University of Bayreuth
Chair of Environmental Chemistry and Ecotoxicology

Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) in an Aquatic Ecosystem - Distribution and Fate

Dissertation
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Table of Content

List of Publications and Author's Contribution

The following cumulative doctoral thesis "Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) in an Aquatic Ecosystem - Distribution and Fate" is based on the scientific publications listed below. Three of them are published and two others have been submitted for publication. Author's contribution to each of them is given below.

Publication I

Weremiuk, A.M., Gerstmann, S., Frank, H. 2006. Quantitative Determination of Perfluorinated Surfactants in Water by LC-ESI-MS/MS. *J. Sep. Sci.* **29**, 2251 – 2255.

Own contribution: idea (50 %), field work (80 %), laboratory work and data evaluation (70 %), writing (70 %).

Publication II

Becker, A.M., Gerstmann, S., Frank, H. 2008. Perfluorooctane Surfactants in Waste Waters, the Major Source of River Pollution. *Chemosphere*, **72**, 115-121.

Own contribution: idea (70 %), field work (80 %), laboratory work and data evaluation (85 %), writing (70 %).

Publication III

Becker, A.M., Suchan, M., Gerstmann, S., Frank, H. 2008. Perfluorooctanoic Acid and Perfluorooctane Sulfonate Released from a Waste Water Treatment Plant in Bavaria, Germany. Submitted for publication to *Environ. Sci. Poll. Res.*.

Own contribution: idea (90 %), field and laboratory work (30 %), data evaluation (50 %), writing (80 %).

Publication IV

Becker, A.M., Gerstmann, S., Frank, H. 2008. Perfluorooctanoic Acid and Perfluorooctane Sulfonate in the Sediment of the Roter Main River, Bayreuth. *Environ. Poll. In Press.*

Own contribution: idea (80 %), field work (70 %), laboratory work and data evaluation (90 %), writing (80 %).

Publication V

Becker, A.M., Gerstmann, S., Frank, H. 2008. Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Two Fish Species Collected from the Roter Main River, Bayreuth, Germany. Submitted to *Bull. Environ. Contam. Toxicol*.

Own contribution: idea (80 %), field work (20 %), laboratory work and data evaluation (90 %), writing (80 %).

List of Abbreviations

APPI-MS – atmospheric pressure photoionisation mass spectrometry
API – atmospheric pressure chemical ionisation
ASE – accelerated solvent extraction
BLfU – Bayerischer Landesamt für Umwelt, Bavarian State Office for Environment
dw – dry weight
EFC – electrochemical fluorination
ESI-MS/MS – electrospray ionisation coupled to tandem mass spectrometry detection
FTOHs – fluorotelomer alcohols
GC-ECD – gas chromatography coupled with electron capture detection
GFF – glass fibre filter
HPLC – high performance liquid chromatography
LC – liquid chromatography
LC-MS – liquid chromatography coupled to mass spectrometry
LOD – limit of detection
LOQ – limit of quantification
MeOH – methanol
na – not analysed
nd – not detected
NMR – nuclear magnetic resonance
NY – New York State
OECD – Organisation for Economic Co-Operation and Development
PFOA – perfluorooctanoic acid/ perfluorooctanoate
PFOS – perfluorooctane sulfonate
PFCs – perfluorocarboxylates
PFSs – perfluorinated surfactants
PFE – pressurised fluid extraction
POSF – perfluorooctanesulfonyl fluoride
PTFE – polytetrafluoroethylen
SPE – solid phase extraction
SPME – solid phase microextraction
TOF – time-of-flight
TOC – total organic carbon

UK – United Kingdom

USA – United States of America

ww – wet weight

WWTP – waste water treatment plant

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Summary

Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) belong to the family of perfluorinated surfactants (PFSs). They are widely distributed and persistent in the environment. For over 50 years, they have been used in numerous applications including paper and textile treatment, production of fluoropolymers, cosmetics and insecticides formulations, and fire fighting foams. They can enter the environment via direct and indirect emission sources such as manufacturing processes, use of commercial products containing PFSs, release of waste waters or degradation of precursor substances.

Due to their physical-chemical properties, i.e. relatively good solubility, low volatility and stability under environmental conditions, water bodies are important sinks for these chemicals. The aim of this doctoral thesis was to elucidate sources, distribution and fate of PFOA and PFOS in an aquatic ecosystem that is not directly affected by fluorochemical production activity. The presented study was mainly focused on the river Roter Main, Bayreuth, Germany, and gives a good picture of the behaviour of PFOA and PFOS in such an aquatic ecosystem.

In order to achieve the main goal, suitable analytical procedures for reliable quantification of trace amounts of the target analytes in different environmental matrices such as water, liquid and solid wastes, sediments, biological tissues, were developed or optimised. Each method included a solid-phase extraction step for analytes' preconcentration and removal of interfering matrix, followed by quantitative determination via high performance liquid chromatography coupled to electrospray ionisation tandem mass spectrometery (HLPC-ESI-MS). Due to the possibility of ionisation suppression, isotope dilution or standard addition method was applied.

Analysis of waste waters collected from four different waste water treatment plants (WWTPs) located in Upper Franconia, Bavaria, Germany, showed that the largest plant (Bayreuth) receiving waste waters of mostly commercial and industrial origin released the highest amount of PFOA and PFOS, whereas the smallest plant (Himmelkron) treating waste waters of only domestic source released the least. The monitoring of waste waters from the WWTP Bayreuth enabled to estimate the typical mass flows of PFOA and PFOS into river waters as about 1 and 5 g/day, respectively, showing that a plant of a medium-size, moderately industrialised city can be a major source of river pollution.

Detailed investigation of PFOA and PFOS concentrations in liquid and solid wastes collected at different stages of the treatment process showed additional fluxes of these compounds inside the plant, likely due to the decomposition of their precursors.

Analysis of sediments collected from the river receiving treated waste waters showed a significant increase in concentrations of both analytes downstream the outlet of the plant (up to 3- and 4-fold for PFOA and PFOS, respectively). PFOS concentrations were up to 40-fold higher in sediments than in river water, showing its higher adsorption potential in comparison to PFOA (max. sediment/water = 6).

Once in the river, PFOS, and to a lower extent PFOA, can bioaccumulate in aquatic organisms. Although partially removed from water, they are still bioavailable for benthic organisms inhabiting the river thus entering the food chain. This was reflected in higher levels found in river goby - in comparison to chub - feeding on invertebrates living in the sediment.

At the starting point of this doctoral thesis little information was available about environmental contamination with PFOA and PFOS in Germany, and it is the first study performed in Bavaria giving such a detailed picture of sources and fate of PFSs in a river ecosystem.

Zusammenfassung

Perfluorooctanoat (PFOA) und Perfluorooctansulfonat (PFOS) sind perfluorierte Tenside (PFT), die in der Umwelt weit verbreitet und persistent sind. Seit über 50 Jahren werden sie vielfältig verwendet, z.B. zur Oberflächenbehandlung von Papier und Textilien, zur Produktion von Fluorpolymeren, in kosmetischen Produkten, in Insektiziden und zur Bildung stabiler Schäume z.B. in Löschmitteln. Sie können auf direktem und indirektem Weg in die Umwelt gelangen: aus Produktionsprozessen, bei der Verwendung kommerzieller, PFT-enthaltender Produkte, über Abwasser oder durch die Zersetzung von Vorläufer-Verbindungen.

Ihre physikalischen und chemischen Eigenschaften, ihre relativ gute Löslichkeit, niedrige Flüchtigkeit und Stabilität unter Umweltbedingungen, machen Gewässer zu wichtigen Senken für diese Verbindungen. Ziel der vorliegenden Doktorarbeit war es, Quellen, Verteilung und Verhalten von PFOA und PFOS in einem aquatischen Ökosystem zu untersuchen, an dem keine Fluorchemikalien produziert werden. Die vorliegende Studie konzentrierte sich hauptsächlich auf den Roten Main, Bayreuth, Deutschland, und gibt einen Einblick in das Verhalten von PFOA und PFOS in einem typischen Fluss-Ökosystem.

Um das Hauptziel zu erreichen, wurden geeignete analytische Verfahren zur zuverlässigen Quantifizierung der Zielverbindungen im Spurenbereich in den verschiedenen Umweltmatrices, Wasser, Abwasser, Klärschlamm, Sediment, tierische Gewebeproben, entwickelt oder optimiert. Alle Methoden beinhalten eine Festphasen-Extraktion zur Aufkonzentrierung der Analyten und zum Entfernen störender Matrix; die Quantifizierung erfolgte mittels Hochleistungs-Flüssigchromatographie und Elektrospray-Ionisations-Tandem-Massenspektrometrie (LC-ESI-MS/MS). Zur Korrektur der möglichen Ionisations-Unterdrückung wurde die Isotopen-Verdünnungs-Technik oder die Standard-Additions-Methode verwendet.

Abwässer vier verschiedener Kläranlagen in Oberfranken, Bayern, Deutschland, wurden untersucht. Die größte Anlage (Bayreuth), in der überwiegend kommerzielle und industrielle Abwässer geklärt werden, trägt die höchsten Mengen von PFOA und PFOS in den Vorfluter ein, die kleinste Anlage (Himmelkron), in der nur häusliches Abwasser geklärt wird, trägt die geringsten Mengen ein. Für die Kläranlage Bayreuth wurden die täglichen Massenfrachten von PFOA und PFOS bestimmt, die in den Roten Main gelangen, sie betrugen ca. 1 g/Tag PFOA und 5 g/Tag PFOS. Außerdem können PFOA und PFOS während des Klärprozess aus Vorläufer-Verbindungen gebildet werden und sich zwischen wässrigen und festen

Stoffströmen verteilen. Abwässer aus kommunalen Kläranlagen können also eine Hauptquelle der Umweltverschmutzung an PFOA und PFOS sein.

Die Analyse von Sedimenten des Vorfluters zeigte eine signifikante Zunahme beider Analyten flussabwärts des Auslaufs der Kläranlage (3-fach für PFOA, 4-fach für PFOS). PFOS-Konzentrationen in Sedimenten waren bis zu 40-mal höher als im Flusswasser, daran zeigt sich sein, im Vergleich zu PFOA, höheres Adsorptionspotential (PFOA: max. Sediment/Wasser = 6).

Einmal im Vorfluter reichern sich PFOS und – in geringerem Maße – PFOA in der Biota dieses Lebensraumes an. Auch adsorbiert ans Sediment sind sie für bentische Organismen bioverfügbar, wie die höhere Belastung der Gründlinge – im Vergleich zu Döbeln – zeigt, die am Grund der Gewässer leben und sich von Invertebraten ernähren, die sie aus dem Bodensediment filtrieren.

Zu Beginn dieser Doktorarbeit gab es nur wenige Informationen zur Umweltbelastung mit PFOA und PFOS in Deutschland; dies ist die erste Studie, die sich detailliert mit Quellen und Verhalten dieser Verbindung in einem aquatischen Ökosystem in Bayern beschäftigt.

1 Introduction

1.1 Perfluorinated Surfactants (PFSs) – Background, Production and Use

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) belong to the wide group of chemicals called perfluorinated surfactants (PFSs). Recently, they have received a lot of attention due to their wide distribution in the environment (chapter 1.4). The presence of strong C-F bonds makes them chemically and thermally very stable, resistant to hydrolysis, photolysis, microbial degradation or metabolism (Kissa, 2001) resulting, on the one hand, in their suitability for industrial and commercial applications and on the other hand, in their persistence in the environment.

PFSs are fully anthropogenic; since the late 1940s they have been synthesised either via electrochemical fluorination (ECF) or telomerisation (Schulz et al., 2003). Commercial production of PFOA and PFOS in the United States has almost entirely been based on ECF (Figure 1) (Schultz et al., 2003). During this inexpensive process, a mixture of isomers and homologues with even and odd numbers of carbon atoms in the chain is obtained; about 30 % of the products are branched (Giesy & Kannan, 2002). Perfluorooctanesulfonyl fluoride (POSF) is the basic unit of the whole group of perfluoroalkyl sulfonate derivatives, as the POSF-based polymers degrade ultimately to PFOS (Giesy & Kannan, 2002). The telomerisation process is used for the production of perfluoroalkyl carboxylates (PFCs) and fluorotelomer alcohols (FTOHs) yielding exclusively linear products of even numbers of carbon atoms (Figure 2) (Kissa, 2001). FTOHs are volatile and can degrade to PFOA.

Production volumes of these chemicals are difficult to estimate. According to Prevendouros et al. (2006), estimated total global production of PFCs was 4400 – 8000 tons between 1975 – 2004. In 2005, the Organisation for Economic Co-Operation and Development (OECD) published results of a survey conducted in 2003 on the production of perfluorinated chemicals, based on responses received from 10 OECD-member countries (OECD, 2005), stating total volumes imported and/or manufactured in the OECD were between 100 and 200 tons/a for PFOA and related chemicals and 30 tons/a for PFOS and related chemicals. At the end of the last decade, the total global capacity for the production of perfluoroalkylated substances by ECF process was estimated to be 4650 metric tons/year (UK Stage 4 Final Report, 2004).

As the PFSs can lower the surface tension of water more efficiently than hydrocarbon-based surfactants and are applicable under conditions that would be too severe for conventional surfactants they have been used in numerous applications including treatment of textiles

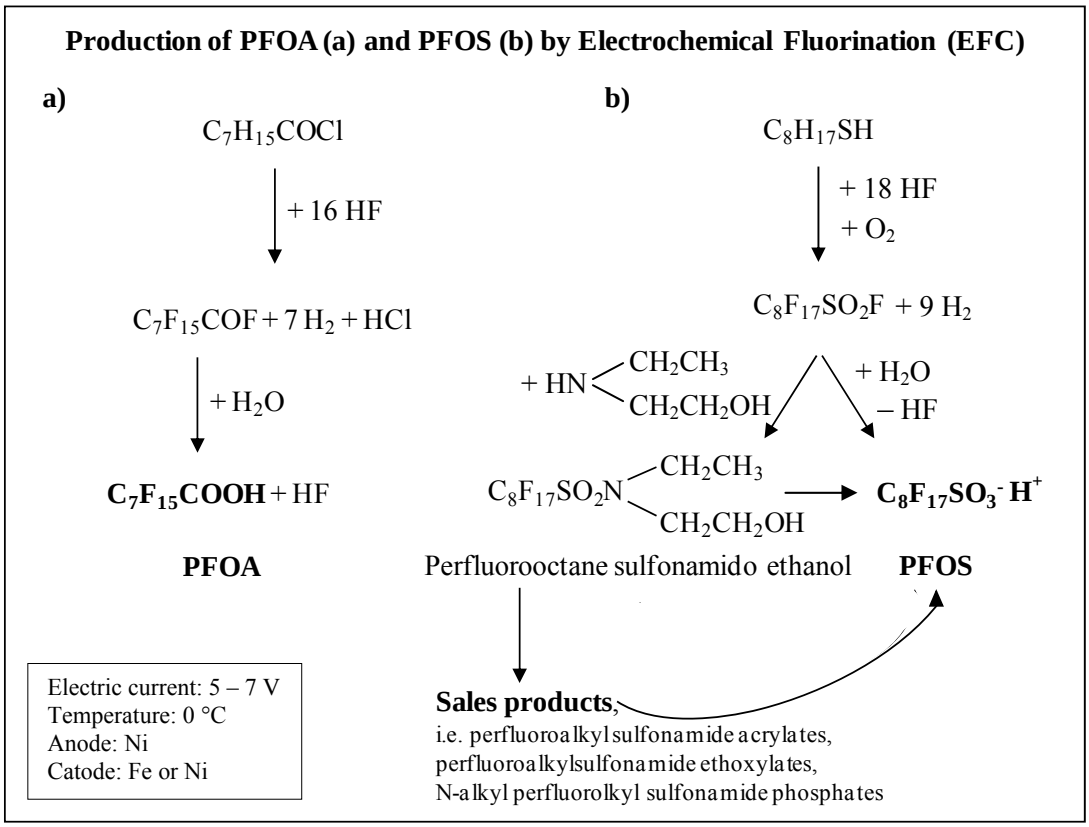


Figure 1. Electrochemical Fluorination Process.

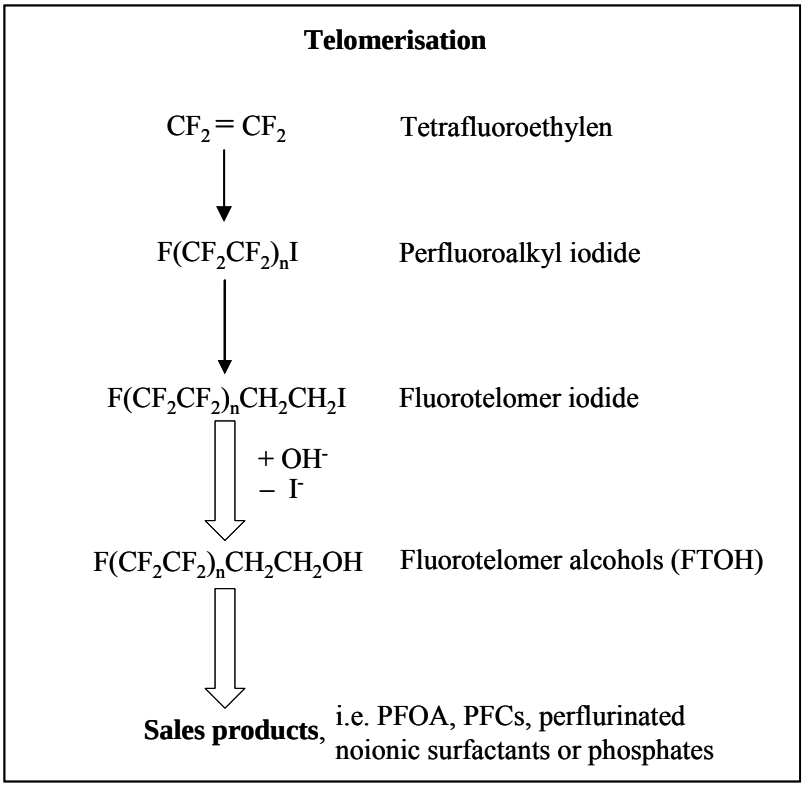


Figure 2. Telomerisation Process.

and paper, formulation of cosmetics and insecticides, production of fluoropolymers, for fire fighting foams, as industrial surfactants, additives, and coatings (Kissa, 2001). Currently, PFOS and related chemicals are used in fire fighting foams, aircraft hydraulic fluids, chromium plating and as anti-reflective or photoresist agents in semiconductor photolithography and as anti-static, surfactant or adhesion-control agents in photographic processes (OECD, 2005). Products containing PFOA have also been used in the industrial sector for metal coatings, textile treatment, as additive for resins, for aqueous dispersion, or for glass fibre impregnation (OECD, 2005).

1.2 Physico-Chemical Properties of PFOA and PFOS

PFOA and PFOS are synthetic organic chemicals consisting of a fully fluorinated carbon chain and a carboxylic group or sulfonate group, respectively (Figure 3).

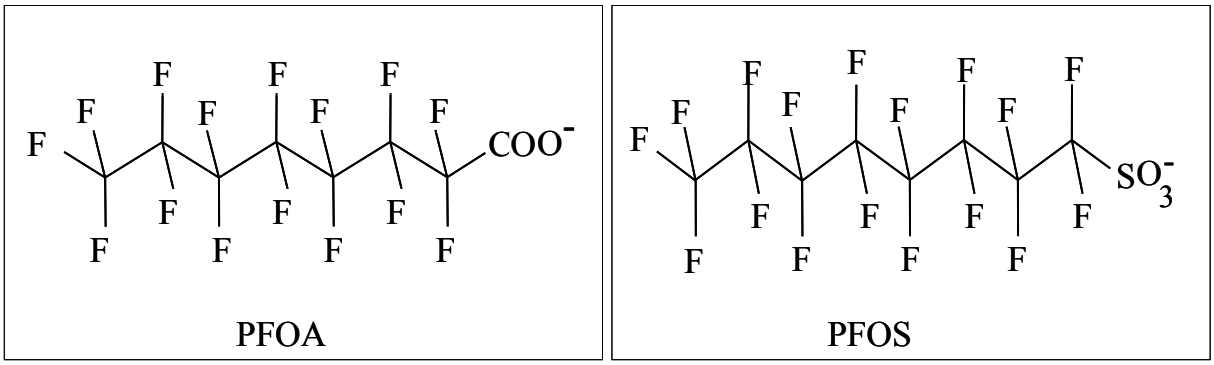


Figure 3. Structural formulae of PFOA and PFOS.

They do not occur naturally in the environment, and due to their low pK_a values, they are present in solutions as anions at pH 7 (Table 1). For PFOA both forms, the free acid and the anion, are present in the environment whereas PFOS only occurs in its anionic form.

PFOA and PFOS form multiple layers in octanol/water mixtures, making determination of the octanol-water partition coefficient (K_{ow}) extremely difficult (Environment Agency, 2004; Prevendouros et al., 2006); it is also believed that the K_{ow} in this case does not allow to estimate the environmental partitioning of these compounds (Ellis et al., 2002).

Henry's law constant is expected to be very low for perfluorooctanoate and relatively high for the acid form of PFOA, so its volatilisation from water is pH dependant. PFOA and PFOS are not expected to be volatilised significantly at environmental conditions; therefore they will be bound to particles in the atmosphere (Prevendouros et al., 2006; OECD, 2002).

Table 1. Physical and chemical properties of PFOA and PFOS.

	PFOA [1)]	**PFOS** [2)]
Molecular weight, g/moL	414.07	538.2
Vapour pressure at 25 °C, Pa	4.2	3.31×10^{-4}
Solubility in pure water at 25 °C, mg/L	9500 [3)]	680
Melting point, °C	45 – 50	> 400
Boiling point, °C	189 – 192	not measurable
pK_a	2 – 3	- 3.27 (calculated)

[1)] free acid (Prevendouros et al., 2006; Boit, 1975);
[2)] potassium salt (OECD, 2002; Environment Agency, 2004);
[3)] solubility of the perfluorooctanoate.

In general, the limited amount of relevant physico-chemical property data makes the application of classical methods for estimating the partitioning of these analytes in the environment difficult.

1.3 Sources of PFOA and PFOS in the Environment

PFOA and PFOS have been reported to enter the environment directly during their production, and during manufacturing, processing and dispersion of fluoropolymers (Hansen et al., 2002; Prevendouros et al., 2006), production and use of fire fighting foams (Moody & Field, 1999; Moody et al., 2002; Hansen et al., 2002; Moody et al., 2003; Prevendouros et al., 2006) and commercial products (Boulanger et al., 2005 a) containing PFSs. Also industrial and commercial waste waters have been suggested as likely sources (3M, 2001; Boulanger et al., 2005 a; Schultz et al., 2006 a, b; Sinclair & Kannan, 2006; Loganathan et al., 2007).

Another possible source of PFOA and PFOS is the atmospheric degradation of volatile precursors that are widely distributed (Martin et al., 2002; Stock et al., 2004; Shoeib et al., 2005, Jahnke et al., 2007 a, b, c). FTOHs have been observed to undergo atmospheric oxidation (Hurley et al., 2004; Ellis et al., 2004; Young et al., 2007) as well as metabolic (Hagen et al., 1981) or microbial (Lange, 2002; Diglasan et al., 2004) degradation to form perfluorocarboxylic acids. Similarly, atmospheric (D'eon et al., 2006; Martin et al., 2006) or microbial degradation (Lange, 2000) of perfluorooctane sulfonamido ethanols is likely to yield PFOS.

1.4 Environmental Concentrations of PFOA and PFOS

Over the years, researchers investigating the environmental fate of halogenated compounds have mostly focused on brominated or chlorinated compounds. Fluorinated chemicals received less attention because their analysis was more complicated, they were believed to have less impact on wildlife or humans, and there were no or very little regulations. In 1976,

Donald Taves from the University of Rochester in New York speculated about widespread contamination of human tissues with trace amounts of organic fluorocompounds originating from commercial products (Renner, 2001). Before perfluorinated surfactants became of interest another chemical - trifluoroacetate (TFA) – gained the attention of researchers. This atmospheric degradation product of partially fluorinated ethanes introduced as alternatives for banned chlorofluorocarbons used in refrigeration was shown to be persistent in the hydrosphere and accumulate in higher plants, i.e. conifers (Frank et al. 1995, Frank et al. 1996, Frank & Jordan 1999, Liekens et al. 1997). Later on, the fluorinated compounds, PFOA and PFOS were the first PFSs to receive a lot of attention as they were shown to be persistent in the environment, and the latter was shown to be bioaccumulative and toxic (Schultz et al., 2003). In the year 2000, growing concern about this class of chemicals resulted in the announcement of the largest producer, 3M company, to phase out the production of PFOS (Renner et al., 2001). Since then, a number of papers reporting environmental concentrations of PFOA and PFOS have been published. The following paragraphs give an overview of the levels of these two analytes in different environmental compartments.

