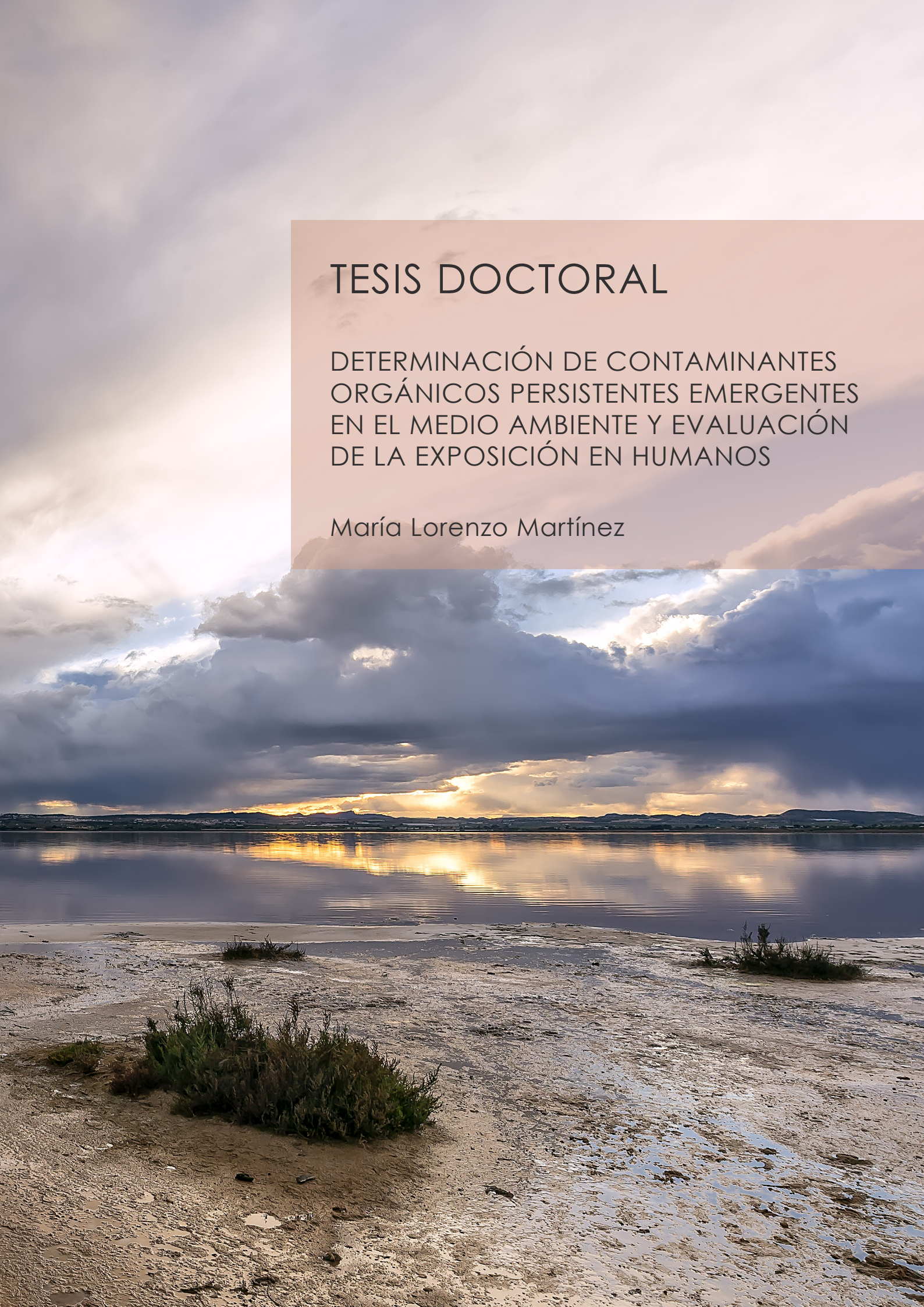
TESIS DOCTORAL

DETERMINACIÓN DE CONTAMINANTES ORGÁNICOS PERSISTENTES EMERGENTES EN EL MEDIO AMBIENTE Y EVALUACIÓN DE LA EXPOSICIÓN EN HUMANOS

María Lorenzo Martínez



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María Lorenzo Martínez

Tesis Doctoral Internacional

Programa de doctorado 3056 en Química

Determinación de contaminantes orgánicos persistentes emergentes en el medio ambiente y evaluación de la exposición en humanos

—

Determination of emerging persistent organic pollutants in the environment and human exposure assessment

—

Determinació de contaminants orgànics persistents emergents en el medi ambient i avaluació de l'exposició en humans

Memoria presentada para optar al título de Doctora por
María Lorenzo Martínez

Dirigida por:

Dra. Yolanda Picó García

Catedrática
Facultat de Farmàcia
Universitat de València

Dr. Julián Campo Velásquez

Investigador contratado
Centro de Investigaciones sobre Desertificación – CSIC

Burjassot, 26 de enero de 2018

MINISTERIO
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Julián Campo Velásquez, Investigador Contratado, y Yolanda Picó García, Catedrática del área de Nutrición y Bromatología en el Departamento de Medicina Preventiva de la Universitat de València, Doctores en Desertificación y Farmacia, respectivamente, por la Universidad de Valencia, e investigadores del Centro de Investigaciones sobre Desertificación (CIDE):

I N F O R M A N :

Que la Licenciada *María Lorenzo Martínez* ha estado trabajando bajo nuestra dirección durante más de tres años en la elaboración de la tesis doctoral que lleva por título **"DETERMINACIÓN DE CONTAMINANTES ORGÁNICOS PERSISTENTES EMERGENTES EN EL MEDIO AMBIENTE Y EVALUACIÓN DE LA EXPOSICIÓN EN HUMANOS"** por lo que autorizamos su presentación para optar al Grado de Doctora con Mención Internacional.

Moncada, 26 de enero de 2018

Dr. Julián Campo Velásquez

Dra. Yolanda Picó García

cide@uv.es

CARRETERA DE MONCADA A NÁQUERA
KM 4,5.
46113 MONCADA (VALENCIA)
TEL: 96 342 41 62
FAX: 96 342 41 60
http://www.uv.es/cide/

Julián Campo Velásquez, Investigador Contratado y Yolanda Picó García, Catedrática del área de Nutrición y Bromatología en el Departamento de Medicina Preventiva de la Universitat de València, Doctores en Desertificación y Farmacia, respectivamente, por la Universitat de València, e investigadores del Centro de Investigaciones sobre Desertificación (CIDE):

I N F O R M A N :

Que la tesis doctoral con mención internacional que lleva por título **"DETERMINACIÓN DE CONTAMINANTES ORGÁNICOS PERSISTENTES EMERGENTES EN EL MEDIO AMBIENTE Y EVALUACIÓN DE LA EXPOSICIÓN EN HUMANOS"** ha sido organizada en 10 capítulos de los cuales 6 han sido publicados y 2 aceptados en revistas incluidas en el SCI o JCR:

- *Lorenzo, M.*, Campo, J., Picó, Y. (2015). **Optimization and comparison of several extraction methods for determining perfluoroalkyl substances in abiotic environmental solid matrices using liquid chromatography-mass spectrometry.** *Analytical and Bioanalytical Chemistry* **407**, 103-115. [**JCR (WOS)** IF 3.125 (2015) en el área de Química Analítica 15/77 Q1].
- *Lorenzo, M.*, Campo, J., Farré, M., Pérez, F., Picó, Y., Barceló, D. (2016). **Perfluoroalkyl substances in the Ebro and Guadalquivir river basins (Spain)**. *Science of The Total Environment* **540**, 191-199. [**JCR (WOS)** IF 4.900 (2016) en el área de Ciencias Ambientales 22/229 Q1 (primer decil)].
- Campo, J., *Lorenzo, M.*, Pérez, F., Picó, Y., Farré, M., Barceló, D. (2016). **Analysis of the presence of perfluoroalkyl substances in water, sediment and biota of the Jucar River (E Spain). Sources, partitioning and relationships with water physical characteristics.** *Environmental Research* **1461**, 98-106. [**JCR (WOS)** IF 3.835 (2016) en el área de Ciencias Ambientales 44/229 Q1].
- *Lorenzo, M.*, Campo, J., and Picó, Y. (2016). **Ultra-high-pressure liquid chromatography tandem mass spectrometry method for the determination of 9 organophosphate flame retardants in water samples.** *Methods X* **3**, 343-349 [**SJR (Scopus)** 0.561 (2016)].
- *Lorenzo, M.*, Farré, M., Blasco, C., Onghena, M., Picó, Y., Barceló, D. (2016). **Perfluoroalkyl substances in Breast milk, infant formula and baby food from Valencian Community (Spain).** *Environmental Nanotechnology, Monitoring & Management* **6**, 108-115. [**SJR (Scopus)** 0.561 (2016)].
- Campo, J., *Lorenzo, M.,* Cammeraat, E.L.H., Picó, Y., Andreu, V. (2017). **Emerging contaminants related to the occurrence of forest fires in the Spanish Mediterranean**. *Science of the Total Environment* **323**, Part A, 156-165. [**JCR (WOS)** IF 4.900 (2016) en el área de Ciencias Medioambientales 22/229 Q1 (primer decil)].
- *Lorenzo, M.*, Campo, J., Pico, Y. **Determination of organophosphate flame retardants in soil and fish using ultrasound-assisted extraction, solid-phase clean-up and liquid chromatography-tandem mass spectrometry.** *Journal of Separation Science* (*Aceptado, disponible on-line www.onlinelibrary.wiley.com/doi/10.1002/jssc.201701461*) [**JCR (WOS)** IF 2.557 (2016) en el área de Química Analítica 30/76 Q2].

cide@uv.es

CARRETERA DE MONCADA A NÁQUERA
KM 4,5.
46113 MONCADA (VALENCIA)
TEL: 96 342 41 62
FAX: 96 342 41 60
http://www.uv.es/cide/

— *Lorenzo, M.*, Campo, J., Picó, Y. **Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems,** *TrAC-Trends in Analytical Chemistry* (Aceptado) [**JCR (WOS)** IF 8.442 (2017) en el área de Química Analítica 1/77 Q1 (primer decil)].

Los artículos publicados han sido incluidos en la tesis el formato original de la revista. En todos ellos, María Lorenzo ha realizado la mayor parte o todo el trabajo experimental, vigilando y supervisando estrechamente los experimentos, así como ha procedido al análisis de los resultados y la elaboración de los manuscritos en directa colaboración con nosotros.

Moncada, 26 de marzo de 2018

Dr. Julián Campo Velásquez

Dra. Yolanda Picó García

cide@uv.es

CARRETERA DE MONCADA A NÁQUERA
KM 4,5.
46113 MONCADA (VALENCIA)
TEL: 96 342 41 62
FAX: 96 342 41 60
http://www.uv.es/cide/

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PRESENTACIÓN DE LA MEMORIA

OBJETIVOS Y ESTRUCTURA

El interés por la presencia de contaminantes emergentes en los distintos compartimentos ambientales ha crecido exponencialmente en las últimas décadas. Estos contaminantes se definen como sustancias químicas que pueden haber estado presentes en el medio ambiente desde hace tiempo, pero solo han sido identificadas recientemente gracias al desarrollo de técnicas analíticas más sensibles. Por tanto, la mayor parte no se encuentran reguladas y sus efectos en el medio ambiente y la salud humana a gran escala son desconocidos (Mandaric et al., 2016).

El destino final para la mayoría de los contaminantes emergentes es el agua o los ecosistemas acuáticos, a los que pueden llegar por distintas vías: eliminación de forma incorrecta, liberación a través de los sistemas de aguas residuales domésticos, mediante la agricultura y la industria o tras su paso por estaciones depuradoras de aguas residuales (WWTP, por sus siglas en inglés) que no los eliminan eficazmente (Campo et al., 2014).

Las investigaciones sobre la incidencia y distribución de contaminantes emergentes en los ecosistemas acuáticos son recientes. La mayoría de estos estudios se basan en los de uso doméstico o agrícola, tales como fármacos (Carmona et al., 2014), drogas (Andrés-Costa et al., 2017) o insecticidas (Ccanccapa et al., 2016), dejando de lado otros de origen industrial que, sin embargo, son más persistentes y, por tanto, susceptibles de acumularse en el medio ambiente, como son los compuestos en los que mayoritariamente se centra esta tesis: sustancias perfluoroalquiladas y retardantes de llama. Los estudios realizados sobre los ecosistemas acuáticos y la evaluación del riesgo se han centrado en aspectos concretos y limitados. Sin embargo, no existe un estudio global sobre el impacto del desarrollo humano y su relación con la presencia de los contaminantes orgánicos persistentes emergentes (ePOP) propuestos en esta tesis, así como sus posibles efectos sobre las poblaciones que se abastecen de sus aguas o los organismos que habitan en ellas.

Así mismo, se necesita un mayor esfuerzo en el desarrollo de metodologías analíticas eficientes y versátiles para la detección y cuantificación de estos contaminantes y sus posibles metabolitos a las concentraciones traza (ng g^{-1} o ng l^{-1}) o ultratraza (pg g^{-1} o pg l^{-1}) a las que se encuentran en matrices ambientales, tales como aguas, suelos, sedimentos y biota.

Por todo ello, el **objetivo global** de esta tesis doctoral consiste en establecer la presencia y distribución de los ePOP objeto de estudio en distintos ecosistemas acuáticos, y forestales, tratando de establecer si la presencia de estas sustancias supone un riesgo para la fauna de estas áreas, así como para las poblaciones humanas de su entorno.

Los **objetivos específicos** a desarrollar en la presente tesis doctoral son los siguientes:

1. Desarrollar la metodología analítica necesaria para la determinación de los ePOP por cromatografía líquida de alta eficacia (HPLC), cromatografía de gases (GC) y espectrometría de masas (MS), o espectrometría de masas en tándem (MS/MS) con triple cuadrupolo (QqQ).

2. Optimizar procedimientos para la extracción de sustancias perfluoroalquiladas (PFAS), retardantes de llama fosforados (PFR) y bromados (BFR) de distintas matrices ambientales: agua superficial, agua residual, suelo, sedimento y biota.

3. Estudiar la incidencia de PFAS y PFR en ecosistemas acuáticos de zonas mediterráneas con especial hincapié en las cuencas hidrológicas de los ríos Ebro, Turia, Júcar, Segura y Guadalquivir, así como en el parque natural de la Albufera por tratarse de un humedal de importancia internacional relacionado con los ríos Turia y Júcar, y cuya calidad del agua se encuentra muy influenciada por las presiones antrópicas.

4. Evaluar la amenaza que suponen determinados ePOP para la fauna acuática a través de la predicción del riesgo, mediante el cálculo de los cocientes de peligrosidad (HQ) para tres niveles tróficos, y la determinación de las concentraciones a las que estos compuestos se encuentran en distintas especies de peces.

5. Estudiar la presencia y movilidad de PFR, BFR, PFAS e hidrocarburos aromáticos policíclicos (PAH) en suelo y sedimento de zonas afectadas por incendios forestales, dado su uso como agentes inhibidores de la combustión (PFR, BFR y PFAS) o su formación debida a la incompleta combustión de la materia orgánica (PAH).

6. Evaluar la presencia de PFAS en leche humana, leche de fórmula y otros alimentos infantiles así como la exposición a través de la dieta durante los dos primeros años de vida.

7. Estimar la exposición individual a ePOP semivolátiles en ambientes interiores mediante el uso de broches de polidimetilsiloxano (PDMS) como muestreadores pasivos de aire.

El **plan de trabajo** diseñado para el desarrollo de los objetivos ha sido el siguiente:

En primer lugar, las PFAS y los PFR fueron seleccionados como dos de las principales familias de ePOP en los que se centraría esta tesis debido a su creciente uso a nivel global, a menudo como sustitutos de otros contaminantes emergentes. Otras familias de contaminantes más conocidas por el mundo científico, como los BFR, PAH y ftalatos, fueron también estudiadas durante el desarrollo de la tesis debido a la preocupación que todavía suscita su presencia en el medio ambiente.

Tras la selección de los contaminantes emergentes persistentes, se desarrollaron y validaron los métodos analíticos necesarios para determinarlos en diferentes matrices ambientales. Los métodos de extracción se basaron en la utilización de fase sólida (SPE), extracción sólido-líquido (SLE) —asistida por ultrasonidos (USE) o por agitación mecánica—, digestiones alcalinas y la extracción líquida presurizada (PLE). La determinación se basó en el análisis por HPLC-QqQ-MS/MS o GC-MS.

Por otra parte, se establecieron las diferentes estrategias de muestreo en las cuencas de los tres ríos principales de la Comunidad Valenciana ─Turia, Júcar y Segura─, en el parque natural de La Albufera de Valencia y en algunas WWTP presentes en estas áreas. Debido a la gran cantidad de muestras necesarias para el desarrollo de esta tesis doctoral, otras campañas de muestreo como las de los ríos Ebro, Guadalquivir y Júcar, la de la zona forestal afectada por el incendio y la de la leche materna, leche de fórmula y alimentos infantiles, fueron planificadas y realizadas por otros colaboradores con anterioridad a mi incorporación al grupo de investigación.

Posteriormente, los métodos desarrollados fueron aplicados a las muestras recogidas para detectar la presencia de ePOP, evaluar la calidad de los recursos hídricos, edáficos y biológicos, y así identificar los focos de contaminación y las áreas más sensibles.

En último lugar, se realizó una evaluación del riesgo que suponen los ePOP seleccionados para la fauna acuática, estimando el riesgo que suponen las concentraciones

encontradas para los distintos niveles tróficos. Esta evaluación de riegos se enlaza con la última parte de la tesis, focalizada en establecer la exposición de los seres humanos a estas sustancias y los posibles riesgos para su salud. Por una parte, se incidió en la exposición infantil a través de la dieta, dado que este grupo de población es especialmente sensible a los efectos de estos compuestos. Por otro lado, se estudió la exposición personal a ePOP semivolátiles a través del aire inhalado en ambientes interiores mediante el uso de muestreadores pasivos de aire personales (PPAS), ya que las distintas actividades individuales suponen variaciones importantes en las concentraciones inhaladas.

Esta tesis doctoral se presenta estructurada en cinco secciones, dentro de las cuales se encuentra toda la información científica generada. Esta investigación se refleja a través de los distintos capítulos.

La **sección 1** consiste en una revisión general que clasifica y describe la importancia de los compuestos seleccionados, y desarrolla un análisis crítico de los retos analíticos que supone su determinación en los ecosistemas acuáticos. Adicionalmente, se exponen las carencias de conocimiento sobre el tema y las novedades con las que contribuye este trabajo:

- **Capítulo 1**. *Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems*.

En la **sección 2** se muestran las metodologías analíticas desarrolladas para determinar los ePOP en distintas matrices ambientales: suelo, sedimento, agua superficial, agua residual y biota, a través de la HPLC-QqQ-MS/MS. Los resultados obtenidos se presentan en tres capítulos que incluyen, además, la aplicación de la metodología desarrollada en matrices ambientales de los ríos Segura y Turia, así como en muestras de aguas residuales de Valencia:

- **Capítulo 2**. *Optimization and comparison of several extraction methods for determining perfluoroalkyl substances in abiotic environmental solid matrices using liquid chromatography-mass spectrometry*.
- **Capítulo 3**. *Ultra-high-pressure liquid chromatography tandem mass spectrometry method for the determination of 9 organophosphate flame retardants in water samples*.

- **Capítulo 4**. *Determination of organophosphate flame retardants in soil and fish using ultrasound-assisted extraction, solid-phase clean-up and liquid chromatography-tandem mass spectrometry*.

En la **sección 3**, se aplican las metodologías desarrolladas en diversos ecosistemas acuáticos incluyendo los de los ríos Ebro, Guadalquivir y Júcar. En éstos, además, se han estudiado los efectos potenciales adversos de los contaminantes mediante el cálculo del HQ. La toxicidad aguda y crónica de los compuesto se predijo a partir de la relación cuantitativa estructura-actividad (QSAR) mediante estimaciones teóricas que relacionan los valores del coeficiente de reparto octanol-agua (K_{OW}) con la toxicidad. Estos datos se utilizaron para calcular el HQ en diferentes niveles tróficos de los ecosistemas acuáticos (algas, daphnias y peces). Los distintos métodos analíticos se aplicaron también en muestras del parque natural de La Albufera, así como en un ecosistema forestal afectado por un incendio, con el fin de obtener una visión de conjunto de la distribución y destino de los contaminantes objetivo. Esta sección está compuesta por cuatro capítulos:

- **Capítulo 5**. *Perfluoroalkyl substances in the Ebro and Guadalquivir river basins (Spain)*.
- **Capítulo 6**. *Analysis of the presence of perfluoroalkyl substances in water, sediment and biota of the Jucar River (E Spain). Sources, partitioning and relationships with water physical characteristics*.
- **Capítulo 7**. *Occurrence, distribution and behavior of emerging persistent organic pollutants (POPs) in a Mediterranean wetland protected area*.
- **Capítulo 8**. *Emerging contaminants related to the occurrence of forest fires in the Spanish Mediterranean*.

La **sección 4** muestra la aplicación a la exposición en humanos a través de dos capítulos. El primero se centra en la exposición de la población infantil de la Comunidad Valenciana a PFAS a través de la dieta (leche materna, leche de formula y alimentación infantil) comparando las ingestas diarias estimadas (EDI) con las ingestas diarias tolerables (TDI) establecidas por la *European Food Safety Authority* (EFSA, 2008). El segundo, presenta el trabajo realizado durante una estancia en la *University of Toronto* (Canadá), en el que se procedió a la caracterización y evaluación de broches de PDMS como muestreadores pasivos de aire para determinar la concentración y la exposición individual a distintos contaminantes presentes en el aire inhalado en ambientes interiores.

- **Capítulo 9**. *Perfluoroalkyl substances in Breast milk, infant formula and baby food from Valencian Community (Spain).*
- **Capítulo 10**. *Polydimethylsiloxane brooch as a personal passive air sampler for semi-volatile organic compounds.*

En la s**ección 5** se recoge un resumen general de los resultados obtenidos en las **secciones 2**, **3** y **4** y su discusión. Finalmente, se presentan las **conclusiones** alcanzadas durante el desarrollo de la presente tesis doctoral, y un apartado de **anexos** que incluye un índice de tablas, figuras y abreviaturas utilizadas en el transcurso de la misma.

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AIM AND STRUCTURE

The interest in the presence of emerging pollutants in the environment has grown exponentially in the last decades. These pollutants are defined as chemical substances that could be present since long time ago, but they have only been recently identified thanks to the development of more sensitive analytical techniques. Therefore, most of them are not regulated and their effects on the environment and human health are unknown on a large scale (Mandaric et al., 2016).

The final destination for most of the emerging pollutants is water or aquatic ecosystems. They arrive in different ways: improper disposal, discharge through domestic wastewater systems, agricultural and industrial spills or after passing through wastewater treatment plants (WWTP) that do not effectively remove these contaminants (Campo et al., 2014).

Research on the incidence and distribution of emerging pollutants in aquatic ecosystems is recent. Most of these studies are based on emerging contaminants from domestic or agricultural use, such as pharmaceuticals (Carmona et al., 2014), drugs of abuse (Andrés-Costa et al., 2017) or pesticides (Ccanccapa et al., 2016). However, the study of other contaminants of industrial origin that are more persistent and therefore susceptible to accumulate in the environment is still scarce. This thesis is mainly focused on two of them: perfluoroalkyl substances and flame retardants. Most studies on aquatic ecosystems and risk assessment have focused on specific and limited aspects. However, there are not global studies on the impact of human development and their relationship with the presence of emerging persistent organic pollutants (ePOPs) in these ecosystems, as the ones selected in this thesis, as well as on their possible effects on populations supplied with their water or on organisms living in them.

Likewise, a greater effort is needed in the development of efficient and versatile analytical methodologies for the detection and quantification of these contaminants, and their possible metabolites, at trace (ng g^{-1} or ng l^{-1}) or ultratrace (pg g^{-1} or pg l^{-1}) levels they occur in environmental matrices, such as waters, soils, sediments and biota.

Therefore, the **general objective** of this thesis is to establish the presence, and distribution of target ePOPs in different aquatic and forest ecosystems, trying to establish

whether the presence of these substances poses a risk to wildlife and to human populations of their surroundings.

The **specific objectives** to be developed in this thesis are as follows:

1. Develop the analytical methodology needed for the determination of target ePOPs by high performance liquid chromatography (HPLC), gas chromatography (GC) and mass spectrometry (MS), or tandem mass spectrometry (MS/MS) with triple quadruple (QqQ).

2. Optimize procedures for the extraction of perfluoroalkyl substances (PFAS), phosphorus (PFR) and brominated (BFR) flame retardants from different environmental matrices: surface water, wastewater, soil, sediment and biota.

3. Study the incidence of PFASs and PFRs in aquatic ecosystems from Mediterranean areas with special emphasis on the hydrological basins of the Ebro, Turia, Jucar, Segura and Guadalquivir rivers, as well as the Albufera Natural Park. The last one is a wetland of international importance related to the Turia and Jucar rivers, affected by different anthropogenic pressures that greatly influence on water quality.

4. Evaluate the threat that certain ePOPs pose to aquatic fauna through the risk prediction by the calculation of the hazard quotient (HQ) for three trophic levels and the determination of the concentrations of these compounds in different fish species.

5. Study the presence and mobility of PFRs, BFRs, PFASs and polycyclic aromatic hydrocarbons (PAHs) in soil and sediment from an area affected by forest fire. Their origin is either their use as combustion inhibiting agents (PFRs, BFRs and PFASs) or its formation due to the incomplete combustion of organic matter (PAHs).

6. Evaluate the presence of PFASs in breast milk, infant formula and baby food as well as the infant exposure to PFAS through diet on their first two years of life.

7. Estimate the personal exposure to semi-volatile ePOPs in indoor environments by using polydimethylsiloxane (PDMS) brooches as passive air samplers.

In order to develop these objectives, the following **work plan** has been designed:

Firstly, PFASs and PFRs were selected as two of the main ePOP groups on which this thesis will focus due to their global increasing use, often as substitutes for other emerging pollutants. BFRs, PAHs and phthalates were also studied in this thesis because of the concern that their presence in the environment still raises.

After the selection of ePOPs, the analytical methods to determine them in different environmental matrices were developed and validated. The extraction methods were based on the use of solid phase extraction (SPE), solid-liquid extraction (SLE) ─assisted by ultrasound (USE) or by mechanical agitation─, alkaline digestion and pressurized liquid extraction (PLE). The determination was based on the analysis by HPLC-QqQ-MS/MS or GC-MS.

Moreover, the different sampling strategies were established in the three main river basins of the Valencian Community ─Turia, Jucar and Segura─, in the Albufera Natural Park and in some surrounding WWTPs. Due to the large number of samples needed for the development of this thesis, the other sampling campaigns were carried out by other members of the research group.

The developed methods were applied to the collected samples in order to detect the presence of ePOPs, to evaluate the quality of water, soil and biological resources and, thus, to identify the contamination source and the most sensitive areas.

Finally, a risk assessment of selected ePOPs for aquatic fauna was made, estimating the risk posed by the concentrations found in water to the different trophic levels. This is linked to the last part of the thesis, focused on establishing the human exposure to these substances and the potential health risk. On the one hand, exposure to children through diet was emphasized, given that this population group is especially sensitive to the effects of these compounds. On the other hand, the inhalation exposure to semi-volatile ePOPs in indoor environments was studied through the use of passive personal air samplers (PPAS), since personal activities influence the exposure.

This thesis has been structured into 5 sections and presented through 10 chapters.

Section 1 consists of a general review that classifies and describes the importance of the selected compounds. It develops a critical analysis of the analytical challenges

involved in their determination in aquatic ecosystems. Additionally, the lack of knowledge on the subject and the novelties are presented in this work:

- **Chapter 1**. *Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems*.

Section 2 shows the developed analytical methodologies to determine ePOPs in different environmental matrices: soil, sediment, surface water, wastewater and biota by HPLC-QqQ-MS/MS. The results obtained are presented in three chapters that also include the application of these methodologies in environmental matrices of the Segura and Turia rivers, as well as in wastewater samples from Valencia:

- **Chapter 2**. *Optimization and comparison of several extraction methods for determining perfluoroalkyl substances in abiotic environmental solid matrices using liquid chromatography-mass spectrometry*.
- **Chapter 3**. *Ultra-high-pressure liquid chromatography tandem mass spectrometry method for the determination of 9 organophosphate flame retardants in water samples*.
- **Chapter 4**. *Determination of organophosphate flame retardants in soil and fish using ultrasound-assisted extraction, solid-phase clean-up and liquid chromatography-tandem mass spectrometry*.

