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Accumulation of perfluoroalkyl substances in human tissues

Francisca Pérez [a], Martí Nadal [b], Alícia Navarro-Ortega [a], Francesc Fàbrega [b], José L. Domingo [b], Damià Barceló [a,c], Marinella Farré [a,*]

[a] Water and Soil Quality Research Group, Dept. of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[b] Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, Reus, Spain
[c] Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, Emili Grahit, 101, 17003 Girona, Spain

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ABSTRACT

Perfluoroalkyl substances (PFASs) are environmental pollutants with an important bioaccumulation potential. However, their metabolism and distribution in humans are not well studied. In this study, the concentrations of 21 PFASs were analyzed in 99 samples of autopsy tissues (brain, liver, lung, bone, and kidney) from subjects who had been living in Tarragona (Catalonia, Spain). The samples were analyzed by solvent extraction and online purification by turbulent flow and liquid chromatography coupled to tandem mass spectrometry. The occurrence of PFASs was confirmed in all human tissues. Although PFASs accumulation followed particular trends depending on the specific tissue, some similarities were found. In kidney and lung, perfluorobutanoic acid was the most frequent compound, and at highest concentrations (median values: 263 and 807 ng/g in kidney and lung, respectively). In liver and brain, perfluorohexanoic acid showed the maximum levels (median: 68.3 and 141 ng/g, respectively), while perfluorooctanoic acid was the most contributively in bone (median: 20.9 ng/g). Lung tissues accumulated the highest concentration of PFASs. However, perfluorooctane sulfonic acid and perfluorooctanoic acid were more prevalent in liver and bone, respectively. To the best of our knowledge, the accumulation of different PFASs in samples of various human tissues from the same subjects is here reported for the very first time. The current results may be of high importance for the validation of physiologically based pharmacokinetic models, which are being developed for humans. However, further studies on the distribution of the same compounds in the human body are still required.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a large group of surface-active organic compounds. Because of their chemical and thermal stability, as well as their hydrophobic and lipophobic nature, they have been used for over 50 years in a number of industrial and commercial applications (Zhao et al., 2012). PFASs are highly resistant to breakdown. Therefore, they are persistent in the environment, being able to accumulate in living organisms and biomagnified through the trophic web (Loi et al., 2011; Powley et al., 2008). Moreover, there is a growing concern related to their potentially harmful effects on human health (Vieira et al., 2013). Due to these reasons, the U.S. industry undertook voluntary actions to phase out production of perfluorooctane sulfonic acid (PFOS) between 2000 and 2002, and in 2007 the United States Environmental Protection Agency (US EPA) published the Significant New Use Rules (SNURs) to restrict the production of PFOS and related substances (Lindstrom et al., 2011). Moreover, in 2006, the major PFAS producers committed the Stewardship Program to phase out the global emissions and products containing perfluorooctanoic acid (PFOA) for 2015. Despite these measures, hundreds of other different PFASs are currently being produced and used. Thus, although the production of PFOA is being phased out by the companies participating in the Voluntary Stewardship Program, environmental contamination and human exposure from PFOA and higher homologue chemicals (e.g. PFNA, PFDA, etc.) are anticipated to continue for the foreseeable future due to a number of reasons: its persistence, their formation from precursor compounds, and the potential for continued production by other manufacturers in the U.S. and/or overseas (Lindstrom et al., 2011).

In 2008, the European Food Safety Authority (EFSA, 2008) established a series of Tolerable Daily Intakes (TDIs) values for PFOS and PFOA at 150 and 1500 ng/kg/day, respectively. PFOS was subsequently included as a persistent organic pollutant (POP) under the Stockholm Convention (UNEP 2010). In 2009, the US EPA Office of Water established the provisional health advisory values for PFOS and PFOA at 200 and 400 ng/L, respectively. It must be highlighted that, although TDIs and the water provisional health advisory were calculated in different basis, in both cases short-term exposure was considered as the relevant period of exposure. This was consistent with PFOA and PFOS toxicity data, which in turn rely upon subchronic exposure experimental values. However, long-term exposures must be considered for the accurate assessment of their potential risk on human health, taking into account that their

* Corresponding author. Tel.: +34 93 400 61 00.
E-mail address: mfuqam@cid.csic.es (M. Farré).