1.4.1 Air and Precipitation

As mentioned before (chapter 1.2), PFOA and PFOS have relatively good water solubility and tend to stay in the aqueous phase as well as to bind to particles present in the atmosphere; these properties are reflected in still limited data on their concentration in the gas-phase of the atmosphere (Table 2).

Only lately, Kim and Kannan (2007) published concentrations of PFOA and PFOS in the gas-phase of air samples collected at Albany, USA. Both analytes were present in the particulate phase of air samples collected in the USA, Germany and Canada (Jahnke et al., 2007; Kim & Kannan, 2007; Stock et al., 2007) in the range of pg/m^3, the highest concentrations were observed in Kyoto, Japan (Harada et al., 2005 c).

Much higher levels (ng/L) of these two analytes have been reported for rain and snow in the USA and Canada, and even in Arctic snow (Loewen et al., 2005; Scott et al., 2006 b; Kim & Kannan, 2007; Young et al. 2007).

Table 2. PFOA and PFOS levels in air [pg/m^3] and precipitation [ng/L];
(G) - gas phase, (P) - particulate phase, na - not analysed, nd - not detected.

Sample	Location	PFOA	PFOS	Reference
Air (G)	Albany, NY, USA	1.89 – 6.53	0.94 – 3.0	Kim & Kannan, 2007
Air (P)	Albany, NY, USA	0.76 – 4.19	0.35 – 1.16	Kim & Kannan, 2007
Air (P)	Kyoto, Japan	na	0.6 – 5.3	Sasaki et al., 2003
Air (P)	Kyoto, Japan	72 – 880	20 – 170	Harada et a., 2005 c
Air (P)	Hamburg, Germany	< 0.2 – 2.6	0.4 – 1.6	Jahnke et al., 2007 d
Air (P)	Cornwallis Island, Canada	1.4	5.9	Stock et al., 2007
Rain	Albany, NY, USA	< 0.75 – 7.27	< 0.25 – 1.51	Kim & Kannan, 2007
Rain	Turkey Lakes, Canada	< 0.5 – 3.1	na	Scott et al., 2006 a
Rain	North East Canada	< 0.1 – 89	na	Scott et al., 2006 b
Rain	Winnipeg, Canada	nd	0.59 ± 0.04	Loewen et al., 2005
Snow	Albany, NY, USA	< 0.75 – 19.6	< 0.25 – 1.93	Kim & Kannan, 2007
Snow	Arctic	< 0.012 – 0.147	0.003 – 0.086	Young et al., 2007

1.4.2 Aquatic Samples

Up to now, PFOA and PFOS concentrations have been measured in tap water, ground water, surface run-off from a location of fire-fighting activity, precipitation (1.4.1), fresh-, salt-, and waste water. An overview of these data is given in Table 3.

So far, the most detailed studies of their concentrations in tap water have been published in Germany (Skutlarek et al., 2006; Brunner, 2007). The maximum PFOA concentration was observed in the river Ruhr, caused by inappropriate disposal of PFS-containing waste that resulted in contamination of the drinking water in the region (Skutlarek et al., 2006). Elevated PFOA values in tap water registered in the Altötting District could be traced back to waste waters from a fluorination process released to the local river (Brunner et al., 2007). The maximum levels observed in drinking water in Osaka City, Japan, were lower than the maximum concentrations mentioned above (Saito et al., 2004).The earliest publication on PFSs in the environment revealed very high PFOA concentrations (up to µg/L) in ground waters at the Naval Air Station and the Tyndal Air Force Base, USA, that followed a fire fighting activity (Moody & Field, 1999). A few years later, similar contamination of ground water resulting

Table 3. PFOA and PFOS levels in aquatic samples [ng/L];
* - after fire fighting activity, nd - not detected, na - not analysed.

Sample	Location	PFOA			PFOS			Reference
Tap water	Osaka and Tohoku area, Japan	0.7	–	40	nd	–	12	Saito et al., 2004
	Ruhr, Germany	< 2	–	519	<2	–	22	Skutlarek et al., 2006
	Altötting District, Germany	< 1	–	230	<1	–	4	Brunner, 2007
Ground water *	Naval Air Station Fallon, Tyndall Air Force Base	nd	–	6570	na			Moody & Field, 1999
	Air Force Base, Michigan, USA	nd	–	105000	8000	–	110000	Moody et al., 2003
Surface runoff	Albany, New York, USA	0.51	–	29.3	<0.25	–	14.6	Kim & Kannan, 2007
River water	Tennessee River, Decatur, USA	<25	–	598	16.8	–	144	Hansen et al., 2002
	Japan	0.1	–	456	0.24	–	37	Saito et al., 2004
	Kyoto area, Japan	7.9	–	110	<5.2	–	10	Senthilkumar et al., 2007
	Moehne, Ruhr, Germany	< 2	–	3640	<2	–	193	Skutlarek et al., 2006
	Cape Fear Basin, North Carolina, USA	12.6	–	287	30	–	132	Nakayama et al., 2007
	Delälven. Vindelälven, Kalix Älv, Sweden	< 0.65			na			Mclachlan et al., 2007
	Pearl and Yangtze River, China	0.85	–	260	0.15	–	99	So et al., 2007
	Oder, Vistula, Poland	3.0	,	3.8	na			McLachlan et al., 2007
Lake water	Canadian Arctic	0.5	–	16	0.9	–	90	Stock et al., 2007
	Albany, New York, USA	3.72	–	15.8	nd	–	9.30	Kim & Kannan, 2007
Sea water	Hong Kong, South China	0.24	–	320	0.04	–	730	So et al., 2004
	Japan	1.9	–	448	0.61	–	28	Saito et al., 2004
	Pacific Ocean	0.015	–	0.142	0.001	–	0.078	Yamashita et al., 2005
	Atlantic Ocean	0.100	–	0.439	0.009	–	0.073	Yamashita et al., 2005
	Charleston, USA	9.5	±	13	12	±	15	Houde et al., 2006 a
	Sarasota Bay, USA	3.6	±	9.2	0.9	±	1.1	Houde et al., 2006 a
Waste water	WWTP, Charleston, USA	59	±	26	30	±	13	Houde et al., 2006 a
	WWTPs nationwide, USA	2.5	–	97	1.1	–	400	Schultz et al., 2006 a
	WWTPs, New York State, USA	58	–	1050	3	–	68	Sinclair & Kannan, 2006
	Pacific Northwest, USA	4.4	–	24	6.1	–	34	Schultz et al., 2006 b
	WWTP Kentucky, USA	8.3	–	334	7.0	–	993	Loganathan et al., 2007
	WWTP Georgia, USA	1.0	–	141	<2.5	–	77	Loganathan et al., 2007

from the use of fire fighting foams was reported by Moody et al. (2003).

The highest concentrations of PFOA and PFOS in river waters were observed in the river Moehne (Germany) due to criminal disposal of contaminated wastes (Skutlarek et al., 2006), followed by levels measured in the Tennessee River (USA) where the contamination originated from a manufacturing plant (Hansen et al., 2002). High PFOA levels have also been noted in Japan (Saito et al., 2004, Sethilkumar et al., 2007), China (So et al., 2007) and in North Carolina, USA (Nakayama et al., 2007). In comparison, Swedish and Polish rivers showed only low levels of pollution with PFOA and PFOS (McLachlan et al., 2007). Moderate levels of PFOA and PFOS have been determined in lakes of the Canadian Arctic (Stock et al., 2007) or in lakes near Albany, NY, USA (Kim & Kannan, 2007).

Currently, low levels (pg/L) of PFOA and PFOS are found in salt water, e.g. Pacific and Atlantic Ocean (Yamashita et al., 2005), with the highest concentrations at the coasts of China and Japan (Saito et al., 2004; So et al., 2004).

Waste waters from various waste water treatment plants (WWTPs) in the USA have been reported to contain concentrations ranging from 1 to over 1000 ng/L PFOA and from 1 to 1000 ng/L PFOS (Houde et al., 2006 a; Schultz et al., 2006 a, b; Sinclair & Kannan, 2006; Loganathan et al., 2007).

1.4.3 Solid Matrices

Relatively low concentrations of PFOA and PFOS (Houde et al., 2006 a; Nakata et al., 2006) were found in the marine sediment with the exception of the harbour of Barcelona, Spain (Alzaga et al., 2005), where fire fighting foams were used.

Moderate levels of PFOA and PFOS have been determined in freshwater sediments (stationary and river waters) in the USA and Japan (Higgins et al., 2006; Senthilkumar et al., 2007). Elevated PFOS concentrations have been observed in sediment samples collected from the Resolute Lake implying other sources than atmospheric deposition, namely contamination by the outflow from a nearby lake receiving raw sewage and waste waters from the airport (Stock et al., 2007). Levels of PFOA and PFOS in solid matrices other than biota and foods are summarized in Table 4.

So far, the highest published concentrations of these analytes in solid matrices have been found in dust collected from Japanese houses (Moriwaki et al., 2003) and in sludge samples collected at WWTPs in the USA and Germany (Higgins et al., 2006; Loganathan et al., 2007; BLfU, 2007 a) reflecting their presence in waste waters and their partitioning between water and sludge during the treatment process.

Table 4. PFOA and PFOS levels in solid matrices [µg/kg];
ww - wet weight, dw - dry weight, na - not analysed, nd - not detected.

Sample	Location	PFOA	PFOS	Reference
Marine sediment, ww	Charleston Harbour area, USA	0.2 ± 0.2	0.4 ± 0.5	Houde et al., 2006 a
	Sarasota Bay, USA	0.06 ± 0.02	0.2 ± 0.2	Houde et al., 2006 a
	Tidal Flat, Ariake Sea, Japan	0.84 – 1.1	0.09 – 0.14	Nakata et al., 2006
	Barcelona Harbour, Spain	8 – 12	na	Alzaga et al., 2005
Still water sediment, dw	San Francisco Bay Area, USA	nd – 0.625	nd – 3.07	Higgins et al., 2006
	Lakes Resolute, Char, Amtituk, Canada	<0.3 – 7.5	0.02 – 85	Stock et al., 2007
River sediment, dw	Kyoto area rivers, Japan	<0.1 – 3.9	<0.33 – 6.4	Senthilkumar et al., 2007
	San Francisco Bay Area, USA	nd – 0.230	nd – 1.3	Higgins et al., 2006
Vacuum cleaner dust, dw	Japan	69 – 3700	11 – 2500	Moriwaki et al., 2003
Sludge (WWTP), dw	San Francisco Bay Area, USA	nd – 29.4	14.4 – 2610	Higgins et al., 2006
	Northwest Pacific, USA	<3 – 12	2.5 – 160	Schultz et al., 2006 b.
	Kentucky, USA	33 – 219	8.2 – 993	Loganathan et al., 2007
	Georgia, USA	7.0 – 130	<2.5 – 77	Loganathan et al., 2007
	Bavaria, Germany	<2.0 – 230	<10 – 6720	BLfU, 2007

A specific and important group of solid matrices whose PFOA and PFOS levels has been determined are food samples (Table 5). Although still scarce, these data are of high importance because they help to estimate human exposure to these compounds.

PFOA concentrations in food samples (Gulkowska et al., 2006; Tittlemier et al., 2007; Ericson et al., 2008) were mostly below the limit of quantification (LOQ); the highest PFOA concentrations were found in microwave popcorn, probably resulting from packaging material treated with fluorinated compounds, in roast beef from Canada (Tittlemier et al., 2007) , and in sea food (Gulkowska et al., 2006). PFOS has been shown to have elevated concentrations in sea food (Gulkowska et al., 2006) and in other animal-derived foods (Tittlemier et al., 2007).

Table 5. PFOA and PFOS levels in food samples [µg/kg ww];
Values in parentheses indicate that the concentration measured was above limit of detection (LOD) but below LOQ.

Sample	Location	PFOA	PFOS	Reference
Seafood	China	<0.25 – 1.67	0.33 – 13.9	Gulkowska et al., 2006
Beef steak	Canada	<0.5	2.7	Tittlemier et al., 2007
Roast beef		2.6	<0.6	
Ground beef		<0.4	2.1	
Pizza		(0.74)	<1	
Microwave popcorn		3.6	(0.98)	
Vegetables [1)]	Spain	<0.027	0.022 ± 0.006	Ericson et al., 2008
Pork[2)]		<0.053	0.045 ± 0.029	
Chicken[3)]		<0.067	0.021 ± 0.001	
Dairy products[4)]		<0.040	0.121 ± 0.050	
Whole milk		0.056 ± 0.002	<0.014	

[1)] lettuce, tomato, green bean, spinach;
[2)] sausage, hot dog, steak, hamburger, ham;
[3)] breast, thights, sausage;
[4)] cheese, yoghurt, cream caramel, custard.

1.4.4 Biota

1.4.4.1 Concentrations in Biological Samples

A summary of PFOA and PFOS concentrations in biota is given in Table 6. So far, biological samples seem to be the best examined ones for this class of chemicals. Data of wild life samples from all trophic levels can be found in the literature, starting from benthic algae from two rivers in the USA (Kannan et al., 2005) up to predators such as polar bears inhabiting Greenland (Bossi et al., 2005 a).

The first report on the global distribution of PFOS in wildlife was published by Giesy & Kannan (2001). Their study contained a wide range of organisms including fish, birds, and mammals from North America, Europe, the Arctic, and the North Pacific Ocean, showing that animals from industrialised regions were higher contaminated than those from remote locations.

Houde et al. (2006 a) reported low levels of PFOA and PFOS in zooplankton collected from Sarasota Bay, USA. Several studies published between 2002 and 2006 focused on invertebrates (Kannan et al., 2002; Martin et al., 2004; Nakata et al., 2006; So et al., 2006). Lugworm inhabiting the tidal flat of the Ariake Sea, Japan, was the only organism showing higher concentrations of PFOA than of PFOS (Houde et al., 2006).

Table 6. PFOA and PFOS levels in biota [µg/kg wet weight, µg/kg dry weight[§] or µg/L*];
na - not analysed, nd - not detected.

Species (Sample type)	Location	PFOA	PFOS	Reference
Benthic algae	Rivers: Raisin, St. Clair, Calumnet, USA	<0.2	2.4 – 3.1	Kannan et al., 2005
Zooplankton	Sarasota Bay, USA	0.3 ± 0.5	0.2 ± 0.07	Houde et al., 2006 a
Lugworm (whole body)	Tidal Flat of Ariake Sea, Japan	70 – 98	<0.3 – 1.3	Nakata et al., 2006
Mysis, Diporeia (whole body)	Great Lakes, USA	2.5 – 90	13 – 280	Martin et al., 2004
Oyster (whole body)[§]	Gulf of Mexico, Chesapeake Bay, USA	na	<42 – 1225	Kannan et al., 2002 a
Oyster (whole body)	South China, Japan	<1.2 – 4.3	0.64 – 3.8	So et al., 2006 a
Clam (soft tissue)	Tidal Flat of Ariake Sea, Japan	<3.0 – 11	<0.3	Nakata et al., 2006
Brown trout (eggs)	Lake Superior, Michigan, USA	<18	49 – 75	Kannan et al., 2005
Carp (muscles)	Saginaw Bay, Michigan, USA	<36	59 – 297	Kannan et al., 2005
Chub (muscles)	Rivers Alz, Main, Germany	<0.5 – 15	1.6 – 6.1	BLfU, 2007 b
Smallmouth bass (liver)	New York State, USA	<1.5 – 7.7	10 – 142	Sinclair et al. 2006
Jack mackerel (liver)	Kyushu Prefecture, Japan	10	1.6	Senthilkumar et al., 2007
Eel (liver)	Flanders, Belgium	na	17 – 9031	Hoff et al., 2005
Green frogs (liver)	Michigan, USA	<72	<35 – 290	Giesy & Kannan, 2001
Sea turtle (plasma)*	Southeastern Coast, USA	0.5 – 8.1	1.4 – 97	Keller et al., 2005
Common merganser (liver)	Niagara River, New York, USA	<7.5	146 – 715	Sinclair et al. 2006
Albatross (liver)	Southern Ocean	<0.6 – 7.8	<0.5 – 21	Tao et al., 2006
Bottlenose dolphins (plasma)*	Charleston, Sarasota Bay, USA	3.4 – 43	340 – 914	Houde et al., 2006 a
Harbour porpoise (liver)	Black Sea, Ukraine	na	33 – 1790	Van De Vijver et al., 2006
Long finned pilot whale (liver)	Faroe Island, Denmark	nd	28	Bossi et al., 2005 a
Mink (liver)	Lalamazoo River watershed, USA	<2 – 3.3	1280 – 59500	Kannan et al., 2005
Sea otter (livers)	California Coast, USA	<5.0 – 147	<1.0 – 884	Kannan et al., 2006
Elephant seal (whole blood)*	Antarctic	na	<0.08 – 3.52	Tao et al., 2006
Baikal Seal (liver)	Baikal, Russia	<1.5 – 3.9	2.6 – 38	Ishibashi et al., 2008
Panda (serum)*	China	0.32 – 8.2	0.76 – 74	Dai et al., 2006
Polar bear (liver)	Greenland, Denmark	<12	1245 – 1325	Bossi et al., 2005 a

Relatively high concentrations of whereas have been measured in mysis and diporeia from the Great Lakes, USA (Martin et al., 2004).Oysters and clams collected in Japan (Nakata et al., 2006; So et al., 2006 a), were only slightly burdened oysters collected from the Gulf of Mexico, and Chesapeake Bay, USA, had very high concentrations of PFOS (Kannan et al., 2002 a).

In several studies the attention was directed to fish species inhabiting fresh- and salt-water basins; some examples from the literature are presented in Table 6. The highest PFOA concentrations were measured in muscle tissue of chub from the river Alz in Germany (BLfU, 2007 b) having also a high water concentration (up to 7.5 µg/L of PFOA, BLfU, 2007 c). The lowest PFOS levels in fish, were found in liver of jack mackerel from Kyushu Prefecture, Japan (Senthilkumar et al., 2007) and in muscle tissue of chub from the rivers Alz and Main, Germany (BLfU, 2007 b), the highest in liver tissue of eel collected in the Ieperlee Canal at Boezinge, Flanders, Belgium (Hoff et al., 2005), an industrialised area with no production sites for fluorochemicals. Relatively high concentrations of PFOS were measured in eggs of brown trout from Lake Superior, Michigan, USA (Kannan et al. 2005).

The only amphibian species analysed for PFSs' is green frog in whose liver up to 290 µg/kg PFOS were found (Giesy & Kannan, 2001). In sea turtles from the southeastern coast of the USA PFOA and PFOS concentrations in their plasma ranged from 0.5 – 8 and 1.4 – 100 µg/L, respectively (Keller et al., 2005).

Fish-eating birds are another group of animals to which a lot of attention has been paid; to estimate their contamination with PFSs, typical concentrations in liver of common merganser and albatross are given in Table 6 (Sinclair et al., 2006; Tao et al., 2006).

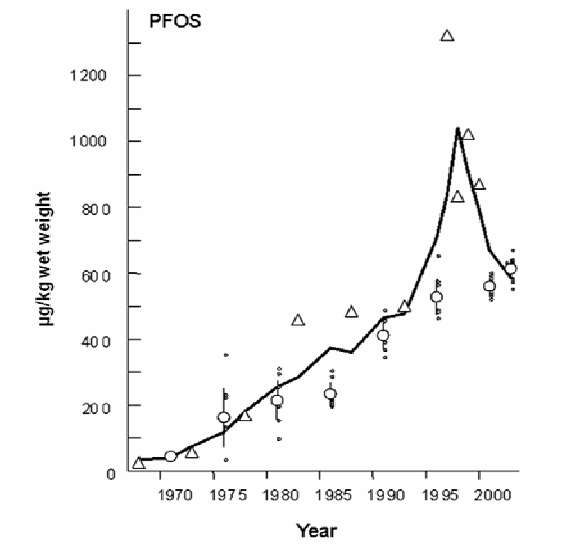
Studies on marine and terrestrial mammals have also been conducted, showing that plasma and liver levels of PFOA and PFOS cover a wide range (Table 6). The highest PFOA concentration was found in sea otters from the California Coast, USA (Kannan et al., 2005). Relatively high PFOA concentrations have also been determined in the plasma of bottlenose dolphins from the Niagara River, NY, USA (Houde et al., 2006 a). PFOS concentrations in mammals were mostly much higher than those of PFOA. The lowest PFOS concentrations in mammals listed in Table 6, were found in whole blood of elephant seal from Antarctica (Tao et al., 2006), liver of long finned pilot whale from Denmark (Bossi et al., 2005 a), liver of Baikal seal, Russia (Ishibashi et al., 2008), or blood plasma of Chinese pandas (Dai et al., 2006), the highest PFOS concentrations in liver of mink from the Kalamazoo River watershed, USA (Kannan et al., 2005).

1.4.4.2 Temporal Trends

A number of studies on the temporal trends of PFSs have been conducted in the last few years. As the first one Kannan et al. (2002 b) reported PFOS concentrations in livers of sea eagles collected between 1979 and 1999 from inland and coastal regions of eastern Germany and Poland. Although concentrations increased from the 1970s and 1980s (25 µg/kg ww) to the 1990s (40 µg/kg ww), no clear temporal trend could be observed. Martin et al. (2004) found an increase in PFOS concentrations (4-fold) in trouts from the Lake Ontario over the whole collection period (1980 – 2001).

A study on PFOS concentration in guillemot eggs from the Baltic Sea between 1968 and 2003 showed a significant increase, on average 7 – 11 % per year (Holström et al., 2005). A sharp peak was observed in 1997 followed by a decrease until 2002 (Figure 4).

Figure 4. Temporal trend of PFOS in guillemot eggs, Baltic Sea, 1968 – 2003 (Holström et al., 2005).



An increasing trend of PFOS concentrations in liver tissue of ringed seals in central-east Greenland collected between 1986 and 2003 has been described by Bossi et al. (2005 b). Smithwick et al. (2006) reported a statistically significant increase in PFOA concentrations in polar bear liver tissue in one of two groups investigated, whereas PFOS increased significantly in both groups.

Eggs of herring gulls from two coastal colonies in Northern Norway collected between 1983 and 2003 (Verreault et al., 2007) showed a 2-fold-increase in PFOS from 1983 to 1993, remaining constant until 2003. Lately, Ishibashi et al. (2008) presented a comparison of PFOS

liver concentrations in Baikal seals collected in 1992 and 2005 (Ishibashi et al., 2008) showing a 2.4-fold increase.

1.4.5 Humans

Table 7 gives some examples of PFOA and PFOS levels in humans. For obvious reasons most of the studies report blood levels of these analytes; lately however, also other data have become available, i.e. in cord blood, breast milk, or liver.

Data for human plasma samples (Fromme et al., 2007) collected from adults in southern Bavaria, Germany, in 2005, suggest that the current exposure of the population in this region is lower than in the USA (Olsen et al, 2003; Kannan et al., 2004).

PFOS was the predominant contaminant among 10 PFSs in blood serum samples from 85 voluntary donors from local universities and hospitals of nine cities in China, its concentration being significantly higher in the male group (Yeung et al., 2006).

Kannan et al. (2004) conducted a study on human blood samples collected in several countries, showing that after PFOS, PFOA was the most abundant PFSs, with the highest concentrations observed in Korea and USA, moderate in Poland, and relatively low in Colombia, Brazil, Italy, Belgium, India, Malaysia, and Japan. PFOS predominated in samples from the USA, Poland and Korea, was moderate in Belgium, Malaysia, Brazil, Colombia, and Japan, and the lowest in India. Kärmann et al. (2006 a) showed that blood of Swedish people is equally burdened with low levels of PFOA and medium levels of PFOS. Pooled serum samples from 3802 male and female Australian residents living in urban (around Sydney) and rural (outside major metropolitan centres) areas in 2002 – 2003 showed a positive relation between age and increasing PFOS concentration (Kärrman et al., 2006 b). The male group had higher PFOA and PFOS levels but no substantial difference was found between rural and urban population.

An investigation of PFOA and PFOS levels in blood of four subpopulations: dockers, farmers, individuals declaring high intake of Baltic Sea fish, general sector of the citizens of the Gulf of Gdansk and the Baltic Sea, Poland, showed that Baltic fish adds to the human body burden with PFOS and to a lesser extent with PFOA (Falandysz et al. 2006).

Between 2004 and 2005, PFOA and PFOS were detected in >99 % of cord serum samples of 299 singletons delivered in Baltimore, USA, suggesting that *in utero* exposure to these compounds is ubiquitous in this population of babies (Apelberg et al., 2007). A similar observation but only with regard to PFOS was made for cord serum samples of Japanese fetuses (Inoue et al., 2004).

Table 7. PFOA and PFOS levels in humans [µg/L or µg/kg wet weight*];
P - plasma, S - serum, M - males, F - females, W – whole, nd - not detected.