In **Section 3**, the methodologies developed are applied in various aquatic ecosystems, including Ebro, Guadalquivir and Jucar rivers. In these rivers, in addition, the potential adverse effects of the pollutants have been studied by calculating the HQ. The acute and chronic toxicity of these compounds was predicted using the quantitative structure–activity relationship (QSAR) by means of mathematical relationships between the octanol-water partition coefficient (K_{OW}) values and the corresponding toxicity. These data were used to calculate the HQ at three different trophic levels (algae, daphnia and fish). The different analytical methods were also applied to samples from the Albufera Natural Park, as well as to samples from a forest ecosystem affected by a fire, in order to obtain an overview of the distribution and fate of target pollutants. This section is composed of four chapters:

- **Chapter 5**. *Perfluoroalkyl substances in the Ebro and Guadalquivir river basins (Spain)*.
- **Chapter 6**. *Analysis of the presence of perfluoroalkyl substances in water, sediment and biota of the Jucar River (E Spain). Sources, partitioning and relationships with water physical characteristics*.

- **Chapter 7**. *Occurrence, distribution and behavior of emerging persistent organic pollutants (POPs) in a Mediterranean wetland protected area.*
- **Chapter 8**. *Emerging contaminants related to the occurrence of forest fires in the Spanish Mediterranean.*

Section 4 shows the human exposure assessment through two chapters. The first one focuses on infant exposure to PFAS through the diet (breast milk, infant formula and baby food) by comparing the estimated daily intake (EDI) with the tolerable daily intake (TDI) established by the European Food Safety Authority (EFSA, 2008). The second one presents the work carried out at the University of Toronto (Canada), which consists on the characterization and evaluation of PDMS brooches as passive air samplers to determine the concentration and personal exposure to semi-volatile ePOP in indoor environments.

- **Chapter 9**. *Perfluoroalkyl substances in Breast milk, infant formula and baby food from Valencian Community (Spain).*
- **Chapter 10**. *Polydimethylsiloxane brooch as a personal passive air sampler for semi-volatile organic compounds.*

Section 5 contains a general summary of the results obtained in **Sections 2**, **3** and **4** and their discussion. Finally, we present the **conclusions** reached during the development of this thesis and a section of **annexes** with complementary information (index of tables, index of figures and index of abbreviations).

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Sección 1

INTRODUCCIÓN

CAPÍTULO 1

Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems.

Parte de este capítulo ha sido aceptado en la revista *TrAC-Trends in Analytical Chemistry* y firmado por los autores:
María Lorenzo, Julián Campo, Yolanda Picó.

Highlights

- Recent advances in sample preparation for ePOPs discussed.
- Approaches to eliminate matrix components in ePOPs analysis assessed.
- Performance for the detection of ePOPs by mass spectrometers compared.
- Challenges and future outlooks for ePOPs analytical methods development outlined.

Abstract

Emerging persistent organic pollutants (ePOPs) include polybrominated diphenyl ethers (PBDEs) and perfluorooctane sulfonyl fluoride/perfluorooctane sulfonate (POSF/PFOS), which are newly listed in the Stockholm Convention. Other ePOPs, which have not been regulated, include organophosphate (PFRs) and novel brominated flame retardants (NBFRs) and the rest of perfluoroalkyl substances (PFASs). Often ePOPs data relating to occurrence, toxicity, impact or environmental behavior are insufficient or inadequate because the lack of proper analytical methods to obtain them. Thus, a critical review of the analytical procedures proposed in the last six years (2011–2017) for determining ePOPs by chromatographic methods in the different compartments of the aquatic ecosystems is presented. The overall analytical procedure, from sampling to final determination is emphasized presenting recent developments in the extraction, pre-concentration, and instrumental detection methods needed for the accurate quantification of ePOPs in environmental samples. Finally, this review examines the basic challenges we face in order to anticipate future direction and urgent needs of this field.

Keywords: persistent organic pollutants; perfluoroalkyl substances, novel brominated flame retardants, organophosphorus flame retardants; aquatic ecosystem; sample treatment; extraction; analytical determination

Abbreviations

1-MP: 1-methyl-piperidine; AA: ammonium acetate; ACE: acetone; ACN: acetonitrile; AcOH: acetic acid; AF: ammonium formate; APCI: atmospheric pressure chemical ionization; APPI: atmospheric pressure photoionization; CI: chemical ionization; cLC: capillary liquid chromatography; DCM: dichloromethane; DDA: data dependent analysis; DI: direct injection; DIA: data independent analysis; dSPE: dispersive solid-phase extraction; EI: electron impact; ECNI: electron capture chemical ionization; ePOP: emerging persistent organic pollutant; ESI: electrospray ionization; EtAc: Ethyl acetate; FA: formic acid; FUSLE: focused ultrasound solid-liquid extraction; GC: gas chromatography; GCB: graphitized carbon black; GPC: gel permeation chromatography; HBCD: hexabromocyclododecane; HEX: hexane; HPLC: high performance liquid chromatography; HRMS: high-resolution mass spectrometry; HS-SPME: headspace solid-phase microextraction; ICP: inductively coupled plasma; IDA: information dependent acquisition; IMS: ion mobility mass spectrometry; LC: liquid chromatography; LDTD: laser diode thermal desorption; LLE: liquid-liquid extraction; LLP: Liquid-liquid partition; MAE: microwave assisted extraction; MeOH: methanol; MS/MS: tandem mass spectrometry; MS: mass spectrometry; MSPD: matrix solid-phase dispersion; MTBE: methyl tert-butyl ether; NBFR: novel brominated flame retardant; NCI: negative chemical ionization; nLC: nano liquid chromatography; PBDE: polybrominated diphenyl ether; PFAS: perfluoroalkyl substance; PFBA: perfluorobutanoic acid; PFBS: perfluorobutane sulfonate; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; PFP: pentafluorophenyl; PFR: organophosphate flame retardant; PLE: pressurized liquid extraction; POP: persistent organic pollutant; POSF: perfluorooctane sulfonyl fluoride; PRM: parallel reaction monitoring; PSA: primary secondary amina; QqLIT: hybrid triple quadrupole linear ion trap; QqQ: triple quadrupole; QqTOF: quadrupole time-of-flight; QuEChERS: quick, easy, cheap, effective, rugged and safe extraction method; RP: reversed phase; SEC: size exclusion chromatography; SLE: solid-liquid extraction; SPE: solid-phase extraction; SPLE: selective pressurized liquid extraction; SPM: solid particulate matter; SRM: selected reaction monitoring; TBAS: tetrabutyl ammonium hydrogen sulphate; TBBPA: tetrabromobisphenol-A; TCEP: tris(2-chloroethyl) phosphate; TCIPP: tris(2-chloroisopropyl) phosphate; TDCPP: tris(1,3-dichloro-2-propyl) phosphate; TEHP: tris(2-ethylhexyl) phosphate; TFC: turbulent flow chromatography; TMPP: tricresyl phosphate; TPhP: triphenyl phosphate; TPP: tripropyl phosphate; t-SIM: targeted selected ion monitoring; UHPLC: ultra-high performance liquid chromatography; UPC^2: ultra-performance convergence chromatograph; USE: ultrasound assisted extraction; WAX: weak anion exchange; WW: wastewater

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1. Introduction

According to the Stockholm Convention on persistent organic pollutants (POPs) [1], these compounds are resistant to chemical, biological, and photolytic environmental degradation. POPs are stable and persistent, long-distance transportable, bioaccumulative, biomagnifiable in the food chain and could pose significant impact on human health and the environment. Exposure to POPs can cause serious health problems including certain cancers, birth defects and dysfunctional immune and reproductive systems, among others. Tracing the occurrence, distribution and fate of POPs in the environment is challenging because they can occur in different phases (e.g., as a gas or attached to airborne particles) and can be exchanged among environmental compartments. Sediments can be considered as a sink of many POPs, once they are released into waterbodies, they may also come into contact with particulate matter in suspension or they can be bioaccumulated in fish and other aquatic organisms producing side effects. Initially, twelve POPs coined as the “dirty dozen” were recognized as causing adverse effects on humans and the ecosystem. These are legacy POPs, the behavior and toxicity of which are well-known and have either been banned or strictly regulated under the United Nations Environment Programme [1], the European Union [2], the United States Environmental Protection Agency and Environment Canada [3]. However, they are still found in the environment and used in some developing countries.

Currently, there is a rising concern about the presence of new organic synthetic compounds in the environment, the so-called new or emerging contaminants. In many cases, these compounds are present in the environment since long time ago but they have not been identified until the development of new and more sensitive analytical methods. Therefore, most of them are not regulated and their effects on the environment and human health are unknown. These emerging contaminants also included emerging POPs (ePOPs) that are either, very recently or not yet regulated. Polybrominated diphenyl ethers (PBDEs) and perfluorooctane sulfonyl fluoride/perfluorooctane sulfonate (POSF/PFOS) were newly added to the list of Stockholm Convention in 2009, and hexabromocyclododecanes (HBCDs) were listed as candidate POPs. ePOPs include these substances as well as several others widely used in industrial processes and consumer products, such as perfluoroalkyl substances (PFASs), non-PBDEs or novel brominated flame retardants (NBFRs), organophosphate flame

retardants (PFRs) and sort-chain chlorinated paraffins that have been proposed as a replacement alternative for banned formulations [4, 5]. **Table 1** classifies ePOPs according to their chemical structure and physico-chemical properties. These compounds have a wide range of physical-chemical properties as water solubility, polarity, volatility, etc. As a whole ePOPs exhibit properties different from legacy POPs. These new POPs are often more polar, less volatile and belong to a sort of different compound classes that come from different sources. This renders to an analytical determination much more demanding and difficult, particularly for the assessment of the aquatic ecosystems introducing a number of analytical matters that need to be solved. Moreover, ultra-trace analysis of these contaminants in aquatic environments is problematic due to the complexity and diversity of natural matrices, including biotic ones that are lipid-rich (the Achilles' heel within efficient extraction).

Due to the high amount of ePOPs, this review focuses on NBFRs, PFRs and PFASs because of their widespread use. Previous reviews on analytical aspects of these ePOPs in several matrices can be found for NBFRs (data up to 2011) [4, 6], PFRs (data up to 2008) [7] and PFASs (data up to 2012) [8, 9]. These reviews are partial, need an update or are not focused on aquatic ecosystems. One book chapter by Guo and Kannan [10] presented an overview of the methodology to analyze traditional and new POPs in environmental matrices, but the wide coverage and the higher number of studies on the formers had as a counterpart that methods related to the latter were scarce and little representative. Then, this critical review that provide a broader coverage on analytical challenges for ePOPs would be useful. In it, we outline the most recent extraction techniques, clean-up procedures and instrumental analysis of ePOPs in aquatic environment matrices published since 2011 offering a global overview of the analysis of the ePOPs. The review also discusses the advantages and disadvantages of these techniques as well as future prospects related to the extraction and determination of ePOPs.

2. Sample extraction and clean-up

Blank contamination is an important issue to take into account during the sample preparation process because of the ubiquity of ePOPs in laboratory material and equipment,

and their presence in indoor air and dust. Some strategies to avoid or reduce blank contamination as (i) heat, wrap and rinse the non-volumetric material before use, (ii) minimize surface contact during sample handling, (iii) work in a cleanroom (iv) reduced the use of plastic materials or (v) perform a pre-extraction of materials that are used have been reported [7, 11-14]. In the case of instrumental contamination, the replacement of some pieces by other fabricated with different materials as well as the insertion of a trap column before the injector are preferred solutions. Reduction of time and method steps during extraction, clean-up and evaporation processes is mandatory. In the case of water samples, direct injection (DI) is another choice to overcome any contamination through sample lab handling. However, DI seems to suffer lower sensitivity than solid-phase extraction (SPE) methods as well as a higher matrix effect [15]. Despite these precautions, signal of some analytes is still present in the laboratory blanks, and minimize it as much as possible is one of the pending issues in the analysis of ePOPs.

Current extraction and clean-up procedures for the analysis of ePOPs are summarized in **Table 2** and **Table 3** for water and any other aquatic environmental matrices, respectively, and discussed in the following sections.

2.1 Extraction and clean-up of aqueous samples

Manual or automated solid phase extraction (SPE) is the most common used strategy to concentrate and extract aqueous samples. This strategy can be versatile and adapted to the analyte characteristics. Main sorbents chosen for PFASs SPE are the weak anion-exchangers like Oasis WAX [13, 16-18] and Strata-X AW [13, 19] because these compounds are negatively charged at environmental pH. Polymeric reversed phases or hydrophilic-lipophilic sorbents as Oasis HLB [20, 21] and Strata-X [22] are also employed. PFASs are the most polar, water soluble and less volatile ePOPs. Their concentrations in water reach the ng L^{-1} level and then, SPE could be performed passing sample volumes between 100 mL and 1 L. For elute PFASs, the principal options are either methanol (MeOH) alone [16], with the addition of 0.1% NH_4OH to ensure ionization and reduce compounds' sorbent retention [17, 18, 22] or both [23].

One important trend in sample extraction framed in the principles of "green analytical chemistry" is the miniaturization of the extraction procedures. PFASs have also been

extracted by headspace solid phase microextraction (HS-SPME) after the formation of their ester derivatives to form volatile derivatives able to both, HS extraction and further gas chromatography (GC) determination. The ester derivatives are non-polar and volatile, then properly extracted by HS-SPME. This method allows a simple, automated and solvent-free extraction which is advantageous when the analysis of aqueous matrices is carried out by GC [24]. Only scarce miniaturization procedures have been reported to the moment due to the robustness of SPE and the low analyte concentrations present in water.

The other ePOPs are increasing apolar, less water soluble (almost insoluble) and more volatile, and as a consequence their concentrations in water are in the pg L^{-1} level. The low concentrations could mostly be detected using high sample volumes. For analyzing large water volumes of 10–200 L, in the case of PFRs, some non-traditional sorbents such as SERDOLITH PAC 3, an hydrophobic resin similar to Amberlite, has been used [25]. The cartridges are usually washed with ultra-pure water [16, 17, 19, 20, 25, 26]. In the case of PFRs, predominant eluents are acetonitrile (ACN) [21], dichloromethane (DCM) [25], or their mixtures [26].

Within this group of large water volume techniques, Gu et al. [27] developed a method to determine tetrabromobisphenol-A (TBBPA) and HBCDs that extracted 6 L of seawater with an automated SPE system, using styrene divinyl benzene disk (SDB-XC, 3M). Once the analytes retained, the disk was dried in an oven and the analytes eluted by pressurized liquid extraction (PLE) using a mixture of DCM and hexane (HEX) (1:1, v/v). PFRs, NBFRs and other POPs were also extracted from high water volumes (up to 160 L) using a XAD-2 resin packed on a glass column. Then, resins were extracted by Soxhlet with a mixture of HEX and acetone (ACE) (1:1, v/v) over a period of 30 h [28]. The use of so high sample volumes also conditions an increase in the potential matrix interference compounds. Then, the need of an additional clean-up step is quite common. For example, in the previous mentioned method, after extraction, matrix interferences were eliminated by passing the samples through a silica gel column eluted with HEX, HEX:DCM (1:1, v/v) and ACE:DCM (7:3, v/v). Similarly, to extract PFASs from 1 L wastewater influent, Munoz et al. 2015 [19] applied an extra clean-up step using graphite cartridges for wastewater samples.

Despite the large volumes of organic solvents required in this technique, liquid-liquid extraction (LLE) using DCM is reported in different aqueous matrices for the analysis of PFRs [25], TBBPA and their main derivatives [29]. LLE even through long and tedious successfully extract these compounds from river water, sea water and tap water samples with satisfactory recoveries.

2.2 Extraction and clean-up of biotic and abiotic solid matrices

Solid-liquid extraction (SLE) technique is the most common procedure to extract ePOPs from solid matrices such as sediment, sewage sludge, soil and solid particulate matter. SLE includes mechanical agitation [22, 30-33] and ultrasound assisted extraction (USE). Martín et al. [33] validated a multi-residue analytical method or 36 emerging pollutants including PFASs and HBCDs that was applied to marine echinoderms and sediments and consisting in mechanical agitation with ACN. USE is the most common procedure for the extraction of ePOPs from abiotic matrices [23, 34, 35] as well as in works whose methods combine both types of biotic and abiotic matrices [13, 22, 27, 36-38]. Some authors applied USE for combined extraction of PFRs and PFAS [11] or PFRs and NBFRs [39]. MeOH and acetic acid (AcOH) are the main organic solvents to extract PFASs while less polar solvents as DCM and HEX are more employed for the analysis of PFRs and NBFRs. Some authors applicate several cycles to improve the efficiency of extraction [12, 13, 23, 27, 30, 34, 36-38, 40]. However, although there are also works that employ USE [12, 40, 41], extraction procedures for biota samples also exploit other alternatives since they are the most complex matrices. These can be as simple as modified quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method —a user-friendly alternative to traditional SLE— for the extraction of PFASs from homogenized benthic invertebrates [42].

Focused ultrasound solid-liquid extraction (FUSLE) is a “green” extraction method based on the application of high power focused ultrasonic waves using a micro-tip immersed directly in the extraction mixture. It has been successfully applied for determining PFAS from sewage sludge and biota using a few milliliters of a mixture ACN:H_2O (9:1, v/v) at 0˚C [43, 44]. FUSLE demonstrated to be a fast, low-cost and efficient extraction technique.

Different solid supports or dispersing agents as diatomaceous earth [45], primary secondary amina (PSA) [46] and a mix of silica and Na_2SO_4 [47] have been used prior to SLE to break-up the sample matrix and improve the solvent-sample interaction. One format widely used to extract PFASs and NBFRs from marine biota is matrix solid-phase dispersion (MSPD) based on this principle. The homogeneous mixtures have been desorbed with different organic solvents as ACN, DCM and ACE:HEX 1:1 (v/v). Villaverde-de-Saa et al. place a layer of Na_2SO_4 and silica gel [45] or a combination of silica, acidified silica and florisil [46] to perform an in-line clean-up.

More exhaustive and continuous techniques as PLE [48], microwave assisted extraction (MAE) [49], Soxhlet [50] or their automatized version Soxtec [51] are applied for the extraction most non-polar compounds with mixtures ACE:HEX or DCM:HEX. PFRs and NBFRs have more affinity for lipids and they need more energy be extracted. PLE is widely used in biotic matrices for the extraction of PFRs and NBFRs, sample amount were lower in the extraction of biotic samples (0.2-10 g) than in abiotic ones (1-15 g). The extractions solvents comprise DCM and mixtures as ACE:HEX, ACE:DCM and HEX:DCM [37, 48, 52-55]. In the case of sediment, selective pressurized liquid extraction (SPLE) that includes an in-line clean-up step within the stain steel cell can be applied. However, this methodology could not be applied for biota because it requires a more complex clean-up and lipid content determination [48].

Analysis of ePOPs in solid matrices requires the removal of particulate matter and co-extracted matrix compounds that can interfere in their determination, especially if liquid chromatography (LC) and mass spectrometry (MS) or tandem MS (MS/MS) is used. Clean-up strategies are also detailed in **Table 3**. In solid sample extracts, SPE has been used as the main clean-up strategy [11, 13, 22, 23, 31, 34, 36, 39, 43, 44, 48, 52, 55]. As for water samples extraction, in the case of PFASs the most used sorbents are the weak anion-exchangers [23, 31, 43, 52] and polymeric reversed phases [22, 34, 36]. Couderc et al. [31] applied SPE with WAX stationary phase followed by a second SPE composed of graphitized carbon black (GCB). GCB is considered both a reverse phase and anion-exchanger sorbent due to its structure and somewhat positively charged surface. Cavaliere et al. [11] use GCB to retain from nonpolar to very polar endocrine-disrupting compounds, that include PFASs and PFRs. Neutral and very weak acids are eluted with a small volume of a mixture of DCM:MeOH. Weak and strong acids

are eluted with DCM:MeOH but with formic acid (FA) or ammonium formate (AF), respectively. Zacs and Bartkevics [13] evaluate four SPE cartridges with weak anion-exchange properties to retain perfluorooctanoic acid (PFOA) and PFOS from environmental samples. Enviro-Clean CUPSA cartridges show less effectiveness in comparison with Oasis WAX, Presep PFC-II and Strata X-AW SPE columns. They also applied the SPE cartridge in combination with SPE column filled with GCB to improve the clean-up of the extracts. Although significant changes in terms of signal suppression or enhancement are not reported, analytes, chromatographic peak shape and stability of retention times were observed.

PFRs and NBFRs are mostly retained in lipids, and polar sorbents in the normal phase, such as silica gel [27, 47, 49-51, 54, 56, 57], alumina [55] and florisil [35, 39, 53] are employed alone or in combination [12, 48] for the removal of these lipids from biotic matrices. The most common elution solvent is the mixture of HEX:DCM [35, 50, 55, 56]. Several authors use 2-3 elution fractions and combine the extracts [49, 51, 54, 57].

Size exclusion chromatography (SEC) or gel permeation chromatography (GPC) can be applied to reduce matrix interferences in biota samples previous to PFR and NBFR analysis [54]. Styrene divinylbenzene copolymer are the most commonly columns (e.g. Bio-Beads S-X3). The disadvantages of GPC are its high solvent-consumption and inability to remove lipid-related substances (additional clean-up with Florisil [35], silica gel [49] or multilayer silica column [51] is required).

A more “green” clean-up technique suitable for lipid-rich matrices is dispersive solid-phase extraction (dSPE) based on the addition of sorbent material into an extract aliquot. Different dSPE sorbents tested for the isolation of PFRs from fish samples showed that the highest efficiency was obtained by ENVI-Carb dSPE, however, this cartridge adsorbed some benzene ring-containing PFRs [e.g. triphenyl phosphate (TPhP) and tricresyl phosphate (TMPP)]. On the other hand, PSA bonded silica (100, 200 and 300 mg) showed high recoveries for all PFRs and acceptable efficiency of adsorption of lipids from fish [40]. dSPE after mechanical agitation was used to the clean-up of marine biota and marine sediment by using 800 mg of C18 sorbent [33].

New techniques as turbulent flow chromatography (TFC) are used for on-line sample pretreatment. This technique uses high solvent flow rates (4-6 mL min^{-1}) through columns packed with large particle size sorbents. TFC is a useful technique to discard large sample compounds such as proteins, peptides and lipids. Once analytes are trapped on the turbulent flow column they are subjected to a back-flushing elution that desorb them to the analytical column for chromatographic separation. TFC has been used mainly in studies involving the extraction of biota samples [22, 37, 41].

3. Determination

To detect and get an accurate quantification of ePOPs in the environmental samples, compounds must be isolated from each other. Because of their higher sensitivity, selectivity and efficiency, chromatographic based separations, especially those using GC and high-pressure LC are clearly the most widely used for ePOPs separation. **Tables 2** and **3** overview the most relevant determination techniques from 2011 onwards.

3.1 Liquid chromatography-mass spectrometry

Most of the analytical methods to determine PFASs and PFRs in environmental samples are based on LC coupled to MS. Reversed phase LC is usually employed for the ePOPs separation. Bonded silica (C18) is the preferred non-polar stationary phase column. Few studies use high-density diol [17], C8 [58], hydrophobic alkyl chain with diol called mixed-mode HILIC-1 [26] and pentafluorophenyl (PFP) [41]. Regarding the mobile phase composition, water is usually employed as base and polar solvents such as MeOH, ACN and their mixtures are added in fixed or varying proportions. 1-Methyl-piperidine (1-MP), ammonium acetate (AA), AF and FA are the most common buffer components added to the mobile phase to improve peak shapes [36].

Recent advances related to separation were also implemented within the field of ePOPs determination, the application of capillary LC (cLC) and nano LC (nLC) [42, 59] to the determination of PFASs in water as well as the application of the new supercritical fluid columns (not exactly LC but related) developed for these compound separation are already a reality [17].

As already mentioned, one important issues background contamination coming from the LC system. Several authors applied a trap column on the high performance LC (HPLC) system in order to distinguish background contamination coming from the equipment to that of the samples [36]. Trap columns used to distinguish the PFRs in the sample of those coming from the instrument also required washing and blank solvent injections before use to remove residual contamination [11]. Capillaries and tubes of the HPLC system that can contain PFASs or PFRs are provisionally replaced when possible [13].

Most used source is electrospray ionization (ESI) as the technique for identification of ePOPs and only few studies apply atmospheric pressure chemical ionization (APCI) to analyze PFRs [40] and atmospheric pressure photoionization (APPI) to analyze NBFRs [51]. Zacs and Bartkevics [13] analyze PFOA and PFOS in various environmental samples and evaluates ESI, APCI and APPI. Although ESI systems are more susceptible to matrix interferences in comparison with APCI and APPI techniques, ESI was chosen for the sample ionization because it provides the highest instrumental sensitivity.

Among the different mass analyzers available, LC coupled to triple quadrupole (QqQ) MS/MS is considered one of the most used detection and quantification techniques for ePOPs [11, 16, 18, 22, 23, 26, 27, 30, 31, 33, 34, 36-38, 40, 41, 43, 45, 52]. It should be taken into account that the low environmental concentrations of these compounds make sensitivity one of the most important parameters to detect these compounds and this mass analyzer achieves the lowest limits of detection.

However, MS/MS sometimes shows inadequate selectivity and some authors propose the use of a high resolution MS (HRMS) technique, quadrupole time-of-flight (QqTOF)-MS or Orbitrap-MS, as an alternative tools for (ultra-)trace analysis of PFASs and NBFRs in complex environmental matrices [13, 19, 38, 51]. **Fig. 1** shows the identification of perfluorobutanoic acid, one of the compounds for which QqQ is not selective enough since it only produces one product ion [60]. HRMS provides accurate mass and then, unequivocal identification. MS and MS/MS data acquisition in HRMS can be in data dependent or independent analysis (DDA or DIA). Zacs et al. [51] also prove the efficiency of three different detection modes: full scan, targeted selected ion monitoring (t-SIM), and DIA using the parallel reaction monitoring (PRM) mode (similar to MS^E depending on the branch of instrument). They found high

background noise and significant interference on the chromatograms when analyze complex matrices by using t-SIM and full scan modes and then selected PRM mode. On the contrary, the same authors report that PRM mode is less sensitive when analyze NBFRs in fish samples [51]. Except for two studies that use full scan acquisition [18, 51], the rest of them use selected reaction monitoring (SRM).

Although separation by LC or GC followed by MS detection are the most widespread detection mode, there are some special cases, such as TBBPA and its derivatives are not properly determined neither by GC-MS (thermolability) or LC-MS (lack of analyte ionization in the source). Liu et al. [29] established a new and sensitive method for these compounds by coupling HPLC separation with inductively coupled plasma (ICP)-MS detection technique. In comparison with the traditional quantification methods, such as MS/MS or DAD detector, the ICP-MS has obvious advantages in sensitivity for the quantification of TBBPA and their derivatives, especially TBBPA-BAE and TBBPA-BDBPE. **Fig. 2** shows the proper peak shape and sensitivity obtained for these compounds.

Furthermore, the evolution of MS interface coupled to LC has bring a new direct analysis, that is able to determine the compounds directly in the extract eliminating the LC separation has already been tested. PFASs were already successfully determined by laser diode thermal desorption method (LDTD) [19]. Other novelties within the field of MS coupled to LC such as the incorporation of ion mobility mass spectrometry (IMS) were not reported. This techniques would be little useful for the most non-polar ePOPs. However, it can have prospects to determine PFASs since they are ionic substances.

3.2 Gas chromatography-mass spectrometry

The selection of GC instead of LC depends on the analyte properties, PFR and NBFRs are commonly determined using gas chromatography-mass spectrometry (GC-MS) because they are thermostable and volatile. However, PFASs commonly required derivatization because even that they are volatile enough to be chromatographed by GC, their ionic nature provide wide and tailing peak difficult to quantify. A rapid and sensitive determination of perfluorocarboxyl acids in aqueous matrices by GC-QqQ-MS was achieved transforming the carboxylic groups in their esters [24].

In GC, electron ionization (EI) has been described as the most commonly used GC technique for the identification of any type of substance. However, ePOPs are more labile than legacy ones and then, softer ionization techniques are preferred, for example electron capture negative chemical ionization (ECNI) is commonly used for the analysis of NBFRs [28, 39, 50, 54, 55]. Recently, the new combination of GC with atmospheric pressure chemical ionization sources (APCI) has open a new horizon within the analysis of these compounds [20, 47, 57].

