:1] with the follow solvents: A) miliQ water, B) acetonitrile, C) n-hexane and D) sodium dodecyl sulphate (SDS) at 1% in miliQ water. All the mixtures were vortexed 1 min, mixed in an ultrasonic bath 20 min and centrifuged at 10000 rpm, 20 min. 0.35 ml of the supernatant from the experiments A and D were transferred into a PP vial. 0.35 ml of the supernatant of the experiments B and C were transferred into a PP vial, dried under a gentle stream of N_2 and reconstituted with 0.35 ml of miliQ water. All the extracts were filled up with 0.15 ml of MeOH and ClPFHxPA was introduced as internal standard. The samples were analyzed by SRM and Scan mode (in positive and negative mode) by LC-QqLit instrument. Some of the obtained chromatograms in SRM mode can be seen in Figure 4. The results obtained with the different extraction experiments show how the level of PFDPA (in intensity terms) increased following the order: miliQ water ~ n-hexane < ACN < SDS. Using ACN as extracting agent we obtained the precipitation of the external proteins retained in the membrane or biofilm generated. The PFDPA came from the destruction of this membrane. The use of SDS as a disrupting cellular agent, the higher level of PFDPA came from extracellular and intracellular source. These results could indicate that part of the compound was adsorbed by the organisms using this one as a source of phosphate or due the similarity between PFDPA and one of the phospholipids used in the extracellular membrane. This hypothesis is in agreement with previous review by Pearsons et al. (17) which suggested that bacteria will adapt to utilize this ones as source of energy. Another possibility is that this compound could be included in cells and excreted material could generate a membrane complex arising from the outer membrane of the cell envelope as in

previous published work by Rothfiel et al. (18) in the study of growing cultures of *Escherichia coli* and *Salmonella typhimurium* and the excretion process of lipopolysaccharides, phopholipids and proteins into the culture fluid. As it can be seen in the Figure 3, the level of PFDPA increased between 15th and 30th day of experiment. These could indicate that the cells started to excrete PFDPA or the death of the responsible organism.

In order to identify the organisms responsible of PFDPA adsorption, microscope analysis was carried out. In addition, algae organisms were discarded because the experiments were carried out in the dark. However, due to the complexity of the matrix, the identification of the organisms was not feasible.

Results obtained using TPPO as an agent to adapt the organisms presents in effluent water from WWTP to phosphonic degradation showed the same generated membrane but in this case the levels of PFDPA didn't follow a coherent adsorption being randomly distributed between the glass and the adsorption in the organisms or membrane.