Sample	Group	Location	PFOA	PFOS	Reference
Blood (P)	adult M,F	Germany	0.5 – 19	2.1 – 55	Fromme et al., 2007
Blood (S)	adult M,F	China	-	32 – 310	Yeung et al., 2006
	Red Cross donors, M, F	Colombia	3.7 – 12	4.6 – 14	Kannan et al., 2004
		Brazil	<20	4.6 – 35	
		Italy	<3	<1 – 10	
		Poland	9.7 – 40	16 – 116	
		Belgium	<1 – 13	4.5 – 27	
		India	<3.0 – 3.5	<1 – 3.1	
		Malaysia	<10	6.2 – 18.8	
		Korea	<15 – 256	3.0 – 92	
		Japan	<6.8 – 12.3	4.1 – 40	
		USA	<3.0 – 88	<1.3 – 164	Olsen et al., 2003, Kannan et al., 2004
	rural, M, F	Australia	5.0 – 9.9	2.7 – 103	Kärrman et al., 2006 b
	urban, M, F	Australia	6.7 – 8.5	5.5 – 25	
Blood (W)	dockers, M	Gdansk, Poland	1.2 – 5.8	5.2 – 24	Falandysz et al., 2006
	farmers, M,F		1.2 – 6.2	6.6 – 25	
	fish-dish fans, M, F		1.7 – 8.7	14 – 84	
	reference, M, F		1.3 – 5.2	6.7 – 46	
	donors, M, F	Sweden	2.1 – 5.1	14.8 – 34.1	Kärrman et al., 2006 a
Cord blood		Japan	1.6 – 5.3	nd	Inoue et al., 2004
Breast milk		USA	0.3 – 7.1	nd – 35	Apelberg et al., 2007
		China	0.05 – 0.21	0.05 – 0.36	So et al., 2006 b
Liver*	transplant donors	USA	<0.03 – 0.62	<0.03 – 0.16	Tao et al., 2008
		USA	<18 – 47	<4.5 – 57	Olsen et al., 2003

So et al. (2006) analysed samples of breast milk from 19 mothers from Zhoushan, China, finding PFOA and PFOS to be the most abundant PFSs. No statistically significant correlation between their concentration and infants' weight was found. Breast milk samples from mothers in Massachusetts, USA (Tao et al., 2008), showed generally lower concentrations than those from China. PFOA concentrations in the milk of mothers nursing for the first time were significantly higher than those of mothers that had previously nursed (Tao et al. 2008).

A study including 30 samples of human liver donor tissue from the USA showed that PFOA was mostly below <18 µg/kg, PFOS was below <4.5 µg/kg in 50 % of samples (Olsen et al., 2003).

Generally, in all studies described above PFOS was the predominant contaminant, and it was usually higher in males than in females. No clear conclusions or correlation between PFSs levels and age, region (urban – rural) or daily intake of these compounds by infants could be drawn, so further investigations are needed.

1.5 Toxicity

1.5.1 Bioaccumulation and Biomagnification Potential

Several studies enabling calculation of bioaccumulation (BAFs), bioconcentration (BCFs), or biomagnification factors (BMFs) have been conducted. A short summary is given in Table 8.

The highest BCF for PFOS was evaluated for liver of common shiner from Etobicoke Creek, Canada, after an accidental spill of fire-fighting foam (Moody et al., 2002). The authors suggested that this value could be influenced by the presence of precursors that were not determined in water but could be possibly degraded to PFOS in fish liver.

BCF calculated for turtles inhabiting the Ai River system, Japan, based on PFOA and PFOS concentrations in their sera and in the surface water were 3.2 and 10.9 (geometric mean), respectively (Morikawa et al., 2006), suggesting a higher bioconcentration potential of PFOS.

Kannan et al. (2005) reported BCFs of 1000 for PFOS in benthic, algae, amphipods, and zebra mussels and of 2400 in round gobies (whole body) in relation to surface water of the Great Lakes, USA, whereas PFOA was not detected in the tissue of any benthic organism, despite its presence in water.

Table 8. A literature overview of BAF, BCFs, BMFs, and TMF.

Sample	Location	PFOA	PFOS	Reference
BAF (common shiner liver/water)	Etobicoke Creek, Canada	-	6300 – 125000	Moody et al., 2002
BCF (turtles serum/surface water)	Ai River system, Japan	0.8 – 15.8	500 – 3800	Morikawa et al., 2006
BCF (zebra mussels/water)	Great Lakes, USA	-	1000	Kannan et al. 2005
BCF (smallmouth, largemouth bass liver /surface water)	New York State, USA	184	8850	Sinclair et al., 2006
BMF (mink liver /carp tissue)	Michigan, USA	-	11 – 23	Kannan et al., 2002 c
BMF (chinook salmon liver /round gobies liver)	Great Lakes, USA	-	10 – 20	Kannan et al., 2005
BMF (eagle or mink liver /salmon liver)	Great Lakes, USA	-	5 – 10	Kannan et al., 2005
BMF (smallmouth, largemouth bass liver /common mergansers liver)	New York State, USA	-	8.9	Sinclair et al., 2006
BMF (striped mullet, whole body/zooplankton)	Charleston, USA	-	23	Houde et al., 2006 a
BMF (dolphin, whole body estimate/Atlantic croaker, whole body)	Charleston, USA	2.3	2.2	Houde et al., 2006 a
TMF (lake trout)	Lake Ontario, USA	0.58	5.9	Martin et al., 2004

Sinclair et al. (2006) estimated a BCF of 8850 for PFOS and one of 184 for PFOA based on the liver concentration of smallmouth and largemouth bass and the surface waters from the NY State lakes, and a BMF of 8.9 for PFOS for fish eating birds (common merganser, liver) calculated with respect to the fish liver.

Laboratory studies performed by feeding mink with carp contaminated with PFOS (240 – 300 µg/kg ww) collected from Saginaw River, Michigan, USA, resulted in BMFs ranging from 11 to 23 depending on the portion of carp included in the diet. BMFs of PFOS based on data obtained from a field study of mink and bald eagle livers (predators) relative to chinook salmon liver (prey) resulted in values from 5 to 10 (Kannan et al. 2005). BMFs of PFOS from 10 to 20 were found for liver of chinook salmon (predator) relative to liver of

river goby (prey). Houde et al. (2006 a) concluded that there is no agreement between BAFs and BMFs from laboratory and field studies.

Trophic magnification factors (TMFs) calculated for PFOA and PFOS in lake trout from a food web from Lake Ontario, USA, were 0.58 and 5.9, respectively, showing that bio-accumulation occurred at the top of the food web for PFOS but not for PFOA (Martin et al., 2004).

1.5.2 Ecotoxicity

A wide range of toxicological studies with PFOA and PFOS have been performed on animals during the last 15 years.

PFOA was suggested to act as hepatocarcinogen through peroxisome proliferation (i.e. rats fed with 0.01 % w/w PFOA; Kawashima et al., 1995; Kennedy et al. 2004; Kudo et al., 2005) and/or at the level of gap junctions (at 350 µM in rats; Upham et al., 1998) and its half-life in male and female rats was calculated to be 5.6 and 0.08 days, respectively (Ohmori et al., 2003).

PFOS was shown to produce cumulative toxicity in rats and primates (i.e. ≥0.2 % PFOS in diet), possibly caused by changes in fatty acid transport and metabolism, membrane function, peroxisome proliferation, and mitochondrial biogenetics (Haughom & Spydevold 1992, Schulz et al., 2003), and to affect the neuroendocrine system in these animals (injection of 10 mg/kg body weight; Austin et al., 2003). LD_{50} for juvenile mallards fed with PFOS in their diet for 5 days was determined to be 750 mg PFOS/kg body weight (Newsted et al., 2006), and its half-lifes in mallard blood serum and liver were estimated to be 6.9 and 17.5 days, respectively. According to Austin at al. (2003) subchronic exposure of rats to PFOS (at 10 mg/kg body weight) leads to significant weight loss accompanied by hepatotoxicity and reduction of serum cholesterol and thyroid hormones.

Few researchers tried to evaluate if toxic effects observed in laboratory animals may also be the ultimate outcome for wild life exposed to reported environmental concentrations. For example, Hoff et al. (2003) reported that PFOS levels observed in tissues of wild life populations (i.e. 300 µg/kg in muscle of carp or 2.6 mg/kg in eagle plasma; Giesy & Kannan 2001) could induce a clear rise in serum transaminase levels thus indicating a disruption of hepatocyte membrane integrity. In another study, a positive correlation between PFOS liver concentration (0.5 – 180 mg/kg ww) and increased liver weight, and liver microsomal lipid peroxidation levels and a negative one with the serum alanine aminotransferase activity was observed for wood-mice (Hoff et al., 2004). Lately, a significant positive correlation between

PFOS hepatic concentration measured in carp and eel (0.011 – 9 mg/kg) collected in Flanders, Belgium, and the serum alanine aminotransferase activity, and a negative correlation between serum protein content and serum electrolyte concentrations (carp) were reported (Hoff et al., 2005).

Both analytes were shown to be transferred from mothers to young rodents (mice, rats) during pregnancy or/and lactation (i.e. at 0.8 mg/kg/day dietary administration; Hoff et al., 2004, Hinderliter et al, 2005, Luebker et al., 2005). Moreover, Luebker et al. (2005) showed that late-stage fetal development may be affected in rat pups exposed *in utero* to PFOS (dietary administration 0.8 mg/kg/day) and may contribute to the observed increased mortality. Exposure to either of the analytes affected a number of genes in 6-week-old chickens (PFOA at ≥ 0.1 g/L, PFOS at 0.02 g/L; Yeung et al., 2007).

Harada et al. (2005 a) reported that both PFOA and PFOS (>5 mg/L) may change membrane surface potential, thereby having an impact on calcium channels. This is in good agreement with the suggestion of Hu et al. (2003) that PFOS (≥5 mg/L) can cause alterations in cell membrane properties.

Additionally, precursors of both analytes can be converted into PFOA and PFOS *in vivo*, such adding to the total burden of these compounds, i.e. the telomere alcohol 8:2. (C_8F_{17}-C_2H_4OH) is transformed into PFOA (Kudo et al., 2005).

1.5.3 Human Toxicity and Health Risk Assessment

Little is known about human toxicity of PFOA and PFOS, and it is uncertain if the effects observed in animals also occur in humans. For example, Burris et al. (2002) reported the estimated half-life serum elimination of PFOA in humans to be approximately 4 years, Olsen et al. (2005) gave half-life values of 3.8 and 5.4 years for PFOA and PFOS, respectively, based on an investigation performed on 26 pentioners from two fluorochemical manufacturing plants, whereas experiments performed on rats resulted in half life values of only up to 5.6 days for PFOA (Ohmori et al., 2003) and 7.5 days for PFOS (OECD, 2002).

The renal clearance of PFOA and PFOS measured in young (20 – 40 years) and old (>60 years) people that had lived in Kyoto more than 10 years suggested that no active excretion of these compounds takes place (Harada et al., 2005 b).

A mortality study performed on workers employed in jobs posing high exposure risk showed an increased number of deaths from bladder cancer; however, doubts remain if this could be attributed to fluorochemical exposure and/or to non-occupational exposures (Alexander et al., 2003).

It is certain that human beings are exposed to PFSs via a number of different pathways: starting form the *in utero* exposure, breast feeding, drinking water, contaminated food up to inhalation of contaminated dust or use of PFS-treated articles, or occupational exposure. Therefore, studies were undertaken to estimate overall human exposure to these compounds and ultimately gain a deeper insight into the potential toxicity of PFOA and PFOS to human beings.

Sasaki et al. (2003) estimated the daily intake of PFOS via inhalation of outdoor dust particles to be up to 100 pg/day, in Oyamazaki, Japan, suggesting that human exposure to PFOS from outdoor air is almost negligible. In contrast, Harada et al. (2006) reported much higher contamination of air with PFOA in Oyamazaki, Kyoto, Japan, ranging from 72 to 879 pg/m^3 resulting in the considerably higher daily intake of 3.4 ng. Moriwaki et al. (2003) analysed dust from Japanese homes (Table 4, Page 10) concluding that humans may be chronically exposed to these compounds through absorption of indoor dust. Saito et al. (2004) revealed that more than one million inhabitants of Osaka have been exposed to PFOA through drinking water (10.8 ng/day, assuming a daily water intake of 2 L).

A positive correlation between PFOS concentration in maternal blood and cord blood was shown by Shoeib et al. (2004) supporting the assumption that the human fetus is exposed to this compound during pregnancy, however, PFOS does not pass completely into the fetal circulation and it is not known to cause any adverse effects. In 2006 (b), So et al. reported the daily intake of PFOA and PFOS through mothers' breast milk to be 0.017 and 0.03 µg/kg/day, respectively, in Zhoushan, China suggesting a potential risk of PFOS to some infants.

Washburn et al. (2005) analysed the PFOA content of selected consumer articles containing fluoropolymers or fluorotelomer-based products (mill-treated carpets, apparel, treated non-woven medical garments, non-stick cookware, and thread seal tape) showing that the aggregated exposure to consumer articles increases serum concentrations from 0.05 to 0.25 ppb for adolescents and adults. The same range as observed in professionals involved in installation, application, or maintenance of such articles. In general, neither use of the said articles nor professional exposure (installation, application, or maintenance of such articles) were considered to have the potential of causing adverse health effects.

Based on a dietary survey in Zhoushan, China, and sea food analyses, the average daily intake of PFOA and PFOS from sea food was estimated to be below the benchmark dose, e.g. from 0.00001 (mollusc) to 0.0002 µg/kg/day (fish) for PFOA, and from 0.00005 (mollusc) to 0.003 µg/kg/day (fish) for PFOS (Gulkowska et al., 2006). Faladysz et al. (2006)

indicated that fish from the Baltic Coast is an important source of PFOS and to a lesser extent of PFOA for the Polish people.

An analysis of composite food group samples from the 2004 Total Diet Study of the UK consumers, concluded a daily intake of 0.07 µg/kg bodyweight PFOA and 0.1 µg/kg bodyweight PFOS, results not raising any immediate toxicological concerns (U.K. Food Standard Agency, 2006). A food intake of PFOA and PFOS of 1.1 ng/kg bodyweight/day has been reported for the population of Tarragona County, Catalonia, Spain (Ericson et al., 2008). Food composite samples collected between1992 and 2004 as part of the Canadian total diet study resulted in a much higher estimated daily dietary intake of total perfluorocarboxylates and PFOS (250 ng/day; 4 ng/kg body weight), suggesting that food is a more important source for Canadians than air, water, dust, treated carpeting, and apparel (Tittlemier et al., 2007). The differences between the three studies might be due to different eating habits reflected by different food included in the respective studies.

1.6 Analytical Methods for PFOA and PFOS Determination

1.6.1 Historical Analytical Methods

Due to their relatively low volatility, good solubility in water and lack of chromophores the analysis of PFSs is a challenging task. The total fluorine content can be determined applying a non-destructive or a destructive method. One of the first methods used to determine the total organofluorine content was neutron activation and X-ray fluorescence (Giesy & Kannan, 2002); unfortunately, these techniques are characterised by low sensitivity and do not provide structure-specific information.

Total organic fluorine in environmental and biological samples was also analysed by oxyhydrogen flame combustion followed by determination by fluoride ion-selective electrode (Sweetsner, 1956; Kissa, 1986). Besides being non-specific the method requires rigorous conditions for quantitative mineralization and has a possible laboratory safety hazard due to the explosive mixture of oxygen and hydrogen.

The methylene blue active substance test, has been used to detect fluorinated surfactants in ground water samples (Levine et al., 1997) but it does not allow to differentiate between hydrocarbon and fluorocarbon surfactants.

For the first time, the use of gas chromatography (GC) coupled with electron capture detection (ECD) for PFOA determination was described by Belisle & Hagen (1980). Later, Ylien et al. (1985) and Moody & Field (1999) described methods employing GC followed by

mass spectrometric detection (MS). Unfortunately, all those methods demand a derivatisation step and are unsuitable for perfluoroalkyl sulfonates whose methyl esters are unstable.

Moody et al. (2001) described nuclear magnetic resonance (^{19}F NMR) for the quantitative determination of PFSs in water samples based on the terminal CF_3 group. Due to the fact that this group is common to all fluoroalkyl chemicals it is not clear whether quantification of individual PFSs in a mixture is possible.

Strauss et al. (2002) described the use of attenuated total reflectance-Fourier transformation infrared spectroscopy for PFOS determination in aqueous samples without sample pre-treatment.

In 1998, Ohya et al. described a method for quantitative determination of PFOA among other perfluorinated carboxylic acids in biological samples by HPLC coupled to a fluorescence detector. Unfortunately, this method has limited specificity due to matrix interferences.

Hansen et al. (2001) reported quantitative, compound-specific analysis of low levels of PFSs in biological samples by HPLC electrospray ionisation tandem mass spectrometry (ESI-MS/MS) that did not require a derivatisation step. Since then, this method has been established as the most common one to determine PFSs in environmental samples. Preconcentration and removal of matrix to avoid interferences are needed for most environmental samples but the method's great advantage of enabling differentiation between various PFSs compensates for that. Methods applied for PFOA and PFOS determination in different environmental matrices are introduced in the following subchapters.

1.6.2 Analysis of Air Samples

PFOA and PFOS are mostly present in their anionic form in the environment having rather low volatility. Therefore, most publications on this topic refer to the analysis of PFOA and PFOS concentrations in particulate matter.

A method to determine PFOS in air-borne particulate matter in high-volume air samples (1400 m^3) employing enrichment on a quartz membrane filter, preconcentration and precleaning of the extract on Presp-C Agri columns (250 mg, Wako Pure Chemicals, Osaka Japan) equipped with membrane filter cartridges (pour size 0.45µm) followed by HPLC-MS was first published by Sasaki et al. (2003). Later, the same method was applied also for PFOA determination in air-borne particulate matter (Harada et al., 2005 c). Mean recoveries of the method were 89 % (LOQ: 0.46 ng) for PFOA and 97 % for PFOS (LOQ: 0.3 ng). Berger at al. (2005) described a method for determination of both ionic surfactants, i.e. PFOA and PFOS,

present in particulate matter, employing enrichment on glass-fibre filters, extraction with MeOH and analysis by HPLC coupled to negative mode ESI time-of-flight (TOF) high resolution MS. LOQs calculated for each sample batch ranged between 0.45 and 56 pg/m^3.

Kaiser et al. (2005) described a method for PFOA analysis in occupationally exposed low-volume (480 L) air samples (ambient air collected around a manufacturing facility) utilising Occupational Safety and Health Administration Versatile Samplers equipped with glass-fibre filters and polystyrene resin sorbents, methanol (MeOH) as extraction solvent, and determination by HPLC-MS was described Due to its validation for a high concentration range (0.5 – 47 µg/m^3) this method is not suitable for environmental air samples. Applying the same sampling setup with the addition of a high-volume cascade impactor Barton et al. (2006) reached a LOQ of 70 ng

1.6.3 Analysis of Aqueous Samples

In 2001, Moody et al. published an analytical technique for the determination of PFSs, among others PFOA and PFOS, employing solid phase extraction (SPE) preconcentration and precleaning step and determination by HPLC coupled to negative ESI-MS/MS, operating in a multiple reaction (MRM) mode for increased sensitivity. The percent recoveries for PFOA and PFOS were 93 % and 68 %. Relatively high LOQs (PFOA: 1.0 µg/L, PFOS 1.7 µg/L, for 100-mL sample) were suitable for the samples analysed as they were highly contaminated (in mg/L) due to the use of fire fighting foams.

Herbert et al. (2002) reported a method suitable for PFOS determination at sub-ppm concentrations in water samples applying direct injection of a sample acetonitrile (AcN) mixture (1:1) to ESI-MS operated in the negative mode. Due to a rather high LOD 5 µg/L the method is useful for samples containing less complex matrices and for cases in which less precise determination of PFOS concentration is acceptable.

Yamashita et al. (2004) were the first to address a background contamination problem during PFSs analysis when describing a method for parts-per-quadrillion PFSs levels determination in seawater. The presented method was similar to those applied previously to fresh water samples (Hansen et al., 2002; and Taniyasu et al., 2003) and included SPE as sample preparation step and quantification by HPLC-ESI-MS/MS. Tseng et al. (2005) employed a sample preconcentration procedure similar to that described by Moody et al. (2001) and HPLC ion trap negative ESI-MS for determination of PFSs in water samples reaching LOQs of 2 and 0.5 ng/L for PFOA and PFOS, respectively.

An automated on-line extraction method using turbulent flow chromatography followed by HPLC coupled to atmospheric pressure photoionisation mass spectrometry (APPI-MS) for PFOS determination in river water samples was described by Takino et al. (2003). The APPI technique showed its advantage over ESI because no matrix effects were observed. On-line enrichment of PFOS from river water samples resulted in significant reduction of sample preparation time, but the achieved LOQ of 18 ng/L was relatively high.

Sinclair & Kannan (2006) published a method for waste water samples analysis similar to that described by Taniyasu et al. (2003) reaching LOQs of 2.5 ng/L for both PFOA and PFOS. Later, a large-volume-injection LC-ESI-MS/MS was successfully used for waste water samples analysis by Schulz et al. (2006 a) (LOQ = 0.5 ng/L for both analytes). Determination of PFSs in waste- and river water samples by mixed hemimicelle-based SPE before HPLC-ESI-MS/MS was presented by Zhao et al. (2007). LODs were 0.07 and 0.2 ng/L for PFOA and PFOS, respectively, and the method was found to be appropriate for PFSs analysis.

Rain water sample analysis utilising a preconcentration step on tubes filled with C_{18} silica gel followed by HPLC-ESI-MS/MS accomplishing LOD of 7.2 ng/L for PFOA and 0.4 ng/L for PFOS was presented by Loewen et al. (2005). Scott et al. (2006 a) reported a method for the determination of C_2-C_9 perfluorocarboxylates in rain samples by preparing the 2,4-difluoroanilides of the acids and analysing by GC-MS avoiding SPE procedure, LODs were 0.5 ng/L.

1.6.4 Analysis of Solid Matrices

The first study reporting an analytical method for PFOA and PFOS in solid matrices other than biological ones was published by Moriwaki et al. (2003). Dust samples collected in Japanese homes were extracted with MeOH by ultrasonic agitation, and the filtrated extract (cellulose acetate filter) was analysed by HPLC-ESI-MS/MS. LODs were 50 and 10 µg/kg for PFOA and PFOS, respectively, and were sufficient for dust samples analysis.

Larsen et al. (2005) compared pressurised solvent and reflux extraction methods employing five different solvents (AcN, chloroform, ethanol, MeOH, and water) for the determination of PFOA in polytetrafluoroethylene (PTFE) polymers using HPLC-ESI-MS/MS. Pressurised solvent extraction with ethanol, water, or MeOH turned out to be the best choice for this purpose (recoveries: 80 – 120 %).

Powley et al. (2005 a) extracted PFOA from the surface of commercial cookware heated up with water under simulated cooking conditions followed by a SPE step. Additionally, rectangular pieces of pans (1.5 x 7.5 cm) were extracted by ASE with a water/ethanol

mixture. All extracts were analysed by HPLC-ESI-MS/MS and the LODs were 10 ng/m^2 for both extraction methods.

Begley et al. (2005) extracted PFOA from materials being in direct contact with food such as PTFE-coated cookware or paper by either shaking with MeOH (50 °C, PTFE) or sonication with ethanol/water mixture (50:50 %). Extracts were subjected to HPLC-ESI-MS/MS analysis. A similar procedure was applied by Stadallius et al. (2006) to extract PFOA from paper and textile samples and a LOD of 1 µg/kg for paper and of 2 µg/kg for textile samples was achieved.

Matrix-free analytical methods for the determination of perfluorinated carboxylic acids in soil, sediment and sludge were presented by Powley et al. (2005 b). First, a sodium hydroxide (NaOH) solution was added to soil/sediment/sludge sample, analytes were extracted by shaking with AcN. Extracts were neutralised and purified by addition of graphitised carbon, acidified (acetic acid), centrifuged, and injected into a HPLC-ESI-MS/MS system. The LOQ for PFOA for each matrix was 1 µg/kg and recoveries were consistently and reproducibly quantitative. Another method, comprising liquid solvent extraction (aqueous acetic acid and MeOH), cleanup via SPE, and injection of the extracts with added internal standards into HPLC-ESI-MS/MS, enabling quantitative determination of PFSs, including both PFOA and PFOS, in sediment and sludge was reported by Higgins et al. (2005). LODs of the method were analyte and matrix dependent coming to 0.01 (sediment) and 1.0 µg/kg (sludge) for PFOA and 0.1 (sediment) and 0.9 µg/kg (sludge) for PFOS.

1.6.5 Analysis of Biota and Human Samples

A method described by Hansen et al. (2001) is the first one allowing simultaneous determination of the two analytes PFOA and PFOS, as well as other PFSs in biological matrixes such as serum and liver tissue and it is still the most commonly used one. It employs, use of an ion-pairing reagent (tetrabutylammonium hydrogen sulphate) followed by extraction with methyl *tert*-butyl ether, and determination via HPLC-ESI-MS/MS. LODs obtained for sera and liver were 1.0 µg/L and 5.0 µg/kg, respectively, for PFOA and 1.7 µg/kg and 8.5 µg/kg for PFOS. Later, Sottani et al. (2002) combined the same extraction method with HPLC atmospheric pressure ionisation tandem mass spectrometry (API-MS/MS) to determine PFOA in human serum but obtained a higher LOD of 10 µg/L.