Portolés et al. [20] compare both EI and chemical ionization (CI) modes and they found that EI yielded highly fragmented mass spectra with the absence of the molecular ion. They also performed separation with GC-QTOF and GC-QqQ operating in APCI mode for the analysis of PFASs precursors. The same authors also tested these three interfaces for the analysis of NBFRs obtaining similar results [47].

It is noteworthy within this field, that recent advances within GC separation, as GCxGC or fast GC have not been tested yet. One probable reason for this behavior is that the main problem due to the thermolability of ePOPs in comparison with traditional ones was the low sensitivity due to the high fragmentation obtained by EI. Once this problem is solved by application of the new APCI interface, it is expected that new methods involving these innovative trends will be developed.

4. Conclusion and future prospects

Additional problem in quantitation of ePOPs is that they occur at very low concentration level (sub-ng L^{-1} range) in water, what means that a highly sensitive method is needed to their accurate quantitation. This issue moves to work with high sample volume and as a consequence, the method usually require time consuming and expensive clean-up before analysis in order to achieve the high level of sensitivity required. The abiotic a biotic environmental matrices in which ePOPs are commonly examined are often complex. These matrixes such as sediments, soils and biota are problematic because usually contain interfering compounds such as humic and fulvic acids, organic matter, lipids, proteins, pigment, etc. (depending on the sample). Hence, the sample preparation and extraction of ePOPs is time consuming. It also means that these compounds need efficient separation and retention away from interfering compounds, which can cause further problems with

detection. Since LLE and SPE require higher volumes of eluting solvents and are time consuming, extractions based on SPME have gained increased attention. However, the need for complete and exhaustive sample preparation is still a great challenge to provide promising efficiency, accuracy, and precision for a wide range of analytes and microextraction approaches are still scarce. Much work needs still to done to adapt ePOPs extraction to the ongoing trends of “green methods” more ecofriendly.

MS techniques have turned into indispensable tools for **ePOPs determination in aquatic ecosystems**, due to its high accurate mass determination for compositional analysis, the ability of MS/MS fragmentation techniques for structural identification, and the possibility to couple with separation techniques like LC or GC. Recently, a promising methodology to **quantify brominated ePOPs** has also been published combining **LC, ICP and MS**. The particularity and somewhat less non-polar character of ePOPs in comparison to legacy POPs has marked that many of the determinations apply LC and that, in the case of GC the implementation of interfaces that produce a soft fragmentation of molecules such as CI and APCI is favored. In this regard, the recent developed alternative techniques to LC, GC and their combination with MS, such as multidimensional approaches (including LC×LC and GC×GC), IMS or direct analysis techniques could offer complementary selectivity and thus, information that would help to increase the ePOPs coverage. These innovative techniques are not well implemented yet. However, it is expected that examples start to appear in a recent future.

Acknowledgements

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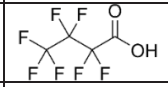
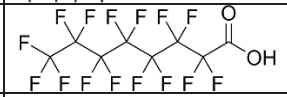
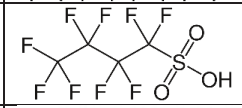
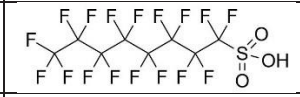
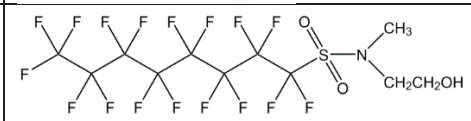
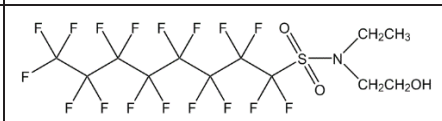
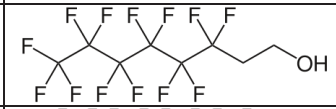
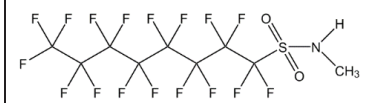
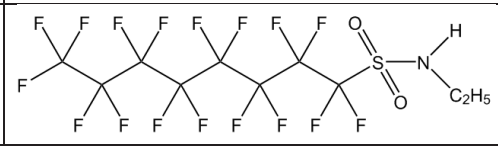
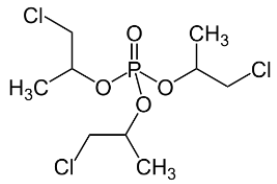
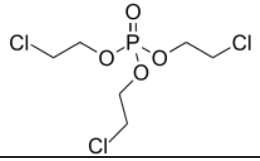
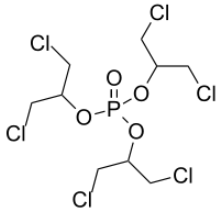
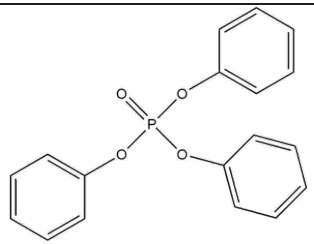
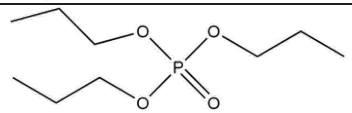
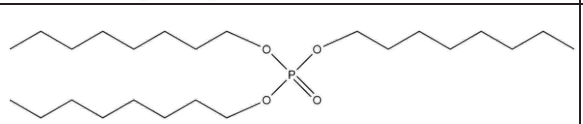
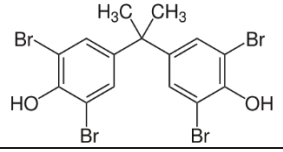
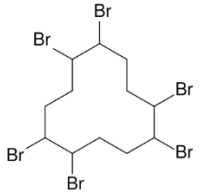
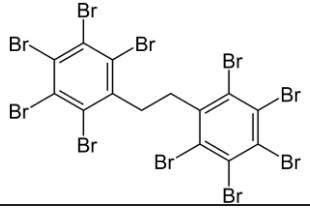
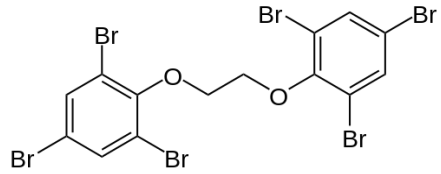
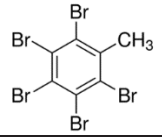
Table 1. Chemical structure and physico-chemical properties from selected ePOPs.

Family name	Category	Main examples	Structure	Log K_{OW}	pK_a
Perfluoroalkyl substances (PFASs)	Perfluoroalkyl carboxylic acids (PFCAs)	Perfluorobutanoic acid (PFBA)		-0.62 [(a)]	<1.6 [(b)]
		Perfluorooctanoic acid (PFOA)		1.79 [(a)]	0.5 [(b)]
	Perfluoroalkyl sulfonic acids (PFSAs)	Perfluorobutane sulfonate (PFBS)		0.14 [(a)]	<0.3 [(b)]
		Perfluorooctane sulfonate (PFOS)		2.56 [(a)]	<0.3 [(b)]
	Perfluoroalkyl sulfonamide ethanols (FOSEs)	2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (N-MeFOSE)		3.81 [(a)]	Not available
		2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (N-EtFOSE)		4.17 [(a)]	Not available
	Fluorotelomer alcohols (FTOHs)	6:2 FTOH		1.62 [(a)]	Not available
		8:2 FTOH		2.82 [(a)]	Not available
	Perfluoroalkyl sulfonamides (FOSAs)	N-methylperfluoro-1-octanesulfonamide (N-MeFOSA)		3.17 [(a)]	Not available
		N-ethylperfluoro-1-octanesulfonamide (N-EtFOSA)		3.53 [(a)]	Not available

Organophosphate flame retardants (PFRs)	Halogenated	Tris(2-chloroisopropyl) phosphate (TCIPP)		2.59 (c)	Not available
		Tris(2-chloroethyl) phosphate (TCEP)		1.44 (c)	Not available
		Tris(1,3-dichloro-2-propyl) phosphate (TDCPP)		3.3 (c)	Not available
	Non-halogenated	Triphenyl phosphate (TPhP)		4.59 (c)	Not available
		Tripropyl phosphate (TPP)		1.89 (c)	Not available
		Tris(2-ethylhexyl) phosphate (TEHP)		9.49 (c)	Not available

Novel brominated flame retardants (NBFRs)	Tetrabromobisphenol-A and derivatives	TBBPA		9.69 (c)	7.5-8.5 (c)
	Hexabromocyclodecanes	HBCD		7.92 (c)	Not available
	Others	Decabromodiphenyl ethane (DBDPE)		11.1(c)	Not available
		1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE)		8.31(c)	Not available
		Pentabromotoluene (PBT)		6.25(c)	Not available

Data obtained from (a) [61], (b) [62], (d) [63].

Table 2. Sample extraction techniques from water samples.

Matrix	Analytes	Extraction	Clean-up	Separation	Determination	Recoveries	Sensitivity (ng L^{-1})	Ref.
WW and surface water (200 mL)	PFASs (n=2)	SPE Enviro-Clean CUPSA, Oasis WAX, Presep PFC-II and Strata-X-AW Wash: FA and MeOH Elution: MTBE:MeOH 1% NH_4OH 9:1	------	Kinetex-C18 reversed-phase (50 × 3.0 mm, 1.7 µm) ACN (10 mM AF) and MeOH/ACN (1:1) Gradient, 0.3 mL min^{-1}	HPLC-(ESI)Orbitrap-MS in PRM	WW: 99-116% Surface: 98-111%	0.1-0.5 [a]	[13]
WW, surface water, ground and drinking	PFASs (n=9)	Ultracentrifugation 30,000 xg Direct injection	------	Waters Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 µm) and C18 (100 × 4.6 mm) H_2O:MeOH (2 mM AA) 95:5 and MeOH (2mM AA) Gradient, 0.5 mL min^{-1}	UHPLC-MS/MS in SRM	Not reported	0.014-0.44 [b]	[15]
WW effluent	PFASs (n=52)	SPE Oasis WAX 60 mg Wash: MeOH, MeOH (0.1% NH_3), H_2O Elution: MeOH	------	MZ-Aqua Perfect C18 (50 × 2.1 mm, 5 µm) MeOH:H_20 5:95 (5 mM AA) and 95:5 MeOH:H_20 (5 mM AA) Gradient, 0.3 mL min^{-1}	HPLC-(ESI)MS/MS(QTrap-QqQ) in scheduled SRM	53-149%	100-1000 [b] 200-20000 [c]	[16]
Surface water (200 mL)	PFASs	SPE OASIS WAX-SPE cartridge (150 mg) Wash: 0.1% NH_4OH, MeOH and H_2O Elution: MeOH (0.1% NH_4OH)	------	C6-C14 PFASs: Waters Acquity BEH C18 (2.1 × 75 mm, 1.7 µm) H_2O (2 mM AA) and MeOH C2-C14 PFASs: Waters Acquity UPC2Torus DIOL (3.0 mm × 150 mm, 1.7 µm) supercritical CO_2 and 0.1% NH_4OH in MeOH Gradient, 1.3 mL min^{-1}	C6-C14 PFASs: UPLC-MS/MS C2-C14 PFASs: UPC^2-MS/MS	84-96%	200-500 [c]	[17]
Surface water (200 mL)	PFASs (n=8)	SPE Oasis WAX Wash: MeOH and 25mM AcOH:AA Elution: MeOH 0.1% NH_4OH	------	ACQUITY UPLC C18 (100 x 2.1 mm, 1.7 µm) H_2O (1 mM AA) and MeOH (1 mM AA) Multi-step gradient elution, 0.3 mL min^{-1}	LC-(ESI)Orbitrap Tribrid HRMS in full scan MS^1/dd-MS^2 and	63-103% (Orbitrap)	Orbitrap: 0.007-0.06 [b] QqQ: 0.01–0.05 [b]	[18]

					LC-(ESI)MS/MS(QqQ) in SRM			
WW and surface water (0.25-1 L)	PFASs (n=15)	SPE Strata X-AW Wash: H_2O Elution: 2x MeOH: H_2O (NH_4OH 0.1%)	Graphite cartridges (WW)	Deposition solvent: EtAc Flow rate 2 L min^{-1}	LDTD/APCI-HRMS(Orbitrap) in full scan	38-106% (WW)	WW: 0.3-4 Surface water: 0.03-0.2	[19]
Surface water and WW (1 L)	PFASs precursors (n=8)	SPE Oasis HLB (500 mg) Wash: MeOH:H_2O 5:95 Elution: EtAc	------	TraceGold TG-WaxMS (30 m × 0.25 mm, 0.25 µm)	GC-(APCI)MS/MS(QqQ) in SRM mode GC-(APCI)QTOF-MS in SRM mode GC-(EI and CI) EI and CI compared and EI selected	80-97%	1-5 fg [(d)]	[20]
Environmental water (200 mL)	PFRs (n=12)	Filtered and SPE Oasis HLB Wash: ACN Elution: ACN	------	Waters BEH C8 (2.1 mm × 50 mm, 1.7 µm) H_2O (0.1% FA) and ACN (0.1% FA) Gradient, 0.2 mL min^{-1}	LC-(ESI)MS/MS(QqQ) in SRM	40-110%	2-6 [(b)]	[21]
Surface water (250 mL)	PFASs (n=21)	SPE Strata-X 200 mg Elution: MeOH (0.1% NH_4OH)	------	Kinetex XB-C18 (50 x 4.6 mm, 1.7 µm) H_2O (10 mM AF):MeOH (10 mM AF) Gradient, 0.2 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM	55-94%	0.01-2.00 [(c)]	[22]
Surface water (250 mL)	PFASs (n=29)	SPE Strata-X AW 200 mg Wash: H_2O Elution: MeOH, MeOH (0.1% NH_4OH) and DCM: C_3H_8O (0.1% NH_4OH) 7:3	------	Waters Acquity BEH C18 (2.1 × 50 mm, 1.7 µm) H_2O (2 mM AA):MeOH 9:1 and MeOH, Gradient, 0.65 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM	15-187%	4-10 [(c)]	[23]
Surface water	PFASs (n=7)	Derivatization with phosphate buffer, pyridine, propanol and propyl chloroformate to form the esters. HS-SPME Carboxen/polydimethylsilo	------	SPME variables optimization: Varian VF-5MS (30 m × 0.25 mm, 0.25 µm) Calibration and quantification analyses:	SPME variables optimization: GC-(EI)MS(ion-trap)/MS in full scan	81.5-123.7%	0.08-6.6 [(b)] 0.17-14.3 [(c)]	[24]

		xane (CAR/PDMS) fiber, 10 min, room temperature		Thermo TR-5MS (30 m × 0.25 mm, 0.25 µm)	Calibration and quantification analyses: GC-(NCI)MS/MS(QqQ) in SRM			
Surface water (250 mL)	PFAS (n=21)	SPE Strata-X 200 mg Elution: MeOH (0.1% NH_4OH)	------	Poroshell EC-C18 (50 x 30 mm, 2.7 µm) Water (10 mM AF) and MeOH (10 mM AF) Gradient, 0.3 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM and UHPLC-(ESI)QqTOF-MS in IDA	QqTOF-MS: 67-99%	QqTOF-MS: 0.1-50 [c]	[60]
Drinking water (intake of treatment plant and treated) (250 mL)	PFASs (n=7)	Automatic SPE Elution: EtAc:isobutanol 9:1 Derivatization with isobutyl chloroformate:isobutanol	------	DB-5 MS (30 m × 0.25mm, 0.25 µm)	GC-MS(q) in SIM	94-98%	0.1-0.5 [b]	[64]
Surface water	PFRs (n=13)	Dissolved phase: SPE SERDOLITH PAC 3 (500 mg) Wash: H_2O Elution: 5x DCM or LLE 2x DCM Particulate phase: USE 2x DCM 15 min	------	HP-5MS (30 mm x 0.25 mm, 0.25 µm)	GC-(EI)MS/MS(QqQ) in SRM	45-77%	2.7-26.6 [b]	[25]
Surface water	PFRs (n=14)	SPE Envi-18 (500 mg) Wash: H2O Elution: DCM:ACN 1:3	------	Acclaim mixed-mode HILIC-1 (2.1 × 150 mm, 5.0 µm) H_2O and ACN Gradient, 0.25 mL min^{-1}	HPLC-(ESI)MS/MS(QqQ) in SRM	58.6-116.2%	0.3-16 [b] 1-35 [c]	[26]
River water (160 L)	PFRs (n=15) and NBFRs (n=8)	Particle phase: Filtration fiber filter (0.7 µm) Dissolved phase: XAD-2 resin glass column	Silica gel column Elution: HEX, HEX:DCM	DB-5MS Ultra Inert (30 m x 0.25 mm, 0.25 µm) for PFRs and RTX-1614 (15 m x 250 µm, 0.1 µm)	GC-(EI)MS(q) for PFRs and GC-(ECNI)MS for NBFRs	PFRs: 57-117% NBFRs: 71-86%	PFRs: 0.02-0.12 NBFRs: 0.00007-0.002	[28]

		XAD-2 and filters extracted by Soxhlet 30 h/800 mL HEX:ACE 1:1	1:1 and ACE:DCM 7:3					
Seawater (6 L)	NBFRs (n=4)	Automated SPE SDB-XC disks, 55˚C, 10 min Disks eluted by PLE DCM:HEX 1:1	------	Reverse-phase column C18 (4.6 mm × 150 mm, 1.8 µm) MeOH and 10 mM AA Gradient, 0.3 mL min^{-1}	LC-(ESI)MS/MS(QqQ)	58-91% (internal standard)	0.03-0.09 (b)	[27]
Seawater, tap water and surface water	NBFRs (n=7)	Filtration (0.45 µm membranes) and LLE 3x DCM	------	ZORBAX Eclipse Plus C18 (150 × 4.6 mm, 5 µm) MeOH (0.1% AcOH) and H_2O (0.1% AcOH) Gradient, 1 mL min^{-1}	HPLC-ICP-MS/MS	67.7-112%	0.71-1.16 (b)	[29]

(a) Method reporting level, (b) method limit of detection, (c) method limit of quantification, (d) instrumental limit of quantification

Table 3. Sample extraction techniques from solid biotic and abiotic matrices

Matrix	Analytes	Extraction	Clean-up	Separation	Determination	Recoveries	Sensitivity (ng g^{-1})	Ref.
Biota, sediment and sewage sludge (0.5 g)	PFASs (n=2)	USE 2x (MeOH and 0.2 M NaOH) 15 min	SPE Enviro-Clean CUPSA, Oasis WAX, Presep PFC-II and Strata-X-AW Wash: FA and MeOH Elution: MTBE:MeOH 1% NH_4OH 9:1	Kinetex-C18 (50 × 3.0 mm, 1.7 µm) ACN (10 mM AF) and MeOH/ACN (1:1) Gradient, 0.3 mL min^{-1}	HPLC-(ESI)MS(Orbitrap) in PRM	Sediment: 98-116% Sludge: 88-98% Fish: 97-109%	0.04-0.12 [a]	[13]
Sediment (5 g) and fish (2 g)	PFASs (n=21)	Sediment: Mechanical agitation and USE AcOH and MeOH Fish: Alkaline digestion (MeOH + NaOH)/4 h + DI	Sediment: SPE (250 mL) Strata-X 200 mg Elution: MeOH (0.1% NH_4OH) Fish: TFC online enrichment with C18 (2.1 x 20 mm, 12 µm)	Sediment: Kinetex XB-C18 (50 x 4.6 mm, 1.7 µm) H_2O (10 mM AF):MeOH (10 mM AF) Gradient, 0.2 mL min^{-1} Fish: Hypersil Gold PFP (50 x 3 mm, 5 µm) Water (20 mM AA) and MeOH (20 mM AA) No linear gradient, 0.4 mL min^{-1}	Sediment: UHPLC-(ESI)MS/MS(QqQ) in SRM Fish: TFC-LC-(ESI)MS/MS(QqQ) in SRM	Sediment: 44-100% Fish: 16-135%	Sediment: 0.04-8.00 [c] Fish: 0.02-2.26 [c]	[22]
Sediment, soil and sludge (0.1-1 g)	PFASs (n=29)	USE 2x (MeOH:1% AcOH 9:1, vortex, sonicated 15 min/60˚C)	SPE Strata-X AW 200 mg Wash: H_2O Elution: MeOH, MeOH (0.1% NH_4OH and DCM:2-propanol (0.1% NH_4OH) 7:3	Waters Acquity BEH C18 (50 x 2.1, 1.7 µm) and Acquity BEH C18 (2.1 × 5 mm, 1.7 µm) H_2O (2 mM AA):MeOH 9:1 and MeOH Gradient, 0.65 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM	Sediment/soil: 60-108% Sludge: 57-114%	Sediment/soil: 2 [c] Sludge: 20 [c]	[23]
Sediment (1 g)	PFASs (n=21)	Mechanical agitation 2x (ACN/0.2M NaOH 3:1)	LLP ion-pair extraction TBAS + 2x MTBE	Restek Ultra C18 (50 x 2.1 mm, 3 µm) H_2O (10 mM AA) and MeOH (10 mM AA) Gradient, 0.25 mL min^{-1}	LC-(ESI)MS/MS(QTrap-QqQ) in SRM	73-120%	0.004-0.2 [b]	[30]
European eel (1 g muscle)	PFASs (n=17)	Mechanical agitation 15 min MeOH (0.01 M KOH)	SPE WAX stationary phase (150 mg) Wash: AA and MeOH Elution: MEOH:NH_4OH 99.5:0.5 and 2nd SPE GCB Envicarb (500 mg)	Gemini C18 reverse phase (50 × 2.0 mm, 3 µm) MeOH and H_2O (20 mM AA) Gradient, 0.6 mL min^{-1}	LC-(ESI)MS/MS(QqQ) in SRM	65-125%	0.006-1.259 [c]	[31]

			Elution: MeOH:glacial AcOH					
Sediment	PFASs and isomers/ enantiomers (n=9)	Mechanical agitation ACN:H_2O 3:2	Derivatization of non-volatile PFASs with diazomethane	Columns in tandem: DB-5MS (30 m × 0.25 mm, 0.25 µm) and BGB-172 Analytik chiral (30m × 0.25 mm, 0.25 µm)	GC-(NCI)MS	Not reported	Not reported	[32]
Soil and sediment (5 g)	PFASs (n=20)	USE MeOH 3x 15 min	SPE Strata-X 33 µm 200 mg Wash: MeOH (0.1% NH_4OH), MeOH, H_2O Elution: MeOH (0.1% NH_4OH)	Kinetex-C18 (50 × 2.1 mm, 1.7 µm) H_2O (10 mM AF) and MeOH (10 mM AF) Gradient, 0.2 mL min^{-1}	UHPLC-(ESI) MS/MS(QqQ) in SRM	Sediment: 69-103% Soil: 70-109%	Sediment: 0.02-0.45 (c) Soil: 0.01-6 (c)	[34]
Mollusk (1-10 g)	PFASs (n=11)	USE H_2O:ACN 1:9 + FA 15 min	Online TFC Columns: Thermo Fluoro XL, (50 × 0.5 mm) and Thermo Cyclone (50 × 0.5 mm)	Thermo Hypersil GOLD PFP (50 × 2.1 mm, 1.9 µm) Mobile phases not reported Isocratic, 2 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM	40-133%	0.03-0.3 (b) 0.1-0.9 (c)	[41]
Benthic invertebrates (12-20 mg)	PFASs (n=2)	Modified QuEChERS 0.5 mL ACN + 0.5 mL H_2O + 0.2 mL HEX + citrate buffer	------	C18 PepMap 100 (15 cm × 75 µm, 3 µm) H_2O (0.1 mM AA) and ACN:MeOH:H_2O (0.1 mM AA) 45:45:10 Gradient, 0.3 mL min^{-1}	NanoLC-(nanoESI)MS/MS(QqLIT) in SRM	92-109%	1.4-3.5 (c)	[42]
Biota (0.5 g)	PFASs and precursors (n=24)	FUSLE ACN:H_2O 9:1/2.5 min/10% power/0°C ice water bath	SPE Evolute-WAX (200 mg) Wash: FA (2%) and H_2O:MeOH 95:5 Elution: ACE 0.1% NH_4OH	ACE UltraCore 2.5 Super C18 (2.1 mm × 50 mm, 2.5 µm) H_2O:MeOH (95:5) and MeOH:H2O 2 mM AA and 5 mM 1-MP Gradient, 0.3 mL min^{-1}	HPLC-(ESI)MS/MS(QqQ) in SRM	Liver: 66-111% Mussel: 77-119% Muscle: 83-146%	Liver: 0.1-4.1 (b) Mussel: 0.1-3.8 (b) Muscle: 0.2-3.2 (b)	[43]
Sewage sludge (0.5 g)	PFASs (n=7)	FUSLE ACN/2x 20 s/pulsation 0.5/65% power/0°C ice water bath	PLE MeOH; 2 cycles; 1 min; 70˚C	Waters Acquity BEH C18 (50 x 2.1, 1.7 µm) ACN (0.1% FA) and H_2O (0.1% FA) Gradient, 0.45 mL min^{-1}	UPLC-(ESI)MS/MS(QTOF)	69-104%	Not reported	[44]

Mollusk (0.5 g)	PFASs (n=8)	MSPD Diatomaceous earth Elution: ACN	Na_2SO_4 + silica	Ascentis Express C18 (50 × 2.1 mm, 2.7 µm) H_2O (5 mM AA) and MeOH (5 mM AA) Gradient, 0.4 mL min^{-1}	LC-(ESI)MS/MS(QqQ) in SRM	64-126%	0.05-0.3 [b] 0.2-1.0 [c]	[45]
Sediment and fish (2 g)	PFASs (n=21)	Sediment: USE 1st H_2O (1 % AcOH), 2nd MeOH ((1 % AcOH) and 3rd H_2O (1 % AcOH) Fish: Alkaline digestion (MeOH + NaOH)	SPE Strata-X 200 mg Elution: MeOH (0.1% NH_4OH)	Poroshell EC-C18 (50 x 30 mm, 2.7 µm) Water (10 mM AF) and MeOH (10 mM AF) Gradient, 0.3 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM and UHPLC-(ESI)QqTOF-MS in IDA	Sediment: 62-100% Fish: 60-95% (QqTOF)	Sediment: 0.1-2 Fish: 0.01-5 (QqTOF)	[60]
Sediment (2 g)	PFASs (n=11) and PFRs (n=2)	USE ACE:MeOH 1:1 30 min and agitation/3h	SPE GCB cartridge Elution: DCM:MeOH 4:1 (10mM AF)	Hypersil Gold (50 × 2.1 mm, 1.9 µm) H_2O and MeOH Gradient, 0.3 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM	75-110%	0.04-2.4 [b] 0.14-7.9 [c]	[11]
Fish (0.25 g)	PFRs (n = 16)	USE ACE:HEX 1:1 2x 15 min	Scavenging tandem basic alumina (5 g) and C18 (2 g) cartridges Wash: ACN Elution: ACN	Purosphere Star RP-18 (125 mm × 2.0 mm, 5 µm) H_2O (0.1% FA) and MeOH (10 mM AA) Gradient, 0.25 mL min^{-1}	LC-MS/MS(QqLIT) in SRM	48-113%	0.3-51.6 [b] 1.12-172 [c]	[12]
Soil and fish (1 g)	PFRs (n=9)	USE MeOH 3x 15 min	SPE Strata-X 33 µm 200 mg Wash: DCM:MeOH 1:1, MeOH, H_2O Elution: MeOH	Kinetex-C18 (50 × 2.1 mm, 1.7 µm) H_2O (0.1% FA) and MeOH (0.1% FA) Gradient, 0.2 mL min^{-1}	UHPLC-(ESI)MS/MS(QqQ) in SRM	Soil: 50-121% Fish: 47-123%	Soil: 0.01-0.1[b] Fish: 0.01-0.15 [b]	[36]
Sediment (1 g) and fish (0.5 g)	PFRs (n=14)	Sediment: PLE (1 g) ACE:HEX 1:1 Fish: USE (0.5 g) ACE:HEX 1:1 2x 15 min	Online TFC Columns: CycloneTM-P (0.5x50mm) and C18-XL (0.5×50 mm) Solvents: H_2O (0.1% FA) and MeOH (AA)	Purosphere Star RP-18 (125 mm × 2.0 mm, 5 µm) H_2O (0.1% FA) and MeOH (0.1% FA) Gradient, 0.25 mL min^{-1}	LC-(ESI)MS/MS(QqQ) in SRM	Sediment: 47-108% Fish: 47-98%	Sediment: 0.02-1.25 [b] 0.05-3.44 [c] Fish: 0.44-19.3 [b] 0.97-24.8 [c]	[37]