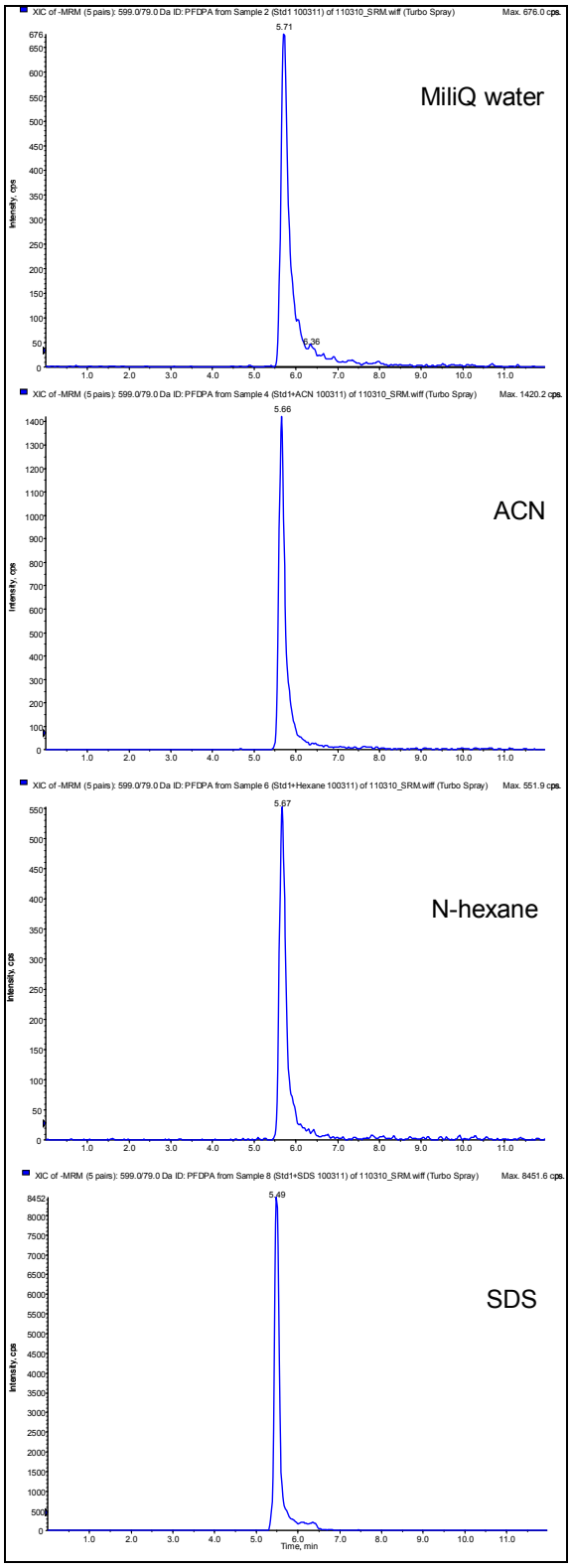

Figure 4: Chromatograms in SRM mode of experiments using miliQ water, acetonitrile, n-hexane and SDS.

Algae adsorption experiments

The algae adsorption experiments didn't conclude any type of adsorption although disappearance of algae green colour in bottle experiments with PFDPA was observed.

Due the disappearance of green colour it was proposed to measure the absorbance of the most concentrated experiment bottles for every analyte. The results showed that the absorbance of PFDPA was four times lower than the absorbance of the other two PFPAs. These preliminary results could indicate that the presence of PFDPA inhibits algae growth or maybe it only inhibits the chlorophyll generation.

More studies are required in order to establish the process of the inhibition of the green colour in PFDPA experiments.

Validation parameters of PFPAs water extraction

Different quality parameters were studied in order to assess the applicability of the method in miliQ water and WWTP effluent water including: method limits of detection and quantification (MLOD and MLOQ, respectively), the percentage of recovery at three different spiking levels (15, 30 and 60 ng/L) and the reproducibility intraday at every spiked level expressed as a percentage of relative standard deviation (%RSD). Results are presented in tables 3 and 4.

Table 3: quality parameters calculated in miliQ water

	MiliQ water							
	MLOD (ng/L)	MLOQ (ng/L)	15 ng/L		30 ng/L		60 ng/L	
			% Rec	% RSD	% Rec	% RSD	% Rec	% RSD
PFHxPA	0.64	2.1	82	3	63	8	81	20
PFOPA	0.35	1.2	76	12	80	6	88	23
PFDPA	0.50	1.7	124	4	58	1	100	17

Table 4: quality parameters calculated in WWTP effluent water

	WWTP effluent water							
	MLOD (ng/L)	MLOQ (ng/L)	15 ng/L		30 ng/L		60 ng/L	
			% Rec	% RSD	% Rec	% RSD	% Rec	% RSD
PFHxPA	0.46	1.5	107	13	110	3	82	13
PFOPA	0.44	1.5	76	22	65	11	61	28
PFDPA	0.83	2.8	107	12	76	5	56	10

%Rec: percentage of recovery (n=3)
%RSD: reproducibility intraday (n=3)
MLOD: method limit of detection
MLOQ: method limit of quantification

Good quality parameters were obtained for the analysis of PFPAs in clean waters (miliQ water optimization) and dirty ones (effluent WWTP water optimization). The selected compounds were detected below 1 ng/L in all waters and percentages of recoveries in the range of spiked levels were between 58 – 124% in miliQ water and between 56 – 107% in WWTP effluent water. The reproducibility intraday was below 30% in all the cases.

PFPAs in water samples

The analysis of miliQ water as a blank of all analytical procedure and all the solvents used during extraction and clean-up procedure extracted in parallel in order to discriminate possible cross contamination concluded that no PFPAs were present in the solvents or included during analytical procedure.