Kannan et al. (2005) analysed samples of fish, mussels, amphipods, and algae by solvent extraction (AcN), followed by SPE and HPLC-ESI-MS/MS determination, obtaining LOQs ranging from 1 to 10 µg/kg ww. Later, So et al. (2006) also used SPE as a preconcen-

tration and clean-up step after alkaline digestion of mussels and oysters tissues. These methods, in comparison to the one described by Hansen et al. (2001), reduce matrix interferences to a great extend.

A time and cost efficient screening method for the analysis of PFSs in biota samples based on the extraction of target compounds from homogenised samples into a solvent mixture used as mobile phase in HPLC, i.e. MeOH/aqueous ammonium acetate (50:50) and determination by HPLC-TOF-MS was reported by Berger & Haukås (2005). This method showed LODs of 1.3 and 0.3 µg/kg ww for PFOA and PFOS, respectively.

An automated SPE clean up followed by HPLC-ESI-MS/MS was developed by Kuklenyik et al. (2004) for measuring trace levels of 13 PFSs in serum and milk. LODs were 0.1 (PFOA) and 0.4 µg/L (PFOS) for serum analysis and 0.2 (PFOA) and 0.3 µg/L (PFOS) for milk samples. So et al. (2006) modified this method by using weak-anion exchange SPE extraction and applied it to human milk samples achieving LOQs of to 21 and 1 ng/L for PFOA and PFOS, respectively.

Determination of PFOA and PFOS in human plasma after protein precipitation with AcN by large volume injection capillary column switching LC coupled to ESI-MS was presented by Holm et al. (2004). Advantages of this method were a simplified sample preparation procedure, its speed (separation and detection within 10 minutes), and low LODs of 0.2 and 0.5 µg/L for PFOA and PFOS, respectively, in untreated plasma.

2 Aim of the Work

The aim of this doctoral thesis was to find the sources of PFOA and PFOS to river ecosystems that are not directly affected by fluorochemical activity, to determine their distribution pattern, and their fate in such an ecosystem. In order to achieve the aim reliable analytical methods for the analysis of PFOA and PFOS in different environmental samples are necessary. A sample extraction including analytes' preconcentration step and removal of matrix interferences followed by HPLC-ESI-MS/MS is a method that is typically used for PFOA and PFOS determination in different environmental samples (see paragraph 1.6). While developing or optimising such analytical protocols, special attention has to be paid to possible contamination sources during sample preparation and to matrix interferences disturbing ionisation efficiency that might result in falsification of results (**Publication I**).

The release of treated waste water has been identified as a possible source of PFSs to aquatic ecosystems (Boulanger et al., 2005; Schultz et al., 2006 a; Sinclair & Kannan, 2006; Schultz et al., 2006 b). However, prior to this doctoral thesis no such data have been published in peer-reviewed literature for Germany or Europe. The mass flows of the analytes from a typical waste water treatment plant in Germany were assessed, and the fate/behaviour of the target analytes during the waste water treatment was investigated (**Publications II and III**).

Once released to aquatic ecosystems, PFOA and PFOS cannot be decomposed under environmental conditions and will partition between different compartments such as water, sediment and may bioaccumulate in living organism. To provide a better understanding of their fate in such systems, of the extent to which they can adsorb on sediment or bioaccumulate in biota, a detailed study on the example of the Roter Main River (**Publication II & III**), its sediments (**Publication IV**) and fishes (**Publication V**) was performed.

3 Optimised Analytical Procedures

3.1 Sample Preparation Procedures

3.1.1 River and Waste Water

River and waste water samples, collected in 2-L polypropylene (PP)-bottles were transferred into 250-mL PP-bottles, centrifuged (12000 rpm, 10 min, 20 °C, High-Performance Centrifuge, Avanti J-25, Beckman, USA) and filtered ($597^1/_2$, Schleicher & Schuell, Dassel, Germany). Next, SPE was performed according to the optimised method described in detail in **Publication I** (river water, 500 mL) and **Publication II** (waste water, 250 mL). Samples were stored at 4 °C in the dark not longer than two weeks, and allowed to reach room temperature prior to analysis.

SPE was performed on C_{18}-cartridges (200 mg, 6 mL, Oasis HLB Waters Corp., Milford, USA) preconditioned with deionised water and MeOH. Waste-water-loaded cartridges were washed with deionised water/MeOH, dried under vacuum, and analytes were eluted with MeOH into 5-mL PP-tubes. Extracts were dried under a gentle nitrogen stream, residues were dissolved in 500 µL each of a mixture of aqueous ammonium acetate/AcN, solutions were filtered (membrane filters, 0.45 µm, Roth, Karlsruhe, Germany) and transferred to PP-snap ring vials (0.75 mL, Supelco, Bellefonte, USA) with polyethylene caps (NeoLab, Heidelberg, Germany) for analysis.

If isotope dilution technique was applied, ^{13}C-labelled PFOA and PFOS standards were added to the samples after the filtration step but before preconcentration with SPE as described in **Publication III**.

3.1.2 Sludge and Sediment

Dewatered sludge, grit and sediment samples were freeze-dried in aluminium boxes precleaned with hexane and MeOH. Sludge was ground with mortar and pestle, transferred to PP-bottles and stored at room temperature until analysis, whereas sediment and grit were sieved (mesh size 0.63 and 2 mm, respectively).

Such prepared solid samples (100 mg of sludge, 1 g of grit or sediment) were extracted in triplicate according to the method described previously by Higgins et al. (2005) which was slightly modified as described in **Publication II** (sludge, grit) and **Publication IV** (sediment). Shortly, the method involves sonication of the homogenised sample at elevated temperature (60 °C) with diluted acetic acid (1 %), followed by sonication with a mixture of MeOH and

(1 %) acetic acid (90:10, vol-%). Obtained extract fractions were preconcentrated and precleaned by SPE using C_{18}-cartridges (200 mg, 6 mL, Oasis HLB Waters Corp., Milford, USA).

Mass labelled internal standards (0.5 ng/g ^{13}C-PFOA and ^{13}C-PFOS each) were added to sediment samples prior to extraction.

3.1.3 Fish

Extraction of fish tissue samples was performed according to a published method (So et al., 2006 a), slightly modified as described in **Publication V**. Analytes were extracted from the homogenised, spiked sample (150 ng each ^{13}C-PFOA and ^{13}C-PFOS) by shaking with methanolic KOH solution (0.01 N). A small portion of the obtained extract was added to deionised water (100 mL), preconcentrated and precleaned by SPE. Depending on the available amount of fish sample the method was slightly different when applied to internal organs of fish – a whole organ was homogenised with methanolic KOH solution (see **Publication V**).

3.2 HPLC-ESI-MS/MS

Sample extracts were analysed by HPLC-ESI-MS/MS according to the developed method described in **Publication I**. Aliquots of 10 µL were injected onto a 150 x 2.0 mm (5 µm) Prontosil C_{18} column (Bischoff, Leonberg, Germany) and analytes were eluted with a mobile phase consisting of 40 vol-% aqueous ammonium acetate (10 mM, pH 4.1), and 60 vol-% AcN. Column temperature was 40 °C, total run time 6.5 min. The column was interfaced with an electrospray ionisation source to a tandem mass spectrometer (API 300, Applied Biosystems, Foster City, USA) operated in the negative ionisation and multiple reaction mode.

3.3 Quantification

Quantification was accomplished via standard addition method (described in detail in **Publication I**; for correction of ionisation suppression) or isotope dilution technique. The latter includes addition of a defined amount of mass labelled analytes (both ^{13}C-labelled PFOA and PFOS) to the sample prior to extraction. Based on the ratio non-labelled (PFOA, PFOS) to labelled (^{13}C-PFOA, ^{13}C-PFOS) analytes peak area and knowing the amount of the labelled standard (internal standard) concentrations of target chemicals can be determined with high precision.

3.4 Sampling Campaigns

3.4.1 River Water, Waste Water and Sludge

During this doctoral work, two studies on surface and waste waters were performed. During the first study, concentrations of PFOA and PFOS were investigated within 4 different WWTPs, located in Upper Franconia, Bavaria, Germany, and in the respective rivers receiving treated waters. Waste water samples were collected at different steps of the treatment process employed in the following plants Bayreuth, Kulmbach, Himmelkron, and Ramsenthal. At the same time, surface water samples upstream and downstream the outlet of the plants were taken from rivers Roter Main (WWTP Bayreuth), Weißer Main (WWTPs Kulmbach and Himmelkron), and Trebgast (WWTP Ramsenthal). A detailed description of the plants (average daily flow, number of inhabitants, waste water origin, and waste water treatment), the rivers (average daily flow) and the sampling campaign is given in **Publication II**.

The second study focused on the WWTP Bayreuth that showed the highest mass loadings of PFOA and PFOS of the 4 WWTPs included in the first study and is a source of the Roter Main contamination with PFOA and PFOS. In order to better assess the average mass loading from the plant, waste waters were monitored for PFOA and PFOS from 14 March to 14 June 2007 every other week on Wednesday and Friday at 10 a.m.. Between 11 and 15 June 2007 at 8 a.m. and 2 p.m. river water samples were collected 1 km downstream the plant twice a day. A detailed description of this sampling is given in **Publication III**.

3.4.2 Sediment

On 19 October 2006, sediment samples from the Roter Main were collected at four different locations: a) 1 km upstream, b) 50 m downstream, c) 500 m downstream, and d) 1 km downstream the WWTP. At each location, 11 individual samples from the upper sediment (~ 15 cm) were collected with a PP-tube (Ø = 10 cm) and transferred into 250-mL PP-bottles. Water samples (n = 3) were also collected at each location. Details of the sampling campaigns are presented in **Publication IV**.

3.4.3 Fish

On 28 August 2007, two fish species, i.e. chub (*Leuciscus cephalus*) (n = 6) and river goby (*Gobio gobio*) (n = 5), were collected from the river Roter Main approximately 3 km downstream the WWTP Bayreuth. The fish was caught by electro-fishing performed by the

employees of the Bavarian Fishery Association. Collected species represented two different fish families having different feeding strategies: chub feeds on water insects, larvae, snails, mussels and worms, and reaches a size up to 40 cm, whereas goby is a bottom-feeding fish that can reach up to 15 cm.

Caught fishes were immediately cooled and transported to the laboratory for further analysis. Chubs were dissected and heart, liver, kidneys, gonads, and muscle tissue were stored separately; only muscles and inner organs were separated of the river goby due to its small size. Details about the collected fish including their size, weight as well as weight of inner organs, and storing procedure are provided in **Publication V**.

4 Results and Discussion

4.1 Optimisation of Analytical Protocols

Elucidating possible sources of sample contamination with PFOA and PFOS during the whole analytical protocol, including sampling, sample preparation, and measurement was a very important step at the beginning of the work (**Publication I**). As PFOA and PFOS are widely used, i.e. for production of laboratory equipment (Yamashita et al., 2004), use of such equipment i.e. Teflon tubing or caps with Teflon septum was avoided. Although Yamashita et al. (2004) reported contamination of some nylon filters, no traces of the analytes were found on paper and nylon filters used for sample or extract filtration in the present doctoral thesis. Tests of solvents used in different analytical protocols showed traces of PFOA (2 ng/L) in MeOH but in no other solvent used; all of them were free of PFOS. To avoid contamination from needles, valves or adapters employed during the SPE procedure a meticulous cleaning procedure was developed (experimental section of **Publication I**). In comparison to tap, bidistilled or Millipore water, deionised water had a low stable concentration of PFOA (0.22 ± 0.05 ng/L) and was chosen whenever water was necessary, i.e. for cartridge conditioning or as a solvent for different solutions.

Methods of PFOA and PFOS determination in different environmental matrices such as surface or waste water, sludge, sediment, fish tissues, employing sample extraction, preconcentration and precleaning step followed by HPLC-ESI-MS/MS determination, were developed and/or optimised. Background contamination with the target analytes of each of the employed procedures was carefully evaluated. Procedural blanks for river (500 mL) and waste (250 mL) water samples contained 0.03 and 0.06 ng/L PFOA, respectively (**Publication I, Publication II**), whereas for sludge (**Publication II**), sediment (**Publication IV**) and fish tissues (**Publication V**) they were below LOD.

Table 9. Recoveries [%] of the extraction procedures employed during the study.

Sample type	Recovery		Reference
	PFOA	PFOS	
River and waste water	79 ± 8	74 ± 6	Publication II
Sediment	73 ± 9	101 ± 10	Publication IV
Sludge	95 ± 8	79 ± 7	Publication II
Fish	88 ± 10	86 ± 10	Publication V

Table 10. LOQs of the extraction procedures employed during the study.

Sample type, sample size	LOQ		Reference
	PFOA	PFOS	
River water, 500 mL	0.06 ng/L	0.12 ng/L	Publication II
Waste water, 250 mL	0.12 ng/L	0.24 ng/L	Publication II
Sediment, 1 g	0.025 µg/kg	0.05 µg/kg	Publication IV
Sludge, 100 mg	0.24 µg/kg	0.60 µg/kg	Publication II
Fish, (0.16 – 7 g)	0.5 – 9 µg/kg	1.0 – 18 µg/kg	Publication V

Recoveries and LOQs of extraction procedures applied to particular sample types analysed during the study are listed in Tables 9 and 10.

An important aspect while using HPLC-ESI-MS/MS for the determination of trace analytes is a possible ionisation suppression by coeluting matrix components (**Publication I)**. Therefore, to obtain reliable results, all sample preparation procedures used during this doctoral study included a SPE step, designed not only for analyte preconcentration but also for matrix removal; and either standard addition method (i.e. **Publication II**) or isotope dilution technique (**Publications III-V**) were employed for quantification.

4.2 River, Waste Water and Sludge

Results of surface water samples collected from three rivers (Roter Main, Weißer Main, Trebgast) and waste waters from 4 different WWTPs (Bayreuth, Kulmbach, Himmelkron, Ramsenthal) have been presented in **Publication II**. The same publication includes also the results for sludge samples collected from the WWTP Bayreuth and the detailed study of mass flows of the analytes through this plant during the treatment process. **Publication III** focused only on the WWTP of Bayreuth and the Roter Main. Results obtained during both studies are summarized below.

4.2.1 River waters

PFOA was found above LOD (0.03 ng/L) in almost all river samples (exceptions: samples collected upstream of the WWTP Bayreuth, Roter Main, March 2007). In all rivers concentrations were relatively low upstream and increased downstream behind the outlets of plants. Its presence upstream of the direct source in the studied cases mainly rural areas might be due to its former use in plant protection formulations, atmospheric deposition, and other small WWTPs located on the tributaries entering the river.

The highest PFOA concentrations in river waters were measured downstream the WWTP Bayreuth in the Roter Main, on average 9 ± 4 ng/L (April 2005 – June 2007). PFOA concentrations found in this river during the whole study period (<0.06 – 18 ng/L) are comparable to those reported for the Guangzhou River, China (So et al., 2007), but much lower than concentrations found in he rivers Yangtze, China (260 ng/L, So et al., 2007), Tennessee, USA (140 – 600 ng/L, Hansen et al., 2002), or Moehne, Germany (3640 ng/L, Skutlarek et al., 2006).

PFOS was detected in all but one river water sample (Weißer Main, upstream the WWTP Himmelkron), its highest values were observed downstream the WWTP Bayreuth Roter Main. In all rivers an increase in PFOS concentration in river water downstream the respective WWTP was observed, although these values were slightly lower than expected when assuming full mixing of river and waste waters. Between April 2005 and June 2007, the average PFOS concentration in the Roter Main 1 km downstream the plant of Bayreuth was 31 ± 18 ng/L. PFOS concentrations (<0.12 – 35 ng/L) found in Roter Main during the research period (April 2005 – June 2007) were higher than those reported for the North American rivers Hudson (1.5 – 3.4 ng/L), Niagara (3.3 – 6.7 ng/L, Sinclair et al, 2006), St. Clair (1.9 – 3.9 ng/L) or Rising (3.5 ng/L, Kannan et al., 2005), comparable to those found in Ruhr, Germany (2.5 – 43 ng/L, Skutlarek et al., 2006) or in Guangzhou, China (0.9 – 100 ng/L, So et al., 2007), but lower than those in the rivers Tama, Japan (157 ng/L, Saito et al., 2003), or Moehne, Germany (193 ng/L, Skutlarek et al., 2006).

Monitoring of the Roter Main revealed an increase in mass flows of both analytes within its waters up to 80 % from morning to early evening (on 11, 13, and 14 June). The mass flow of PFOA and PFOS followed the same pattern suggesting the same origin.

4.2.2 Liquid and solid wastes

PFOA was found above LOQ (0.06 ng/L) in all waste water samples collected from 4 different WWTPs in Upper Franconia, Germany (**Publication II**, table 2) with the highest concentrations determined in the effluent of the biggest plant serving the highest number of inhabitants (WWTP Bayreuth; 20 – 250 ng/L; **Publication I and II**). It was also found in all solid wastes collected from this plant (**Publication II**, table 3), with the highest concentration in waste activated and sewage sludge.

The highest PFOS concentrations were found in waste waters of the same WWTP (14 – 400 ng/L, **Publication I and II**), the highest values were measured in the primary treatment tank in March 2007. Its concentrations in solids were up to 120 µg/kg, and up to 7-fold

higher than PFOA concentration in the respective sample (**Publication II**, table 3). PFOS was below LOD in influents of two plants (Ramsenthal and Himmelkron) included in the study but could be measured in the respective effluents (**Publication II**, table 2).

Detailed study of the analytes mass flows within liquid and solid wastes of the plant of Bayreuth showed that PFOA concentrations were up to 20-times higher in the effluent in comparison to the respective influent, and only 10 % of its total mass flow was removed together with sludge. The total mass flow of PFOS within solid and liquid wastes increased within the plant 3-fold, but in contrary to PFOA about 50 % of it was adsorbed onto sludge (**Publication II,** Fig. 1).

During the whole study, the daily mass loading of PFOA to the receiving river from the WWTP Bayreuth was estimated to be 1.2 ± 0.5 g/day, it was the highest of all investigated plants, but lower than that reported for a smaller plant in rural Kentucky, USA (1.8 – 2.7 g/day, Loganathan et al., 2007) or for a large American plant (~ 45 g/day, Sinclair & Kannan, 2006). The amounts released from other studied plants were substantially lower than those from Bayreuth: ~ 0.5 g/day WWTP Kulmbach, ~ 0.05 g/day WWTP Ramsenthal and ~ 0.03 g/day WWTP Himmelkron. A similar trend was observed in regard to mass loadings of PFOS, the highest was found for WWTP Bayreuth (4.7 ± 2.3 g/day, during whole study), the lowest for WWTP Himmelkron (0.02 g/day). The amount of PFOS released from the plant of Bayreuth was much higher than those reported for US-WWTPs, i.e. 0.6 (Schultz et al., 2006 b; Loganathan et al., 2007) or 1 g/day (Sinclair & Kannan, 2006).

4.3 Sediments

Results summarised below have been described in detail in **Publication IV**.

PFOA concentrations in sediment samples collected from Roter Main were often below LOQ, especially at locations upstream the WWTP. PFOA sediment levels downstream the plant were up to 3-fold higher than those upstream and the highest level, 175 ng/kg dw, was registered 50 m downstream the plant. Its sediment concentrations were approximately 2-fold higher relative to the respective water samples and up to 6-fold higher than the average water concentration measured in the Roter Main between April 2005 and June 2007 (0.1 km upstream: 3 ng L^{-1}, 1 km downstream: 9 ng L^{-1}, **Publication II & III**). In general, PFOA concentrations found in the Roter Main were lower than those reported for four rivers from the San Francisco Bay, USA (<LOD – 1300 ng/kg, Higgins et al., 2005), Tidal Flat Areas of the Ariake Sea (840 – 1100 ng/kg, Nakata et al., 2006), or for Japanese rivers: Kamo, Uji, Tenjin (1300 – 3900 ng/kg, Senthilkumar et al., 2007).

PFOS sediment concentrations were up to 17-fold higher than those of PFOA in the respective sediment sample, and 20- to 40-fold higher than in the respective water sample or than in the average water concentration determined in the river water between April 2005 and June 2007 (0.1 km upstream: 2.0 ng/L, 1 km downstream: 30 ng/L, **Publication II & III**), which is due to its stronger adsorption potential. PFOS sediment concentration increased after the outlet of the plant up to 4-fold and was comparable to those of the Ariake Sea (90 – 140 ng/kg, Nakata et al., 2006) or of rivers from the San Francisco Bay (160 – 230 ng/kg, Higgins et al., 2005) but lower than reported for the rivers Tenjin or Osaka in Japan (3800 – 11000 ng/kg, Senthilkumar et al., 2007).

No correlation between the level of either analyte and the total organic content (TOC) was observed, although such a correlation has been suggested (Higgins & Luthy, 2006). Johnson et al. (2007) suggested that adsorption of these PFSs to sediments with low TOC content is partially controlled by electrostatics, and thus their fate and transport in an aquatic system can also be influenced by inorganic materials, as well as pH of water and sediment.

4.4 Fish

PFOA concentrations in different tissues of the chubs were in most cases below LOQ, only in gonads it was found more frequently (in four out of six; <0.4 µg/kg ww up to 9.7 µg/kg ww). PFOA concentrations in river gobies were generally higher, up to 3.0 µg/kg ww in inner organs, and up to 9.8 µg/kg ww in muscles.

Overall, PFOA concentrations measured in fish collected from the Roter Main were lower than in eel, barb, carp, nase, or greyling (muscles: 6.4 – 53 µg/kg ww, livers: 2.6 – 840 µg/kg ww, BLfU, 2007 b) collected from the highly contaminated river Alz, Germany (up to 7.5 µg/L of PFOA, BLfU, 2007 c) but higher than in largemouth or smallmouth bass from the River Raisin, St. Clair and Calumet, USA (muscle <2 µg/kg ww, Kannan et al., 2005), in trout from Lake Ontario (whole body: 1µg/kg ww, Martin et al., 2004), or in white fish purchased on a Catalan market in Spain (muscles: <0.065 µg/kg ww, Ericson et al., 2008).

PFOS concentrations measured in the caught fish were higher than those of PFOA, and ranged from 7.5 µg/kg ww (muscles) up to 250 µg/kg ww (gonads) in chubs, and from 69 µg/kg ww (muscles) up to 406 µg/kg ww (inner organs) in gobies, probably due to its higher water and sediment concentrations (25 ng/L and 240 ng/kg, respectively).

Average PFOS concentrations in different tissues of chub were highest in liver, and kidneys, followed by gonads and heart; lowest values were measured in muscles. This is in a

good agreement with previously published data showing that PFOS accumulates mainly in liver (Giesy et al., 2001; Kannan et al., 2005), kidneys (Martin et al., 2004; Van de Vijver et al., 2005) and gonads (Martin et al., 2003). Concentrations found in chub liver are comparable to those of smallmouth bass from New York State lakes (10 – 140 µg/kg ww, Sinclair et al., 2006), chinook salmon (30 – 170 µg/kg ww) or whitefish (33 – 81 µg/kg ww) of the Great Lakes (Kannan et al., 2005) but lower than those in eel, perch, roach from rivers Main and Alz (liver: 15 – 4300 µg/kg ww, BLfU, 2007 b) or in carp or gibel carp from Flanders, Belgium (10 – 9030 µg/kg ww, Hoff et al., 2005). PFOS muscles concentrations were similar to those found in fish from other Bavarian rivers (BLfU, 2007 b).

PFOS levels in river goby were 3- to 4-times higher than in chub, in average in muscle tissues 80 ± 17 µg/kg ww and in combined inner organs (liver, kidneys, heart, gonads, intestines) 300 ± 80 µg/kg ww. According to Higgins et al. (2006), PFSs are readily bioavailable in sediments, this is in a good agreement with the fact that PFOS-concentrations were higher in river gobies, feeding mainly on invertebrates living in the sediment that was shown to contain PFOS levels 22-fold higher than water.

BAF for PFOA between muscle tissue of river gobies and its concentrations in the river was calculated to be 740, it was higher than that reported for rainbow trout exposed to this chemical under laboratory conditions by Martin et al. (2007, BAF = 4). BAFs of PFOS calculated for chubs' livers or river gobies' inner organs relative to the average water concentration (median Roter Main, 1 km downstream the plant, March - June 2007 = 27 ng/L) were 4650 and 10900, respectively. BAFs are in a good agreement with those calculated for coastal fish from Japan (8540, Taniyasu et al., 2003) or the Niagara River, USA (8850, Houde et al., 2006).

5 Conclusions and Recommendations

In order to obtain reliable data while analysing traces of PFSs, such as PFOA and PFOS, potential sources of background contamination must be elucidated and eliminated or at least minimised when elimination is not possible. Using HPLC-ESI-MS/MS for determination, special attention has to be paid to possible interferences arising from co-eluting sample matrix. Thus, employed analytical procedures should be designed to ensure effective removal of such interferences, furthermore, suitable quantification methods, such as isotope dilution technique or standard addition, have to be employed.