Lipid-rich biota (applied in fish) (1 g)	PFRs (n=13)	USE 2x (DCM:HEX 1:1) + NaCl + $MgSO_4$ 10 min	dSPE: PSA 100 mg, PSA 200 mg, PSA 300 mg, Z-Sep+ 300 mg, DSC-18 300 mg and ENVI-Carb 300 mg	Waters Cortecs UPLC C18 (2.1 x 50 mm, 1.6 µm) coupled with ACQUITY UPLC in-line filter kit. H_2O and MeOH Gradient, 0.5 mL min^{-1}	UHPLC-(APCI+)MS/MS(QqQ) in SRM	Egg: 54-108% Liver: 66-113%	Egg: 0.02-0.13 [(b)] 0.06-0.29 [(c)] Liver: 0.03-0.14 [(b)] 0.05-0.5 [(c)]	[40]
Fish (1 g)	PFRs (n=14)	MAE HEX:ACE 1:1; 1200 W; 130˚C; 20 min	GPC Bio-Beads S-X3 Elution: DCM:HEX and Silica gel column (4 g) Elution: 1st fraction: HEX, 2nd fraction: DCM/HEX 3:7, 3rd fraction: ACE:EtAc 1st, 2nd and 3rd fractions combined	DB-5 MS column (30 m × 0.25 mm, 0.25 µm)	GC-(EI)MS(q) in SIM	39-105%	0.006-0.021 [(b)]	[49]
Sediment and fish (200 mg lipid)	PFRs (n=9)	PLE DCM:ACE 1:1; 3 cycles; 70˚C	SPE NH_2 cartridge (500 mg) Washed: DCM and HEX Eluted: DCM:HEX 8:2 and DCM	Luna C18 (150 mm × 3 mm, 3 µm) Mobile phases not reported	HPLC-(ESI)MS/MS(QqQ) in SRM	Sediment: 71-130% Fish: 43-134%	0.2-29 [(b)]	[52]
Soil and sediment (15 g)	PFRs (n=11)	PLE ACE:HEX 1:1; 40 min; 35˚C	Florisil column (1 g) Eluted: ACN	ZORBAX Eclipse Plus C18 RRHD (100 mm × 2.1 mm, 1.8 µm) H_2O (10 mM AA) and MeOH (10 mM AA) Gradient, 0.3 mL min^{-1}	UHPLC-(ESI)MS/MS in SRM	Soil: 56-104% Sediment: 59-103%	0.01-5 [(c)]	[53]
Biota and sediment (0.5 g)	PFASs (n=6) and NBFR (n=1)	Mechanical agitation 2x ACN	dSPE C18, hand-shaked and centrifuged 5 min/4050 xg	HALO C18 Rapid Resolution (50 × 4.6, 2.7 µm) H_2O (AcOH:AA) and MeOH (AcOH:AA) Gradient, 0.6 mL min^{-1}	HPLC-(ESI)MS/MS(QqQ) in SRM	80-114%	0.008-4 [(b)] 0.03-13 [(c)]	[33]
Sediment (1.5 g) and sludge (0.1 g)	NBFR (n=9) and PFRs (n=10)	USE 2x EtAc:cyclohexane 5:2	Florisil cartridges (5-10 g) Wash and elution: EtAc:cyclohexane 5:2	DB-5MS (15 m × 0.250 mm, 0.10 µm)	GC-(ECNI)MS/MS(QqQ) in SRM	Sediment: 48-140% Sludge: 64-140%	Sediment: 0.2-80 [(b)] Sludge: 3.7-575 [(b)]	[39]

Bivalve and sediment (5 g)	NBFR (n=4)	USE 2x DCM:HEX 1:1	Multilayer silica column 1.5% deactivated silica and 44% H_2SO_4 silica Florisil column (used for bivalve when necessary)	RP C18 (4.6 mm × 150 mm, 1.8 µm) MeOH and 10 mM AA Gradient, 0.3 mL min^{-1}	LC-(ESI)MS/MS(QqQ)	65-96%	Bivalve: 0.013-0.084 [(b)] Sediment: 0.023-0.074 [(b)]	[27]
Marine sediment and biota (5-10 g)	NBFR (n=12)	USE HEX:DCM 1:1	GPC (only biota) Bio-Beads Eluent: HEX:DCM 1:1 Extra clean-up for GC: Deactivated Florisil (8 g) Eluent: HEX:DCM 1:1 and PTFE Cartridge Filter (0.2 µm) for LC	HT-5MS (15m x 0.25, mm 0.10 µm) for GC and ZORBAX Eclipse Plus C18 (2.1 x 100mm^2, 1.8 µm) for LC H_2O and ACN:MeOH 30:70 Gradient, 0.4 mL min^{-1}	GC-EI-MS/MS(QqQ) in SRM and LC-ESI-MS/MS(QqQ) in SRM	Biota: 41-106% Sediment: 31-105%	0.0001-0.33 [(b)]	[35]
Mollusks (0.5 g)	NBFR (n=6)	MSPD PSA Elution: DCM	Silica, acidified silica (10% H_2SO_4) and florisil (5% H_2O)	DB-5HT (15 m × 0.25 mm, 0.10 µm	GC-(NCI)MS(Q)	46-120%	0.003–0.6 [(b)] 0.01–2.1 [(c)]	[46]
Marine biota (6-200 g)	NBFR (n=2)	MSPD Silica gel: Na_2SO_4 4:1 Elution: ACE:HEX 1:1	Multilayer silica column neutral silica, 44% H_2SO_4 silica and 56% KOH silica Elution: HEX	DB-1HT (15 m × 0.25 mm, 0.1 µm)	GC-(APCI)MS/MS(QqQ) in SRM and GC-(EI)MS/MS(QqQ) Lower LOD for APCI	Not reported	1-25 fg [(b)]	[47]
Sediment, sludge, fish and dolphin blubber (1-1.5 g)	NBFR (n=3)	Sediment: SPLE Sludge, biota: PLE HEX:DCM 1:1; 2 cycles; 10 min; 100˚C	Sludge: Silica (2 g) and alumina (5 g) cartridges Biota: fat removal with H_2SO_4 and SPE with alumina cartridges (5 g)	DB-5MS (15 m × 0.1 mm, 0.1 µm)	GC-(NCI and EI)MS/MS(QqQ) in SRM EI selected as the best ionization mode	Sediment: 103-105% Sludge: 52-66% Fish: 58-80% Dolphin blubber: 71-76%	Sediment: 0.03-0.1 [(b)] Sludge: 0.4-0.9 [(b)] Fish: 0.2-9.6 [(b)] Dolphin blubber: 0.1-1.1 [(b)]	[48]
Sediment	NBFR (n=2)	Soxhlet ACE:HEX (1:1)	Multilayer silica column Elution: HEX:DCM 1:1	DB-XLB (30 m x 0.25 mm, 0.25 µm)	GC-(ECNI)MS in SIM	70-130%	0.056-0.006 [(b)]	[50]

Fish	NBFR (n=14)	Soxtec (automated soxhlet extraction) DCM:HEX 1:1	GPC Bio-Beads S-X3 cycloHEX:EtAc 1:1 and multilayer silica column 44% H_2SO_4 silica and deactivated silica Elution: HEX:DCM 1:1 and ACE	Kinetex reverse-phase C18 (100 x 2.1 mm, 2.6 µm) MeOH:H_2O 60:40 and MeOH:toluene 95:5 Gradient, 0.4 mL min^{-1}	HPLC-(APPI)MS(Orbitrap) in full scan	83-117%	0.001-0.25 (c)	[51]
Sediment, fish and seal blubber (10 g)	NBFR (n=13)	HBCD diastereomers: PLE DCM; 2 cycles; 5 min; 100˚C Other NBFRs: PLE DCM:HEX 1:1 ; 3 cycles; 5 min; 100˚C	HBCD diastereomers: SEC Envirosep-ABC (350 x 21.1 mm) Elution: DCM and Silica column (2 g) Elution 1st fraction: HEX Elution 2nd fraction: HEX:DCM Elution 3rd fraction: DCM Other NBFRs: SEC Envirogel polymer column (30 x 1.9 cm, 15 µm) Elution: DCM	HBCD diastereomers: Acquity BEH C18 (150 mm x 2.1, 1.7 µm) H_2O:MeOH 3:1 and MeOH:ACN 1:1 Gradient, 0.2 mL min^{-1} Other NBFRs: DB-5MS (15 m×0.25 mm, 0.1 µm)	HBCD diastereomers: UPLC-(ESI)MS/MS(QTrap) in SRM Other NBFRs: GC-(ECNI)MS	Sediment: 81-123% Fish: 43-120% Seal blubber: 54-76%	Not reported	[54]
Fish (3 g)	NBFR (n=3)	PLE HEX:DCM 1:1; 2 cycles; 10 min; 100˚C	Neutral alumina cartridge (5 g) Wash: HEX Elution: HEX:DCM	DB-5MS (15 m×0.25 mm, 0.1 µm)	GC-(ECNI)MS/MS(QqQ) in SRM	51-98%	0.2-2.1 (b) 0.4-6.8 (c)	[55]
Fish (10 g) and fish feed (1 g)	NBFR (n=6)	Modified QuEChERS 5 mL H_2O + 10 mL EtAc + 4 g $MgSO_4$ + 2 g NaCl	Silica gel column (1 g or 5 g) Elution: HEX:DCM (3:1)	Rxi-17Sil MS (30 m × 0.25, 0.25 µm)	UGC-(EI)MS/MS(QqQ) in full scan	82-101%	0.5- 1 (c)	[56]
Seal and egg	NBFR (n=21)	Lipid extraction in open glass column HEX:DCM 1:1 in an open glass column	Multilayer silica column Elution: HEX and HEX:DCM 1:1	Rtx-1614 (15 m × 0.25 mm, 0.1 µm)	GC-(APCI)MS/MS(QqQ) in SRM	44-131%	0.001-0.01 (b)	[57]

(a) Method reporting level, (b) method limit of detection, (c) method limit of quantification.

Figures

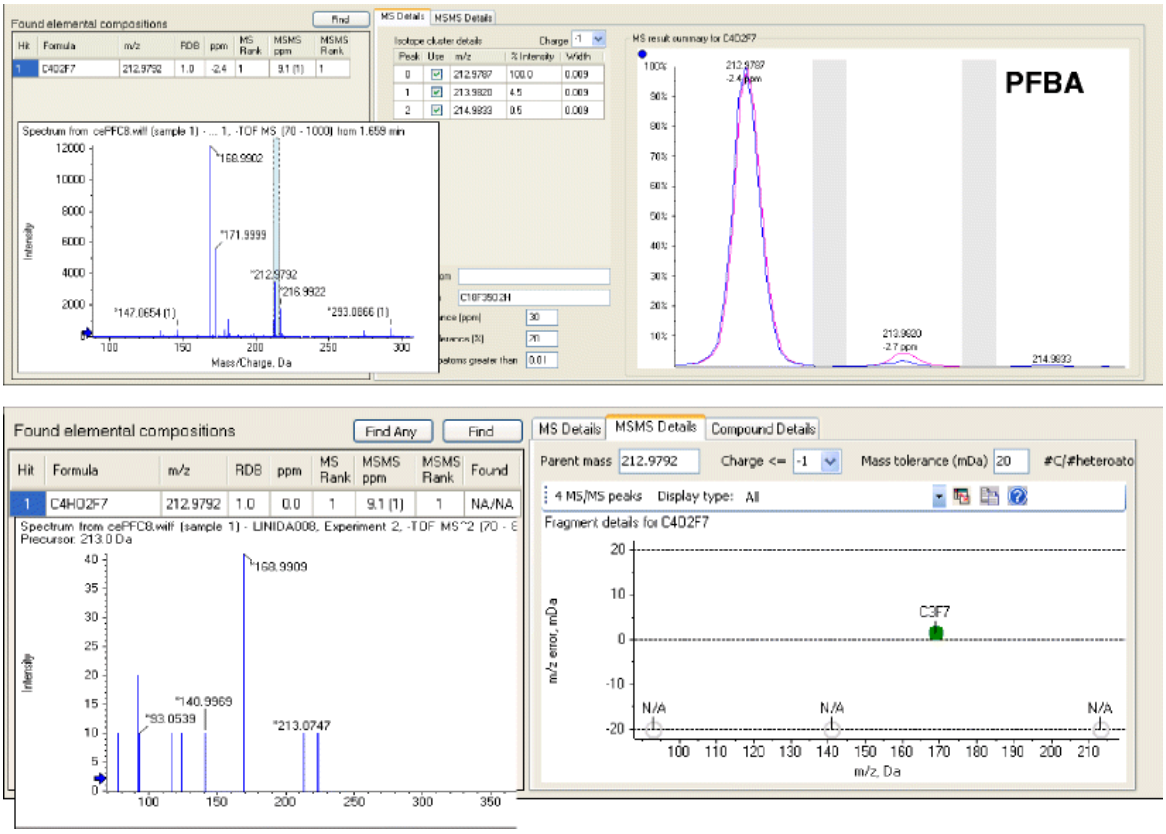


Fig. 1. Water sample: PFBA was identified using Formula Finder and combining TOF-MS and TOF-MS/MS information. Reproduced with permission from Ref. [60]. Copyright (2015) Springer.

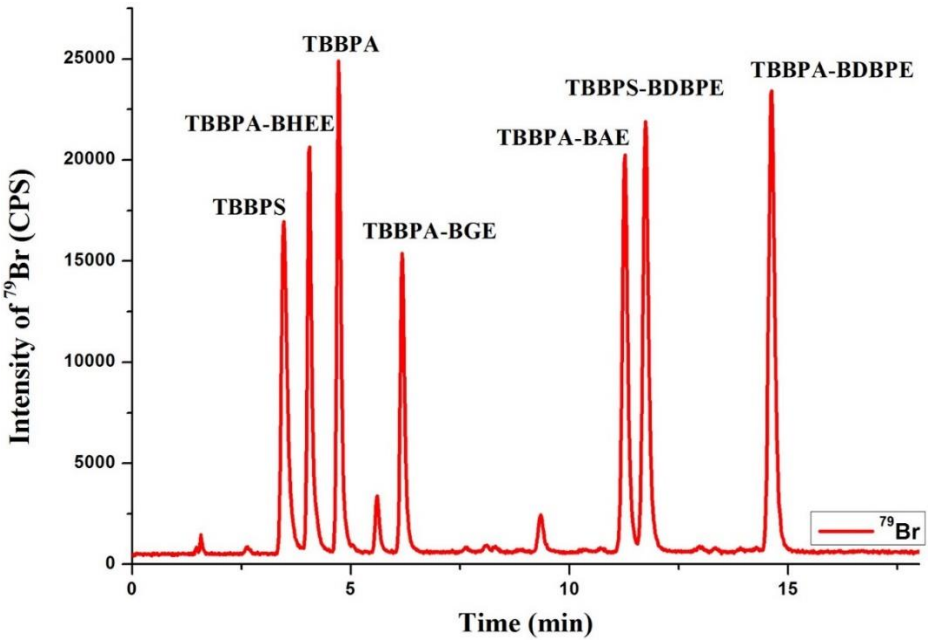


Fig. 2. HPLC-ICP-MS chromatography of mixed standard solutions of TBBPA, TBBPA-BHEE, TBBA-BGE, TBBPA-BAE, TBBPA-BDBPE, TBBPS, and TBBPS-BDBPE (100 µg L^{-1}) under the optimized conditions. Reprinted from Ref. [29]. Copyright (2017) with permission from Elsevier.

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Sección 2

DESARROLLO DE METODOLOGÍA ANALÍTICA

CAPÍTULO 2

Optimization and comparison of several extraction methods for determining perfluoroalkyl substances in abiotic environmental solid matrices using liquid chromatography-mass spectrometry.

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María Lorenzo, Julián Campo, Yolanda Picó.

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RESEARCH PAPER

Optimization and comparison of several extraction methods for determining perfluoroalkyl substances in abiotic environmental solid matrices using liquid chromatography-mass spectrometry

María Lorenzo[1] · **Julián Campo**[1,2] · **Yolanda Picó**[1]

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Abstract In this study, four extraction methods of perfluoroalkyl substances (PFASs) in soils and sediments were validated and compared in order to select the one that provides the best recoveries and the highest sensitivity. The determination of PFASs was carried out by liquid chromatography-tandem mass spectrometry. The extraction methods compared were based on (i) an aqueous solution of acetic acid and methanol (recoveries 44–125 %, relative standard deviation (RSD) <25 %), (ii) methanol (34–109 %, <25 %), (iii) sodium hydroxide digestion (24–178 %, <49 %), and (iv) ion pair (35–179 %, <31 %). The best results were obtained with methanol extraction, which recovered a greater number of PFASs and provided values between 45–103 % in sediment and 34–109 % in soil with RSDs <25 % and limits of quantification (LOQs) between 0.02–0.31 and 0.01–6.00 ng g^{-1}, respectively. The selected method was successfully applied to Segura River sediments and soil samples taken near the Turia River. This study demonstrates the presence of PFASs in the studied rivers of the Valencian Community (0.07–14.91 ng g^{-1} in Segura River sediments; 0.02–64.04 ng g^{-1} in Turia River soils).

Keywords Perfluoroalkyl substances · LC-MS/MS · Extraction method · Soil · Sediment · River basin

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✉ María Lorenzo
maria.lorenzo@uv.es

[1] Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE-UV, GV, CSIC), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

[2] Environmental Forensic and Landscape Chemistry Research Group, Desertification Research Centre (CIDE-UV, GV, CSIC), Carretera Moncada-Náquera, Km 4.5, 46113 Moncada, Valencia, Spain

Introduction

Since 1950, perfluoroalkyl substances (PFASs) are used in a number of industrial and commercial applications as surfactants and stain repellents [1]. Examples of products containing PFASs or precursors are antifire foam, alkaline detergents, paints, nonstick cookware, carpets, upholstery, textile fibers, shampoos, floor polish, smoke inhibitors, semiconductors, pesticide formulations, food packaging, tapes, denture cleaners, etc. [2, 3]. PFASs are in the environment due to (i) industrial use and release, (ii) consumer products containing them, and (iii) biotic or abiotic degradation of larger derivatives and polymers containing perfluoroalkylated moieties. The precursors are widely used commercially and reach the environment through the raw materials used in factories or by-products containing them [2]. Another problem with PFASs is that conventional wastewater treatments show a limited efficiency to eliminate them, so they accumulate in sludge or are released into the water via the effluent [4, 5].

During the last decades, numerous studies have detected its presence in food [6], water [7, 8], sediment [9], sewage sludge [5, 10], animals, and humans [11, 12]. Consequently, concern has increased because of the stability, persistence, and bioaccumulative characteristics of these compounds, which can have adverse effects on humans and wildlife [13]. Data from human studies reveal that PFASs are present in breast milk [14], semen [15], umbilical cord [16], urine [17], blood, and serum [18]. Some toxicological studies show that PFASs

can cause liver cancer and affect reproduction or weight of newborns [19, 20, 3]. Furthermore, once PFASs enter the human body, they are barely removed [21].

Because of its remarkable ubiquity, perfluorooctanesulfonate (PFOS) was included in 2009, Annex B of the Stockholm Convention on persistent organic pollutants (POPs) to limit their production [22], and in the Annex III of substances subject to review for possible identification as priority substances or priority hazardous substances of Directive 2008/105/EC [23]. Other institutions such as the United States Environmental Protection Agency [24] and Canadian environmental authorities [25] have signed agreements with companies to restrict the use of perfluorooctanoic acid (PFOA). The use of PFASs is only limited in some developed areas like the USA and Europe and its production is moving to other countries such as China, where they had a PFOS production level of 100 t in 2012 [26]. If these regulatory efforts are not coordinated, economic factors may shift the production of these materials to countries that prioritize economic development to environmental concerns [27]. Although lower blood levels of PFASs are being observed in populations of the countries where they have been regulated (e.g., the American Red Cross data indicate a 75 % decline in PFOS concentrations from 2000–2001 to 2010 [28]), these are being replaced by short-chain PFASs that are now found at increasing levels in the environment and humans [29].

At present, there are few well-documented cases about how soil or sediment can play a critical role in the distribution of PFASs in the environment and subsequent human exposure. The main limiting factor to expand the number of studies is the complexity of these matrices, the low PFAS concentrations [30], and the interferences from fluoro-containing materials that compromise quantification. These shortcomings make necessary to develop standardized extraction methods with broad applicability. Promising methods such as microwave-assisted extraction (MAE) and pressurized liquid extraction (PLE) are not always appropriate for PFASs. The vessels where the sample is digested in MAE are mainly made with Teflon (source of PFASs). Moreover, conventional PLE system, even that was proposed several times for the determination of PFASs [31], has also some parts of the instruments made of Teflon, like the rings of the stain steel cells. The replacement of these pieces by homologous made of other nonfluorinated materials is complicated. Common extraction procedures are based on four different methods: (i) acetic acid and methanol extraction [32, 33], (ii) only methanol extraction [34], (iii) sodium hydroxide (NaOH) digestion [35], and (iv) ion pairing with tetrabutylammonium hydrogen sulfate [36]. Methods using acetic acid and/or methanol were developed for application in environmental samples, whereas those applying NaOH digestion and the ion pair were initially designed for biological matrices and later adapted for the environmental ones. The choice of a suitable sample preparation technique is essential for the accurate and reliable characterization of PFASs in trace or ultratrace concentrations. Because of the chemical peculiarities of these compounds, a number of important factors must be considered: (i) background contamination (laboratory materials made of or containing perfluoroethylene or perfluoroalkyl compounds) that is a source of interferences for the analysis of PFASs, (ii) selection of the analyte isolation and preconcentration technique, as well as (iii) careful optimization of the corresponding operational parameters. Other difficulties that complicate the analysis of soil and sediment are the long extraction and purification steps, as well as matrix effects that can make the quantification of some compounds very complicated. Only one report is available comparing ion-pairing and methanol methods [36].

Thus, there is a need to undertake a systematic study to generate data that can be valuable for monitoring the occurrence of PFASs in the environment. The objective of this study was to carry out this systematic evaluation for 20 PFASs including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs), and fluorotelomer unsaturated carboxylic acids (FTUCAs) in two environmental abiotic matrices (soil and sediment). To our knowledge, this is the first time that the performance of the four methods is compared for such a wide range of PFASs. The target compounds have been determined by ultra high-performance liquid chromatography system (UHPLC) coupled to tandem mass spectrometry (MS/MS). Recoveries, precision, sensitivity, and matrix effects of the four extraction methods were assessed. The methods that provide the best results were implemented to carry out a survey on the presence of PFASs in soil and sediment samples.

Material and methods

Chemicals

PFCAs (PFBA, PFHxA, PFHpA, PFOA, ipPFNA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA), PFSAs (PFBS, PFHxS, PFHpS, PFOS, ipPFNS, PFDS), and FTUCAs (FOUEA), as well as internal standards isotopically labeled (MPFASs) with ^{13}C and ^{18}O (MPFBA, MPFHxA, MPFHxS, MPFOA, MPFOS, MPFNA, MPFDA, MPFUnDA, MPFDoDA), were used. All were from Wellington Laboratories (Guelph, Ontario, Canada) at concentration of 50 μg mL^{-1} in methanol, with the exception of ipPFNA that was at 45 μg mL^{-1}. The meaning of the acronyms and other useful information are detailed in the Electronic Supplementary Material (ESM) (Tables S1, S2, and S3). Stock standard and solutions were prepared in methanol and stored in polypropylene tubes at 4 °C.

Glacial acetic acid ($C_2H_4O_2$, 99.9 %) was purchased from Sigma-Aldrich (St. Louis, MO, USA); hydrochloric acid (HCl, 37 %) was from Merck, KGaA (Darmstadt, Germany); formic acid (CH_2O_2, 94.5 %) was from Amresco (Solon, OH, USA); anhydrous ammonia (NH_3, 99.99 %) and tetrabutylammonium hydrogen sulfate (TBAS, 97 %) were from Sigma-Aldrich (St. Louis, MO, USA); ammonium formate (CH_5NO_2, 97 %) was from Alfa Aesar (Karlsruhe Germany); and sodium hydroxide micropills (NaOH, 98.8 %) were from Poch (Gliwice, Poland). Methyl *tert*-butyl ether (MTBE, 99 %) and sodium carbonate (Na_2CO_3, 100.2 %) were obtained from VWR (Radnor, PA, USA). Deionized water was from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA) and LC-MS grade methanol was purchased from Panreac (Darmstadt, Germany).

Sample collection and pretreatment

Soil and sediment samples were collected from riverine areas of the Valencian Community (East of Spain). A total of 21 soil samples from the Turia River basin collected in 2012 and 26 in 2013 were analyzed. Soil samples of the upper 20-cm horizon layer were collected. From each sampling point, of 1 m^2, two subsamples were taken. Once in the laboratory, soil samples were dried and passed through a 2-mm Ø sieve, and then, the subsamples of each sampling point were homogenized to create a composite one. The composite soil samples were extended in a layer of approximately 1 cm thickness on polypropylene trays and air-dried in darkness at 20 °C to a moisture content of approximately 3 % water. Then, soil samples were stored in a sealed plastic bag at 4 °C. Sediments were taken with a Van Veen Grab sampler from the lower part of the Segura River in December 2013. A total of 12 samples were collected at six sampling points (2 samples per point). Sediment samples were transported in boxes packed with ice and stored at −20 °C in a freezer upon arrival at the laboratory. In the following 48 h after the collection, the sediment samples were freeze-dried (−75 °C, 10 mTorr, 48 h) in a VirTis Sentry 2.0 Freeze Dryer from SP Scientific (Warminster, PA, EEUU), sieved (125 μm), and stored in aluminum containers at −20 °C until analysis. The materials were carefully checked to prevent introduction of contamination. The location of the sampling points can be found in Fig. 1. The coordinates of all sampling points and a brief description of them are given in the ESM (Table S4, Fig. S1a, b).

Extraction methods

Procedural and instrumental blank contamination is a major challenge in most of the laboratories performing trace analysis of PFASs and the possible sources of contamination as well as techniques for reducing the contamination are not well-established yet [37]. In the present study, strict controls were carried out to ensure that the material and reagents are free of PFAS contamination. Fluoropolymer parts of the instrument were exchanged and background signals of the analytes were not observed in solvent blank injections. Procedural blank contamination was reduced by avoiding the use of fluoropolymer materials in the lab during sample preparation and extraction and by rigorously rinsing all equipment with methanol before use. Very low levels of procedural blank contamination were occasionally observed for PFOS. However, the blank contamination was negligible compared to quantified PFOS concentrations in the soil and sediment. MPFASs were added to soil and sediment samples as internal standards to obtain a concentration in the final extract of 25 ng mL^{-1}.

Matrix effects and sensitivity are also important issues associated to the amount of sample processed in each extraction procedure. As the optimization of the different methods was not carried out in the laboratory, the amount of sample used in each method was that reported as optimal by other authors [32–36]. These differences have an effect in sensitivity and matrix effect that is discussed in the "Results and discussion" section.

Acetic acid and methanol extraction

This method is based as previously described by Higgins et al. [32] and has already been detailed elsewhere [33]. Briefly, the homogenized sediment or soil samples (1 g) were transferred to 50 mL polypropylene centrifuge tubes and 10 mL of 1 % acetic acid solution was added. Each tube was vortexed, placed in a 40 °C ultrasonic bath for 15 min, and centrifuged at 956 rcf for 2 min. The supernatant solution was transferred to a second polypropylene tube. The extraction was repeated twice with 2.5 mL methanol (MeOH) and 1 % acetic acid mixture 90:10 (*v*/*v*) and with 10 mL of 1 % acetic acid solution. All extracts were combined in the second tube, adjusted to a volume of 250 mL with Milli-Q water, and cleaned up by solid-phase extraction (SPE) (see "Cleanup method").

Methanol extraction

In this procedure, 5 g of soil or sediment was extracted three times using 10 mL of methanol, vortexed, sonicated for 15 min, and centrifuged at 956 rcf for 15 min. Finally, after reducing the volume to 5 mL under nitrogen purging, 20 μL of formic acid and 100 mL Milli-Q were added and the sample was cleaned up by SPE (see "Cleanup method").