Table 5: results of water monitoring

	PFHxPA			PFOPA		
Sample Name	**Average (ng/L)**	**SD**	**%RSD**	**Average (ng/L)**	**SD**	**%RSD**
Mineral Water 1	< MLOD			< MLOD		
Mineral Water 2	< MLOD			< MLOD		
Frankfurt Tap Water 1	<MLOD			< MLOQ		
Frankfurt Tap Water 2	< MLOD			< MLOD		
Frankfurt Tap Water 3	< MLOD			< MLOD		
Idstein Tap Water 1	< MLOD			< MLOD		
Potable water 1	27	0.01	11	< MLOD		
Water after charcoal treatment	46	0.01	13	< MLOD		
Rhine Sand Trap water	53	0.01	16	< MLOD		
Nidda river 1	23	1.8	8	< MLOD		
Nidda river 2	< MLOD			9.4	0.6	6
Nidda river 3	51	4.1	8	< MLOD		
Nidda river 4	< MLOD			6.6	0.3	5
Nidda river 5	63	13	21	< MLOD		
Pfuhlgraben, Wehrda	< MLOQ			< MLOD		
Usa, Friedberg, Unterhal	< MLOQ			< MLOD		
Erlenbach, Bad Vilbel	< MLOQ			< MLOD		
Landgraben, Trebur, Brue	2.8	0.7	25	< MLOD		
Horlof, Niederflorstadt	< MLOQ			< MLOD		
Wetter, Assenheim	2.6	0.1	5	< MLOD		
Solz, Sorga	< MLOQ			< MLOD		
Rosbach, Nieder-Woellsta	< MLOQ			< MLOD		
Wehre, Niederhone	< MLOQ			< MLOD		
Schwarzbach, Astheim, Me	3.0	0.5	16	< MLOD		
Beinesgraben, Bauschheim	2.5	0.5	20	< MLOD		
Zellersbach, Roehrigshof	2.6	0.8	30	< MLOD		
Bach aus Pferdsdorf, Wil	< MLOQ			< MLOD		
Wickerbach, Floer Sheim	< MLOQ			< MLOD		
Weihe, Untersuhl	< MLOD			< MLOD		
Hauptgraben, Astheim	< MLOD			< MLOD		
Eitra (Fischbach), Bodes	< MLOD			< MLOD		
Solz, Weiterode	< MLOQ			< MLOD		
WWTP effluent 1	< MLOD			3.9	0.3	7
WWTP effluent 2	< MLOQ			< MLOQ		

MLOD, MLOQ: method limit of detection and quantification, respectively

As can be seen in table 5, three over thirty-four analyzed samples showed positive levels for PFOPA in the range of 3.9 – 9.4 ng/L. The most contaminated samples were from Nidda River and from WWTP effluent.

In the case of PFHxPA the levels were established between 2.5 – 63 ng/L. These compounds have been reported before by Eon et al. (10) at levels between 0.88 and 3.4 ng/L of PFOPA in Canadian Surface water and between 0.76 and 2.5 in effluent water. These levels are comparables to levels reported in this work although these last ones presented higher range and included positive results in the analysis of PFHxPA.

The main difference between the concentration obtained for PFHxPA and PFOPA could be explained using the physicochemical parameters calculated previously in this work. As can be seen in the section of "Adsorption experiments in sludge", the percentage of adsorption established at equilibrium for PFOPA was between 45% in activated sludge and 80% in sludge. This indicated that the major part of PFOPA introduced into the WWTP system would be found in sludge or activated sludge of WWTP at higher levels than in water. In the case of PFHxPA no equilibrium between water and sludge (or activated sludge) was observed. Our results indicted that this compound could be randomly distributed between water and soil fractions and this can support our water results.

PFDPA was analyzed in all water samples but the results were below MLOQ in all cases.

Conclusions

This work reports some physicochemical parameters of a new class of perfluorinated acids detected before in Canada rivers and WWTP effluents. However, higher concentrations of PFHxPA and PFOPA were found in the present study of a German river and in a WWTP effluent.

The degradation experiments concluded that PFPAs were not biodegraded, although accumulation or adsorption of PFDPA was assessed. More studies are required in order to elucidate the fate and environmental impact of this compound. Also complementary study has to be performed in order to assess the type or possible type of organisms that can adsorb this compound and the mechanism of adsorption.

The identification of these related products could be a tool to help the registration, evaluate, authorize and restrict, if it would be necessary, these chemical substances although further studies are necessary.

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