Data obtained during the present study showed that at sites without direct fluoroproduction activity, treated waste waters are a major source of river pollution with PFOA and PFOS. The highest release of both analytes from 4 Upper-Franconian WWTPs was observed for the plant serving the largest population, having the highest average daily flow, and treating waste waters mostly of industrial and commercial origin. The lowest mass loading of PFOA and PFOA was observed for the smallest plant treating waste waters of only domestic source. Degradation of their precursors during the waste water treatment process pose an additional flux of PFOA and PFOS inside the plants, while PFOA passes a plant almost undiminished to enter receiving waters, almost a half of PFOS is adsorbed onto sludge. Monitoring of waste waters released from the plant of Bayreuth enabled to estimate the average daily release from this facility to be 1.2 ± 0.5 g PFOA and 4.7 ± 2.3 g PFOS.

Once released to the river, PFOA and PFOS partition into the sediment, which is reflected in their higher concentrations registered downstream the plant, with the latter showing the higher adsorption potential. Even adsorbed on sediments, they are still bioavailable for the biota inhabitating the given ecosystem, which in turn is reflected in their higher levels found in the fish species feeding on the invertebrates sucked up from the sediment.

PFOS released from the WWTP, present in waters and sediments of the river, is bioaccumulated in aquatic organisms, its tissue distribution in fish follows the pattern: liver > kidneys > gonads, heart >> muscles. PFOA has a lower bioaccumulation potential.

In order to get a wider perspective of PFS's sources, fate and distribution in an environment as the Roter Main, optimisation of the presented analytical methods to enable investigation of a broader spectra of analytes, such as precursors of PFOA and PFOS, longer and shorter chain PFSs is necessary. Analysis of such precursor chemicals could help better understand processes taking place during the waste water treatment resulting in an additional flux of PFOA and PFOS.

Due to the fact that neither of the two analytes of interest can undergo degradation in the environment, the best solution to avoid further contamination of the aquatic environment would be prevention of their release. Tang et al. (2006, 2007) suggested that PFOS could be removed from waste waters by reverse osmosis and/or nanofiltration. Application of this or any other technique enabling removal of PFSs from waste waters, preferably before they reach WWTP, should be further investigated.

Moreover, exposure experiments performed with the target analytes on aquatic organisms at environmental concentrations would assist estimation of the extent of the problem that living beings are facing due to PFSs' release.

6 Publication I

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Short Communication

Quantitative determination of perfluorinated surfactants in water by LC-ESI-MS/MS

The surfactants perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), and derivatives of the latter have emerged as globally distributed persistent environmental contaminants. Methods for their reliable quantitative determination at ppt-levels (ng/L) are needed in order to detect their main sources, to elucidate their environmental fate, and to identify potential sinks. The common method for water analysis involves preconcentration by SPE followed by LC coupled to ESI MS/MS (LC-ESI-MS/MS). All sample preparation steps must be carefully optimized in order to arrive at reliable quantitative data. Two major aspects are important: (i) during SPE, contaminations may arise from materials containing traces of PFOA/S; (ii) during LC-ESI-MS/MS, ionization yields are suppressed by matrix components and depend upon the analyte concentrations in the extracts. The levels of PFOA/S in the river Roter Main near Bayreuth have been determined using the optimized method.

Keywords: LC-ESI-MS/MS / Polyfluorinated surfactants

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1 Introduction

The perfluorinated surfactants (PFS) perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), and derivatives of the latter have been manufactured for over 50 years. At the end of the last decade, their annual production was about 4650 metric tons [1]. Due to their unique molecular properties, *i.e.*, being both water- and fat-repellent, they have been used for treatment of textiles and paper, in cosmetics, insecticide formulations, fire fighting foams, hydraulic fluids, in the photographic industry, for metal plating, and for production of semiconductors [2, 3]. PFS are persistent and anthropogenic; they are stable toward acids, bases, oxidants, and reductants, and they are resistant toward microbial degradation [2]. As their Henry's law constants and their volatilities are low, they partition to the atmosphere only to a very low degree, the only compartment in which they conceivably could be remineralized. They are globally distributed and ubiquitous [4, 5], and are found in the hydrosphere and biosphere [6 – 18].

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Abbreviations: PFOA, perfluorooctanoate; **PFOS,** perfluorooctane sulfonate; **PFS,** perfluorinated surfactants; **PP,** polypropylene; **WWTP,** waste water treatment plant

Methods sufficiently sensitive for environmental monitoring are GC-MS after derivatization [19] and liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) of the nonderivatized PFS [6 – 14]; the latter is the more common method.

Two major problems must be considered when trace levels of PFS need to be determined: (a) they are employed for production of numerous plastic equipments used in the laboratory such as vessels, cartridges, or tubings; therefore, care must be taken to keep blank levels at a minimum [20, 21]; (b) during LC-ESI-MS/MS, matrix components and actual concentrations of analytes in the final extract may suppress ESI yields, as observed previously [22 – 24].

In order to cope with the problem of ionization suppression, internal standards such as carboxylic acids [25] or tetrahydroperfluorooctane sulfonate (THPFOS) [4, 10] have been suggested. On the other hand, both are now recognized as problematic: carboxylic acids have lower acidity and surface activity than the PFS, and THPFOS may be present in environmental samples. Short-chain perfluorinated dicarboxylic acids have also been employed but they are less hydrophobic than the analytes [21]. Therefore, mass spectrometric isotope dilution and standard addition are the only reliable methods for quantification of PFS in environmental samples.

2 Experimental

2.1 Chemicals and equipment

Perfluorooctanoic acid (95%, Lancaster Eastgate, UK), perfluorooctane sulfonate potassium salt (98%, Fluka, Buchs, Germany), ammonium acetate (99.0%, Fluka), acetic acid (Fluka), methanol (picograde, Promochem, Wesel, Germany), and acetonitrile (ACN) (picograde, Promochem) were used as obtained.

All the equipments were precleaned by rinsing with bidistilled water and methanol and washed in a laboratory washing machine with alkaline detergent (Neodisher, Dr. Weigert, Hamburg, Germany). SPE connectors, valves, and adapters were sonicated in a beaker with water for 10 min, followed by methanol for another 10 min. This was done three times.

2.2 Sample collection

Water samples were collected at four sites along the river Roter Main at Bayreuth in Northern Bavaria, Germany, in April, June, and July 2005. The first site was 1 km upstream from the local waste water treatment plant (WWTP) near the city center, next to a street with low traffic volume. The second, third, and fourth were 100 m upstream, 100 m downstream and 1 km downstream from the outlet of the WWTP; at these sites, the river is surrounded by meadows.

Samples were collected in 2-L screw-capped high-density polypropylene (PP) bottles (VWR, Darmstadt, Germany). At each location, two 2-L grab samples were taken, immediately transported to the laboratory, and stored at 4°C in the darkness. Prior to preparation, the samples were allowed to reach room temperature.

2.3 SPE

The analytical procedure for extraction of the water samples was similar to a previously described method [7] with the following modifications. The water samples were transferred to 250-mL screw-cap PP bottles, centrifuged (IIigh-Performance Centrifuge, Avanti J-25, Beckman, USA) at 12 000 rpm for 10 min, and filtered using folded paper filters (597$^1/_2$, Schleicher & Schuell, Dassel, Germany). For preconcentration, C18 cartridges were used (6 mL, 200 mg, Oasis HLB Waters, Milford, USA), conditioned with 6 mL of methanol followed by 10 mL of deionized water (1 drop/s). Filtered water samples of 500 mL were passed through cartridges (5 mL/min) under reduced pressure (0.4 bar). Cartridges were washed and dried with air under reduced pressure (0.4 bar) for 30 min, the analytes were eluted with 4 mL of methanol (1 drop/s), and eluates were collected in 5-mL PP tubes. The solvent was evaporated under a gentle nitrogen stream, and the dry residues were redissolved in 500 µL of a (50:50, vol%) mixture of aqueous ammonium acetate (10 mmol/L) and ACN. Extracts were filtered through LC-membrane filters (0.45 µm, Roth, Karlsruhe, Germany) and transferred into a PP autosampler vial (0.75 mL, Supelco, Bellefonte, USA). When necessary, filtered extracts were diluted (1 + 1, 1 + 4, and 1 + 9) by transferring defined volumes (250, 100, and 50 µL) of the extract using Eppendorf pipettes into other PP-vials and adding a 50:50 (vol%) mixture of aqueous ammonium acetate (10 mmol/L) and ACN to a final volume of 500 µL.

2.4 LC/MS/MS

Extracts were analyzed *via* LC-ESI-MS/MS under conditions reported previously [7] with the following modifications: LC separation was done on a C18 column (ACE-EPS, 150 × 2 mm, 5 µm, 120 Å, Prontosil, Bischoff, Leonberg, Germany) with a mobile phase consisting of 40 vol% of aqueous ammonium acetate, 10 mmol/L, pH = 3.1, and 60 vol% ACN. Column temperature was 40°C. The total run time was 6.5 min. Once a week, the HPLC-column was rinsed overnight with ACN/water (50:50, vol%); when highly concentrated extracts (>10 µg/L) were analyzed, this was done daily.

The column was interfaced *via* an ESI source to a tandem mass spectrometer (API 3000 LC/MS/MS; Applied Biosystems/MDS SCIEX, Foster City, USA). Cone voltage was −4.3 kV, and dwell time was 0.2 s; the nebulizer, curtain, and collision gas flow rates were 8, 10, and 4 L/min, respectively; the nebulizer temperature was 350°C. Argon was used as collision gas, and the collision energy was optimized for each compound. The multiple reaction mode (MRM) was employed for quantification, with the parent and daughter ions of *m/z* 413/369 for PFOA, and *m/z* 499/99 + 80 for PFOS; the abundance of the ion *m/z* 80 for SO_3^- was always higher but *m/z* 99 for FSO_3^- was also monitored for fluorine selectivity. The two broad peaks (Fig. 1) between 4.3 and 5.6 min represent branched and linear isomers [22]; for quantification, the areas of both were integrated. The fraction of branched isomers in real samples was usually higher than in the pure commercial standard.

For calibration, a stock solution of PFOA was prepared by dissolving 53 mg of perfluorooctanoic acid (95%) in 100 mL ACN, resulting in a concentration of 500 mg/L. An equally concentrated stock solution of PFOS was prepared by dissolving 55 mg of perfluorooctane sulfonate potassium salt (98%) in 100 mL of ACN. A low-concentrated standard mixture of 10 µg/L each (PFOA and PFOS) was prepared from the stock solutions by appropriate dilutions with a 50:50 (vol%) mixture of aqueous ammonium acetate (10 mmol/L) and ACN. The low-concentrated standard mixture was used for daily preparations of working solutions in the range from 1 to 10 µg/L for

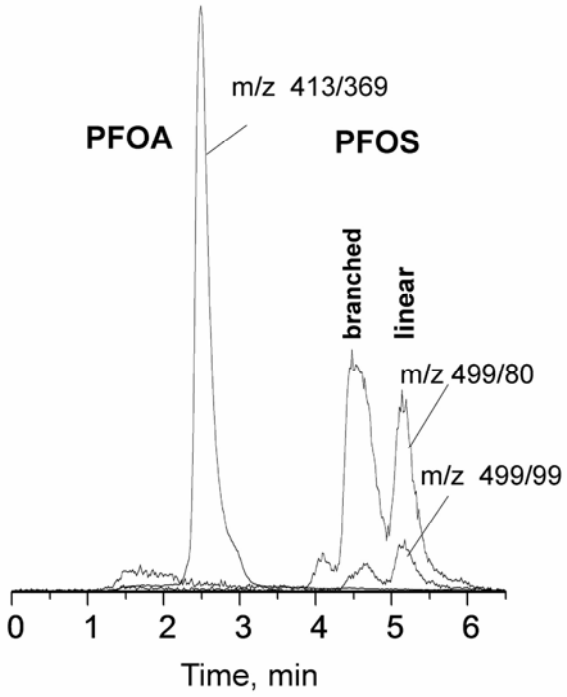

Figure 1. LC-ESI-MS/MS chromatogram of a typical SPE extract of a river water sample (enrichment factor E_f 1000) with concentrations of 41 ± 2 ng/L PFOA and 18 ± 1.7 ng/L PFOS.

calibration, standard addition, and spiking experiments. All these solutions were stored in a refrigerator.

For each batch of samples collected at the same time and at the same location, an individual standard addition calibration consisting of five points was prepared.

2.5 Quality control

Spike and recovery experiments with deionized water and river water samples (June 2005, location 1, PFOA: 1.0 ± 0.2 ng/L, PFOS: 0.8 ± 0.05 ng/L) (500 mL) were performed to determine the accuracy of analysis. Three deionized water samples (500 mL each) were spiked to 3 ng/L PFOA and 3 ng/L PFOS by adding 500 µL of a working standard solution (3 µg/L) with an Eppendorf pipette, and three additional ones were spiked to 1.5 ng/L PFOA and 1.5 ng/L PFOS by adding 250 µL of the same standard mixture. River waters were spiked to three different levels (1, 2, and 3 ng/L) each by adding various volumes of differently concentrated working solutions (250 µL of 2 µg/L, 500 µL of 2 µg/L, and 500 µL of 3 µg/L). Extraction efficiency was determined by analyzing sequentially diluted SPE extracts, because suppression of the ESI yield was less pronounced at low concentrations.

3 Results and discussion

As an early step in quantitative determination of environmental trace chemicals, the potential presence of blank contaminants must be addressed, especially if the analyte is a widely employed chemical. When beginning this project, numerous problems arose from high blanks. As laboratory equipment made from plastic may contain traces of PFS [20], meticulous cleaning as described in the experimental part is necessary. Tubing, adapters, and any other equipment made from Teflon can be major sources of contamination and should not be used.

Solvents employed in the sample preparation procedure were tested by evaporation to dryness, dissolution in ammonium acetate/ACN, and analysis by LC-ESI-MS/MS. Traces of PFOA (≈2 ng/L) have been found in methanol, nothing in ACN; PFOS was not found in either case.

Cartridges complete with needles and valves were tested for the presence of the target analytes by conditioning and elution with methanol. This experiment yielded blanks equivalent to a concentration of 0.03 ng/L PFOA for a 500-mL water sample.

The blank concentrations of PFOA in deionized, Millipore-filtered, and tap water ranged from 0.18 to 0.22 ng/L; for reasons unknown, bidistilled water was higher (0.35 ± 0.04 ng/L). As deionized water showed a relatively low concentration and variability (0.22 ± 0.05 ng/L), it was chosen for conditioning of cartridges, for blank determinations, preparation of mobile phase, and as a solvent for dilution. For conditioning and washing of SPE cartridges, 13 mL of deionized water was used, corresponding to a blank contribution of 0.003 ng/L PFOA for a 500-mL water sample.

SPE recoveries from deionized water spiked at two different levels (1.5 and 3 ng/L) were 99 – 100% (±7% RSD) for PFOA and 83 – 94% (±7% RSD) for PFOS. River water samples spiked with both analytes at low level (1 ng/L) showed recoveries of 97% (±2% RSD) for PFOA and 82% (±2% RSD) for PFOS. Recoveries in river water samples spiked with both analytes at 2 or 3 ng/L were 79 – 83% (±3% RSD) for PFOA and 69 – 72% (±3% RSD) for PFOS.

Prior to injection, extracts were passed through nylon syringe filters to remove particles. Such filters were previously reported [20] to contain PFOA and PFOS, but nothing was found in this case.

When all precautions were taken, the blank contamination resulting from SPE cartridges, reagent water, and solvent used for elution was about ≈0.03 ng/L PFOA for a sample of 500 mL. Thus, blank subtraction, obviously, is only relevant for low-concentrated samples (<1 ng/L PFOA). PFOS blanks were always below the detection limit (0.05 ng/L).

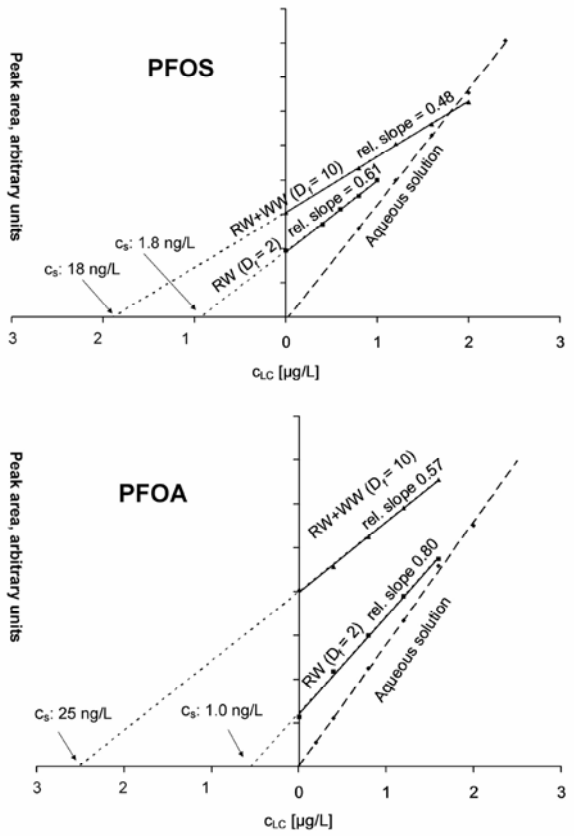

Figure 2. Comparison of calibration curves obtained for PFOA and PFOS: standard compounds in (50:50, vol%) aqueous ammonium acetate (10 mmol/L)/ACN (- - -); diluted (D_f = 2) river water extract (RW) (1 µg/L PFOA, 1.8 µg/L PFOS) by standard addition (—); diluted (D_f = 10) extract of river water containing waste water (RW + WW) (25 µg/L PFOA, 18 µg/L PFOS) by standard addition (—). The slopes of the calibration lines obtained for the solution of pure standard compounds are arbitrarily set to 1.0. The concentrations c_s indicated at the intercepts of the extrapolated regression lines with the abscissa are calculated taking the enrichment factor (E_f = 1000) and the extract dilution D_f into account.

With an enrichment factor of 1000 by SPE, the LODs (S/N 3) for surface water were 0.025 ng/L for PFOA and 0.05 ng/L for PFOS, and LOQs (S/N 6) were 0.05 and 0.1 ng/L, respectively.

Ionization yields – and thus the concentrations calculated for a given sample – depend on the actual concentration of analytes in the extracts, especially of the weak acid PFOA. Upon coelution of matrix components and at high analyte concentrations, ESI yield is suppressed. This is due to the fact that the surface charge on a primary spray droplet is insufficient to fully ionize all analytes and matrix molecules.

Therefore, the slopes of the calibration curves obtained by standard addition for extracts of river water and river water containing waste water may be up to two-fold lower (Fig. 2) than those from pure solution of commercial standards. Extracts with higher concentrations of analytes and consisting of more complicated matrix experience stronger signal suppression; therefore, such extracts need to be diluted to have a concentration in the final extract injected to LC-ESI-MS/MS of not more than 2 µg/L. As the suppression of ionization may be highly variable between sample batches, always a standard addition sequence was performed.

Analyte concentrations in the measured extracts (c_{LC}) were calculated according to

$$c_{LC} = A/S \text{ (µg/L)}$$

where A is the peak area of the MRM chromatogram and S is the slope of the calibration line obtained by standard addition. The concentration in the water sample (c_s) was calculated as

$$c_s = (c_{LC} \times D_f)/(E_f \times R) \text{ (ng/L)}$$

with D_f being the dilution factor (1 + 0 = 1, 1 + 1 = 2, 1 + 4 = 5, *etc.*), E_f the enrichment factor (*e.g.*, 1000 when, from a water sample of 500 mL, an extract of a final volume of 500 µL is obtained), and R the recovery (%/100).

The reproducibility determined by triplicate injections of river water extracts was within 5% for both analytes. The precision of analyzing a river water sample by triplicate extraction was 10%; the combined standard uncertainty (u) of the analytical procedure was 9.2%. The greatest contribution of uncertainty arose from the variability of recovery and from the chromatographic quantification (regression line preparation, peak integration).

The optimized method was applied to the analysis of river water samples (Fig. 3); the dotted lines in Fig. 3 indicate the values obtained when using a pure solution of commercial standards for calibration. Obviously, the higher the concentrations of matrix components and of the analytes in the sample, the greater is the risk of determining erroneously low values and underestimate budgets.

PFOA and PFOS were detected in all samples. Their concentrations (July 2005) upstream of the WWTP were 2.2–2.6 ng/L PFOA and 3.2–3.4 ng/L PFOS. They were five- to six-fold increased (14 ng/L PFOA, 26 ng/L PFOS) 100 m downstream of the WWTP, and about 1 km downstream they were still high (12 ng/L PFOA, 14.5 ng/L PFOS). Obviously, household waste waters are a main source of the general river pollution by PFS [24, 26].

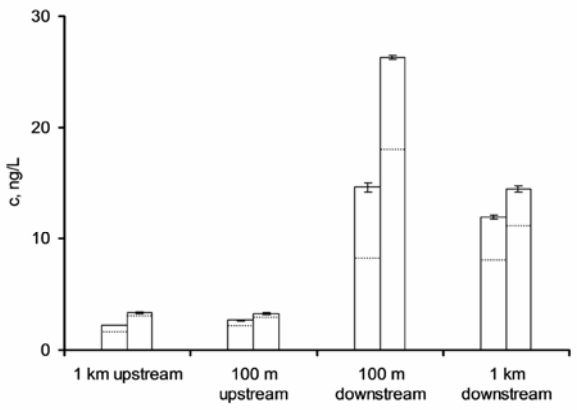

Figure 3. Typical concentrations of PFOA (left columns) and PFOS (right columns) in the river Roter Main before and after the inflow of treated waste water from the WWTP of Bayreuth (July 2005). The dotted lines (· · ·) indicate values obtained when the results are calculated based upon a calibration with an aqueous solution of the pure standards.

4 Conclusions

For quantitative determination of PFS in water samples (tap water, ground water, river water, waste water) by LC-ESI-MS/MS, potential sources of blank contamination must be carefully identified and minimized, as plastic materials used for sample preparation may contain the analytes.

The suppression of ESI – and thus of the analyte signal – depends on analyte concentration and the presence of coeluting matrix compounds; this requires careful optimization to ensure reliable quantification by standard addition or mass spectrometric isotope dilution. Since the ^{13}C-PFOA isotopomer can exhibit ionization yields different from the nonlabeled analyte [27], the standard addition method remains an important tool for evaluation of matrix effects.

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7 Publication II

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Perfluorooctane surfactants in waste waters, the major source of river pollution

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Abstract

Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are persistent and widely distributed in the environment. Recently, the discharge of municipal waste water has been shown to be an important route of such perfluoroalkyl surfactants into the aquatic environment.

The aim of this study was to assess the mass flow of PFOA and PFOS from typical waste water treatment plants (WWTPs) into surface waters. Samples were collected at different stages of treatment of four WWTPs in Northern Bavaria, Germany, and from the rivers receiving the treated waste waters (WW).

The outflow of PFOA from the WWTPs to the rivers was 20-fold higher than the inflow to the plants; about a tenth was removed with the sludge. For PFOS, the increase from inlet to outlet was about 3-fold; almost half of it was retained in the sludge. Both surfactants were released into river water from the WWTP of a medium-sized city with domestic, industrial and commercial waste waters; in domestic waste waters the surfactants were found at much lower levels.

Keywords: Surface waters; Waste waters; Sewage sludge; Mass flow; Perfluorinated surfactants

1. Introduction

The perfluoroalkyl surfactants (PFS) perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are persistent environmental contaminants; they are globally distributed and are found in humans and in wildlife, even at remote locations (Houde et al., 2006). PFOA is also a degradation product of fluorotelomer alcohols (Lange, 2002; Dinglasan et al., 2004), PFOS of perfluorooctane sulfonamides (Lange, 2001); precursors such as 2-(*N*-ethyl perfluorooctane sulfonamido) ethanol or 2-(*N*-ethyl perfluorooctane sulfonamido) acetic acid are transformed into PFOS (Lange, 2000; Boulanger et al., 2005).

Their unique molecular properties, chemical and thermal stability, their water- and fat-repellent properties have made them and their derivatives particularly suitable for treatment of textiles and paper, for cosmetics and insecticide formulations, for fire fighting foams, and in the production of fluoropolymers (Kissa, 2001).

The discharge of municipal waste waters (WW) is one of the principal routes of introducing these chemicals into the aquatic environment, as reported for various water treatment plants (WWTPs) in the United States (3M, 2001; Boulanger et al., 2005; Schultz et al., 2006a, b; Sinclair and Kannan, 2006). Studies on their mass flow were reported by Sinclair and Kannan (2006) and Schultz et al. (2006b). In 2006 a severe pollution of some rivers in Germany due to inappropriate disposal of PFS-containing wastes was reported (Skutlarek et al., 2006) resulting in contamination of drinking water in the same region.