Methanolic NaOH digestion

In the optimized method, 1 g of soil or sediment was mixed with 2 mL of 200 mM NaOH solution in MeOH and

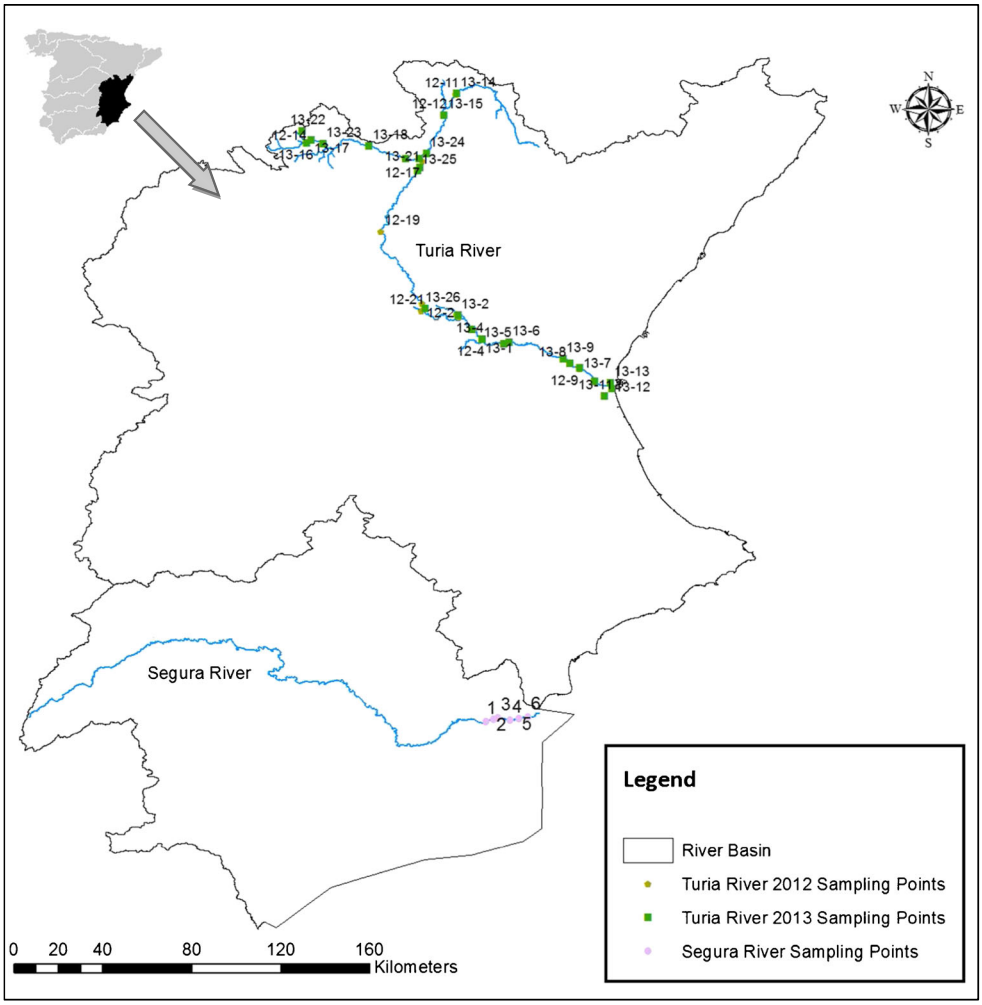

Fig. 1 Location of the sampling points

ultrasonically extracted for 30 min. Then, 20 mL of MeOH was added to the mixture, shaken for 30 min and added with 0.05 mL of 4 M HCl. The mixture was centrifuged (956 rcf) for 15 min and the supernatant transferred to a second tube of 50 mL polypropylene. The process is repeated again but adding 10 mL methanol instead of 20 mL. The total volume of the final extract was 30 mL of MeOH. For analysis, an aliquot of 10 mL of the final extract was taken and reduced under nitrogen purging to 3 mL and adjusted to a volume of 250 mL with Milli-Q water for SPE cleanup (see "Cleanup method").

Ion-pair extraction

Briefly, 0.5 mL of 0.5 M TBAS solution and 4 mL of 0.25 M sodium carbonate buffer (pH 10) were added into a 15-mL polypropylene tube containing 0.5 g of dried soil or sediment. After a thorough mixing, 5 mL of MTBE was added to the solution and was vigorously shaken for 20 min and centrifuged at 956 rcf for 8 min. The supernatant was transferred to a second tube. The addition of MTBE was repeated once. Again the supernatant was transferred to the second tube and then evaporated to dryness under a gentle stream of N_2 and redissolved with 5 mL of MeOH. Finally, it was adjusted to a volume of 250 mL with Milli-Q water and SPE cleanup was performed (see "Cleanup method").

Cleanup method

The extracts were cleaned up by SPE to eliminate acids, salts, and other compounds that can potentially cause matrix-induced ion suppression or enhancement during the PFAS analysis. This was performed by passing the samples through a Phenomenex Strata™ C-18 cartridge according to the procedure described by Taniyasu et al. [38]. In the four methods, the same process was performed, and the cartridges were preconditioned with 4 mL of 0.1 % ammonium hydroxide in MeOH (*v*/*v*), 4 mL of MeOH, and 4 mL of Milli-Q water. Then, the samples were passed through the cartridges by vacuum and the vacuum was kept during 15 min to dry the cartridge. Finally, PFASs were eluted with 4 mL of 0.1 % ammonium hydroxide in MeOH (*v*/*v*) and were recovered in

15 mL polypropylene tubes. Each cartridge was used only once. The tubes were evaporated to dryness under N_2 (2–5 h), redissolved in 250 μL of MeOH, sonicated for 2 min, and transferred to a vial to be injected into the liquid chromatography-tandem mass spectrometer (LC/MS-MS).

Liquid chromatography-tandem mass spectrometry

A 1260 Infinity Ultra High-Performance Liquid Chromatograph (UHPLC) combined with a 6410 Triple Quadrupole (QqQ) Mass Spectrometer (MS/MS) of Agilent Technologies (Santa Clara, CA, USA) with electrospray ionization (ESI) was used. Data were processed using MassHunter Workstation Software for qualitative and quantitative analysis (GL Sciences, Tokyo, Japan).

PFASs were separated with a Kinetex C18 (50×2.1 mm, 1.7 μm) from Phenomenex (Torrance, CA, EEUU). The mobile phases consisted of (A) water and (B) methanol, both containing 10 mM ammonium formate. The following gradients were applied: 0 min (30 % B), 0.5 min (30 % B), 12 min (95 % B), and 20 min (95 % B) and return to the initial conditions. An equilibration time of 12 min was applied to stabilize the column conditions for a new injection. The flow rate was kept at 0.2 mL min^{-1} throughout the run, and the sample volume injected was 5 μL. Analysis was performed in negative ion mode. Data acquisition was carried out in selected reaction monitoring (SRM) to identify and quantify using two precursor→product ion transitions (except for PFBA), retention times, and the ratio of intensities between the two product ions. Fragmentor and collision energies were optimized for each compound individually. Information related to instrumental determination and the optimal conditions are reported in ESM (Tables S5 and S6). Separation achieved is shown in Fig. 2.

Method validation

The validation of the instrumental parameters was performed by determining linearity, instrumental limits of detection (LOD) and quantification (LOQ), and intraday (repeatability) and interday (reproducibility) precision. The linearity was evaluated using eight different concentrations (from the LOQ to 75 ng mL^{-1}) of PFAS standard solutions in methanol. The correlation coefficients (R^2) were superior to 0.99 for each PFAS.

Validation experiments were performed by spiking soil and sediment samples with all selected PFASs at different concentrations. Few microliters of a methanolic solution of PFASs at the appropriate concentrations were thoroughly spread onto the sample with a GL syringe. After homogenization, the spiked samples were left to balance for 20 min. Then, the samples were processed as reported in "Extraction methods," "Cleanup method," and "Liquid chromatography-tandem mass spectrometry" sections. For the assessment of all mentioned parameters, the analyte response was always related to the internal standard responses to compensate for undesirable matrix effects and losses during the extraction step (except in the evaluation of the matrix effect).

Among the different soil and sediment samples, those that have lower levels of PFASs were chosen. In the case of the soil sample, PFASs were not detected. As in the sediment, the sample used had a very low amount of PFOS, visible at levels lower than LOQ. The selected soil is characterized by pH >7, loamy texture, and low levels of organic matter (≈4 %). The sediment sample was also characterized by pH >7 and a percentage of carbonates >30 %. See ESM for more information about the selected soil and sediment sample characteristics (ESM Table S7). These samples are representatives of those analyzed in this study.

The LOD was calculated as the mass of analyte required to produce a signal-to-noise ratio (S/N) of 3:1, where the noise is calculated as three times the standard deviation of the background signal. The LOQ (i.e., the lowest concentration at which the analyte can be reliably detected meeting some predefined bias and imprecision goals [39]) was also established as that value whose S/N was 10:1. Instrumental LOD values were in the range from 0.11 to 1.11 ng mL^{-1} and LOQs were between 0.33 and 3.33 ng mL^{-1} (see ESM Table S8).

The precision was determined by calculating the interday and intraday precision as relative standard deviations (RSD). Repeatability (intraday) was measured as the RSD of the standard concentration of 25 ng mL^{-1} obtained in five consecutive injections performed on the same day, while the reproducibility (interday) was calculated by measuring the concentration of the standards on five different days. Repeatability provided RSDs lower than 9.6 %. In terms of reproducibility, as expected, the RSD was higher, although it was within acceptable limits (<12 %). All values are specified in ESM (Table S8).

To determine the total effect on the signal (matrix effect × recovery), the peak areas of the internal standards in the matrix (soil or sediment) were compared with those obtained in methanol. Recoveries were calculated by spiking both soil and sediment samples with PFASs. To verify the accuracy of the method, five replicates (n=5) of each matrix were performed.

Recovery was determined by subtracting endogenous PFAS levels from the corresponding spiked samples. The soil and sediment samples were not pre-extracted because the extraction procedures not only extract the analytes but also some matrix components. Then, the effect of the matrix in the analyte extraction can be modified by this previous step. This provides absolute recoveries according to the equation:

$$\mathrm{AR} = \frac{\text{area of analyte sample}}{\text{area of analyte external standard}}$$

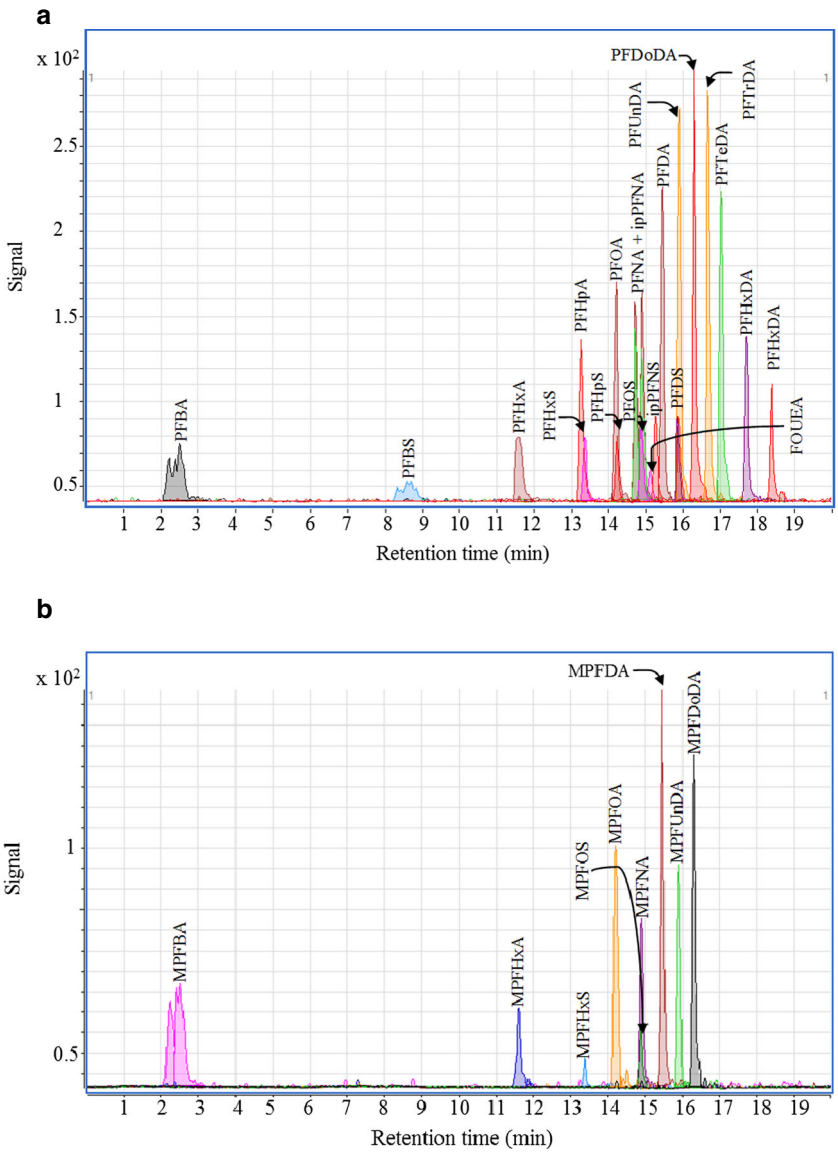

Fig. 2 (**a**) Standard chromatogram of spiked sediment (25 ng mL^{-1}) with PFASs. (**b**) Standard chromatogram of spiked sediment (25 ng mL^{-1}) with MPFASs

The matrix effect in LC-MS analysis was first assessed by Matuszewski et al. [40] who used very simple equations (i), (ii), and (iii) to determine the matrix effect (ME), recovery of extraction (RE), and overall process efficiency (PE) that is the recovery and matrix effect.

$$\text{(i)} : \text{ME}\ (\%) = \frac{B}{A} \cdot 100$$

$$\text{(ii)} : \text{RE}\ (\%) = \frac{C}{B} \cdot 100$$

$$\text{(iii)} : \text{PE}(\%) = \frac{C}{A} \cdot 100 = \frac{\text{ME} \cdot \text{RE}}{100}$$

In which A is the area of the PFAS(s) recorded for the standard solution, B is the area of the PFAS(s) recorded for the sample spiked with the target compound(s) after extraction, and C is the area of the PFAS(s) recorded for the sample spiked with the target compound(s) before extraction. The use of the recovery and matrix effect has been widely applied in the determination of PFASs [31, 33, 35, 36, 41].

Results and discussion

Extraction by acetic acid and methanol

This method detected all target PFASs in both sediment and soil. As shown in Table 1, the PFCA recoveries were in the range from 44 to 112 % in sediment and 61 to 116 % in soil. Regarding PFSAs, recoveries were from 56 to 125 % in sediment and 63 to 114 % in soil. FTUCAs (FOUEA) showed a recovery of 103 % in sediment and 91 % in soil. As for the accuracy of the method, the RSDs were found between 4 and 21 % in sediment and 5 to 25 % in soil. This method was

Table 1 Recoveries (%), RSDs (%), and LOQs (ng g^{-1}) of acetic acid and methanol extraction method

Compound	Sediment			Soil		
	Recovery (%)	RSD (%)	LOQ (ng g^{-1})	Recovery (%)	RSD (%)	LOQ (ng g^{-1})
PFBA	44	19	0.40	92	25	0.71
PFHxA	56	18	0.40	99	8	0.92
PFHpA	92	12	0.20	81	5	0.23
PFOA	112	9	0.18	116	10	0.17
PFNA	111	11	0.75	97	16	0.86
ipPFNA	101	14	0.37	96	5	0.39
PFDA	98	9	0.84	110	9	0.75
PFUnDA	92	16	0.73	92	10	0.73
PFDoDA	104	8	0.07	100	10	0.07
PFTrDA	77	4	0.10	67	5	0.12
PFTeDA	71	5	0.23	74	7	0.23
PFHxDA	79	14	6.00	61	20	0.66
PFODA	84	9	8.00	64	21	2.00
PFBS	125	13	6.00	114	20	3.00
PFHxS	71	12	0.50	63	15	0.56
PFHpS	68	8	0.09	106	9	0.06
PFOS	82	19	0.36	87	18	0.33
ipPFNS	58	10	0.36	65	14	0.32
PFDS	56	13	0.61	81	22	0.42
FOUEA	103	21	0.73	91	22	1.07

already used in our laboratory to extract PFASs from sediment and sludge with similar results [4, 32].

Recoveries of this study were compared with those obtained by Higgins et al. [32], who measured PFCAs (PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTeDA) and PFSAs (PFHxS, PFOS, and PFDS) in sediment using a method also based on acidic extraction. Their reported recoveries ranged from 74 to 98 % for PFCAs and from 79 to 85 % for PFSAs. As in our study, recoveries were lower for long-chain compounds. Higgins et al. [32] suggested that it may be due to different reasons such as inefficient removal of environmental solid matrices, insufficient retention and/or elution during SPE as well as the suppression of the signal due to matrix effects during LC-MS/MS analysis (more pronounced in long-chain PFCAs). LOQs were also calculated (Table 1) and they were on the same order of magnitude than those calculated by Higgins et al. [32] for PFCAs (0.46 vs. 0.31 ng g^{-1}) and PFSAs (0.49 vs. 0.19 ng g^{-1}).

An estimate of the total effect on the signal (matrix effect and recovery) was performed comparing the results obtained for different MPFASs in samples and in methanolic standards (Fig. 3). In general, compounds in matrices suffer a negative effect, i.e., present worse recoveries than those in methanol. Suppression ranged from −19 to −79 % for sediment and from −3 to −78 % for soil, with the short- (MPFBA) and long-chain (MPFDoDA) compounds the most affected. Some low intensity signal enhancement was detected in MPFHxA (28 %) and MPFOA (17 %) in soil and MPFHxS (9 %) in sediment. In general, the effects are lower in soil than in sediment.

Extraction with methanol

Preliminary experiments were carried out to optimize the method. Several variables such as solvent (acetonitrile and methanol), volume of solvent (2, 5, 10, and 20 mL), and sonication time (5, 15, 25, and 60 min) were optimized. The optimum conditions were those reported in the experimental section. As in the extraction by acetic acid, all target PFASs were recovered in both sediment and soil. Recoveries for PFASs were high, from 69 to 103 % in sediment and from 70 to 109 % in soil, with the exception of PFHxA (59 and 56 % in sediment and soil, respectively) (short-chain PFCA) and PFODA (45 and 34 %) (the longest chain PFCA). Good accuracy in both matrices was obtained: 0.3–21 % RSD in sediment and 1–21 % in soil (Table 2). Methanol was already used as extractant by Beškoski et al. [34] for the analysis of some PFCAs (PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA) and PFSAs (PFBS, PFHxS, PFOS, and PFDS) of sediment samples from a wastewater artificial channel of an industrial complex. Beškoski et al. [34] estimated the LOQ for PFCAs (0.06 ng g^{-1}) and PFSAs (0.12 ng g^{-1}), very close to those obtained in the present study (0.02–0.31 ng g^{-1}). There are no

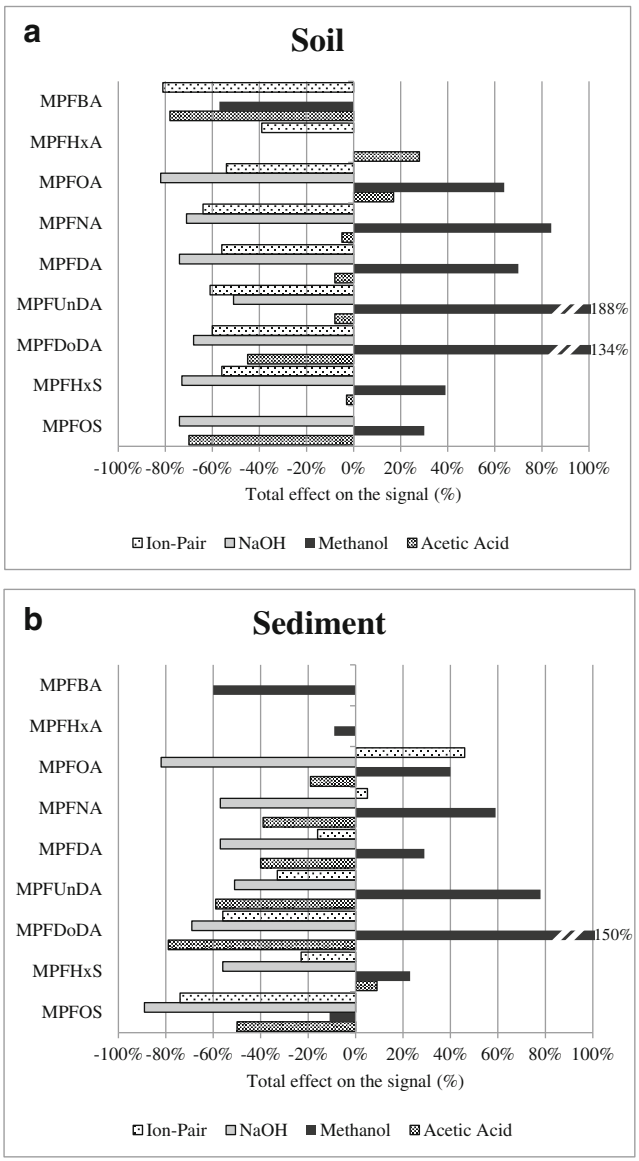

Fig. 3 (**a**) Total effect on the signal (matrix effect × recovery) in soil. (**b**) Total effect on the signal (matrix effect × recovery) in sediment

published data about spike tests or recoveries obtained with this method.

The effect on the signal produced in this method is positive in most of the compounds, with values between 23 and 150 % in sediment and from 30 to 188 % in soil. Negative values were recorded for MPFBA (−57 %) in soil and for MPFBA (−60 %), MPFHxA (−9 %), and MPFOS (−11 %) in sediment. The effect in this method is more pronounced due to the quantity of sample used, which is greater than in the rest of methods (5 g) (Fig. 3).

Extraction by methanolic NaOH digestion

NaOH digestion has been widely used in biological matrices because PFASs tend to bind to proteins and NaOH digestion breaks this bond [42–44]. Several variables were optimized such as NaOH concentration (1, 2, and 5 mM), volume (1, 2, and 5 mL), and extraction time (15, 30, and 60 min). The best results were obtained with 2 mL of NaOH 200 mM for 30 min. Of the 20 compounds listed, a total of 18 PFASs in sediment and 13 in soil were detected. Recoveries in sediment were from 24 to 113 % for PFCAs, from 41 to 125 % for PFSAs (except PFBS, with a recovery of 371 %), and 126 % for FOUEA (FTUCAs), whereas in soil, recoveries were from 48 to 109 % for PFCAs, 86 to 134 % for PFSAs, and 178 % for FOUEA (FTUCAs) (Table 3). In soil, short-chain compound of PFSAs (PFBS) and PFCAs (PFBA, PFHxA, and PFHpA) as well as long-chain PFCAs (PFHxDA, PFODA) were not detected. Sediment recoveries were higher (except for PFODA). The recovery of PFBS in sediments was unusually high, which was explained by a possible interference. The precision (RSD) of the method was 2–36 % in sediment and 2–49 % in soil. Yeung et al. [35] measured the concentration of PFCAs (PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA) and PFSAs (PFBS, PFHxS, PFOS, PFDS) obtaining recoveries from 70.5 to 97 % for PFCAs and 73 to 81.5 % for PFSAs. For the compounds mentioned previously, recoveries in this study were from 42 to 109 % for PFCAs and 90 to 125 % for PFSAs (except PFBS). The precision was good, except for some compounds like PFBS in sediments and FOUEA in soils.

The LOQs were calculated for soils, being in the range from 0.07 to 1.66 ng g^{-1} for PFCAs, 0.07 to 0.52 ng g^{-1} for PFSAs, and 0.77 ng g^{-1} for FTUCAs (FOUEA). The LOQs for sediment were in the range from 0.08 to 1.72 ng g^{-1} for PFCAs, 0.07 to 0.33 ng g^{-1} for PFSAs, and 0.55 ng g^{-1} for FTUCAs (FOUEA) (Table 3). The effect on the signal of the two shortest chain PFCAs (MPFBA and MPFHxA) could not be calculated. For the rest of the compounds, there were losses between −51 and −89 % in sediment and from −51 to −82 % in soil (Fig. 3). Other authors have used this method successfully for sediment (e.g., Yang et al. [45], who obtained recoveries from 31 to 121 % in sediment for PFCAs (PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA) and from 50 to 93 % for PFSAs (PFHxS, PFOS, and PFDS), as well as LOQs in the range from 0.02 to 0.05 ng g^{-1} for both PFCAs and PFSAs).

Extraction using ion pair

The ion-pair method was first developed by Hansen et al. [46] for biological matrices. This extraction procedure is suitable for the analysis of relatively homogenous matrices and is often used when the patterns can be added to similar samples to those under study (e.g., the use of animal sera to quantify PFASs in human sera) (e.g., Llorca et al. [43]). However, when this method is applied to heterogeneous matrices, such as soils and sediments used in this study, it is limited by the

Table 2 Recoveries (%), RSDs (%), and LOQs (ng g^{-1}) of methanol extraction method

Compound	Sediment			Soil		
	Recovery (%)	RSD (%)	LOQ (ng g^{-1})	Recovery (%)	RSD (%)	LOQ (ng g^{-1})
PFBA	102	21	0.13	109	25	0.12
PFHxA	59	20	0.31	56	20	6.00
PFHpA	101	18	0.04	87	21	0.04
PFOA	95	14	0.04	104	14	0.04
PFNA	101	12	0.17	92	20	0.18
ipPFNA	103	6	0.07	96	3	0.08
PFDA	98	6	0.17	105	14	0.16
PFUnDA	103	0.3	0.13	91	9	0.15
PFDoDA	96	9	0.02	94	8	0.02
PFTrDA	97	11	0.02	91	11	0.02
PFTeDA	102	9	0.03	99	3	0.03
PFHxDA	71	15	0.11	70	18	0.13
PFODA	45	6	0.45	34	1	0.59
PFBS	95	12	0.05	75	19	0.06
PFHxS	85	18	0.08	99	14	0.07
PFHpS	75	11	0.02	86	15	0.01
PFOS	96	19	0.06	100	19	0.06
ipPFNS	75	20	0.06	74	20	0.06
PFDS	79	19	0.09	105	10	0.07
FOUEA	69	13	0.28	86	20	0.23

complexity of the matrix, since the compounds present in it can lead to deletion or enhancement of the ion signal between samples. Despite the problems, this method has been used in complex matrices, as in the study by Zhang et al. [36], where sewage sludge was analyzed achieving good results through the implementation of previous SPE processes (not used in biological matrices) and the use of isotopically labeled internal standards. Zhang et al. [36] applied the method for PFCAs (PFHxA, PFHpA, PFOA, PFNA, PFDoDA, PFTeDA), PFSAs (PFBS, PFHxS, PFOS), FTUCAs (FOUEA), and others not analyzed in this paper.

Although an intensive optimization of all method variables was carried out including the amount and concentration of the ion-pairing agent, as well as the volume of MTBE and number of extractions, of the 20 possible PFASs, only 11 were recovered in sediment and 12 in soil samples. As shown in Table 4, the recoveries of the compounds detected were between 69 and 178 % in sediment and from 35 to 131 % in soil. van Leeuwen and de Boer [30] pointed out that the variability in the recoveries of this method is one of its main disadvantages, and it may be due to the complexity of the matrix used. The method precision for PFASs detected was between 5 and 31 % for both soil and sediment. Zhang et al. [36] obtained recoveries from 85 to 153 % for PFASs analyzed (except for PFBS, which value was 52 %), similar to our results.

The total effect on the signal, as in the acetic acid and NaOH methods, was mainly negative (−16 to −74 % in sediment and −39 to −81 % in soil). In the work of Zhang et al. [36], the effects on the signal obtained were calculated for MPFBA (−18 %), MPFHxA (15 %), MPFOA (24 %), and MPFOS (−13 %). LOQ reported in Table 4 ranged from 0.21 to 0.95 in sediment and 0.15 to 3.74 ng g^{-1} in soil. Zhang et al. [36] calculated LOQs for PFCAs (1.4 ng g^{-1}), PFSAs (6 ng g^{-1}), and FTUCAs (1 ng g^{-1}) in sewage sludge, which were clearly higher.

Comparison of methods

The total effect on the compound signal (matrix effect and recovery) was mostly characterized by response suppression (Fig. 3). Suppression effect due to coelution of matrix components has been widely described [47]. Furthermore, recoveries are commonly <100 % that also justifies the lower signals of the internal standards (ISs) in samples than in the methanolic standard. However, the extraction with methanol provides signal enhancement for most of the PFASs in both soil and sediment. The addition of the ISs to the samples corrects for these effects and achieves a proper quantification without the need to use matrix-matched standards.