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presence has been reported in drinking water, ambient air, and food (Domingo et al., 2012a,b; Ericson Jogsten et al., 2012; Ericson et al., 2008, 2009; Post et al., 2009, 2012).

PFASs have been related to different toxicological effects on mammals. In mice, the neonatal exposure to PFOS and PFOA has been linked up to changes in proteins of importance for the neuronal growth and synaptogenesis in the brain developing (Johansson et al., 2009), as well as with neurobehavioral defects and changes in the cholinergic system (Johansson et al., 2008). In addition, perfluorohexanesulphonate (PFHxS) has been related to irreversible neurotoxic effects in neonatal mice, showing a similar behavior to that of other POPs, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and bisphenol A (Viberg et al., 2013). A recent study in human suggested that higher PFOA serum levels might be associated with testicular, kidney, prostate, and ovarian cancers, and non-Hodgkin lymphoma, according to the concentrations of residents in 6 areas with contaminated drinking water supplies (Vieira et al., 2013).

In the human body, the polar hydrophobic nature of fluorine-containing compounds can lead to increased affinity for proteins (Jones et al., 2003; Luebker et al., 2002; Vanden Heuvel et al., 1992; Weiss et al., 2009). A number of PFASs have been detected in human serum, cord blood and breast milk (Domingo et al., 2012a; Ericson et al., 2007; Fromme et al., 2010; Haug et al., 2009a,b; Llorca et al., 2010). As other bioacumulative halogenated contaminants (e.g., polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and PCBs), PFASs can have long persistence in the body. However, they do not tend to accumulate in fat tissue. According to outcomes of animal studies, PFOA and PFOS are mostly excreted through the urine (Cui et al., 2010), but limited observations in humans suggest that only one-fifth of the total body clearance is renal (Harada et al., 2005). The elimination half-life of PFOA in humans was roughly estimated to be 3.5 years, while that of PFOS was approximately 4.8 years (Olsen et al., 2007), according to data from retired workers. Post et al. (2012) recently reviewed studies reporting the elimination half-life values between 2.3 and 3.3 years, following an exposure to contaminated drinking water (Post et al., 2012). Information about sources, environmental fate and toxicokinetics of PFOS and PFOA is largely available, while estimation values in the half-lives of PFBS, PFHxS and PFBA (Chang et al., 2008; Lau et al., 2007). In contrast, data on most of the PFASs currently in use, continues to be very limited. It has been hypothesized that the possible harmful effects associated to PFASs accumulation are of special concern during early stages of life (Maisonet et al., 2012; Post et al., 2012; Schecter et al., 2012). However, their accumulation and distribution in the different human tissues are still poorly understood. The potential accumulation of PFASs with different chain lengths is an issue of great importance for exposure assessment and risk characterization studies. Most current investigations on human accumulation have focused on the occurrence in blood and breast milk, while very few studies have reported levels in other tissues. Kärrman et al. (2009) determined the concentrations of six PFASs in liver samples collected post-mortem in Spain. Mean concentrations of 27 and 1 ng/g of PFOS and PFOA, respectively, were found. In turn, Maestri et al. (2006) found levels of 14 ng/g of PFOS and 3 ng/g of PFOA in a pooled liver samples corresponding to seven subjects from northern Italy, while Olsen et al. (2003) reported mean PFOS and PFOA concentrations of 19 and 47 ng/g, respectively, in 30 subjects from USA. Finally, Pirali et al. (2009) detected PFOA and PFOS in thyroid tissue (median levels: 2 and 5.3 ng/g, respectively), concluding that those compounds are not actively concentrated in the thyroid.