Usually, the origin of PFOA and PFOS in WW is from cleaning and care of surface-treated products (clothing and carpets), by use of cosmetics containing such PFS, from industrial activities and by leaching from plastic products

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(Boulanger et al., 2005). PFOA and PFOS are persistent during WW treatment (Lange, 2000) and they partition between aqueous and solid waste streams (Higgins et al., 2005; Sinclair and Kannan, 2006) as shown for some WWTPs in the USA. The aim of this study was to assess the range of mass flows of PFOA and PFOS from typical WWTPs in Germany.

2. Materials and methods

2.1. Chemicals and equipment

Perfluorooctanoic acid (95%, Lancaster Eastgate, UK), perfluorooctane sulfonate potassium salt (98%, Fluka, Buchs, Germany), acetic acid (100%, Merck, Darmstadt, Germany), aqueous ammonium hydroxide solution (25%, Merck, Darmstadt, Germany), ammonium acetate (99%, Fluka, Buchs, Germany), and methanol (picograde, Promochem, Wesel, Germany) were used as obtained. All equipment was pre-cleaned as described previously (Weremiuk et al., 2006); Teflon equipment was avoided.

2.2. Sampling sites

Grab samples were collected from the different stages of the treatment process of the WWTPs: Bayreuth, (A, river Roter Main), Kulmbach (B, river Weißer Main), Ramsenthal (C, river Trebgast), and Himmelkron (D, river Weißer Main), Northern Bavaria, Germany. These WWTPs have average daily flows of 40 000, 20 000, 2200 and 2300 $m^3\ d^{-1}$. Plant A serves 72 000 inhabitants including breweries, food, plastics and tobacco industries, B 45 000 inhabitants with breweries, food and cosmetics industries; in both cases, approximately two thirds of WW come from commercial and industrial sources. Plant C receives domestic WW from 14 000 inhabitants and, to a small extent, from industrial sources (tire recycling, iron works); plant D receives exclusively domestic waste water from 11 000 inhabitants.

In all four plants, WW is treated similarly, starting with a mechanical step in which large objects (paper, bottles, and branches) are removed by a grate. Passing an aerated grit chamber, sand and gravel are removed. Next, the water enters the aerated primary treatment tank, followed by a slurry tank to allow sedimentation of suspended solids as primary sludge (PS); about half of the latter is pumped to digestion towers. In the following biological stage, phosphate elimination, nitrification and denitrification takes place and aero–anaerobic microorganisms form aggregates. These settle in the final clarification basin; the clear overflow is discharged into the river. The microorganism-rich underflow from the clarification tanks is returned to the active-sludge treatment tank as recirculated activated sludge (RAS); a tenth (waste-activated sludge, WAS) is transferred to a sludge holding tank, mixed with primary sludge, fermented for 4 weeks, dewatered, the water is returned to the active-sludge treatment tank, and the sewage sludge (SS) is incinerated.

Surface water samples were collected from the rivers receiving the WWTP-effluents. The average river flows are 270 000 (Roter Main), 90 000 (Weißer Main), and 50 000 (Trebgast) $m^3\ d^{-1}$.

2.3. Sample collection

Samples were collected on 06 April and 06 July 2005 from plant A and the Roter Main (Table 1), and on 13 July (plant B), 20 July (plant C), 27 July 2005 (plant D) from the WWTPs and the rivers. Average temperatures during these days were between 12 and 20 °C. There was no rainfall 3 days before or during sampling. Effluent samples were collected twice, i.e. on the same day (effluent 1) as the inlet water and 48 h later (effluent 2), the duration of the treatment process. At plant A, deactivated and dewatered sewage sludge samples were collected. A third sampling was performed on 15–17 March 2006 from plant A and the river (Table 1); ambient temperatures were between −5.0 and −2.0 °C, without precipitation during sampling or the 3 days prior to it.

WW samples were stored in 2.0 l polypropylene (PP) bottles at 4.0 °C in the dark. They were allowed to reach room temperature, particulate matter was removed by centrifugation (High-Performance Centrifuge, Avanti J-25, Beckman, USA) at 12 000 rpm for 10 min in 250 ml screw-cap PP-bottles. Removed particulate matter was freeze dried in pre-cleaned (hexane and methanol) aluminium boxes (189 × 86 mm, 540 ml), ground with a pestle, transferred to 50 ml PP-tubes and stored at room temperature until analysis.

Sewage sludge was collected in PP-bags, freeze-dried in pre-cleaned aluminium boxes, ground with mortar and pestle, transferred to PP-bottles and stored at room temperature until analysis.

2.4. Sample preparation

Waste and river water samples were extracted as described before (Weremiuk et al., 2006). First, they were filtered using folded paper filters ($597^1/_2$, Schleicher & Schuell, Dassel, Germany). Aliquots (250 ml) were passed through C18 cartridges (200 mg, 6.0 ml, Oasis HLB Waters Corp., Milford, USA) preconditioned with methanol and deionised water. The waste-water-loaded cartridges were washed with deionised water/methanol (60:40, vol%), dried under vacuum (0.4 bar), and the analytes were eluted with 4.0 ml methanol at a flow rate of one drop s^{-1} and collected in PP-tubes. The extracts were dried under a gentle nitrogen stream, the residues were dissolved in 500 µl each of a mixture of aqueous ammonium acetate, 10 mM/acetonitrile (50:50, vol%), the solutions were filtered (membrane filters, 0.45 µm, Roth, Karlsruhe, Germany) and transferred to PP snap ring vials (0.75 ml, Supelco, Bellefonte,

Table 1
Sampling scheme and river and waste water flows

	Plant					
	A			B	C	D
	I 06 April 2005	II 06 July 2005	III 15 March 2006	II 13 July 2005	II 20 July 2005	II 27 July 2005
River flow upstream of WWTP $m^3 s^{-1}$	264380	86400	276480	129600	51840	129600
Effluent from WWTP $m^3 s^{-1}$	31104	25920	34560	19900[§]	2200[§]	2300[§]
Waste water samples						
Inflow	✓	✓	✓	✓	✓	✓
Grit chamber	✓					
Primary treatment tank	✓	✓	✓	✓		
AS treatment tank	✓	✓		✓	✓	✓
Effluent 1	✓	✓	✓	✓	✓	✓
Effluent 2[*]	✓ (8)	✓ (08)	✓ (17)	✓ (15)	✓ (22)	✓ (29)
River water samples						
0.1 km upstream of WWTP	✓	✓	✓	✓	✓	✓
1 km downstream of WWTP	✓	✓	✓	✓	✓	✓
Sludge samples						
Sewage sludge[*]		✓ (08)	✓ (17)			
Waste activated sludge[*]			✓ (17)			
Primary sludge			✓			

AS – activated sludge, [*]collected 48 h after first sampling, date given in brackets, [§]average flow during dry weather.

USA) with polyethylene caps (NeoLab, Heidelberg, Germany) for analysis.

Freeze-dried sludge and particulate matter were extracted as previously described (Higgins et al., 2005). Briefly, 100 mg ground material was sonicated in 7.5 ml 1.0% (vol) acetic acid, centrifuged, and the supernatant was collected. The pellet was re-suspended in 1.7 ml methanol and dilute (1.0%) acetic acid (90:10, vol%), sonicated, centrifuged again, and the supernatant combined with the first. This was repeated two more times to yield 27.5 ml extract. A final wash with acetic acid (1.0%, 7.5 ml) yielded a total volume of 35 ml. This was passed through a C18 cartridge (200 mg, 6.0 ml, Oasis HLB Waters Corp., Milford, USA) preconditioned with methanol and 1.0% (vol) acetic acid. The analyte-loaded cartridge was washed with deionised water and dried under vacuum for 1 h. The analytes were eluted with 4.0 ml methanol at a flow rate of one drop s^{-1}, the solvent was evaporated, and the dry residue was redissolved in 2.0 ml aqueous ammonium hydroxide (0.01%) and acetonitrile (50:50, vol%). The solution was filtered (membrane filter, 0.45 μm, Roth, Karlsruhe, Germany) and transferred to PP snap ring vials (0.75 ml) with polyethylene caps.

Grit was extracted in the same way with a few modifications: 1.0 g sieved (mesh 2.0 mm), freeze-dried grit was extracted in two cycles consisting of a wash with 10 ml 1.0% acetic acid, followed by 2.5 ml methanol/acetic acid (90:10, vol%), and a final acetic acid (1.0%) wash; all these were combined and the analytes were extracted by SPE.

2.5. Instrumental analysis

The SPE-extracts were analysed by LC–ESI-MS/MS (Weremiuk et al., 2006). Aliquots of 10 μl were injected onto a 150 × 2.0 mm (5 μm) Prontosil C18 column (Bischoff, Leonberg, Germany) and the analytes were eluted with a mobile phase consisting of 40 vol% aqueous ammonium acetate, 10 mM, pH 3.1, and 60 vol% acetonitrile. Column temperature was 40 °C, total run time 6.5 min. The column was interfaced with an electrospray ionisation source to a tandem mass spectrometer (API 300, Applied Biosystems, Foster City, USA) operated in the negative ionisation and multiple reaction mode (MRM).

2.6. Quantification

Quantification of the analytes in river and water samples was based on individual standard addition calibrations (Weremiuk et al., 2006). For determination of recovery from freeze-dried sludge, the samples were spiked directly prior to extraction at two different levels, i.e. 50 μg kg^{-1} ($n = 4$) and 100 μg kg^{-1} ($n = 3$) of each PFOA and PFOS, taking their presence in non-spiked samples ($n = 5$) into consideration. Recoveries from waste water ($n = 6$) and surface water ($n = 6$) samples were 86% (±8% rsd) for PFOA and 74% (±6% rsd) for PFOS, from sludge ($n = 3$) 95% (±8% rsd) for PFOA and 79% (±7% rsd) for PFOS.

Procedural blanks for river and waste water were 0.03 ng l^{-1} and 0.06 ng l^{-1} PFOA at SPE-enrichment factors of 1000 and 500, respectively. The limits of quantification (LOQ, signal to noise ratio 7) were 0.06 and 0.12 ng l^{-1} PFOA, and 0.12 and 0.24 ng l^{-1} PFOS for river and WW, respectively. LOQs for sludge (100 mg sample) and grit samples (1.0 g sample) were 0.24 and 0.024 μg kg^{-1} PFOA, and 0.6 and 0.06 μg kg^{-1} PFOS; procedural blanks were below the limit of detection.

The expanded relative uncertainty U ($k = 2$) was <20% for all types of samples (water, particulate matter, sludge) for PFOA and PFOS. The greatest contribution of uncertainty arose from the variability of recovery and from the chromatographic quantification (regression line preparation, peak integration). The analytical standard deviation, between 1.0% and 13%, was lower than uncertainty. Standard deviations are not shown in the diagrams and tables as they were lower than the fluctuations of PFS concentrations in real samples.

The amounts of PFOA and PFOS released daily from the WWTPs are calculated based on their concentrations in the effluents 1 and 2 during each sampling and on daily flows (plant A – at sampling day; plants B, C, D – average during dry weather) according to the following Eq. (1):

$$ml_a = (c_a \times F) \times 10^{-6} \quad (1)$$

where ml_a is the mass loading of the analyte [g d^{-1}], c_a is the concentration of the analyte [ng l^{-1}], and F is the mechanical daily flow [m^3 d^{-1}].

The mass flow of PFOA and PFOS with solids in WWTPs are calculated based on their concentrations in the dry solids according to the following Eq. (2):

$$ml_b = (c_b \times F_s \times D_s) \times 10^{-6} \quad (2)$$

where ml_b is the mass loading of the analyte [g d^{-1}] in solids, c_b is the concentration of the analyte in a dry weight of solids [µg kg^{-1} dw], F_s is the mechanical daily flow of solids [m^3 d^{-1}], and D_s dry solids content in the sample [kg m^{-3}].

3. Results and discussion

3.1. PFOA/S in WW and solids from WWTPs

PFOA was found in all WW samples (Table 2), highest in the effluent of plant A, second highest in plant B. It was also found in all solid samples from plant A (Table 3), with lowest concentrations in grit and highest in WAS and SS.

PFOS was found at highest concentrations in the effluent of plant A; in the influents of plants B and D it was below detection limit (Table 2) but was found in activated sludge and in the effluent. In plant A (April 2005) and C, a decrease from inlet to outlet was observed, due to adsorption and removal with the sludge. The lowest effluent concentration was found in plant D which receives WW only from households.

PFOS in solids of plant A were high (Table 3) due to its adsorptivity. The lowest levels were found in grit, the highest in WAS and SS. Sewage sludge concentrations were mostly lower than reported for other regions (Environmental Ministry of Baden-Wuerttemberg, 2007).

In general, PFOA concentrations found in WW from WWTPs in Upper Franconia are comparable to those reported for WWTPs in the USA (Boulanger et al., 2005; Higgins et al., 2005; Schultz et al., 2006a, b), only PFOS tends to be higher (Sinclair and Kannan, 2006). The values are much lower than reported for surface and drinking water resulting from the mentioned PFT spill (Skutlarek et al., 2006).

3.2. Mass flow

Daily mass flows on 15–17 March 2006 were calculated for plant A (Fig. 1). PFOA flow in WW did not differ between the grit chamber and the primary treatment tank but increased 10-fold during activated-sludge treatment and again 2.5-fold up to the effluent; it also increased in the particulate phase during primary treatment (2-fold) and activated sludge treatment (7-fold). The overall mass flow of PFOA increased about 20-fold during the WW treatment process; this general trend was also observed

Table 2
PFOA and PFOS concentrations [ng l^{-1}] in waste water at different stages of treatment

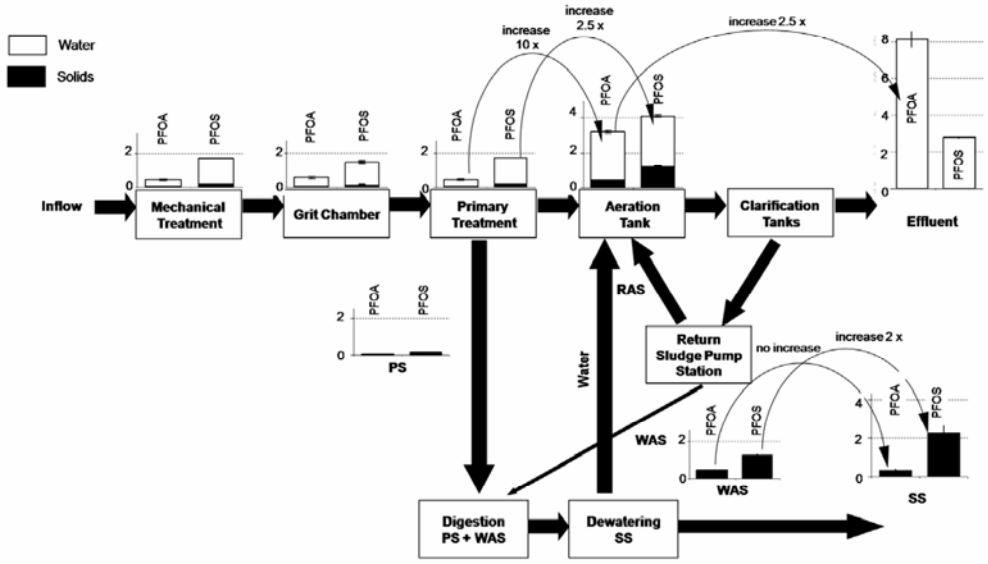
	Plant A			Plant B	Plant C	Plant D
	06 April 2005	06 July 2005	15 March 2006	13 July 2005	20 July 2005	27 July 2005
PFOA						
Inflow	20	40	12	7	4.1	1.8
Grit chamber	n.d	n.d.	15	n.d.	n.d.	n.d.
Primary treatment	18	25	20	10	n.d.	n.d.
AS treatment	n.d	35	78	43	3.8	2.2
Effluent 1	93	50	44	24	8.7	11
Effluent 2	74	48	250	18	5.7	11
PFOS						
Inflow	85	25	44	<1	33	<1
Grit chamber	n.d	n.d.	38	n.d.	n.d.	n.d.
Primary treatment	130	14	35	<1	n.d.	n.d.
AS treatment	n.d	160	80	7.5	7	3.8
Effluent 1	45	140	31	30	12	12
Effluent 2	50	195	86	9.4	7.5	2.4

AS – activated sludge, Effluent 1 – collected together with the inflow, Effluent 2 – collected 48 h after the inflow, n.d. – not determined.

Table 3
PFOA and PFOS [μg kg^{-1} dry weight] in solids from WWTP A

Sampling	PFOA		PFOS	
	15 March 2006	17 March 2006	15 March 2006	17 March 2006
Grit	0.5	n.d	1	n.d
PM – Inflow	4	n.d	25	n.d
PM – Grit chamber	4	n.d	20	n.d
PM – Primary treatment	8	n.d	15	n.d
Primary sludge	7	5	18	24
Waste activated sludge	n.d	23	n.d	62
Sewage sludge	18	11	120	80

PM – particulate matter separated from liquid wastes, n.d. – not determined.



WAS – waste activated sludge, RAS – recirculated activated sludge, PS – primary sludge, SS – sewage sludge

Fig. 1. Mass flows of PFOA and PFOS in plant A (Bayreuth), 15–17 March 2006.

Table 4
PFOA and PFOS concentrations [ng l^{-1}] determined in the effluent of 4 WWTPs and the receiving rivers, calculated concentrations under assumption of complete mixing

	Plant A			Plant B	Plant C	Plant D
	06 April 2005	06 July 2005	15 March 2006	13 July 2005	20 July 2005	27 July 2005
	Roter Main			Weißer Main	Trebgast	Weißer Main
PFOA						
River – 0.1 km upstream of WWTP	0.9	2.8	2.0	1.1	1.3	0.8
Effluent 1	93	50	44	24	8.7	11
River – 1 km downstream, determined	10	14	5	1.7	1.0	1.0
Calculated	10	13	7	3.7	1.1	1.2
PFOS						
River – 0.1 km upstream of WWTP	0.8	6	2.2	3.5	3.3	<0.03
Effluent 1	45	140	31	30	12	12
River – 1 km downstream, determined	6.2	15	5.1	3.3	1.7	0.7
Calculated	6.0	37	5.5	6.2	4.1	1.0

Effluent 1 – Collected on the same day as the river water samples.

in the three other plants (Table 2). The total amounts of PFOA released from the City of Bayreuth to the River Roter Main on 17 March 2006 was calculated as 8.0 g d^{-1} (effluent 2), obviously an exceptionally high amount. The amounts recorded on 06 April 2005 (2.9 g d^{-1}), 08 April 2005 (4.2 g d^{-1}), 06 July 2005 (1.3 g d^{-1}), or 15 March 2006 (1.6 g d^{-1}) seem to represent more typical levels, on average about 2.2 ± 1.3 g d^{-1}. A small amount of PFOA was removed (17 March 2006) with deactivated and dewatered sludge, i.e. 0.3 g d^{-1} (~4.0%).

The amounts of PFOA released to the rivers from the other three WWTPs together with treated WW were considerably lower than those released form plant A: 0.5 g d^{-1} (plant B), 0.05 g d^{-1} (plant C), and 0.03 g d^{-1} (plant D).

The mass flow of PFOS in the liquid phase increased about 3-fold, in the particulate phase about 9-fold. In waste-activated sludge it was 8-fold higher than in primary sludge, and a further 2-fold increase was observed following fermentation. Overall, the total mass flow of PFOS increased about 3-fold during the treatment process. About 3 g d^{-1} of PFOS (55% of the total) was released to the river, about 2.3 g d^{-1} (45%) was removed via the dewatered, deactivated sewage sludge.

The average release of PFOS from plant A was calculated to be 2.8 ± 1.5 g d^{-1}, higher than from the other three WWTPs (B: 0.4 g d^{-1}, C: 0.07 g d^{-1}, D: 0.02 g d^{-1}) or from US-WWTPs (~1 g d^{-1}) (Sinclair and Kannan, 2006; Schultz et al., 2006b).

An average mass loading of about 30 μg person^{-1} d^{-1} of PFOA (110 μg person^{-1} d^{-1} on 17 March 2006) and 40 μg person d^{-1} of PFOS can thus be deduced for Bayreuth, perhaps due to the high fraction of industrial and commercial WW; from household WW (plants C and D) the release of both PFOA and PFOS together is only 1–3 μg person d^{-1}.

3.3. River water

PFOA was detected in all river water samples (Table 4), values being relatively uniform upstream of the WWTPs. The presence of PFSs in this rural area might result from their former use in plant protection formulations, and from atmospheric deposition. Concentrations determined 1 km downstream were as expected when assuming full mixing of WW and river water; they were highest downstream of plant A at low ratios of river flow versus WW discharge ($r = 8.4$ on 08 April 2005, 4.2 on 06 July 2005, 8.7 on 15 March 2006). Low concentrations in the discharged water in combination with a high river/discharge ratio (e.g. $r = 57$, plant D) entailed correspondingly low levels.

This was also observed for PFOS although the values one km downstream were slightly lower than calculated, likely due to adsorption to river bed sediments and removal from the flowing river water.

4. Conclusions

Apparently, the total release of both surfactants is highest from the WWTP with industrial WW (plant A), the second highest from the smaller WWTP (plant B), also receiving commercial and industrial WW. The lowest mass loading to the river was registered for the small plant receiving only domestic WW (plant D). While PFOA increases almost 10-fold due to formation from precursors, passing the WWTP practically undiminished and going fully into the rivers, half of PFOS is retained in sludge and, prevented from entering the aquatic environment.

PFOS concentrations found in sewage sludge in plant A (80, 120 μg kg^{-1}) are below the recently suggested maximum allowed PFOS level for sewage sludge (200 μg kg^{-1}). Nevertheless, this study shows that PFOA and PFOS are constantly released to rivers with municipal WW, its extent depending upon the fraction of industrial contribution.

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8 Publication III

Perfluorooctanoic Acid and Perfluorooctane Sulfonate Released from a Waste Water Treatment Plant in Bavaria, Germany

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Abstract

Background, Aim and Scope:
Perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), and their precursors and derivatives have been employed as surfactants and anti-adhesives. As PFOA and PFOS are environmentally persistent, they have been detected, sometimes at high levels (e.g. 4.4 µg/L River Möhne, Germany), in surface waters and in the aquatic food chain.

The discharge of municipal waste waters is one of the principal routes of PFOA and PFOS to the aquatic environment. In a previous study, their concentration in grab samples collected from the waste water treatment plant (WWTP) of Bayreuth, a city of 72 000 inhabitants in Bavaria, Germany, during two periods showed significant differences. In order to estimate representative released amounts, the surfactants were monitored every second week over a period of three months. In a second campaign, river water receiving the WWTP-effluent was sampled twice a day for 5 consecutive days.

Materials and Methods:
Quantitative analysis was done by stable-isotope dilution, pre-cleaning and pre-concentration by solid phase extraction, and liquid chromatography followed by electrospray ionisation/tandem mass spectrometry.

Results:
The mass flows of PFOA and PFOS through the WWTP were determined. PFOA is fully discharged into the river, while about half of PFOS is retained in the sewage sludge. The average daily mass load of the river Roter Main by the WWTP of Bayreuth is about 1.2 ± 0.5 g PFOA and 5 ± 2 g PFOS, with variations of up to 140 % within one day.

Discussion:
In general, fluctuations in mass flow for both PFOA and PFOS followed a similar pattern suggesting their release from sources where both substances are used in fixed ratio.

Conclusion:
Overall, the total annual release to the rivers of Germany may be in the range of several hundred kilograms of PFOA and several tons of PFOS.

Recommendations and Perspectives:
The release from WWTPs is likely to contribute to high perfluorinated surfactant (PFS) levels in fish; therefore, further ecotoxicological investigation with aquatic organisms is recommended. As perspective, specific techniques for removal of PFSs from the waste water at the point of origin need to be developed and implemented.

Keywords: HPLC-ESI-MS/MS; perfluorooctanoic acid; perfluorooctane sulfonate; river water; waste water; perfluoroalkyl surfactants.

Introduction

Perfluoroalkyl surfactants (PFSs) have high thermal and chemical stability and unique physical and chemical properties. They are employed for a wide range of applications to serve as liquid repellents for paper, leather, textiles, and carpets, as industrial surfactants, additives and coatings, as constituents of fire fighting foams, and as anti-adhesives in the processing of polymers (Kissa 2001). Thus, the application and use of PFS-containing products during manufacturing processes constitute an important source of PFSs in the aquatic environment (Dinglasan et al. 2004).

Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are persistent (Prevendouros et al. 2006) degradation products of industrially used PFTs (Lange 2001), PFOA also of fluorootelomer alcohols (Dinglasan et al. 2004). PFOA and PFOS have been detected frequently in river and lake water (0.1 - 10 ng/L of PFOA) (Prevendouros et al. 2006), sediments (e.g, 0.4 µg/kg PFOA, and 3.8 µg/kg PFOS) (Higgins et al. 2005), sludge (5.6 µg/kg PFOA, and up to 2600 µg/kg PFOS) (Higgins et al. 2005), Oysters (Matagorda Bay, USA, PFOS up to 1.2 mg/kg wet weight) (Kannan et al. 2002), fish (0.5 µg/kg wet weight of PFOS in the liver of eel, river Main near Bamberg, Bavaria, Germany) (Corinna 2006), reptiles (PFOS

0.3 mg/kg wet weight in liver of green frogs, Michigan, USA) (Houde et al. 2006), and mammals (PFOS up to 180 mg/kg wet weight in liver of mice inhabiting a fluorochemical plant, Antwerp, Belgium) (Houde et al. 2006).