Just the methods based on methanolic extraction, acidified or not, were able to extract all the PFASs collected. The only methanol extraction was the most sensitive, providing the lowest LOQ for all compounds (except PFHxA in soil), while the ion pairing was the least sensitive. The acetic acid

Table 3 Recoveries (%), RSDs (%), and LOQs (ng g^{-1}) of methanolic NaOH digestion method

Compound	Sediment			Soil		
	Recovery (%)	RSD (%)	LOQ (ng g^{-1})	Recovery (%)	RSD (%)	LOQ (ng g^{-1})
PFBA	42	2	1.56	n.d.	–	n.d.
PFHxA	n.d.	–	n.d.	n.d.	–	n.d.
PFHpA	53	3	0.35	n.d.	–	n.d.
PFOA	109	20	0.18	109	18	0.18
PFNA	93	12	0.90	94	3	0.88
ipPFNA	113	18	0.33	102	20	0.37
PFDA	76	7	1.08	48	9	1.72
PFUnDA	82	15	0.82	68	10	0.99
PFDoDA	103	20	0.07	94	20	0.08
PFTrDA	110	5	0.07	78	14	0.10
PFTeDA	87	13	0.19	73	2	0.23
PFHxDA	24	11	1.66	n.d.	–	n.d.
PFODA	n.d.	–	n.d.	n.d.	–	n.d.
PFBS	(371)[a]	36	0.23	n.d.	–	n.d.
PFHxS	102	18	0.34	123	3	0.29
PFHpS	90	4	0.07	86	7	0.07
PFOS	125	21	0.23	87	23	0.33
ipPFNS	41	17	0.52	n.d.	–	n.d.
PFDS	81	26	0.42	134	28	0.26
FOUEA	126	23	0.77	178	49	0.55

n.d. not detected

[a] PFBS presented anomalous recovery

extraction was the second most sensitive method for PFCAs and the NaOH digestion for PFSAs. The smaller number of compounds detected using the NaOH digestion can be explained because the basic pH can promote the binding between PFSAs and soil cations preventing their extraction. Finally, the ion-pair method, despite being widely used, provided the worst results. This method was initially developed for biological matrices and problems with accuracy and variability have been frequently noted [30].

Recoveries obtained using methanol extraction with or without acetic acid are also the best ones. The only methanol method was applied to soil and sediment because it was more sensitive. However, both have certain limitations, such as being labor-intensive in the case of methanol and acetic acid (it takes 6 h to prepare a sample) or the long evaporation steps in the case of methanol that can affect volatile PFASs. Figure 2a, b shows a chromatogram of spiked (25 ng mL^{-1}) sediment with PFASs and MPFASs used, respectively.

Application

Based on the results, methanol extraction method was selected for its application in Segura River sediments and Turia River soils, in order to detect PFAS presence. Each sample was analyzed in triplicate. For the correct determination of PFASs in these matrices, before and after each batch of 25–30 samples, calibration lines were constructed. Furthermore, for each of the 15 samples, a quality control was performed by injecting an experimental blank, a procedural blank, and a positive control.

Segura River sediments

Figure 4 presents the PFAS concentrations detected. PFCAs were found in 100 % of the samples at a concentration range of 0.07 to 14.91 ng g^{-1}, being the highest concentration for the shortest chain PFCA (PFBA). Two long-chain PFCAs (PFTrDA and PFTeDA) were also found in one of the sampling points. For PFSAs, PFOS levels up to 2.29 ng g^{-1} were found. Note the presence of FOUEA in one sampling point with a concentration of 2.56 ng g^{-1}.

As shown in Fig. 4, in Segura River sediments, PFBA and PFOS were the most frequently detected (100 % of the samples). PFOA was found in 50 % of the samples and the other compounds were detected only in one sampling point. As pointed out in some recent studies [3, 48], PFBA is the dominant perfluoroalkyl substance replacing PFOA because of

Table 4 Recoveries (%), RSDs (%), and LOQs (ng g^{-1}) of extraction method using ion pair

Compound	Sediment			Soil		
	Recovery (%)	RSD (%)	LOQ (ng g^{-1})	Recovery (%)	RSD (%)	LOQ (ng g^{-1})
PFBA	n.d.	–	n.d.	35	31	3.74
PFHxA	n.d.	–	n.d.	62	25	2.95
PFHpA	178	20	0.21	107	21	0.35
PFOA	115	31	0.34	131	12	0.30
PFNA	100	12	1.66	130	22	1.29
ipPFNA	118	5	0.64	131	5	0.57
PFDA	94	12	1.76	92	15	1.79
PFUnDA	142	5	0.95	99	25	1.36
PFDoDA	83	17	0.17	94	17	0.15
PFTrDA	n.d.	–	n.d.	103	20	0.15
PFTeDA	n.d.	–	n.d.	n.d.	–	n.d.
PFHxDA	n.d.	–	n.d.	n.d.	–	n.d.
PFODA	n.d.	–	n.d.	n.d.	–	n.d.
PFBS	n.d.	–	n.d.	n.d.	–	n.d.
PFHxS	85	26	0.83	147	25	0.48
PFHpS	69	19	0.18	67	19	0.19
PFOS	179	25	0.32	n.d.	–	n.d.
ipPFNS	n.d.	–	n.d.	n.d.	–	n.d.
PFDS	n.d.	–	n.d.	n.d.	–	n.d.
FOUEA	92	24	2.12	n.d.	–	n.d.

n.d. not detected

production restrictions. The presence of these compounds can be explained by their release in WWTP effluents during the last decades, industrial waste (chemical and electrical industries located at the upper part of Segura River basin) and even agricultural (as they may be aids of some pesticides formulations). See ESM (Table S9) for more information about PFAS concentrations in Segura River.

Comparing the concentrations in sediments of the Segura River with those reported in the literature, comparable values were found. A similar study by Campo et al. [49] in sediments from the Llobregat River (Catalonia, Spain) presented similar mean concentrations, although slightly higher for PFOA and PFOS (0.74 and 2.76 ng g^{-1}, respectively). Like in Segura River, PFTrDA (0.19 ng g^{-1}) was found in one sampling point. In both studies, PFBA is the compound found at a higher concentration, although in the Llobregat River (3.67 ng g^{-1}), the average is 3 times lower than that detected in the Segura River (10.47 ng g^{-1}). Other studies in sediments measured concentrations of some PFASs such in L'Albufera of Valencia, with values ranging from 0.03 to 10.9 ng g^{-1} for PFOA and 0.10 to 4.80 ng g^{-1} for PFOS [33]. PFOA values were 10 times higher in L'Albufera than in the Segura River, while PFOS levels were similar in both places. However, other authors have found lower concentrations, such as Yang et al. [45] who measured concentrations of PFOA and PFOS in sediments of Liao River (China) 4 and 8 times lower, respectively, than those measured in the Segura River. Thompson et al. [50] also found concentrations of PFOA 10 times lower and PFOS values slightly higher.

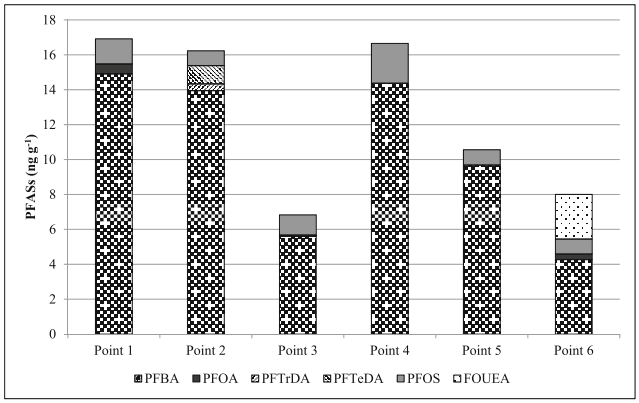

Fig. 4 Accumulated concentration (ng g^{-1}) of PFASs in sediment at different sampling points of Segura River

Turia River soils (2012 and 2013)

In 2012, PFCAs values from 0.02 to 19.97 ng g^{-1} were obtained and from 0.82 to 2.74 ng g^{-1} for PFSAs, and FTUCA was not detected. Based on occurrence in sediments, PFBA is the compound at higher concentrations (maximum of

17.96 ng g^{-1}), followed by PFOA (3.08 ng g^{-1}) and PFOS (2.74 ng g^{-1}). PFHxA was also detected at concentrations below the LOQ of the method (Fig. 5a). PFBA was found in a larger number of sampling points (77 %), followed by PFOA (59 %) and PFOS (14 %). In 2013, slightly higher values than those of the previous year were obtained for PFCAs (from 0.06 to 64.04 ng g^{-1}) and PFSAs (0.69 to 4.15 ng g^{-1}). Again PFBA was the compound at the highest concentration (64.04 ng g^{-1}), followed by PFOA (6.96 ng g^{-1}) and PFOS (4.15 ng g^{-1}) (Fig. 5b). PFBA was also the prevalent (42 %), followed by PFOA (31 %) and PFOS (15 %). However, the occurrence frequency was lower than that obtained in 2012.

There are few articles about PFAS concentrations in soil. The published data only focused on the concentrations of PFOA and PFOS. Li et al. [41], in agricultural soils of Shanghai, found PFOA in the range from 3.3 to 44 ng g^{-1} and 9.2 to 10.4 ng g^{-1} for PFOS. Strynar et al. [51] conducted a pilot study to analyze soil PFASs in six countries and they obtained variable ranges from 0.95 to 12.4 ng g^{-1} for PFHxA, 0.76 to 31.7 ng g^{-1} for PFOA, and 0.58 to 10.1 ng g^{-1} for PFOS. However, no studies have been found in river basins that can be compared with this study. See SI for more information about PFAS concentrations in 2012 (ESM Table S10) and 2013 (ESM Table S11).

Conclusions

Of the four extraction methods tested, the extraction with methanol (recoveries between 34 and 109 %, RSD <25 %, and LOQ 0.01–6.00 ng g^{-1}) and with acetic acid (44–125 %, <25 %, 0.06–8.00 ng g^{-1}) gave appropriate results. Digestion with sodium hydroxide (NaOH) and the ion-pair extraction showed worse recoveries (some PFASs were not extracted) and less sensitivity. The total effect on the signal (matrix effect × recovery) showed that the matrix effect produced in the ionization source is still a challenge in the analysis of PFASs in solid matrices, despite being corrected by the

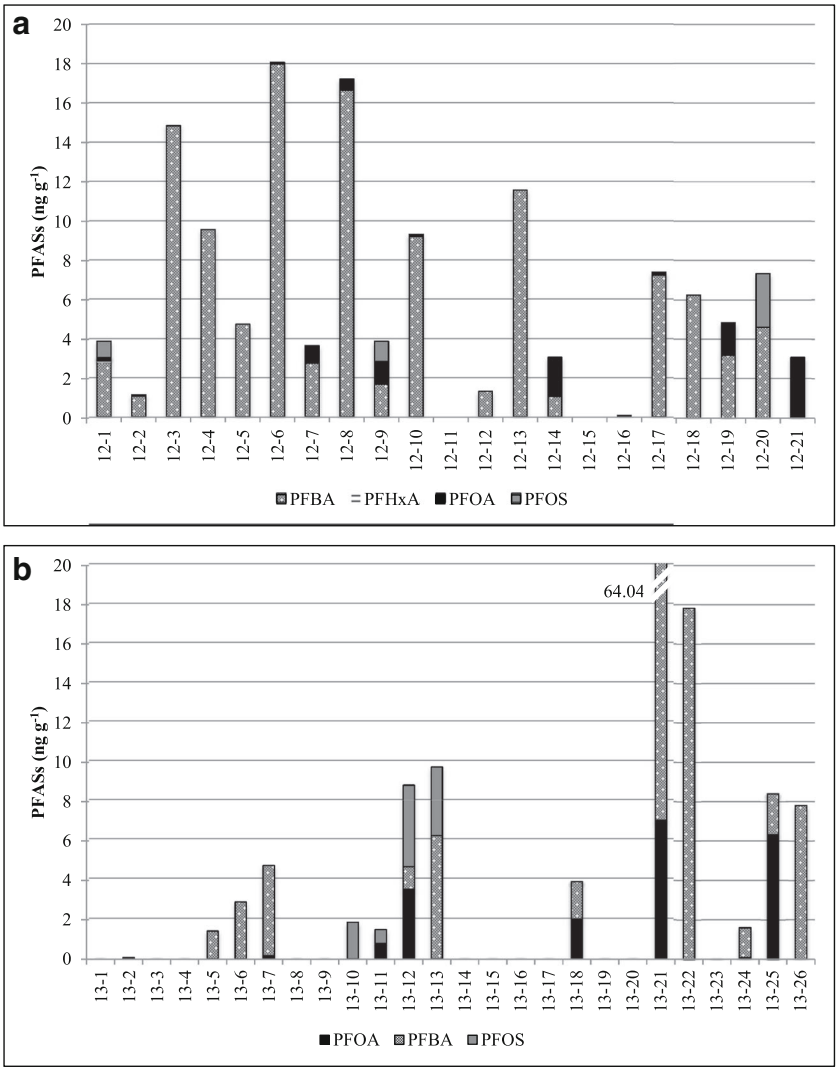

Fig. 5 (**a**) Accumulated concentration (ng g^{-1}) of PFASs in soil at different sampling points of Turia River in 2012. (**b**) Accumulated concentration (ng g^{-1}) of PFASs in soil at different sampling points of Turia River in 2013

use of internal standards (MPFASs). The quantification obtained by both methods was appropriate and the scope of their application can be widened in the future to other similar matrices like sludge samples.

In the monitoring conducted in sediment samples from the Segura River, PFOA, PFBA, PFOS, PFTeDA, PFTrDA, and FOUEA were detected. PFBA, PFOA, and PFOS were detected in soil samples taken at the Turia River basin in 2012 and 2013. The highest concentrations were in both rivers for PFBA, confirming the growing presence of short-chain compounds that replace traditional PFASs. This is the first study about PFAS concentrations in the Segura and Turia Rivers and the first time FOUEA has been detected in sediments.

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Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Optimization and comparison of several extraction methods for determining perfluoroalkyl substances in abiotic environmental solid matrices using liquid chromatography-mass spectrometry

María Lorenzo, Julián Campo, Yolanda Picó

Table S1 Family, structure, name, acronym, CAS number and formula of PFASs

Family (acronym)	Structure	n	Name	Acronym	CAS number	Formula
Perfluoroalkyl carboxylic acids (PFCAs)	$C_nF_{2n+1}COOH$	3	Perfluorobutanoic acid	PFBA	375-22-4	$C_4HF_7O_2$
		5	Perfluorohexanoic acid	PFHxA	307-24-4	$C_6HF_{11}O_2$
		6	Perfluoroheptanoic acid	PFHpA	375-85-9	$C_7HF_{13}O_2$
		7	Perfluorooctanoic acid	PFOA	335-67-1	$C_8HF_{15}O_2$
		8	Perfluorononanoic acid	PFNA	375-95-1	$C_9HF_{17}O_2$
		8	Perfluoro-7-methyloctanoic acid	ipPFNA	Not available	$C_9HF_{17}O_2$
		9	Perfluorodecanoic acid	PFDA	335-76-2	$C_{10}HF_{19}O_2$
		10	Perfluoroundecanoic acid	PFUnDA	2058-94-8	$C_{11}HF_{21}O_2$
		11	Perfluorododecanoic acid	PFDoDA	307-55-1	$C_{12}HF_{23}O_2$
		12	Perfluorotridecanoic acid	PFTrDA	72629-94-8	$C_{13}HF_{25}O_2$
		13	Perfluorotetradecanoic acid	PFTeDA	376-06-7	$C_{14}HF_{27}O_2$
		15	Perfluorohexadecanoic acid	PFHxDA	67905-19-5	$C_{16}HF_{31}O_2$
		17	Perfluorooctadecanoic acid	PFODA	16517-11-6	$C_{18}HF_{35}O_2$
Perfluoroalkane sulfonic acids (PFSAs)	$C_nF_{2n+1}SO_3X$ X = Na, K	4	Potassium perfluorobutanesulfonate	PFBS	29420-49-3	$C_4F_9SO_3K$
		6	Sodium perfluorohexanesulfonate	PFHxS	82382-12-5	$C_6F_{13}SO_3Na$
		7	Sodium perfluoroheptanesulfonate	PFHpS	Not available	$C_7F_{15}SO_3Na$
		8	Sodium perfluorooctanesulfonate	PFOS	1763-23-1	$C_8F_{17}SO_3Na$
		9	Sodium perfluoro-7-methyloctanesulfonate	ipPFNS	Not available	$C_9F_{19}SO_3Na$
		10	Sodium perfluorodecanesulfonate	PFDS	Not available	$C_{10}F_{21}SO_3Na$
Fluorotelomer unsaturated carboxylic acids (FTUCAs)	$C_nF_{2n+1}CF=CHCOOH$	7	2H-Perfluoro-2-decenoic acid	FOUEA	70887-84-2	$C_{10}H_2F_{16}O_2$

Table S2 Molecular structures of PFASs

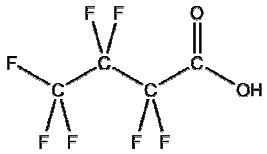
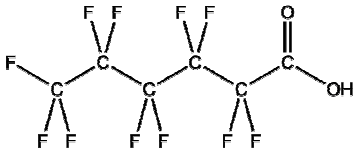
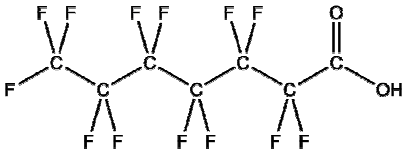
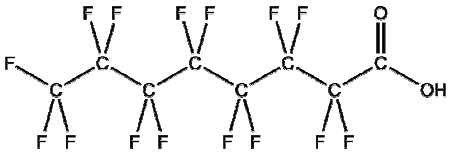
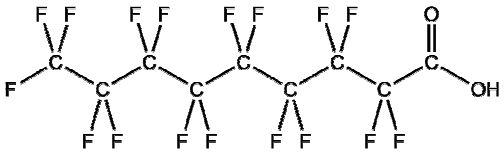
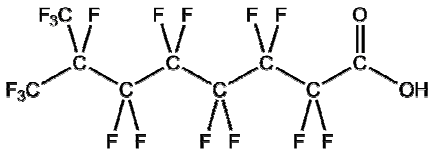
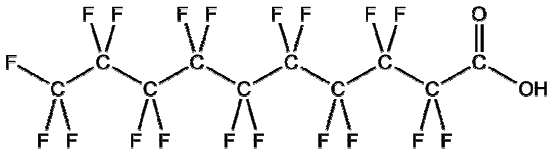
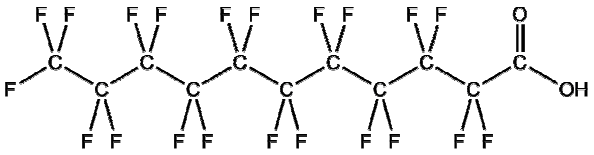
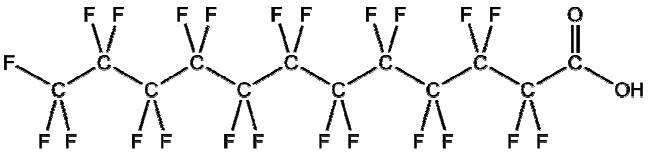
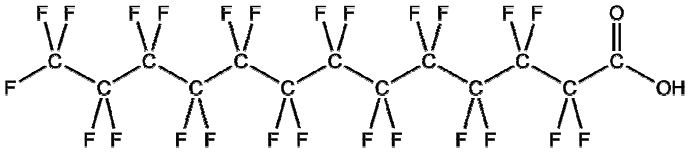
Acronym	Structure
PFBA	
PFHxA	
PFHpA	
PFOA	
PFNA	
ipPFNA	
PFDA	
PFUnDA	
PFDoDA	
PFTrDA	

Table S2 (continued) Molecular structures of PFASs

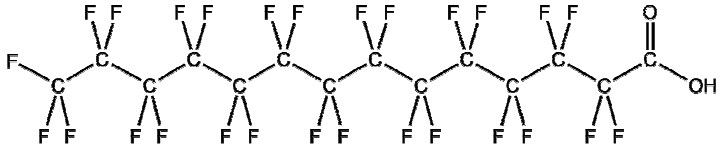
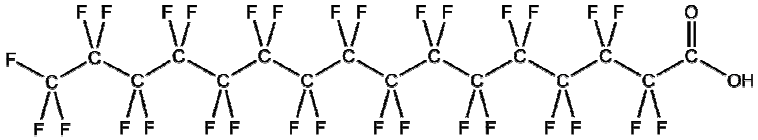
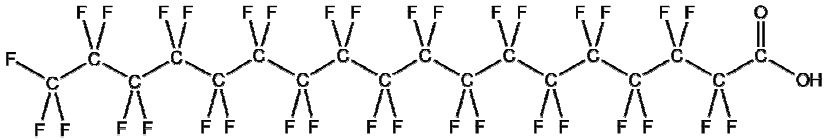
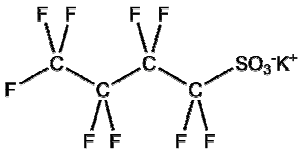
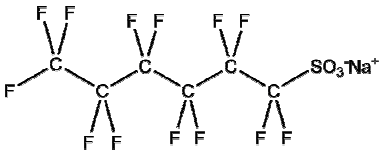
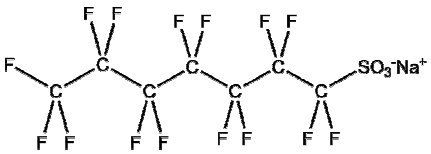
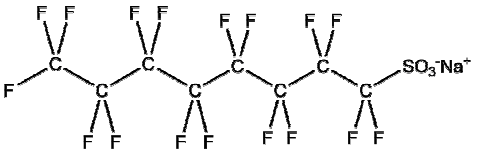
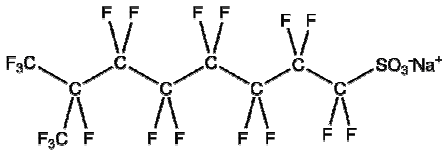
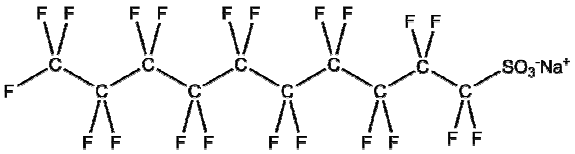
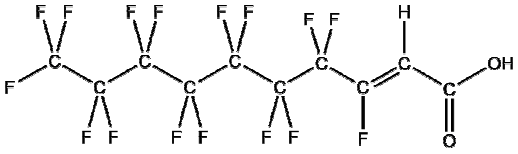
Acronym	Structure
PFTeDA	
PFHxDA	
PFODA	
PFBS	
PFHxS	
PFHpS	
PFOS	
ipPFNS	
PFDS	
FOUEA	

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Table S3 Name, acronym and formula of MPFASs

Name	Acronym	Formula
Perfluoro-n-(1,2,3,4$^{13}C_4$)butanoic acid	MPFBA	$^{13}C_4HF_7O_2$
Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	MPFHxA	$^{13}C_2{}^{12}C_4HF_{11}O_2$
Sodium perfluoro-1-hexane($^{18}O_2$)sulfonate	MPFHxS	$C_6F_{13}S^{18}O_2{}^{16}ONa$
Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	MPFOA	$^{13}C_4{}^{12}C_4HF_{15}O_2$
Sodium perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	MPFOS	$^{13}C_4{}^{12}C_4F_{17}SO_3Na$
Perfluoro-n-(1,2,3,4,5$^{13}C_5$)nonanoic acid	MPFNA	$^{13}C_5{}^{12}C_4HF_{17}O_2$
Perfluoro-n-(1,2$^{13}C_2$)decanoic acid	MPFDA	$^{13}C_2{}^{12}C_8HF_{19}O_2$
Perfluoro-n-(1,2$^{13}C_2$)undecanoic acid	MPFUnDA	$^{13}C_2{}^{12}C_9HF_{21}O_2$
Perfluoro-n-(1,2$^{13}C_2$)dodecanoic acid	MPFDoDA	$^{13}C_2{}^{12}C_{10}HF_{23}O_2$

Table S4 Coordinates of all sampling points

	Point	UTM X[(a)]	UTM Y[(a)]	Reference
Segura River Sampling	1	688383	4216632	Bridge, Benejúzar
	2	690974	4217731	Azud de Alfeitamí, Almoradí
	3	692415	4218261	After WWTP, Algorfa
	4	696728	4217353	Stand near Formentera
	5	700024	4218173	Rojales
	6	703132	4218850	Bridge, Guardamar
Turia River Sampling (2012)	12-1	673997	4398936	Calles
	12-2	674589	4397963	Calles
	12-3	679648	4392454	Near Loriguilla dam
	12-4	682877	4388118	Fuencaliente
	12-5	690376	4386376	Bugarra
	12-6	692308	4386783	Bugarra
	12-7	691432	4386786	Bugarra
	12-8	713610	4377916	La Presa
	12-9	717215	4375932	Manises
	12-10	671242	4497930	Villalba Alta
	12-11	671242	4497930	Villalba Alta
	12-12	667500	4488337	Near Alfambra
	12-13	620809	4475013	Before Tramacastilla
	12-14	622144	4476231	After Tramacastilla
	12-15	642173	4474094	After Gea de Albarracín
	12-16	654549	4468794	Near San Blas
	12-17	660388	4467562	Teruel
	12-18	659446	4468617	Near Parador, Teruel
	12-19	646939	4435616	Ademúz
	12-20	661820	4403623	Before Benagéber dam
	12-21	661622	4400225	Benagéber dam
Turia River Sampling (2013)	13-1	682877	4388118	Fuencaliente
	13-2	674403	4398751	Calles
	13-3	679298	4392436	Chulilla
	13-4	682877	4388118	Chulilla
	13-5	690584	4386265	Bugarra
	13-6	692308	4386783	Bugarra
	13-7	717246	4376190	Orchard, Huerto S. Francisco
	13-8	713585	4377967	Orchard, La Presa
	13-9	711326	4379920	Orchard, Masía de Traver
	13-10	722638	4370253	Orchard, Faitanar
	13-11	726201	4363485	Orchard L'Albufera
	13-12	728582	4366902	Orchard, L'Arbre del Gos
	13-13	728023	4369884	Orchard, Montolivet
	13-14	671584	4498215	Villalba Alta-Alfambra
	13-15	667504	4488313	Villalba Alta-Alfambra
	13-16	620779	4475005	Tramacastilla

Table S4 (continued) Coordinates of all sampling points

	Point	UTM X[(a)]	UTM Y[(a)]	Reference
Turia River Sampling (2013)	13-17	622151	4476223	Tramacastilla
	13-18	642150	4474127	Gea de Albarracín
	13-19	654762	4468671	Vega de Teruel
	13-20	659446	4468596	Vega de Teruel
	13-21	659683	4464556	Vega de Teruel
	13-22	618967	4480511	Tramacastilla
	13-23	626270	4474757	Tramacastilla
	13-24	661825	4471198	Vega de Teruel
	13-25	659043	4463240	Vega de Teruel
	13-26	662818	4401283	Benagéber dam