The main objectives of the present study were the following: 1) to optimize and validate an on-line analytical approach based on turbulent flow chromatography coupled to tandem mass spectrometry (TFC-LC-MS/MS) for determining PFASs in various human tissues; 2) to measure the levels of 21 PFASs in these human tissues in order to elucidate their distribution and accumulation in the human body. The method optimized for the tissue analysis was carefully selected to accomplish the minimum sample size requirements and to reduce sample manipulation. The analytical procedure was validated for different kinds of tissues, and applied for the determination of selected compounds in liver, lung, brain, bone, and kidney samples collected post-mortem from 20 subjects. PFASs values were correlated with the concentrations of some heavy metals (unpublished results) in the same tissue samples, as well as with the levels of PCDD/Fs in adipose tissue from 15 of the same individuals (Nadal et al., 2009). To the best of our knowledge, these are the first data reporting the accumulation of a notable number of PFASs in human tissues, as well as comparing the body burden of these pollutants with that of other environmental contaminants (metals and PCDD/Fs).

2. Materials and methods

2.1. Chemicals and standards

Standard solutions were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). The standard analytes used in this study were: i) PFAC-MXB [98% purity in methanol] containing perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA, perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutanesulphonate (PFBS), perfluorohexanesulphonate (PFHxS), perfluorooctanesulphonate (PFOS) and perflurodecanesulphonate (PFDS); ii) FTA [98% purity in isopropanol] including perfluorohexyl ethanoic acid (FHEA), perfluorooctyl ethanoic acid FOEA, and perfluorodecyl ethanoic acid FDEA; iii) perfluorooctane sulfonamide (PFOSA) [98% pure in methanol]. Identification and quantification were performed using the following internal standards: i) MPFAC-MXA [>98%] containing [$^{13}C_4$]-perfluorobutanoic acid (MPFBA ($^{13}C_4$)), ion [$^{18}O_2$]-perfluorohexanesulfonate (MPFHxS ($^{18}O_2$)), [$^{13}C_2$]-perfluorohexanoic acid (MPFHxA ($^{13}C_2$)), ion [$^{13}C_4$]-perfluorooctanesulfonate (MPFOS ($^{13}C_4$)), [$^{13}C_4$]-perfluorooctanoic acid (MPFOA ($^{13}C_4$)), [$^{13}C_5$]-perfluorononanoic acid (MPFNA ($^{13}C_5$)), [$^{13}C_2$]-perfluorododecanoic acid (MPFDoA ($^{13}C_2$)), [$^{13}C_2$]-perfluorodecanoic acid (MPFDA ($^{13}C_2$)), [$^{13}C_2$]-perfluoroundecanoic acid (MPFUdA ($^{13}C_2$)); ii) MFTA-MXA [>98%] [$^{13}C_2$]-perfluorohexylethanoic acid (MFHEA($^{13}C_2$)), [$^{13}C_2$]-perfluorooctylethanoic acid (MFOEA($^{13}C_2$)), [$^{13}C_2$]-perfluorodecylethanoic acid (MFDEA ($^{13}C_2$)) and iii) [$^{13}C_8$]-perfluorooctanesulfonamide (MPFOSA ($^{13}C_8$)).

Water, methanol, acetonitrile, CHROMASOLV®Plus for HPLC grade, ammonium acetate salt ($AcNH_4$: MW, 77.08; 98%), and formic acid (HFo) were obtained from Sigma-Aldrich (Steinheim, Germany). To remove possible cross contamination, polypropylene (PP) insert vials and inert taps were used.

2.2. Sampling and pre-treatment

Samples from liver, kidney, brain, lung, and bone (rib) were collected in 2008 from 20 subjects who had been living in different areas of Tarragona County (Catalonia, Spain) at least for the last 10 years. Causes of death were varied, including multiple trauma, subdural hematoma, ischemic heart disease, accident or self-injury. Autopsies and extraction of samples were carried out during the first 24 h after the time of death. Additional data from the subjects, such as age (mean: 56; range: 28–83) and smoking habits information, were collected (Table S1; Supporting Information). Tissue samples were stored at −20 °C before analysis. The study protocol was reviewed and approved by the Ethical Committee for Human Studies of the School of Medicine, Universitat Rovira i Virgili, Reus/Tarragona, Spain.

Sample pre-treatment was based on a previously published protocol (Llorca et al., 2010). Briefly, 1 g of each sample was weighed and transferred into a 15 mL PP tube. Then, 2 mL of water were added, and the mixture was shaken. Homogenates were fortified with surrogate