The discharge of municipal waste water is the one of the principal routes of PFOA and PFOS to enter the aquatic environment (Boulanger et al. 2005, Schultz et al. 2006 a, b, Sinclair and Kannan 2006, Loganathan et al. 2007) but strong fluctuations in mass flow were observed at the middle-sized waste water treatment plant (WWTP) of Bayreuth (Upper Franconia, Bavaria, Germany) (Becker et al. 2008). In order to better estimate the typical amounts daily released, PFOA and PFOS were monitored in waste and river water every second week from 14 March to 15 June 2007, and twice a day from 11 to 15 June 2007.

1 Materials and Methods

1.1 Chemicals and Equipment

Perfluorooctanoic acid (95 %, Lancaster Eastgate, UK), [1, 2- $^{13}C_2$]-perfluorooctanoic acid (98 %, Perkin Elmer, Boston, USA), perfluorooctane sulfonate potassium salt (98 %, Fluka, Buchs, Germany), [1, 2, 3, 4-$^{13}C_4$]-perfluorooctane sulfonate sodium salt (99 %, 50 µg/mL-solution in MeOH, Campro Scientific, Berlin, Germany), acetic acid (100 %, Merck, Darmstadt, Germany), ammonium acetate (99.0 %, Fluka, Buchs, Germany), MeOH, and AcN (picograde, Promochem, Wesel, Germany) were used as obtained. The equipment was precleaned as described previously (Weremiuk et al. 2006); Teflon equipment was avoided.

1.2 Sample Collection

Grab water samples were collected in spring 2007 from the municipal WWTP of Bayreuth (Upper Franconia, Bavaria, Germany) serving a population of 72 000 inhabitants and discharging about 1670 m^3/h into the river Roter Main, the latter having an average hourly flow 11 250 m^3. The inflowing waste water first passes a mechanical stage for removal of big objects (bottles or branches), grit and sand, a primary sedimentation basin (~ 2 hour), a biological treatment basin (~ 30 h), and another basin for clarification (~ 16 h) (Becker et al. 2008). The treated waste water is discharged into the river approximately 48 hours after inflow.

From 14 March to 18 May 2007, grab water samples were collected every other week on Wednesday (10:00 h) from the WWTP (4 x 250 mL) and the river (4 x 500 mL) with precleaned 500-mL PP-bottles. On each Friday at 10:00 h, i.e. forty eight hours after the first

sampling (duration of the waste water treatment process), effluent of the WWTP and river water were collected, 0.1 km upstream and 1 km downstream of the WWTP. Waste water temperatures ranged from 13 °C (14 March 2007) to 16.5 °C (4 May 2007). Rain fell during the nights before 20 April, 12 and 13 June.

From 11 to 15 June 2007, river water samples (4 x 500 mL) were collected twice a day at 8:00 and 14:00 h with 500-mL PP-bottles 1 km downstream of the WWTP.

Particulate matter was removed by centrifugation before storage (Loganathan et al. 2007) of the samples at 4°C in the dark, no longer than a week.

1.3 Sample Preparation and Analysis

Solid phase extraction (SPE) was done as described (Becker et al. 2008), modified as follows: to waste water 250 µL of a 100-µg/L mixture of ^{13}C-PFOA and ^{13}C-PFOS each, to river water 100 µL of a 10-µg/L mixture of ^{13}C-PFOA and PFOS each was added. The SPE extracts were dried under nitrogen and the residues were dissolved in 2500 µL (waste water) or 500 µL (river water) of a mixture of aqueous ammonium acetate (10 mmol/L) and AcN (50:50, vol-%). For analysis, the extracts were diluted (river waters 1+1, waste waters 1+9) with the same ammonium acetate/AcN mixture to yield a concentration of 1 µg/L of ^{13}C-labelled standards. The diluted extracts were transferred to PP-snap ring vials, closed with polyethylene caps, and analysed by LC-ESI-MS/MS (Weremiuk et al. 2006). When analytes were below 1 µg/L, non-diluted extracts were used.

1.4 Quantification

For calibration, a stock solution of 98 mg/L ^{13}C-PFOA was prepared by dissolving 10 mg of ^{13}C-PFOA (98 %) in 100 mL AcN, a ^{13}C-PFOS (free acid) stock solution of 1.9 mg/L was prepared by diluting 1 mL of a 50-mg/L-solution ^{13}C-PFOS sodium salt in a 25-mL PP-volumetric flask (Supelco, Bellefonte, USA). Medium- (100 µg/L of each ^{13}C-PFOA and ^{13}C-PFOS) and low-concentrated (10 µg/L of each ^{13}C-PFOA and ^{13}C-PFOS) standard mixtures were prepared from the stock solutions by appropriate dilutions with the ammonium acetate/AcN mixture.

Standard solutions containing non-labelled PFOA and PFOS in a range from 1 to 8 µg/L and 1 µg/L of each ^{13}C-labelled analyte were used for daily calibrations. Calibration curves were constructed by plotting the peak area ratios of analyte and internal standard versus analyte

concentrations. The regression coefficients were higher than 0.995. The limits of quantification (LOQ, signal to noise ratio 7) for river and waste water were 0.06 and 0.12 ng/L PFOA and 0.12 and 0.24 ng/L PFOS, respectively. Procedural blanks using deionised water were 0.015 ng PFOA; PFOS was below the limit of detection (signal to noise ratio 3).

The expanded relative uncertainty U (k = 2) was <20 % for PFOA and PFOS. The analytical standard deviation, between 1 % and 15 %, was lower than uncertainty. Standard deviations are not shown in the diagrams and the tables as they were much smaller than the variation of concentrations in real samples.

The mass flows of PFOA and PFOS in the WWTP were calculated by:

(1) $mfw_a = (c_a \times F_w) \times 10^{-3}$

where mfw_a is the mass flow of analyte in the waste waters [mg/h], c_a the concentration of analyte [ng/L], and F_w the mechanical hourly flow [m^3/h].

The mass flows in river water downstream the WWTP were calculated similarly by:

(2) $mfr_a = (c_a \times F_R) \times 10^{-3}$

where mfr_a is the mass flow of analyte [mg/h], c_a the concentration of the analyte [ng/L], and F_R the river water flow [m^3/h].

2 Results

2.1 Waste Water

PFOA was found in all treated waste water samples (Tab. 1) in concentrations ranging from 20 to 73 ng/L, with an average of 44 ± 19 ng/L, similar to those reported for 2005 and 2006 (60 ± 20 ng/L, Becker et al. 2008). In the WWTP-effluent the mass flows of PFOA were 1.3- to 4.5-fold higher than in the influent (Fig. 1a), ranging from 25 mg/h (16 May 2007) up to 84 mg/h (16 March 2007). Correlation to water flow through the WWTP or to water temperature was not observed.

Concentrations of PFOS (Fig. 1b) were about 10-fold higher than PFOA. Highest levels (300 – 390 ng/L) were found in the primary treatment stage in March and April 2007, lowest (60, 86 ng/L, respectively) in the influent and in the primary treatment stage in May 2007. The

mass flow of PFOS through the WWTP was higher in March and April 2007 (Figure 1b), ranging from 60 (inflow, 2 May 2007) to 640 mg/h (primary treatment, 18 April 2007). The mass flow in the water phase decreased during the treatment process except for the samples of 2 and 4 May 2007 when effluent temperatures were relatively high (17 °C).

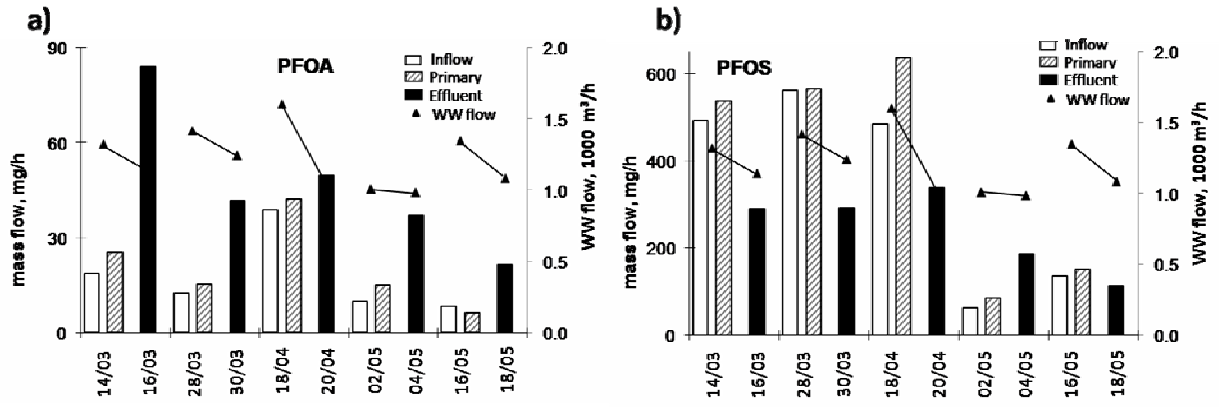

Figure 1. Mass flow of PFOA (a) and PFOS (b) in WWTP of Bayreuth in mg/h, 14 March – 18 May 2007. WW flow registered at the time of sampling.

2.2 River Water

PFOA in river water (Tab. 1) upstream the WWTP was below detection limit (16 and 30 March 2007) or between 1 and 2 ng/L (April and May 2007). The concentrations 1 km downstream the WWTP were as expected for complete mixing of river and waste water or slightly lower. Between 11 and 15 June 2007 they were higher (11 ± 4 ng/L) than in the preceding

Table 1. PFOA and PFOS concentrations [ng/L] determined in the effluent of the WWTP and the receiving river (Roter Main), and calculated values under assumption of complete mixing.

	16 March	**30 March**	**20 April**	**04 May**	**18 May**
River flow, m^3/h	12 300	12 420	7 850	5 470	7 560
PFOA (ng/L)					
River – 0.1 km upstream	< 0.06	< 0.06	2.0	1.0	1.2
Effluent	73.00	39	49	38	20
River – 1 km downstream	8	4	6	6	3.1
Calculated	7	4	8	7.6	3.9
PFOS (ng/L)					
River – 0.1 km upstream	< 0.12	1.0	1.0	1.0	1.5
Effluent	252	241	336	192	106
River – 1 km downstream	31	18	32	27	9.9
Calculated	24	25	44	34	16.4

months (5.4 ± 1.9 ng/L), corresponding to the lower river flow during summer time. The total mass flow in the river was not significantly different for the two periods: 50 ± 30 mg/h from March to May, and 70 ± 20 mg/h in June (Fig. 2). The highest load was registered on Friday morning, 15 June 2007 (96 mg/h), the lowest on Monday morning, 11 June (34 mg/h). On 11, 13, and 14 June, the mass flow increased from morning to early afternoon by up to 80 %.

PFOS concentrations in the river water upstream the WWTP (Tab. 1) were about 1 ng/L, on 16 and 30 March 2007 below quantification limit. Concentrations determined 1 km downstream were strongly raised by the WWTP-release, slightly lower than calculated for full mixing of waste and river water; only in one case it was higher (16 March 2007). Concurrent with those of PFOA, the highest mass load of PFOS (Fig. 2) was registered on Friday morning 15 June 2007 (96 mg/h), the lowest on Monday morning, 11 June (34 mg/h). On 11, 13, and 14 June, the mass flow increased from morning to early afternoon by up to 80 %.

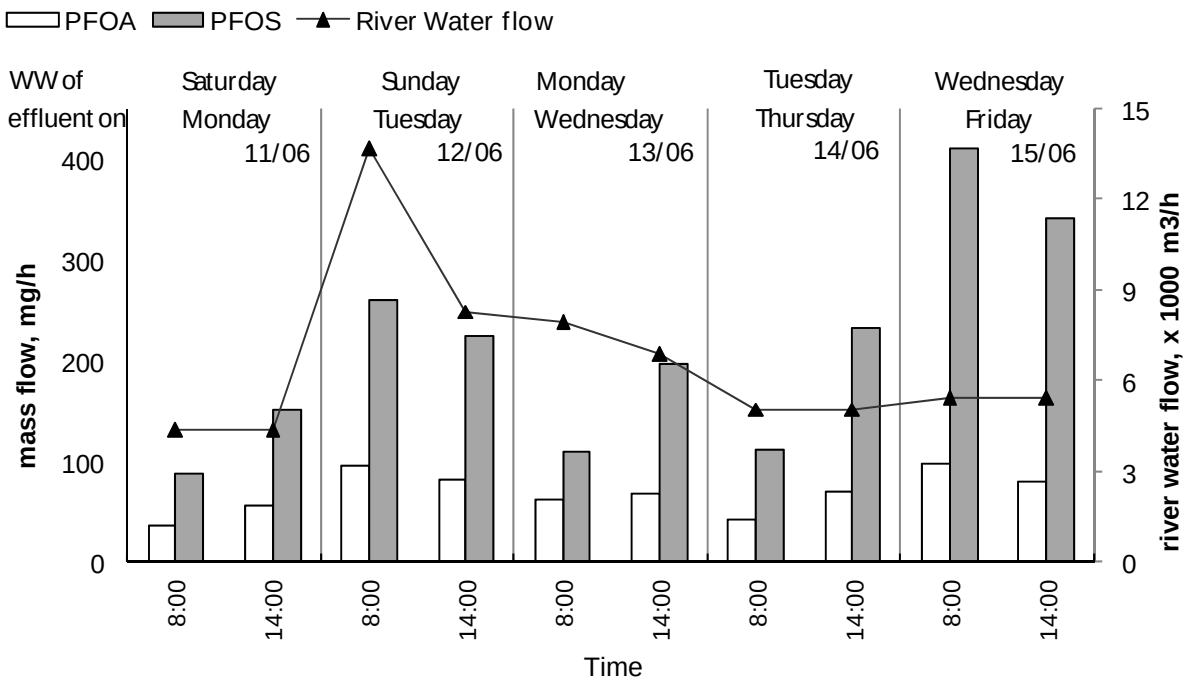

Figure 2. PFOA and PFOS mass flows [mg/h] in river water 1 km downstream the WWTP; samples were collected daily at 8:00 and 14:00 h.

3 Discussion

The mass flow of PFOA in waste water increased eightfold from inlet to outlet on 14 and 16 March 2007, and entailed an average release of 47 ± 23 mg/h PFOA (i.e 1.1 g/day) into the river. This is in good agreement with previous findings (1.4 g/day) (Becker et al. 2008), lower than reported for a plant in rural Kentucky in 2005 (1.8 to 2.7 g/day) serving a population of 15 000 inhabitants with a capacity of 20 000 m^3/day (Loganathan et al. 2007), and much lower than reported (~ 45 g/day) for a large US-plant (130 000 m^3/day) (Sinclair and Kannan 2006).

The mass flow of PFOS in the water phase of a WWTP usually decreases to about half due to adsorption on sewage sludge, influenced by numerous factors such as rate of formation from precursors, relative amounts of sludge, temperature, relative flows etc. (Schultz et al. 2006 a, b, Sinclair and Kannan 2006). The average release of PFOS into the river was calculated as 240 ± 90 mg/h (~ 6 g/day), higher than in summer 2005 (3.3 g/day) (Becker et al. 2008) or reported for US-WWTPs, i.e. 0.6 (Schultz et al. 2006 b, Loganathan et al. 2007) to 1 g/day (Sinclair and Kannan 2006).

In general, fluctuations in mass flow for PFOA and PFOS follow a similar pattern suggesting their release from sources where both substances are used in fixed ratio, most likely as an anti-adhesive additive in certain technical applications.

Overall, the concentrations of PFOA in the river Roter Main receiving treated waste water ranged from 3 – 18 ng/L, comparable to typical concentrations determined in the river Ruhr, Germany (<LOD – 7.5 ng/L) (Lange 2004) or in the river Guangzhou, China (0.85 – 13 ng/L) [17], but much lower than the concentrations in the rivers Yangtze (260 ng/L) (so et al. 2007), Tennessee, USA (140 – 600 ng/L) (Hansen et al. 2002), or Moehne, Germany (3640 ng/L) (Skutlarek et al. 2006). The presence of low concentrations of PFOA upstream the WWTP (<0.06 – 2 ng/L) might result from atmospheric deposition, run-off in the watershed, and from small WWTPs located on tributaries entering the river upstream of the sampling point.

The concentrations of PFOS (10 – 76 ng/L) are comparable to those found in the Ruhr (2.5 – 43 ng/L) (Lange 2004), the river Guangzhou in China (0.9 – 100 ng/L) (So et al. 2007), lower than the concentrations in the river Tama in Japan (157 ng/L) (Saito et al. 2003), the river

Yangtze, China (So et al. 2007), or the river Moehne, Germany (193 ng/L), but higher than in the US-rivers Hudson (1.5 – 3.4 ng/L), Niagara (3.3 – 6.7 ng/L) (Sinclair et al. 2006), St. Clair (1.9 – 3.9 ng/L), or Rising (3.5 ng/L) (Kannan et al. 2005).

4 Conclusions

The reported data together with those from a previous study (Becker et al. 2008) present evidence that the average daily mass load from the treated waste water of Bayreuth into the river Roter Main is about 1.2 ± 0.5 g PFOA and 5 ± 2 g PFOS; large fluctuations are obvious and may depend on industrial activity schemes. Additional releases from WWTPs further downstream are likely to contribute to high PFS-levels in fish (Corinna 2006). Overall, the total annual release to the rivers of Germany may be in the range of several hundred kilograms of PFOA and several tons of PFOS.

5 Recommendations and Perspectives

Once released to the aquatic environment PFOA and PFOS due to their persistence are likely to adsorb on sediments or bioaccumulate in biota inhabiting such water bodies. To assess the impact such mass loading of PFSs from WWTPs can have on aquatic ecosystems, further investigations including sediments, benthic organisms and fish is recommended. In order to obtain a more complete picture of the persistent PFSs released to the Roter Main with waste waters, determination of a wider range of compounds is needed.

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9 Publication IV

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Perfluorooctanoic acid and perfluorooctane sulfonate in the sediment of the Roter Main river, Bayreuth, Germany

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River sediments constitute a sink of perfluorinated surfactants released from the waste water treatment plant.

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ABSTRACT

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are widely distributed in aquatic ecosystems. Their sources are known but few studies about their accumulation potential in river sediments exist. The aim of this study is to assess the concentrations of PFOA and PFOS in sediments in relation to their levels in river water receiving effluent from a waste water treatment plant (WWTP). PFOS accumulates by a factor of about 40 relative to river water, PFOA only up to threefold. In contrast to previous suggestions, in this case the enrichment on sediment is not correlated to the total organic carbon contents.

1. Introduction

Over the last 50 years, perfluorochemicals (PFCs) such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been widely used both in industry and as components of consumer products (Kissa, 2001; Schultz et al., 2003). Due to their specific physical–chemical properties, especially their chemical and thermal stability and their low surface free energy, especially their water- and fat-repellent properties (Kissa, 2001), they have been used in numerous applications. At the same time, their persistence and potential to bioaccumulate led to their wide-spread presence in the global environment (Prevendouros et al., 2006; Houde et al., 2006). Several direct and indirect sources of PFCs-emission to the environment have been reported, such as manufacturing processes in the polymer and electronics industries (Prevendouros et al., 2006), in the degradation of volatile precursors (Ellis et al., 2004; Dinglasan et al., 2004; Lange, 2000, 2002), or in the release of treated waste waters (Becker et al., 2008, submitted for publication; Boulanger et al., 2005; Schultz et al., 2006a,b; Sinclair and Kannan, 2006).

Sediments have been suggested as one of two final sinks of PFCs, the other being the deep oceans (Prevendouros et al., 2006). Most of the earlier studies have focused on water and biological matrices; little information is available on PFC concentrations in soils and sediments. Relatively low concentrations of PFOA and PFOS were found in sediments collected from four rivers in the San Francisco Bay, USA (Higgins et al., 2005), from the Tidal Flat Areas of Ariake Sea (Nakata et al., 2006) or from the rivers Kamo, Uji, and Tenjin in Japan (Senthilkumar et al., 2007).

The aim of this study was to determine the accumulation potential of PFOA and PFOS in sediments relative to the water of the river Roter Main: a well quantified source of these compounds into the river is the municipal waste water treatment plant (WWTP) of Bayreuth (Becker et al., 2008, submitted for publication).

2. Materials and methods

2.1. Chemicals and equipment

PFOA (95%, Lancaster Eastgate, UK), [1, 2-$^{13}C_2$]-PFOA (98%, 10 mg, Perkin–Elmer, Boston, USA), perfluorooctane sulfonate potassium salt (98%, Fluka, Buchs, Germany), [1, 2, 3, 4-$^{13}C_4$]-perfluorooctane sulfonate sodium salt (99%, 50 µg/mL-solution in methanol, Campro Scientific, Berlin, Germany), acetic acid (100%, Merck, Darmstadt, Germany), ammonium acetate (99.0%, Fluka, Buchs, Germany), methanol, and acetonitrile (picograde, Promochem, Wesel, Germany) were used as obtained. The equipment was precleaned as described previously (Weremiuk et al., 2006); Teflon equipment was avoided.

2.2. Sampling location and collection

The investigation area was the river Roter Main flowing through Bayreuth, Upper Franconia, Germany, which has an average daily flow of 270 000 $m^3\,day^{-1}$ and receives treated waste waters of industrial, commercial and domestic origin

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Table 1
Concentrations of PFOA, PFOS [ng kg^{-1} dw] in river sediment samples, Roter Main near Bayreuth, 19 October 2006 (UpS – upstream the WWTP, DwS – downstream the WWTP, $LOQ_{PFOA} = 25$ ng kg^{-1} dw, $LOQ_{PFOS} = 50$ ng kg^{-1} dw)

Sample	Location							
	0.1 km UpS	0.05 km DwS	0.5 km DwS	1 km DwS	0.1 km UpS	0.05 km DwS	0.5 km DwS	1 km DwS
	PFOA				PFOS			
1	<25	<25	<25	<25	<50	264 (2)	56 (19)	176 (7)
2	<25	<25	91 (3)	33 (3)	66 (1)	218 (15)	226 (2)	237 (9)
3	<25	120 (16)	60 (2)	55 (2)	290 (12)	120 (10)	170 (10)	100 (5)
4	<25	–	64 (11)	<25	50 (10)	–	348 (7)	82 (15)
5	<25	<25	48 (4)	53 (13)	230 (8)	153 (14)	72 (4)	143 (29)
6	48 (6)	70 (4)	142 (10)	77 (10)	80 (9)	415 (3)	227 (13)	92 (6)
7	52 (4)	41 (20)	168 (6)	79 (12)	95 (14)	130 (3)	378 (5)	296 (9)
8	<25	135 (17)	141 (6)	105 (20)	116 (9)	381 (6)	235 (11)	170 (2)
9	49 (16)	<25	34 (24)	<25	<50	183 (13)	537 (10)	348 (10)
10	26 (8)	106 (14)	<25	72 (2)	91 (6)	331 (7)	179 (4)	264 (10)
11	46 (17)	175 (5)	80 (12)	50 (14)	76 (10)	506 (10)	301 (4)	307 (5)
Mean	27 ± 18	70 ± 60	85 ± 60	50 ± 30	105 ± 85	280 ± 120	250 ± 150	200 ± 90

Mean is calculated using a half of the LOQ.

from the municipal WWTP, having a daily flow of 40 000 m^3 and serving a population of 72 000 inhabitants. Based on our previous studies it was estimated that about 1.2 ± 0.5 g PFOA and 4.7 ± 2.3 g PFOS are daily released from the plant into the river (Becker et al., 2008, submitted for publication).

On 19 October 2006, sediment samples were collected at four different locations: (a) 1 km upstream, (b) 50 m downstream, (c) 500 m downstream, and (d) 1 km downstream the WWTP. At each location, 11 individual samples from the upper sediment (~15 cm) were collected with a polypropylene (PP) tube (Ø = 10 cm) and transferred into 250-mL PP-bottles. Water samples ($n = 3$) were also collected at each location with 2-L PP-bottles.

2.3. Sample preparation and analysis

Sediment samples were weighed into aluminium boxes precleaned with hexane and methanol, freeze-dried, sieved (0.63 mm mesh), transferred into clean 50-mL PP-bottles, and stored at room temperature until analysis. Their total organic carbon (TOC) content was determined and the samples were extracted as described previously (Becker et al., 2008).

Mixed sediment samples were also prepared for each location by weighing 1 g of each of the 11 individual samples of the respective location into a 50 mL PP-centrifuge tube. Each sample was thoroughly mixed and extracted in triplicate as described above.