(a) UTM coordinates in ETRS89

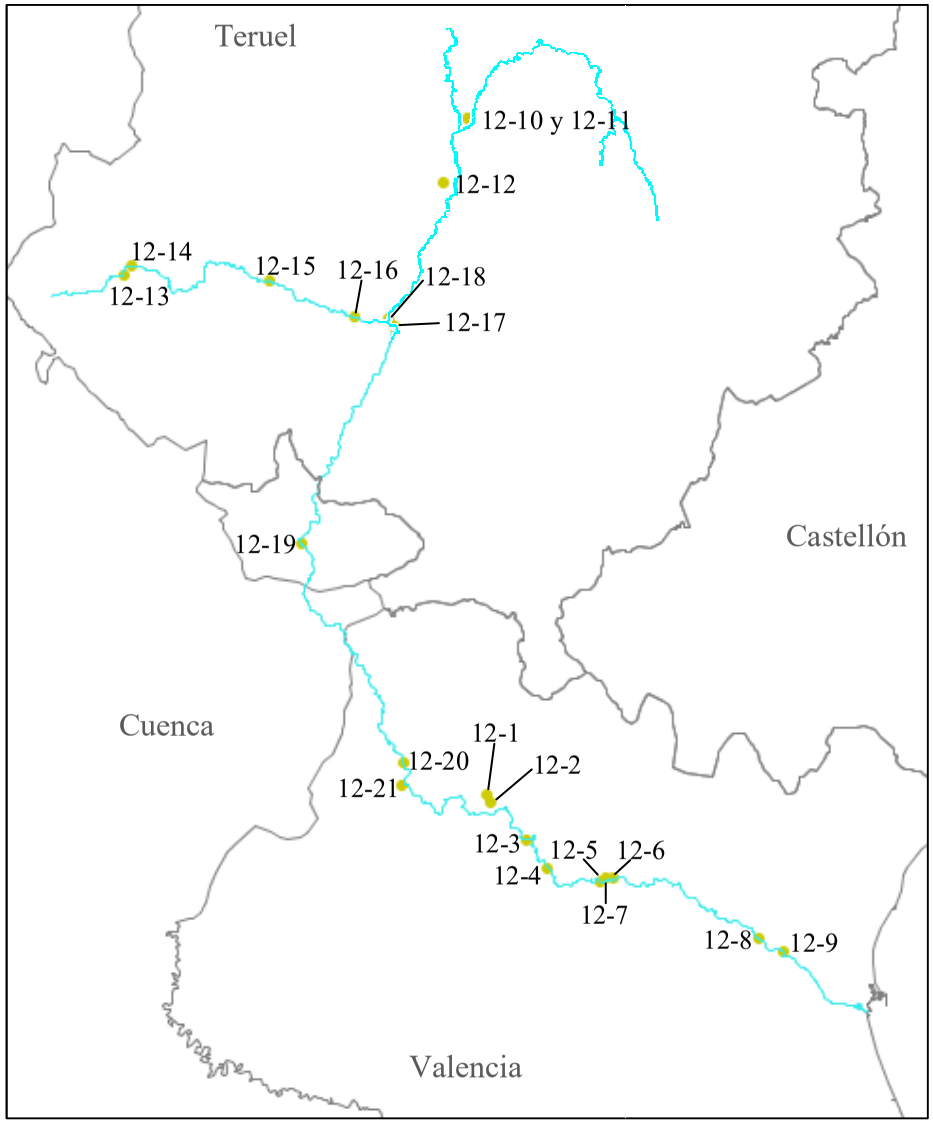

Figure S1a Turia River sampling (2012). Made with gvSIG 2.0

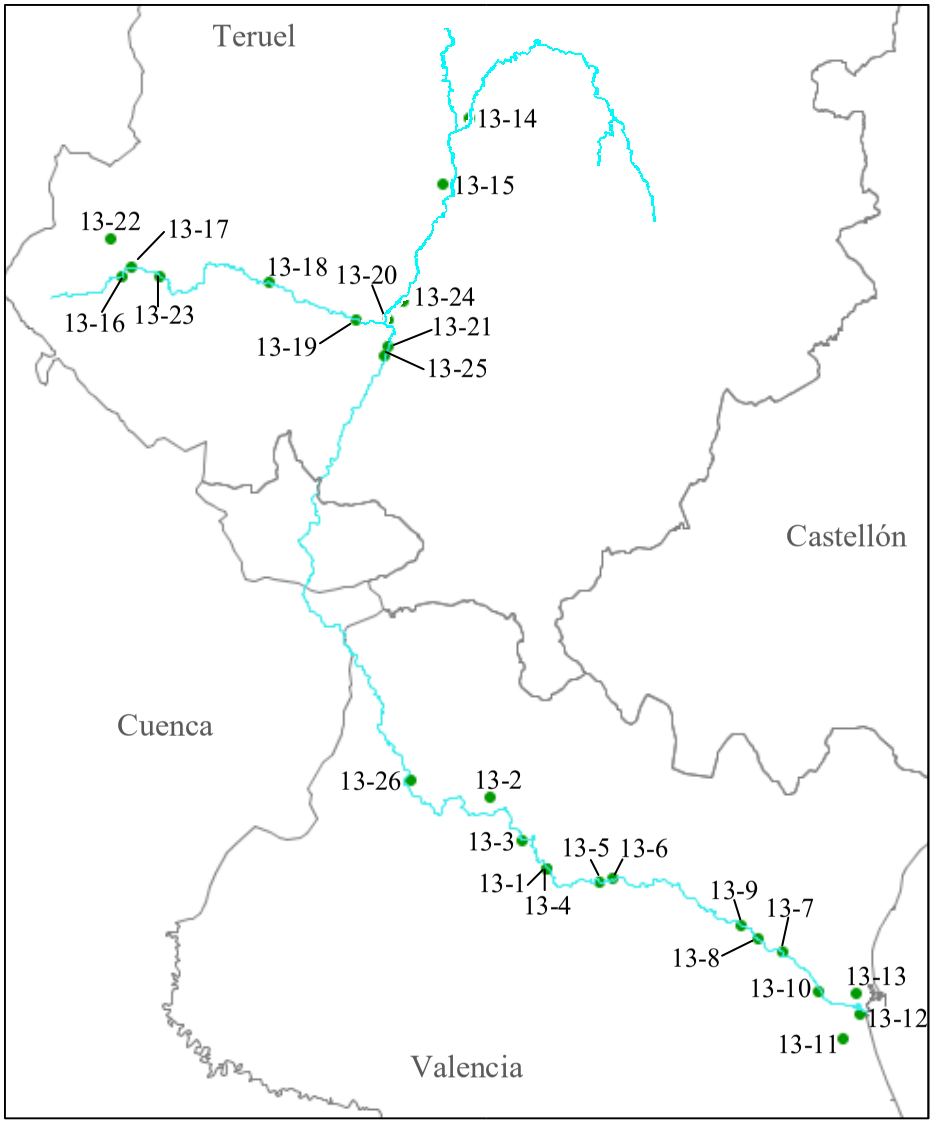

Figure S1b Turia River sampling (2013). Made with gvSIG 2.0

Table S5 Instrumental characteristics used for PFASs determination

LC CONDITIONS	
Analytical column	Kinetex XB-C18: 50.0 × 2.1 mm, 1.7 µm particle size (Phenomenex, Torrance, USA)
Column temperature	30° C
Volume injected	5 µL
Mobile phase	(A) Water – (B) methanol both with 10 mM Ammonium Formate
Flow rate	0.2 mL min^{-1}
Linear gradient	0 min (30 % B), 0.5 min (30 % B), 12 min (95 % B), 20 min (95 % B), and return to the initial conditions (equilibration time 12 min)
TRIPLE QUADRUPOLE MS/MS CONDITIONS	
Ionization characteristics and source	MS/MS performed in selected reaction monitoring mode (SRM) with electrospray ionization (ESI) in negative mode
Gas temperature	300° C
Gas flow	11 L min^{-1}
Nebulizer	30 psi
Capillary voltage	4000 V
Chamber current	1.27 µA
Scan type	MRM, with MS1 and MS2 at unit resolution and cell acceleration voltage of 7 eV

Table S6 Dynamic MRM conditions for LC-MS/MS determination of PFASs

PFASs[a]	t_R [b] (min)	Precursor ion	MS_1 [c]	Frag[d] (V)	CE[e] (V)	MS_2[f]	Frag (V)	CE (V)	MS_2/MS_1 (%) (%RSD)[g]
MPFBA	*1.9*	*217*	*172*	*62*	*0*	-	-	-	-
PFBA	2.3	213	169	66	5	-	-	-	-
PFBS	8.4	299	99	142	38	80	142	26	15.3 (2.3)
PFHxA	11.1	313	269	71	5	119	71	5	10.6 (3.3)
MPFHxA	*11.2*	*315*	*270*	*71*	*5*	*119*	*71*	*5*	*7.1 (0.8)*
PFHpA	12.7	363	319	76	5	169	76	5	68.5 (9.2)
PFHxS	12.8	399	99	169	37	80	169	29	65.9 (10.8)
MPFHxS	*12.8*	*403*	*103*	*164*	*33*	*84*	*164*	*37*	*23.5 (4.3)*
PFOA	13.6	413	369	87	5	169	87	5	46.7 (1.4)
MPFOA	*13.6*	*417*	*372*	*82*	*5*	*169*	*82*	*13*	*22.5 (0.6)*
PFHpS	13.7	449	99	179	37	80	179	57	31.9 (8.9)
ipPFNA	14.2	463	419	87	5	169	87	5	27.0 (1.2)
PFOS	14.3	499	99	190	41	80	190	65	82.2 (3.2)
PFNA	14.4	463	419	82	5	219	82	5	13.2 (0.9)
MPFNA	*14.4*	*468*	*423*	*82*	*5*	*223*	*82*	*9*	*17.6 (1.4)*

Table S6 (continued) Dynamic MRM conditions for LC-MS/MS determination of PFASs

PFASs[a]	t_R [b] (min)	Precursor ion	MS_1 [c]	Frag[d] (V)	CE[e] (V)	MS_2 [f]	Frag (V)	CE (V)	MS_2/MS_1 (%) (%RSD)[g]
MPFOS	*14.4*	*503*	*99*	*180*	*41*	*80*	*180*	*61*	*30.0 (1.1)*
FOUEA	14.6	457	393	167	9	-	-	-	-
ipPFNS	14.8	549	99	195	45	80	195	73	21.6 (1.6)
PFDA	15.0	513	469	89	5	269	89	13	15.3 (2.2)
MPFDA	*15.0*	*515*	*470*	*92*	*5*	*270*	*92*	*12*	*8.4 (0.4)*
PFUnDA	15.5	563	519	104	5	269	104	13	14.1 (0.6)
MPFUnDA	*15.5*	*565*	*520*	*94*	*5*	*269*	*94*	*13*	*4.3 (0.4)*
PFDS	15.5	599	99	80	80	80	80	80	17.6 (1.3)
PFDoDA	16.0	613	569	94	5	269	94	13	9.0 (0.8)
MPFDoDA	*16.0*	*615*	*570*	*112*	*5*	-	-	-	-
PFTrDA	16.4	663	619	104	0	169	104	24	8.1 (1.8)
PFTeDA	16.8	713	669	112	5	169	112	25	7.8 (0.2)
PFHxDA	17.6	813	769	114	8	169	114	28	9.6 (1.1)
PFODA	18.4	913	869	134	10	169	128	29	13.3 (1.1)

(a) Compounds ordered by retention time; (b) t_R = retention time; (c) MS_1 = selected product ion for quantification; (d) Frag = fragmentor; (e) CE = collision energy; (f) MS_2 = selected product ion for qualification; (g) MS_2 /MS_1 (%RSD) = = mean values obtained from the matrix-matched calibration curves and relative standard deviation of the ratio. Italics mean isotopically labelled internal standard

Table S7 Characteristics of selected soil and sediment for method validation

Characteristics	Soil	Sediment
Organic matter (%)	4.29	2.78
Water content (%)	1.80	1.54
Carbonates (%)	22.74	28.25
pH (ClK)	7.35	7.25
Electrical conductivity (dS m^{-1})	0.81	-
Nitrogen ($cmol_c$ kg^{-1})	23.46	-
Sodium ($cmol_c$ kg^{-1})	0.05	0.04
Potassium ($cmol_c$ kg^{-1})	0.42	0.30
Magnesium ($cmol_c$ kg^{-1})	2.18	1.21
Calcium ($cmol_c$ kg^{-1})	10.55	14.25
Cation-exchange capacity ($cmol_c$ kg^{-1})	13.21	15.75
Clay (%)	13.97	26.30
Silt (%)	14.68	
Sand (%)	71.34	73.70

Table S8 Parameters for instrumental validation

Family (acronym)	Compound	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Repeatability RSD (%)	Reproducibility RSD (%)
Perfluoroalkyl carboxylic acids (PFCAs)	PFBA	0.9923	1.11	3.33	1.1	3.1
	PFHxA	0.9942	0.28	0.83	4.4	9.1
	PFHpA	0.9926	0.28	0.83	6	12.0
	PFOA	0.9963	0.28	0.83	5.4	7.1
	PFNA	0.9950	0.56	1.67	0.2	0.6
	ipPFNA	0.9953	0.56	1.67	6.9	10.7
	PFDA	0.9989	0.28	0.83	2.7	4.8
	PFUnDA	0.9939	0.56	1.67	0.1	0.6
	PFDoDA	0.9965	0.11	0.33	2.9	5.3
	PFTrDA	0.9992	0.11	0.33	8.4	10.1
	PFTeDA	0.9909	0.28	0.83	7.4	9.5
	PFHxDA	0.9900	0.56	1.67	2.6	3.5
	PFODA	0.9871	1.11	3.33	1.2	2.3
Perfluoroalkane sulfonic acids (PFSAs)	PFBS	0.9920	1.11	3.33	0.1	0.1
	PFHxS	0.9946	0.56	1.67	1.6	3.8
	PFHpS	0.9956	0.11	0.33	9.6	11.4
	PFOS	0.9953	1.11	3.33	3.8	5.0
	ipPFNS	0.9982	1.11	3.33	6.9	10.8
	PFDS	0.9927	0.28	0.83	0.9	1.6
Fluorotelomer unsaturated carboxylic acids (FTUCAs)	FOUEA	0.9902	1.11	3.33	0.5	1.6

Table S9 PFAS concentrations (ng g^{-1}) in sediment samples from the Segura River

Compound	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6
PFCAs						
PFBA	14.91	13.95	5.60	14.38	9.69	4.27
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	0.57	n.d.	0.07	n.d.	n.d.	0.31
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ipPFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	0.39	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	1.05	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs						
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	1.45	0.85	1.17	2.29	0.88	0.86
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FTUCAs						
FOUEA	n.d.	n.d.	n.d.	n.d.	n.d.	2.56

n.d. = not detected

Table S10 PFAS concentrations (ng g^{-1}) in soil samples from the Turia River during year 2012

Compound	12-1	12-2	12-3	12-4	12-5	12-6	12-7	12-8	12-9	12-10
PFCAs										
PFBA	2.78	1.07	14.81	9.55	4.72	17.96	2.75	16.63	1.68	9.18
PFHxA	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	0.19	0.07	<LOQ	n.d.	n.d.	0.10	0.89	0.57	1.16	0.13
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ipPFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs										
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	0.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.03	n.d.
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FTUCAs										
FOUEA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detected
<LOQ = below limit of quantification

Table S10 (continued) PFAS concentrations (ng g^{-1}) in soil samples from the Turia River during year 2012

Compound	12-11	12-12	12-13	12-14	12-15	12-16	12-17	12-18	12-19	12-20	12-21
PFCAs											
PFBA	n.d.	1.31	11.55	1.05	n.d.	7.22	6.24	3.19	4.61	n.d.	n.d.
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	n.d.	n.d.	n.d.	2.00	0.09	0.15	n.d.	1.65	n.d.	3.08	n.d.
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ipPFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs											
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.74	n.d.	n.d.
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FTUCAs											
FOUEA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detected

Table S11 PFAS concentrations (ng g^{-1}) in soil samples from the Turia River during year 2013

Compound	13-1	13-2	13-3	13-4	13-5	13-6	13-7	13-8	13-9	13-10	13-11	13-12	13-13
PFCAs													
PFBA	n.d.	n.d.	n.d.	n.d.	1.38	2.81	4.49	n.d.	n.d.	n.d.	n.d.	1.15	6.18
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	n.d.	0.06	n.d.	n.d.	n.d.	n.d.	0.16	n.d.	n.d.	n.d.	0.76	3.43	n.d.
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ipPFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs													
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.81	0.69	4.15	3.48
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FTUCAs													
FOUEA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detected

Table S11 (continued) PFAS concentrations (ng g^{-1}) in soil samples from the Turia River during year 2013

Compound	13-14	13-15	13-16	13-17	13-18	13-19	13-20	13-21	13-22	13-23	13-24	13-25	13-26
PFCAs													
PFBA	n.d.	n.d.	n.d.	n.d.	1.88	n.d.	n.d.	64.04	17.84	n.d.	1.50	2.07	7.82
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	n.d.	n.d.	n.d.	n.d.	1.96	n.d.	n.d.	6.96	n.d.	n.d.	0.10	6.33	n.d.
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ipPFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs													
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FTUCAs													
FOUEA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detected

CAPÍTULO 3

Ultra-high-pressure liquid chromatography tandem mass spectrometry method for the determination of 9 organophosphate flame retardants in water samples.

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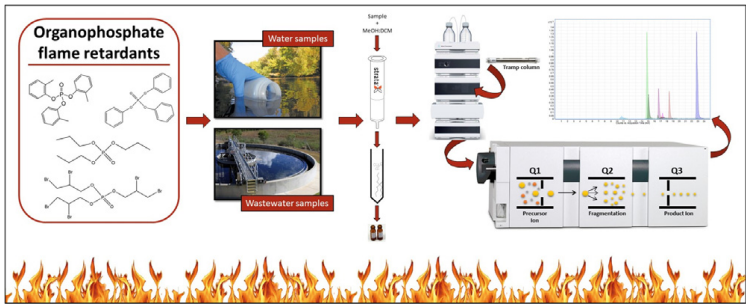
Ultra-high-pressure liquid chromatography tandem mass spectrometry method for the determination of 9 organophosphate flame retardants in water samples

María Lorenzo[a,*], Julián Campo[b,c], Yolanda Picó[a]

[a] Food and Environmental Safety Research Group (SAMA), Desertification Research Centre (CIDE-UV, GV, CSIC), Faculty of Pharmacy, University of Valencia, Burjassot, Valencia, Spain
[b] Environmental Forensic and Landscape Chemistry Research Group, Desertification Research Centre—CIDE (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana), Carretera Moncada—Náquera km 4.5 (Campus IVIA), 46113 Moncada, Valencia, Spain
[c] Earth Surface Science, Institute for Biodiversity and Ecosystems Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

GRAPHICAL ABSTRACT



ABSTRACT

Few methods are available for comprehensive organophosphate flame retardants (PFRs) detection in water and wastewater. Gas chromatography has been employed previously, but this approach is less selective, not amenable for use with deuterated standards and can suffer unfavorable fragmentation. Ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC-QqQ-MS/MS) has become the most promising platform, already applied successfully for analysis of selected PFRs in some environmental matrices like water and wastewater. However, the presence of some interferences from the dissolvent, the equipment and the used materials should be taken into account. The procedure involves:

* Corresponding author.
E-mail address: maria.lorenzo@uv.es (M. Lorenzo).

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- The first determination of PFRs by UHPLC-QqQ-MS/MS using a trap column to distinguish the interferences coming from the instrument and mobile phases.
- The optimization of the LC separation to distinguish all target compounds and their interferences.
- This method coupled to a solid-phase extraction (SPE) improve the detection and quantification of PFRs.

ARTICLE INFO
Method name: Analysis of organophosphate flame retardants in water by SPE and UHPLC–MS/MS using a trap column.
Keywords: Organophosphate flame retardants, Interferences, Trap column, LC–MS/MS, Water, solid phase extraction
Article history: Available online 20 April 2016

Method details

Interference analysis

Ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC-QqQ-MS/MS) is likely the most promising platform for the determination of PFRs in water and wastewater matrices [1–5]. However, the presence of some interferences from the dissolvent, the equipment and the laboratory material can disturb their analytical determination. We made, up to our knowledge, the first determination of PFRs using a trap column to distinguish these interferences. Furthermore, we prove two different solvents, acetonitrile (ACN) and methanol (MeOH), observing the presence of analyte's contamination in both cases. The system is applied to the determination of PFR in water.

Figs. 1 and 2 present chromatograms of a non-spiked river sample and the same sample spiked with the analytes.

Reagents and samples

(a) **Compounds:** the nine PFRs and the four deuterated PFRs used as internal standard (IS) (Table 1**)** were purchased from LGC Standards (Germany).
(b) **Solvents:** methanol (MeOH), dichloromethane (DCM) and acetonitrile (ACN) were bought from VWR (Radnor, PA, USA) and formic acid from AMRESCO (Solon, OH, USA), all of them were of analytical quality.
(c) **Ultra-pure water**: prepared with Milli-Q SP Reagent Water System from Millipore (Bedford, MA, USA).
(d) **Standard solutions**: individual solutions (1 mg/mL) were prepared by dissolving the PFRs in MeOH. Mixed stock solutions containing 10000 ng/mL and 100 ng/mL of each of the nine PFRs were prepared by dilution of the stock solutions with MeOH. Stock and mixed standard solutions were stored in polypropylene tubes at 4 °C.
(e) **Samples**: water samples were obtained from the Turia River (May 2015) from the influent and the effluent of the wastewater treatment plant (WWTP) of Pinedo (March 2015), both near the city of Valencia (Spain). Influent wastewater samples were filtered under vacuum using the ADVANTEC® filters to remove the particulate matter. Analysis of spiked samples and blanks were made in triplicate.

Optimization of sample extraction

(a) Put the Phenomenex Strata-X 33u Polymeric Reversed Phase (200 mg/6 mL) cartridges (Phenomenex, Torrance, Ca, USA) into a 12 port vacuum manifold Supelco Visiprep 57030-U de Sigma-Aldrich (St. Louis, MO, EEUU).
(b) Precondition the cartridge with 6 mL MeOH:DCM (1:1 v/v), 6 mL of MeOH and 6 mL of water, with 350 mba/h·PA vacuum.
(c) Pass the water samples through the cartridges under previous vacuum at a flow rate of 10 mL/min.

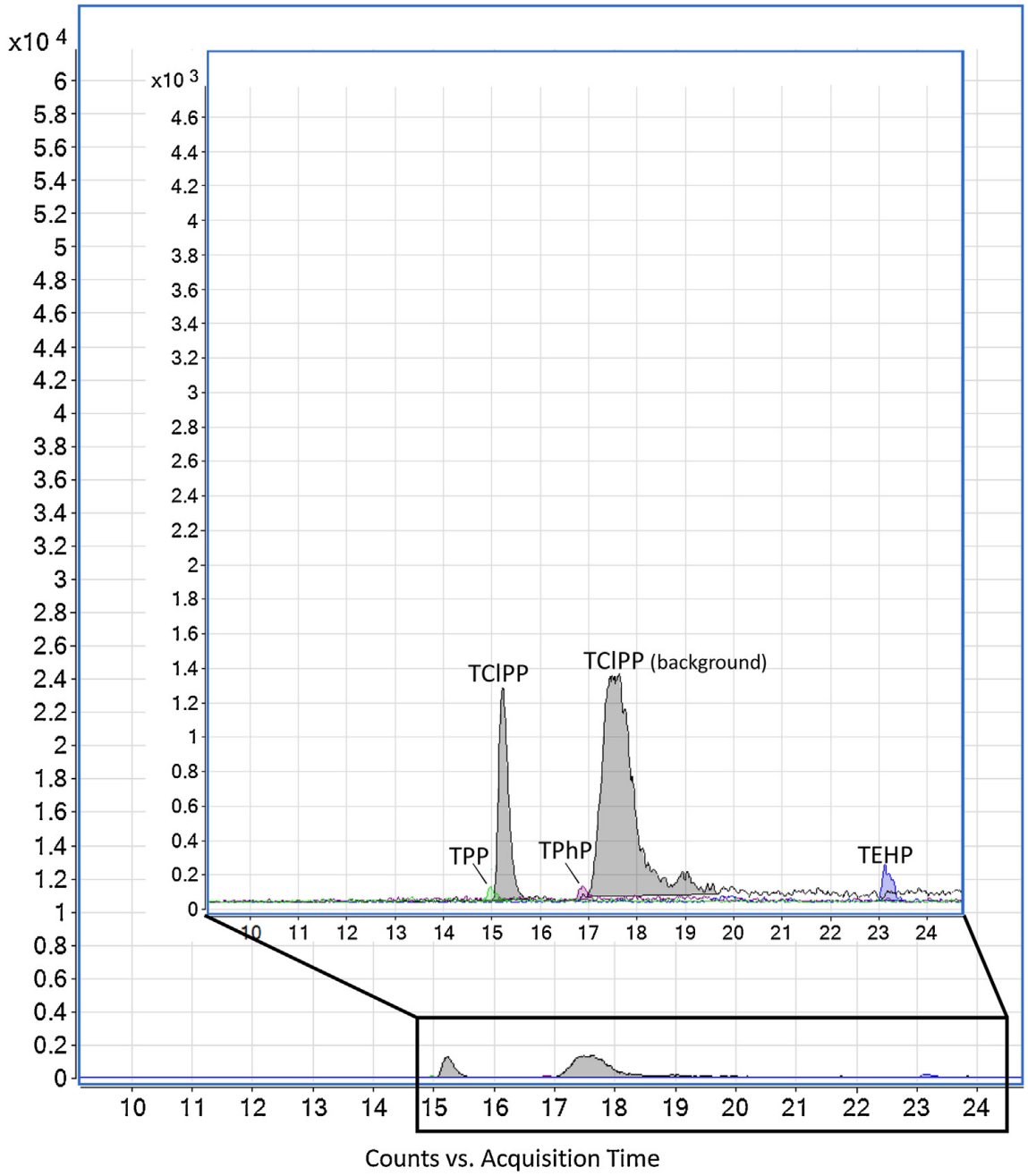

Fig. 1. Chromatogram of a river sample.

(d) Dry the cartridges under vacuum for 15 min.
(e) Elute the analytes on a 15 mL Falcon tube VWR (Radnor, PA, USA) with 10 mL of MeOH:DCM (50:50 v/v).
(f) Evaporate the extracts to dryness at 40 °C using a combined sample concentrator model SBHCONC/1 and a heating plate model SBH130D/3 both manufactured by Stuart (Stafford, UK).
(g) Redissolve the residue in 1 mL of methanol by agitation and ultrasonication for 1 min and pass the extract to 2 mL amber vials with 250 μL insert polypropylene 100/PK + Septum Sil/PTFE, both manufactured by Análisis Vínicos S.L. (Tomelloso, Spain).

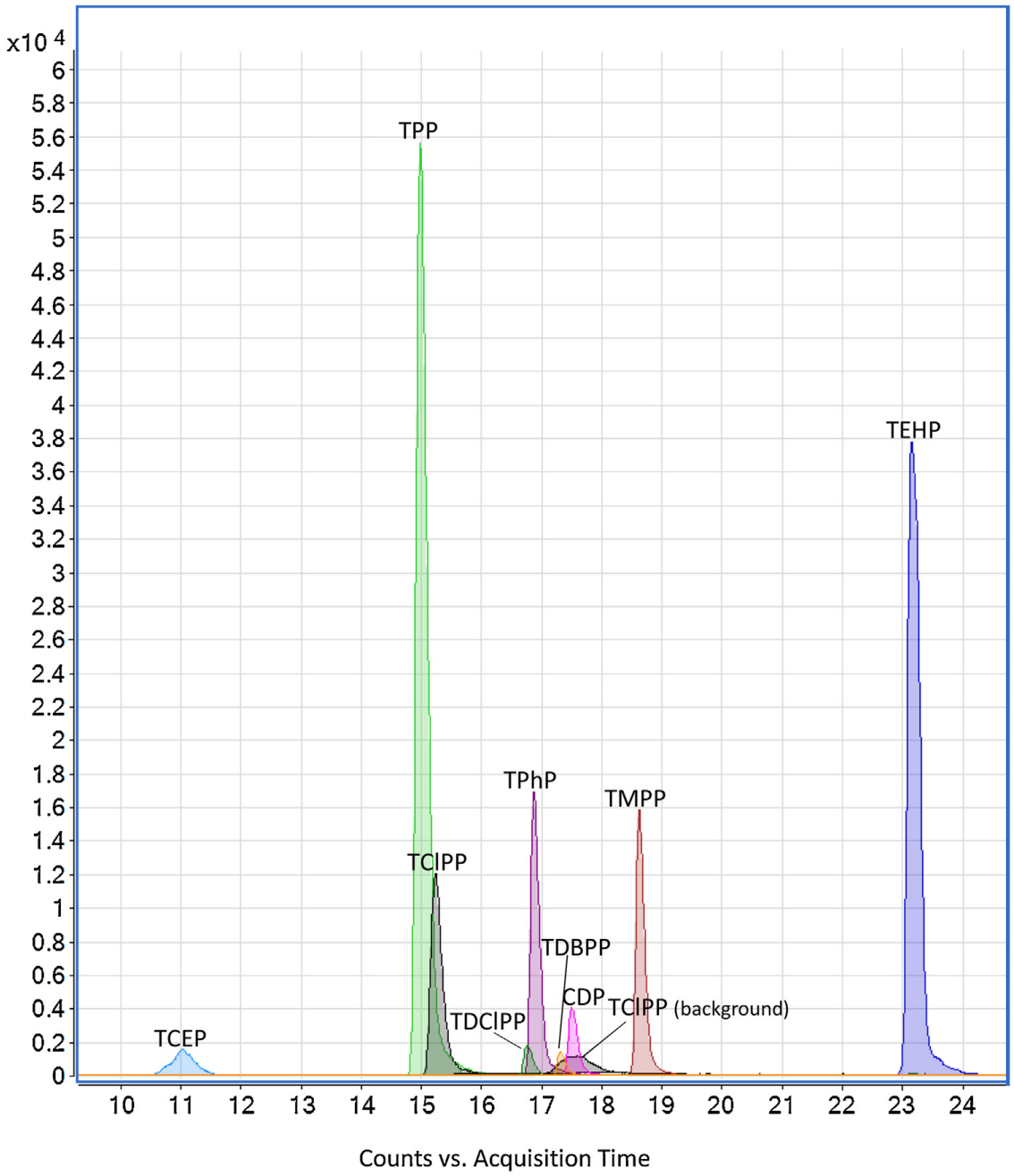

Fig. 2. Chromatogram of the previous river sample spiked at 500 ng/mL.