Briefly, 1 g of sediment was weighed into a new, clean 50-mL PP-centrifuge tube and 50 µL of a standard solution containing 10 µg L^{-1} each of ^{13}C-PFOA and ^{13}C-PFOS were added. Each sample was sonicated in 10 mL 1% (vol.) aqueous acetic acid, centrifuged, and the supernatant was collected. The pellet was re-suspended in 2.5 mL of a mixture of methanol and 1% aqueous acetic acid (90:10, vol.-%), sonicated, centrifuged again, and the supernatant was combined with the first one. The procedure was repeated one more time to yield 25 mL extract. A final wash with acetic acid (1%, 10 mL) yielded a total volume of 35 mL. The analytes were extracted by solid phase extraction (SPE) using a C18 cartridge (200 mg, 6.0 mL, Oasis HLB Waters Corp., Milford, USA), eluted with methanol, the solvent was evaporated, and the residue was redissolved in 0.5 mL of a mixture (50:50, vol.) of aqueous ammonium acetate (10 mmol/L, pH = 4.1) and acetonitrile. The solution was filtered and transferred to PP-snap ring vials (0.75 mL) with polyethylene (PE) caps, and analysed by HPLC-ESI-MS/MS (Weremiuk et al., 2006).

Water samples were prepared and analysed as described previously (Weremiuk et al., 2006; Becker et al., submitted for publication).

2.4. Quantification

For calibration, stock solutions of ^{13}C-PFOA, ^{13}C-PFOS, PFOA, PFOS and two working standard solutions, one containing 10 µg L^{-1} of each ^{13}C-PFOA and ^{13}C-PFOS, the other 10 µg L^{-1} of each non-labelled PFOA and PFOS, were prepared as described previously (Becker et al., 2008, submitted for publication).

For quantitative analysis of sediment extracts, standard solutions containing non-labelled PFOA and PFOS in the range from 0.5 to 2.5 µg L^{-1} and 1 µg L^{-1} of each ^{13}C-labelled analyte were used for calibration. For analysis of water sample extracts, standard solutions containing non-labelled PFOA and PFOS in a range from 1 to 8 µg L^{-1} and 1 µg L^{-1} of each ^{13}C-PFOA and ^{13}C-PFOS were used. Calibration curves were constructed by plotting analyte and internal standard peak area ratios versus analyte concentrations; regression coefficients were higher than 0.995. The limits of quantification (LOQ, signal to noise ratio 7) for sediments were 25 ng kg^{-1} dry weight (dw) PFOA and 50 ng kg^{-1} dw PFOS, for river water 0.06 ng L^{-1} PFOA and 0.12 ng L^{-1} PFOS. Procedural blanks for sediment samples were below the limit of detection (LOD), for water it was 0.015 ng PFOA, PFOS was below LOD.

3. Results and discussion

PFOA concentrations in sediment samples were frequently below LOQ especially at the location upstream the WWTP, but also reached 175 ng kg^{-1} dw (Table 1). Obviously the variability between individual samples is rather high, the widest being observed 0.5 km downstream the WWTP, potentially reflecting incomplete mixing of waste and river waters at this location.

PFOS concentrations in sediment ranged from <50 to 570 ng kg^{-1} dw, with the highest variations 500 m downstream the WWTP (Table 1). PFOS was up to 17-fold higher than PFOA, due to its stronger adsorption potential. A mixed sample of each location was prepared, extracted and analysed. Results of the mixed samples analysis (Table 2) were in a good agreement with the average values calculated for each location (Table 1), and confirmed that concentrations of the analytes downstream of the plant were significantly increased.

TOC of sediment samples ranged between 0.06 and 0.8%. PFOA and PFOS concentrations in river water were between 10 and 23 ng L^{-1}, and between 1.7 and 16 ng L^{-1}, respectively (Table 2).

PFOA concentrations in sediment samples downstream the WWTP were about threefold higher than upstream. Relative to the respective water sample the sediment concentrations were about twofold higher, and relative to the average water concentration

Table 2
Comparison of PFOA and PFOS concentrations in water [ng L^{-1}] and sediment (mixed sample) [ng kg^{-1}] at four locations (Sampling: 19 October 2006, UpS – upstream the WWTP, DwS – downstream the WWTP)

Sampling location	PFOA			PFOS		
	Water	Sediment	Ratio (Sediment/water)	Water	Sediment	Ratio (Sediment/water)
1 km UpS WWTP	10 ± 1	18 ± 5	1.8	1.7 ± 0.3	72 ± 14	42
0.05 km DwS WWTP	23 ± 2	68 ± 2	3.0	16.0 ± 0.3	310 ± 20	19
0.5 km DwS WWTP	23 ± 1	40 ± 14	1.7	14.0 ± 0.5	230 ± 20	17
1 km DwS WWTP	23 ± 3	35 ± 9	1.5	11.0 ± 0.2	240 ± 22	22

measured in the Roter Main between April 2005 and June 2007 (0.1 km upstream: 3 ng L^{-1}, 1 km downstream: 9 ng L^{-1}: Becker et al., 2008, submitted for publication) up to sixfold higher. The river sediment concentrations were lower than reported for four rivers located in the San Francisco Bay, USA (<LOD – 1300 ng kg^{-1} dw) (Higgins et al., 2005), in Tidal Flat Areas of the Ariake Sea (840–1100 ng kg^{-1} dw) (Nakata et al., 2006) or in rivers Kamo, Uji and Tenjin in Japan (1300–3900 ng kg^{-1} dw) (Senthilkumar et al., 2007). PFOS sediment concentrations were 20- to 40-fold higher than in the respective water samples or than in the average water concentration determined in the river water between April 2005 and June 2007 (0.1 km upstream: 2.0 ng L^{-1}, 1 km downstream: 30 ng L^{-1}; Becker et al., 2008, submitted for publication). The sediment levels were comparable to those of the Ariake Sea (90–140 ng kg^{-1} dw) (Nakata et al., 2006) or in four rivers from the San Francisco Bay (160–230 ng kg^{-1} dw) (Higgins et al., 2005), but lower than reported for the rivers Tenjin or Osaka in Japan (3800–11 000 ng kg^{-1} dw) (Senthilkumar et al., 2007).

In this study, a correlation of the PFC content of the sediments to TOC was not found, although suggested previously (Higgins and Luthy, 2006). According to Johnson et al. (2007), it is likely that adsorption of PFOA and PFOS is also partially controlled by electrostatics meaning that also inorganic materials, will influence their fate and transport in aquatic system, as well as other factors, e.g. pH-values of water and sediment, and water temperature.

4. Conclusions

Treated waste water released from local WWTPs is a main source of PFOA and PFOS in rivers (Becker et al., 2008, submitted for publication). Once released, PFCs partition into the sediment: PFOA, carboxylic acid, about 10-fold less than PFOS a sulfonic acid. Higgins et al. (2007) have suggested that they are readily bioavailable; thus, studies with fish from such rivers are required to assess to which extent these compounds enter the aquatic food chain. The data presented here reflect a momentary picture and do not take the dynamic effects of the adsorption process or sediment turnover into consideration; such investigation would require a long term monitoring program over several years.

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10 Publication V

Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Two Fish Species Collected from the Roter Main River, Bayreuth, Germany

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Abstract

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are widely distributed in the environment. The discharge of municipal waste waters has been recognised as one of the major routes of introduction into aquatic ecosystems. The present study deals with the estimation of the accumulation potential of PFOA and PFOS in two fish species with different feeding strategies, i.e. chub (*Leuciscus cephalus*) and river goby (*Gobio gobio*), inhabiting a river receiving treated waste waters from a municipal waste water treatment plant (WWTP).

PFOS was detected in chub (7 - 250 µg kg^{-1} wet weight) and river goby (70 - 400 µg kg^{-1} wet weight) with bioaccumulation factors (BAFs) of 4600 and 11 000. The high BAF in the bottom-feeding river goby shows that river sediments with their high PFOS-concentrations relative to the free water phase play an important role in food chain transfer of PFOS. The tissue distribution of PFOS was as follows: liver > kidneys > heart, gonads >> muscles. PFOA concentrations in both fish were low and in chub mostly below LOQ.

Keywords: perfluorooctanoic acid, perfluorooctane sulfonate, fish, aquatic environment

Introduction

Perfluorinated surfactants (PFSs) such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been the subject of many recent investigations (Houde et al. 2006, Prevendouros et al. 2006). PFOA, PFOS and their derivatives have been widely used in consumer products and industrial applications because of their chemical and thermal stability, and their water- and fat-repellent properties (Kissa 2001).

PFSs enter the environment in various ways, mainly via waste waters (Becker et al. 2008 a, b, Boulanger et al. 2005, Schulz et al. 2006 a, b; Sinclair and Kannan 2006) from industries in which these compounds are used as anti-static agents for surface treatment, moulding or extrusion, as components of fire fighting foams, or as abiotic or biotic degradation products of precursors (Prevendouros et al. 2006) during waste water treatment. Their persistence and potential bioaccumulation have resulted in their ubiquitous distribution in the environment, in wildlife and in humans (Giesy et al. 2001, Kannan et al. 2005; Houde et al. 2006, Prevendouros et al. 2006, Becker et al. 2008 a, b).

PFOA and PFOS have been detected in freshwater fish in the United States (Kannan et al. 2005, Furdui et al. 2007, Moody et al. 2002, Sinclair et al. 2006), in Canada (Tittlemier et al. 2007, Martin et al. 2004), in Japan (Taniyasu et al. 2003), in Belgium (Hoff et al. 2005), and in Germany (Federal Office for Environment 2007 a).

Hepatic PFOS concentrations in eel and carp (17 – 9030 and 11 – 1820 µg kg^{-1} wet weight, respectively) were shown to be positively correlated to serum alanine aminotransferase activity and negatively to serum protein content and serum electrolyte concentrations (Hoff et al. 2005). Recently, fish have been identified as a source of PFSs in humans on the Baltic Coast (Falandysz et al. 2006).

The aim of the present study was a) to assess the accumulation potential of PFOA and PFOS in two fish species with different feeding strategies inhabiting a river with a well quantified source, i.e. a municipal waste water treatment plant (WWTP) (Becker et al. 2008 a, b), and b) to determine their tissue distribution.

1 Materials and Methods

1.1 Chemicals and Equipment

PFOA (95 %, Lancaster, Eastgate, UK), [1, 2- $^{13}C_2$]-PFOA (98 %, Perkin Elmer, Boston, USA), perfluorooctane sulfonate potassium salt (98 %, Fluka, Buchs, Germany), [1, 2, 3, 4- $^{13}C_4$]-perfluorooctane sulfonate sodium salt (99 %, 50 µg/mL-solution in MeOH, Campro Scientific, Berlin, Germany), acetic acid (100 %, Merck, Darmstadt, Germany), ammonium acetate (99.0 %, Fluka, Buchs, Germany), potassium hydroxide (KOH, analytical grade, Roth, Karlsruhe, Germany), MeOH, and AcN (picograde, Promochem, Wesel, Germany) were used as obtained. The equipment was pre-cleaned as described previously (Weremiuk et al. 2006); Teflon equipment was avoided.

1.2 Sample Collection

On 28 August 2007, two fish species, i.e. chub (*Leuciscus cephalus*) (n = 6) and river goby (*Gobio gobio*) (n = 5), were caught in the river Roter Main by electro-fishing by the employees of the Bavarian Fishery Association. The sampling site was located approximately 3 km downstream the WWTP of Bayreuth, Upper Franconia, Germany. The river has an average daily flow of 270 000 m^3 and receives a daily average of 40 000 m^3 treated waste water of industrial, commercial, and domestic origin from a population of 72 000 inhabitants. The daily loading of the river with the WWTP-effluent is 1.2 ± 0.5 g PFOA and 4.7 ± 0.5 g PFOS (Becker et al. 2008 a, b).

The chub is a freshwater fish of the family *Cyprinidae* which lives in rivers with slow and moderately fast flowing waters, in canals and still waters of various kinds, breeding in flowing waters, with a spawning season from April to June. It feeds on water insects, larvae, snails, mussels, worms, and small fishes, reaches a size of 30 to 40 cm, and can be found all over Europe except Scotland, Ireland and Northern Scandinavia. The river goby is a bottom-feeding fish of the *Gobiidae* family widespread throughout Europe, sifting through mud and silt of fast to moderately flowing rivers, sucking up invertebrates. It rarely exceeds 15 cm and spawns between Mai and end of June.

The caught fish were cooled immediately and transported in PP-bags to the laboratory. The chubs were dissected; heart, liver, kidneys, gonads, and muscle tissue were stored separately

in 50-mL PP-centrifuge tubes at -20 °C. Of the river Goby, due to the small size, only muscles and inner organs were separated (table 1).

Table 1. Animal size and weights of organs/tissues taken for analysis, Roter Main, 27 Aug. 2007.

Chub (*Leuciscus cephalus*)								
Fish	**Length, cm**	**Weight, g**	**Age, a**	**Subsample weight, g**				
				Liver	**Kidneys**	**Gonads**	**Heart**	**Muscles**
1	26	172.5	4	2.77	0.22	3.71	0.21	1.00
2	25	161.1	4	1.80	0.16	3.23	0.17	1.00
3	22	110.8	4	1.51	0.21	1.63	0.18	1.00
4	24	129.2	4	1.07	0.73	1.43	0.17	1.00
5	24	129.7	4	2.31	1.10	2.27	0.20	1.00
6	32	367.5	4	4.55	2.48	7.13	0.49	1.00
	River Goby (*Gobio gobio*)							
Fish	**Length, cm**	**Weight, g**	**Age, a**	**Subsample weight, g**				
				Organs		**Muscles**		
1	13	19.05	3	2.44		5.50		
2	13	22.05	3	2.12		4.63		
3	14.5	23.85	3	2.18		4.77		
4	12	13.40	3	1.74		3.34		
5	13.5	17.00	3	1.67		3.51		

1.3 Sample Preparation and Analysis

The tissue samples were extracted according to a slightly modified, published method (So et al. 2006). For example, 5 g thawed muscle tissue was homogenised in a 50-mL PP-bottle with a mechanical homogeniser (Ultra-Turrax, Janke & Kankel GmbH, Staufen, Germany) without addition of solvent. After homogenisation of the tissue of one fish, the homogeniser was thoroughly washed with tap water, bidistilled water, and MeOH; the washes were discarded. Each sample was extracted in triplicate: 1.0 g homogenate was weighed into a new 50-mL PP-centrifuge tube and 150 µL standard solution containing 100 µg L^{-1} each of ^{13}C-PFOA and ^{13}C-PFOS was added. After addition of 30 mL methanolic KOH solution (0.01 N), the mixture was shaken at room temperature for 16 h (22 rpm, Shaker, GFL 3040, Burgwegel, Germany) and centrifuged (High-Performance Centrifuge, Avanti J-25, Beckman, USA, 3000 rpm, 20 °C, 10 min); 2 mL of the supernatant was transferred to a 250-mL PP-bottle, diluted with 100 mL deionised water and mixed thoroughly. The analytes were preconcentrated and precleaned by solid phase extraction (SPE).

The organs (table 1) were mechanically homogenised with 5 mL each of methanolic KOH solution (0.01 N) in a 50-mL PP-bottle. Residues of the sample sticking to the homogeniser were recovered by washing it five times with 5 mL methanolic KOH solution each, combining the washes with the homogenised sample. Between samples, the homogeniser was cleaned as above. Standard solution, 150 µL, containing 100 µg L^{-1} each of ^{13}C-PFOA and ^{13}C-PFOS, was added to each 30-mL sample of tissue homogenate, and the mixture was shaken at room temperature for 16 h (22 rpm). Upon centrifugation (3000 rpm, 20 °C, 10 min), 2 mL each of the supernatant were transferred to three 250-mL PP-bottles containing 100 mL deionised water; the bottles were thoroughly shaken.

The analytes were preconcentrated by SPE as previously described (Weremiuk et al. 2006) but without applying vacuum and omitting the washing of the cartridge to avoid losses. Analysis and quantification was done by HPLC-ESI-MS/MS (Weremiuk et al. 2006).

1.4 Quantification

For calibration, stock solutions of ^{13}C-PFOA, ^{13}C-PFOS, PFOA, PFOS and three working standard solutions containing: a) 100 µg L^{-1} each of both ^{13}C-PFOA and ^{13}C-PFOS, b) 20 µg L^{-1} each of both labelled standards, and c) 10 µg L^{-1} each of both non-labelled standards, were prepared as described previously (Becker et al. 2008 a).

For quantitative analysis, standard solutions containing non-labelled PFOA and PFOS in the range from 0.5 to 15 µg L^{-1}, and 2 µg L^{-1} each of both ^{13}C-labelled standards were used for calibration.

Calibration curves were constructed by plotting analyte and internal standard peak area ratios versus analyte concentrations; regression coefficients were higher than 0.995.

Recoveries from fish samples relative to the ^{13}C-labelled standard were 88 % (± 10 % rsd) for PFOA and 86 % (± 10 % rsd) for PFOS.

The limits of quantification (LOQ, signal to noise ratio 7) for PFOA and PFOS were 1.5 and 3 ng, respectively, divided by the sample weight. Procedural blanks consisting of 50-mL PP-tubes filled with 30 mL methanolic KOH solution and spiked with 150 µL of a standard solution containing 100 µg L^{-1} each of ^{13}C-PFOA and ^{13}C-PFOS were below the limit of detection (LOD).

2 Results and Discussion

PFOA-concentrations in the different organs of the chubs were above LOQ (table 2) only occasionally, i.e. in the heart of chub 1, the liver of chub 6, the kidneys of chubs 2 and 3, and the gonads of chubs 2, 4, 5, 6. PFOA concentrations in the river gobies were generally higher and ranged from < 0.6 to 3.0 µg kg^{-1} wet weight (ww) in the organs, and from 2.0 and 9.8 µg kg^{-1} ww in the muscles (table 3).

Table 2. Concentrations of PFOA and PFOS [µg kg^{-1} ww] in chub tissue.

Fish	Liver	Kidneys	Gonads	Heart	Muscles
			PFOA		
1	< 0.5	< 6.8	< 0.4	21 ± 0.7	< 1.5
2	< 0.8	206 ± 18	9.7 ± 0.5	< 9	< 1.5
3	< 1.0	8.2 ± 1	< 0.9	< 8	< 1.5
4	< 1.4	< 2.1	5.8 ± 0.4	< 9	< 1.5
5	< 0.7	< 1.3	2.0 ± 0.1	< 8	< 1.5
6	3.6 ± 0.2	< 0.6	2.7 ± 0.2	< 3	< 1.5
			PFOS		
1	110 ± 12	66 ± 8	52 ± 1	23 ± 3	7.5 ± 0.5
2	120 ± 9	83 ± 1	66 ± 2	49 ± 1	14.5 ± 0.5
3	113 ± 1	102 ± 8	56 ± 2	103 ± 1	14.6 ± 0.5
4	152 ± 13	137 ± 6	67 ± 8	66 ± 3	11.3 ± 0.9
5	117 ± 9	133 ± 4	57 ± 3	59 ± 5	15.6 ± 0.6
6	123 ± 12	100 ± 1	*247 ± 15	40 ± 1	12.2 ± 0.7
Mean	**123 ± 15**	**103 ± 28**	**60 ± 7**	**57 ± 27**	**13 ± 3**

* Outlier, not included in mean calculation.

Table 3. Concentrations of PFOA and PFOS [µg kg^{-1} ww] of the inner organs and muscles of river goby.

Fish	PFOA		PFOS	
	In. organs	Muscles	In. organs	Muscles
1	< 0.6*	4.5 ± 0.3	290 ± 9	69 ± 6
2	2.4 ± 0.3	7.8 ± 0.1	230 ± 16	76 ± 5
3	3.0 ± 0.2	9.8 ± 0.5	345 ± 40	65 ± 4
4	1.2 ± 0.2	2.0 ± 0.4	205 ± 2	85 ± 6
5	< 0.9*	5.2 ± 0.8	406 ± 40	108 ± 9
Mean	**1.5 ± 1.2**	**5.9 ± 0.8**	**295 ± 80**	**80 ± 17**

* Mean is calculated using half of these values (LOQs).

Overall, PFOA concentrations found in both species were lower than in the liver of jack mackerel purchased on a Japanese market originating from a fish farm (Senthilkumar et al. 2007), or in muscles (6.4 – 53 µg kg^{-1}) or livers (2.6 – 840 µg kg^{-1}) of eel, barb, carp, nase,

greyling from the river Alz (Federal Office for Environment 2007 a) where PFOA water concentration was up to 7.5 µg L^{-1} (Federal Office for Environment 2007 b); they were higher than in muscle tissue of largemouth bass or smallmouth bass from the River Raisin, St. Clair and Calumet (<2 µg kg^{-1}) (Kannan et al. 2005), in whole body homogenate of trout from Lake Ontario (1 ± 0.1 µg kg^{-1}, Martin et al. 2004), or in muscles of white fish purchased on a Catalan market (< 0.065 µg kg^{-1}, Ericson et al. 2008).

Relative to the PFOA concentrations in the river (median March – June 2007: 8 ng L^{-1}, Becker et al. 2008 a), a bioaccumulation factor (BAF) of 740 was calculated for the muscle tissue of river gobies. Martin et al. (2007) reported a BAF of 4 for rainbow trout exposed to PFOA under laboratory conditions, but Houde et al. (2006) pointed out that there is no agreement between laboratory and field BAFs for PFSs.

Higher concentrations of PFOS in river water (median March – June 2007: 25 ng L^{-1}, Becker et al. 2008 a) and sediment (October 2006, mixed sample collected 1 km downstream the WWTP: 240 ng kg^{-1} dry weight, Becker et al. 2008 c) led to higher levels in the caught fish (table 2), especially in liver (123 ± 15 µg kg^{-1} ww), kidneys (100 ± 30 µg kg^{-1} ww), gonads (52 ± 1 µg kg^{-1} ww) and heart (57 ± 27 µg kg^{-1} ww); lowest values (13 ± 3 µg kg^{-1} ww) were in muscle tissue. These results are in agreement with previous findings showing that PFOS accumulates in liver (Giesy et al. 2001, Kannan et al. 2005), kidneys (Martin et al. 2005, Van de Vijver et al. 2005) and gonads (Martin et al. 2003).

River gobies were quite small, and therefore only muscle and combined inner organs (liver, kidneys, heart, gonads, intestines) were analysed. PFOS levels were between 65 and 108 µg kg^{-1} ww (average: 80 ± 17 µg kg^{-1} ww), in pooled organs between 205 and 406 µg kg^{-1} ww (average: 300 ± 80 µg kg^{-1} ww, n = 5). Obviously, the PFOS-concentrations in river goby muscles are about 6-times those of chub, reflecting the fact that the former feed mainly on benthic invertebrates living in the sediment with relatively high PFOS concentrations (October 2006, mixed sample collected 1 km downstream the WWTP: 35 ± 9 ng kg^{-1}; sediment/water concentration ratio = 22, Becker et al. 2008 c). Higgins et al. (2006) suggested that PFSs in sediments are readily bioavailable and can be bioaccumulated from the sediment.

PFOS concentrations in the liver of chub of the Roter Main are similar to those in smallmouth bass liver from New York State lakes (10 – 140 µg kg^{-1}, Sinclair et al. 2006), chinook salmon (30 – 170 µg kg^{-1}) or whitefish (33 – 81 µg kg^{-1}) of the Great Lakes (Kannan et al. 2005) but

lower than in the livers of eel, perch, roach from the river Main or Alz (15 – 4300 µg kg^{-1}) (Federal Office for Environment 2007 a) or in carp or gibel carp from Flanders (Belgium) (10 – 9030 µg kg^{-1}, Hoff et al. 2005). Concentrations in muscles were comparable to those in fish from other Bavarian rivers (Federal Office for Environment 2007 a).

A comparison the PFOS-concentrations in chubs' livers or pooled organs of river gobies (123 or 295 µg kg^{-1} ww, respectively) with the average water concentration of 27 ng L^{-1} (Roter Main, 1 km downstream the WWTP, March - June 2007; Becker et al. 2008 a) resulted in a liver-based BAF of 4600 for chub and 11 000 for river goby; it is assumed that the liver-BAF of the latter species would even be higher if it were analysed separately. The values are in good agreement with those determined for coastal fish from Japan (8540, Taniyasu et al., 2003) or the Niagara River (8850, Houde et al. 2006).

3 Conclusions

PFOS was detected in all chub and river goby samples of the Roter Main downstream the WWTP of Bayreuth. In chub the concentrations were highest in liver, lowest in muscles. PFOA concentrations were about five-fold lower. In the bottom-feeding river goby both PFSs were higher than in chub, due to their different food sources.

This study shows that PFOS released to the river from a municipal WWTP is accumulated in the liver of exposed fish by factor of 10^4 or higher, PFOA by a factor of less than 10^3.

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Ehrenwörtliche Erklärung

Hiermit erkläre ich, dass ich die Arbeit selbst verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

Ferner erkläre ich, dass ich weder an der Universität Bayreuth noch anderweitig versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

(Anna Maria Becker)