Instrumentation

The analysis was performed using:

(a) **UHPLC-QqQ-MS/MS system**: 1260 Infinity ultra-high-performance liquid chromatograph combined with a 6410 triple quadrupole mass spectrometer (MS/MS) with electrospray ionization (ESI) of Agilent Technologies (Santa Clara, CA, USA).
(b) **LC column**: Kinetex C18 (50 × 2.1 mm, 1.7 μm) from Phenomenex (Torrance, CA, EEUU).

Table 1
List of the compounds name, acronym and formula.

Compounds	Acronym	Formula	CAS number
Tris(2-chloroethyl) phosphate	TCEP	$C_6H_{12}Cl_3O_4P$	115-96-8
Tri-*n*-propylphosphate	TPP	$C_9H_{21}O_4P$	513-08-6
Tris(2-chloroisopropyl)phosphate	TClPP	$C_9H_{18}Cl_3O_4P$	13674-84-5
Tris(1,3-dichloro-2-propyl)phosphate	TDClPP	$C_9H_{15}Cl_6O_4P$	13674-87-8
Triphenyl phosphate	TPhP	$C_{18}H_{15}O_4P$	115-86-6
Cresyl diphenyl phosphate	CDP	$C_{19}H_{17}O_4P$	26444-49-5
Tris(2,3-dibromopropyl)phosphate	TDBPP	$C_9H_{15}Br_6O_4P$	126-72-7
Tricresylphosphate	TMPP	$C_{21}H_{21}O_4P$	1330-78-5
Tris-(2-ethylhexyl)phosphate	TEHP	$C_{24}H_{51}O_4P$	78-42-2
Triphenyl phosphate D15 (IS)	M-TPHP	$C_{18}D_{15}O_4P$	1173020-30-8
Tris(1,3-dichloro-2-propyl) phosphate D15 (IS)	M-TDCIPP	$C_9D_{15}Cl_6O_4P$	Not available
Tris-(2-chloroethyl) phosphate D12 (IS)	M-TCEP	$C_6D_{12}Cl_3O_4P$	115-96-8
Tris(2-chloroisopropyl) phosphate D18 (IS)	M-TCIPP	$C_9D_{18}Cl_3O_4P$	13674-84-5

(c) **Trap column**: Zorbax Eclipse Plus C18 (30 × 4.6 mm, 3.5 μm) from Agilent Technologies (Santa Clara, CA, USA). Place this column between the pump and the auto sampler.
(d) **Mobile phases**: (A) water and (B) methanol, both containing 0.1% of formic acid.
(e) **Gradient**: 0 min (30% B), 0.5 min (30% B), 12 min (95% B), 18 min (98% B) and 25 min (98% B) and return to the initial conditions. An equilibration time of 15 min was applied to stabilize the column conditions.
(f) **Flow rate**: 0.2 mL/min and the sample volume injected was 5 μL.
(g) **Analysis**: performed in positive ionization mode.
(h) **Data acquisition**: carried out in selected reaction monitoring (SRM) to identify and quantify using two precursor-product ion transitions, retention times, and the ratio of intensities between the two product ions.

Fragmentor and collision energies were optimized for each compound individually (Table 2).

Validation of the method

The validation of the instrumental parameters (Table 3**)** was performed by determining recoveries, limit of detection (LOD), limit of quantification (LOQ) and linearity over the range to obtain suitable R^2. The quantification was performed using the internal standard method. The mixture of the internal standards was added to water to a concentration of 200 ng/L to get a final concentration in the injected extract of 50 ng/mL. The reported parameters were calculated from distilled, river and wastewater samples spiked with standard solutions prepared in methanol at the appropriate concentrations, in a way that the volume of organic solvent added were never higher than 250 μL. The samples were processed with plastic materials as much as possible to avoid adsorption to glass. There were not river or wastewater samples free of PFRs. Then, to perform these experiments several non-spiked samples were analyzed and the peak area of those compounds were subtracted to that found in the spiked samples.

The LOD was calculated as the mass of analyte required to produce a signal-to-noise (S/N) ratio of 3:1 and the LOQ of 10:1. S/N ratios were calculated using MassHunter Workstation Software (GL Sciences, Tokyo, Japan). LODs and LOQs were estimated from water samples spiked at 4 ng/L for river water and at 100 ng/L in wastewater. Values reported in Table 3 are LODs and LOQs in the injected extracts that would correspond to LODs ranging from 0.12 to 1 ng/L and LOQs from 1.2 to 10 ng/L in water samples. The results obtained for the LODs and LOQs show that the proposed method is sensitive enough for the determination of the PFRs in water samples.

Table 2
Dynamic MRM conditions for UHPLC-QqQ-MS/MS determination of PFRs.

Acronym	Associated IS	Precursor Ion	Product ion	Fragmentor	Collision Energy
TCEP	M-TCEP	287	99	100	15
		285	223	100	10
TPP	M-TPhP	225.1	140.9	84	3
		225.1	98.9	84	15
TClPP	M-TClPP	329	99	80	15
		327	175	80	10
TDClPP	M-TDClPP	432.9	99.1	80	15
		430.9	99.1	80	15
TPhP	M-TPhP	327.1	214.9	117	27
		327.1	151.9	117	43
CDP	M-TPhP	341.1	151.9	167	43
		341.1	90.9	167	39
TDBPP	M-TDClPP	698.6	98.9	120	25
		696.6	98.9	120	25
TMPP	M-TPhP	369.1	165.6	192	31
		369.1	91	192	43
TEHP	M-TPhP	435.4	98.9	113	7
		435.4	71	113	5
M-TPHP	–	342	160	120	47
		342	82	120	47
M-TDCIPP	–	448	102	120	15
		446	102	120	15
M-TCEP	–	299	67	100	20
		297	67	100	20
M-TCIPP	–	347	102	100	20
		345	102	100	20

In italics, the compounds used as IS.

The precision studies were carried out from the evaluation of intra-day and inter-day variations of the areas ratio between the analyte and its IS using water extracts suitably stored. The intra-day precision of peak areas ratio (five replicates at 400 ng/L), expressed by means of the percentage of relative standard deviation (%RSD ($n = 5$)) were lower than 11.4% and the inter-day precision (five replicates) were less than 20%. Recoveries were calculated from samples spiked at 400 ng/L analyzed in quintuplicate.

The calibration of the LC–MS/MS was conducted using seven different concentrations (from the LOQ to 300 ng/mL) of PFR standard solutions in methanol with 50 ng/mL of each deuterated compound used as IS. The use of IS prevent the matrix effects

The robustness of the method was clearly ascertained during the optimization procedure, by establishing the consequences of the deliberate introduction of minor reasonable variations (mostly different water volume analyzed, sample flow-rates during the extraction step, cartridge drying times

Table 3
Recoveries and R^2 values for calibration curves (n = 7), LODs and LOQs (ng/mL) and intra and inter-day precision.

Acronym	Recoveries	R^2	LOD (ng/ml)	LOQ (ng/ml)	Intra-day precision	Inter-day precision
TCEP	98%	0.994	0.03	0.3	7.5	6.6
TPP	95%	0.990	0.25	2.5	5.8	19.6
TClPP	102%	0.990	0.25	2.5	3.0	6.6
TDClPP	96%	0.990	0.03	0.3	4.1	8.2
TPhP	96%	0.998	0.1	1	4.2	18.9
CDP	94%	0.994	0.1	1	2.6	19.9
TDBPP	99%	0.998	0.25	2.5	11.4	17.5
TMPP	101%	0.990	0.1	1	3.2	18.6
TEHP	106%	0.992	0.25	2.5	5.9	20.0

Table 4
Analysis of 250 mL of the river water and the influent and effluent wastewater samples (ng/ml).

Acronym	River water	Influent wastewater	Effluent wastewater
TCEP	<LOQ	19.0	0.8
TPP	4.0	<LOQ	<LOQ
TClPP	72.5	448.9	134.6
TDClPP	<LOQ	24.4	20.5
TPhP	5.6	20.2	9.7
CDP	<LOQ	298.5	23.7
TDBPP	<LOQ	393.9	7.3
TMPP	<LOQ	17.0	2.2
TEHP	12.5	9.7	99.9

and eluent composition) and by the similar results obtained checking different types of water. The results obtained from the variations of ±20% in the different parameters were not significantly different from those achieved by the validated method. These results proved that the proposed method was robust.

Each 10 samples, one instrumental and one procedural blank were analyzed to serve as quality control. Samples analyzed shown clearly two peaks, the first one corresponding to the TClPP found in the river water and the second to the instrumental background, indicating background contamination from the injection system and tubing of the LC–MS/MS can be successfully separated from the sample contamination. Table 4 shows the performance of the system in water samples (river, influent and effluent wastewater).

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mex.2016.04.006.

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Supplementary information

Ultra-high-pressure liquid chromatography tandem mass spectrometry method for the determination of 9 organophosphate flame retardants in water samples

María Lorenzo[1,a], Julián Campo[b,c], Yolanda Picó[a]

[a] *Food and Environmental Safety Research Group (SAMA), Desertification Research Centre (CIDE-UV, GV, CSIC), Faculty of Pharmacy, University of Valencia, Burjassot, Valencia, Spain*

[b] *Environmental Forensic and Landscape Chemistry Research Group. Desertification Research Centre - CIDE (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana). Carretera Moncada - Náquera km 4.5 (Campus IVIA). 46113 Moncada, Valencia, Spain.*

[c] *Earth Surface Science, Institute for Biodiversity and Ecosystems Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands*

[1] Corresponding author Tel. +34 963543092; fax: +34 963544954
E-mail address: maria.lorenzo@uv.es

Additional information

PFRs have become increasingly important in recent times because the significant reduction in the use of polybrominated diphenyl ethers (PBDEs) - persistent organic pollutants, dangerous for the environment. PFRs are a suitable alternative because they unstable and metabolized quickly in living organisms. Since several decades ago, these compounds were exploited in the production of dyes, varnishes, adhesives, synthetic resins, polyvinyl chloride, hydraulic fluids, plastics and textiles [1-3]. The occurrence, fate and metabolism of OFRs has been and is a hot spot of environmental research.

One of the most important problems associated to the analysis of PFRs in environmental matrices is contamination during all stages of the analytical procedure. Some authors have highlighted laboratory contamination (glass, plastic and rubber material, dissolvent, instruments, etc.) as an important problem in the analysis of contaminants not fully solved yet [6-10]. To reduce or eliminate instrumental background contamination in the case of phthalates [11] and perfluoroalkyl substances [12, 13], the UHPLC plumbing was altered inserting a trap column between the pump and the injector, this delay the elution of the compounds coming from the instruments from those coming from the sample extracts eliminating interferences and improving the accuracy of the quantification. Up to our knowledge, the insertion of a trap column to eliminate background contamination by BFRs has not been tested before. Its use allows to obtain a proper validation of the method in water samples.

Wang et al. 2011 first studied the possible blank contamination by PFRs from the sample treatment or mobile phase of the LC [2]. They found blank contamination in both, methanol and acetonitrile, but they select acetonitrile for the mobile phase because it provided much cleaner instrumental background. We also tested both solvents (methanol and acetonitrile) and also found background contamination in the two solvents (Fig. S1) but in our case, the methanol was selected because provides better separation and the interferences can be minimized using the trap column.

PFR extraction from water samples has been carried out by liquid–liquid extraction (LLE) [4] or solid-phase extraction (SPE) [2] but also by microextraction approaches such as solid-phase microextraction (SPME) [14]. Nevertheless, SPE has been the most frequently selected technique because it provides robust results and high sample throughput. In our study, all the selected OFRs were determine in water samples using SPE with Oasis HLB. After elution of the SPE cartridge with methanol-dichloromethane (50:50, v/v), Analytes can be recovered in an extension higher than 94 %, without the need of adjusting the pH of the samples. The performance of the method has been evaluated using environmental water samples with different complexities.

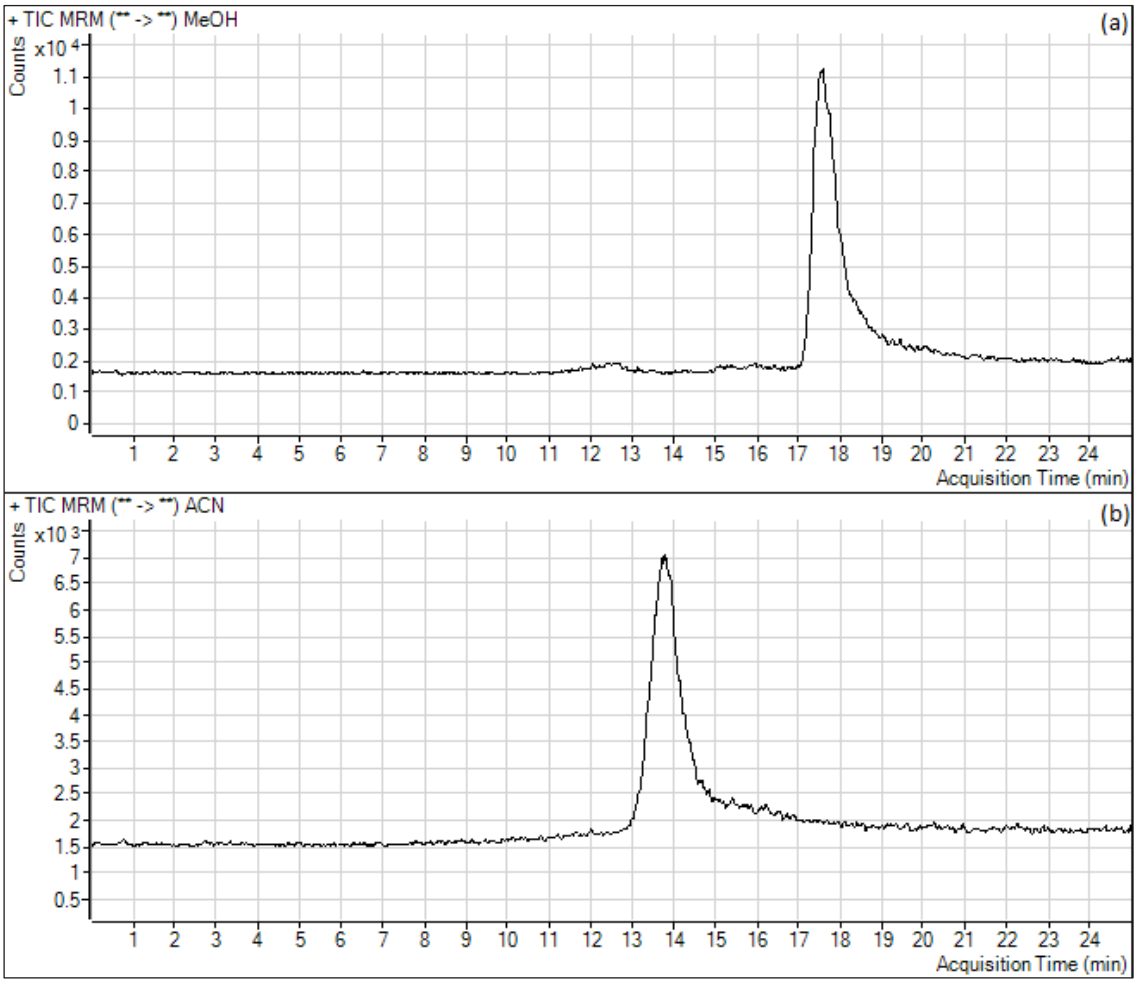

Figure S1. Background contamination in (a) MeOH with MeOH as mobile phase and (b) ACN with ACN as mobile phase.

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CAPÍTULO 4

Determination of organophosphate flame retardants in soil and fish using ultrasound-assisted extraction, solid-phase clean-up and liquid chromatography-tandem mass spectrometry.

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María Lorenzo, Julián Campo, Yolanda Picó.

Abstract

A solid-liquid extraction method combined with HPLC-MS/MS was developed and optimized for extraction and analysis of organophosphorus flame retardants in soil and fish. Methanol was chosen as the optimum extraction solvent, not only in terms of extraction efficiency, but also for its broader analyte coverage. The subsequent clean-up by SPE is required to eliminate matrix co-extractives and reduce matrix effects. Recoveries of the optimized method were within acceptable ranges (70-120%) of 50-121% for soil and 47-123% for biota, both with high precision (RSDs <12% in soil and <23% in biota). The method limits of detection ranged from 0.06 to 0.20 ng g^{-1} dry weight and between 0.02 and 0.30 ng g^{-1} wet weight for soil and biota samples, respectively. However, samples with a high lipid content produce several problems as SPE cartridge clogging that increase variability and analysis time. The method was successfully applied for the determination of organophosphorus flame retardants in soil and fish from L'Albufera Natural Park (Valencia, Spain). Target compounds were detected in all soil and fish samples with values varying from 13.8 to 89.7 ng g^{-1} dry weight and from 3.3 to 53.0 ng g^{-1} wet weight, respectively.

1. Introduction

Flame retardants (FRs) are chemicals added to materials to both prevent combustion and to delay the spread of fire after ignition. Since the ban on some brominated flame retardants (BFRs), organophosphorus flame retardants (PFRs) have been proposed as alternatives [1]. PFRs are widely used as plasticizers and anti-foaming agents in a variety of industries including plastics, furniture, textile, electronics, construction, vehicles and petroleum industries [2]. Usually, PFRs are not bounded chemically to the host material, so they can be easily released by abrasion and volatilization [3]. Public concern has increased in recent years due to the high volume consumed: 205,000 t worldwide in 2005 [4].

There is still insufficient knowledge related to PFRs toxicity [5-10]. However, significant adverse effects were already reported for PFRs. The European Union banned the use or manufacture of tris(2-chloroethyl) phosphate (TCEP) in 2001 that is classified as carcinogenic (cat. 2) and human reproductive toxicant (cat. 1B) [11]. This compound has been replaced progressively by other flame retardants as tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and tris(2-chloroisopropyl) phosphate (TCIPP). The former is also classified as carcinogenic (cat. 2) and the latter, although not yet classified, is also considered a possible carcinogenic [11, 12].

Household furniture, carpets, plastic materials from electronic devices, upholstery, glues and lacquers remain as major sources of PFRs, then, most of the recent research priorities have focused on the migration and occurrence of PFRs in dust and indoor air where they can easily reach higher concentrations and affect human health. The environmental release during the industrial use of PFRs is expected but data on environmental matrices such as soil and fish are still scarce [2]. More common extraction techniques for PFRs

include mechanical agitation and pressurized liquid extraction (PLE) [13, 14]. These techniques can be followed by a purification step, as the gel permeation chromatography (GPC) [15], on-line turbulent flow chromatography (TFC) [16], or the solid-phase extraction (SPE) [17]. Traditionally, the most used technique for the analysis of PFRs has been the GC coupled to flame photometric detector (FPD) [18], mass spectrometry (MS) [19], or tandem mass spectrometry (MS/MS) [20]. LC shares some drawbacks with GC methods, including long chromatographic runs, incomplete separation of some congeners and low throughput. Wang, Liu and Yin [21] developed an early LC coupled to MS/MS method to determine PFRs in water samples with high performance and quick determination. In last years, the use of LC-MS/MS has been generalized for screening PFRs in environmental samples as sediment [22-24], soil [23], biota [22, 24-26], water [17, 21] and wastewater [17, 27]. The main issues in the analysis of PFRs by LC-MS/MS are interferences and background contamination. In this sense, in a previous work on water samples, Lorenzo et al. [17] applied a trap column to distinguish the background contamination coming from the equipment to that of the samples. Unlike other environmental matrices, such as water or air that only show punctual concentrations of PFRs, the analysis of soil and biota allows to measure accumulation over time. These matrices constitute a new analytical challenges due to their own complexity.

The purpose of this study was the development and optimization of an analytical method to determine 9 PFRs in abiotic (soil) and biotic (fish) samples using solid-liquid extraction (SLE) assisted by ultrasound and followed by SPE and HPLC-MS/MS analysis. Moreover, the specific goal of this work was to demonstrate the effectiveness of this analytical methodology for the analysis of environmental samples taken as study area the surroundings of L'Albufera Natural Park (Valencia, East of Spain). To the authors'

knowledge, this is the first proposed method applicable to both matrices, being also a universal method easily extendable to other biotic or abiotic matrices.

2. Material and methods

2.1. Standards and reagents

Tripropyl phosphate (TPP), tris(2,3-dibromopropyl) phosphate (TDBPP), tris(2-ethylhexyl) phosphate (TEHP), tricresyl phosphate (TMPP), TDCIPP, TCEP, TCIPP were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Triphenyl phosphate (TPhP) and cresyl diphenyl phosphate (CDP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The deuterium labelled d_{15}-TDCIPP, d_{15}-TPhP, d_{12}-TCEP, d_{18}-TCIPP were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). CAS number and empirical formula are listed in **Table S1.** Stock standard and working solutions were prepared in methanol and stored at 4 °C. Methanol, dichloromethane, hexane, ethyl acetate and acetonitrile were bought from VWR (Radnor, PA, USA) and formic acid from AMRESCO (Solon, OH, USA), all of them with the highest purity grade. Ultra-pure water was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA).

2.2. Sample collection

Selected soil was sampled at L'Albufera Natural Park (Valencia, Spain) from the upper 20-cm by means of a shovel and transported in pre-rinsed polypropylene container. The soil presented pH>7, clay texture, high content in $CaCO_3$ (>30%) and low level of organic matter (≈ 2%). In the laboratory, the soil sample was spread in a layer of approximately 1 cm thick on polypropylene trays and air-dried in darkness, at 20 °C, to a moisture

content of approximately 3%. Then, the soil sample was passed through a 2-mm Ø sieve and stored in sealed plastic bag at 4 °C until analysis.

Biota samples, European eel *(Anguilla anguilla)* and brown trout *(Salmo trutta),* were selected for analysis because of their high content of adipose tissue (8-31% and 2-15%, respectively) [28] where usually PFRs bio-accumulate, and because of their traditional presence as part of the native fauna in rivers and wetlands of the Valencian Community (Spain). In this study, five specimens of each species were obtained from a feeding center. Once in the laboratory, they were crushed, homogenized and frozen at -20 °C.

2.3. Sample preparation

Different experiments were carried out in order to optimize the sample preparation procedure (**Fig. S1**). For both matrices and prior to the extraction, d_{15}-TDCIPP, d_{15}-TPhP, d_{12}-TCEP and d_{18}-TCIPP were added as internal standards (IS) to obtain a final concentration in the extract injected in the HPLC of 10 ng g^{-1}. Several sample amounts were tested. 1 g of homogenized sample was put in a 50 mL centrifuge tube. Methanol, acetonitrile, dichloromethane, hexane and ethyl acetate as well as different mixtures were tested as extraction solvents. The sample was homogenized with 10 mL of solvent, then, shaken for 3 min and sonicated for another 15 min. This procedure was repeated three times. Three strategies to eliminate suspended material were tested: filtration with Büchner funnel, filtration with traditional paper filter and centrifugation. After that, samples were evaporated to 1 mL and added to 250 mL volumetric flask with ultra-pure water.

To select the optimal cartridge for the SPE clean-up, which allows maximum retention of co-extractives and good elution of the analytes, seven different types were tested: STRATA-X polymeric reversed phase of 60, 200 and 500 mg (Phenomenex), OASIS

HLB 60 mg (Waters), SUPELCO 60 and 500 mg (Sigma-Aldrich) and ThermoSci C8 200 mg (Thermo Scientific). Prior to elution and to ensure removal of water, cartridges were air-dried under vacuum (15 min) or water was displaced adding then 250 µL of methanol. Four volumes of methanol (1:1) were tested (5, 8, 15 and 20 mL). Samples were evaporated to dryness, re-dissolved with 250 µL of methanol and sonicated for 2 min.

2.4. Instrumental analysis

The instrumental analysis follows the previous work [17] with minor modifications. Briefly, a 1260 Infinity HPLC combined with a 6410 MS/MS triple quadrupole of Agilent Technologies (Santa Clara, CA, USA), with electrospray ionization (ESI), was used. Data were processed using MassHunter Workstation Software for qualitative and quantitative analysis (GL Sciences, Tokyo, Japan). Kinetex XB-C18 (50.0 × 4.6 mm, 1.7 µm particle size) was selected as chromatographic column, and ZORBAX Eclipse Plus C18 was selected as trap column and installed between the pump and injector. The mobile phases consisted of (A) water and (B) methanol, both containing 0.1% formic acid. The following gradients were applied: 0 min (30% B), 0.5 min (30% B), 12 min (95% B), 18 min (98% B) and 25 min (98% B), and return to the initial conditions. An equilibration time of 15 min was applied to stabilize the column conditions for a new injection. The flow rate was kept at 0.2 mL min^{-1} throughout the run, and the sample volume injected was 5 µL. Analysis was performed in positive ion mode. Data acquisition was carried out in selected reaction monitoring (SRM) to identify and quantify using two precursor-product ion transitions, retention times, and the ratio of intensities between the two product ions. Fragmentor and collision energies were optimized for each compound individually (**Table S2**).

2.5. Quality assurance and method validation

Background contamination is an important issue to take into account when working with PFRs since they are widely used in lab material and equipment, and their presence is known in indoor air and dust [29]. Different strategies have been developed to minimize blank contamination during the sample preparation: minimizing surface contact during sample handling by reducing clean-up, performing extraction and evaporation steps, working in a cleanroom, pre-cleaning all glassware with a polar and non-polar solvent [13], and rinsing the walls of this glassware with an organic solvent [30, 31]. In this work, the use of any rubber and plastic material was avoided (except polypropylene) to minimize possible contamination of the samples during sampling and sample preparation, and all the glassware was baked at 450 °C for 4 h and rinsed with acetone. The laboratory blanks were extracted using the same methodology described before.

Linearity was determined by a seven-point calibration curve including all the analytes, with concentrations ranging from 0.4 (1.9 for TCEP, TDBPP and CDP) to 300 ng mL^{-1}. The IS was added to obtain a final concentration of 10 ng g^{-1}. The instrumental limits of detection (iLOD) and quantification (iLOQ) were calculated as the minimum amount of analyte dissolved in methanol that gave a signal-to-noise ratio of 3 and 10, respectively. Precision was measured as intra- and inter-assay variability by the relative standard deviation (RSD) of five consecutive injections, while the inter-assay precision was assessed through five injections on five different days at the level of 100 ng mL^{-1} (**Table S3**).

Recoveries of the method were evaluated using 1 g of sample spiked at 10 ng g^{-1} of each PFRs. Three replicates were made with three matrix blank samples in order to evaluate the presence of target PFRs. For the assessment of recovery results, the analyte response

was always related to the internal standard responses to compensate for undesirable matrix effects and losses during the extraction step (except in the evaluation of the matrix effect). The method precision (reproducibility), expressed as RSDs, was evaluated in quintuplicate by intra- day variation using the spiked soil and fish sample with PFRs at 10 ng g^{-1}.

The matrix effect was calculated by comparing the response of target PFRs at 10 ng g^{-1} concentration each, in the dissolvent solution (methanol), with those acquired spiking the same amount of analytes into an extract of soil, *Salmo trutta* or *Anguilla anguilla* obtained through the sample preparation process developed in this study, and using the following equation:

$$\text{Matrix effect} = \left[\frac{\text{Response in matrix}}{\text{Response in methanol}} - 1\right] \cdot 100$$

The method limit of detection (mLODs) and method limit of quantification (mLOQs) values were estimated by injecting in triplicate the extracts obtained from 1 g of spiked soil, *Salmo trutta* or *Anguilla anguilla* and were determined as the amount which gives 3 and 10 times, respectively, the standard deviation of the peak area for the replicates. This was estimated injecting the extract of samples spiked at low concentrations (1 and 5 ng g^{-1}).

3. Results and discussion

3.1. Optimization of extraction

Soil and biota samples were extracted by SLE. The performance of a given SLE application is critically dependent on the properties of the selected solvent, which determines the selectivity and the reliability of the method. Different types of solvent

including methanol, acetonitrile, ethyl acetate, dichloromethane, hexane and several mixtures were tested since they were used in previous studies with good results [25, 32]. **Fig. 1** presents the results obtained for the three pure solvents or mixtures [methanol, dichloromethane-hexane (1:1 v/v), ethyl acetate-acetonitrile (1:1, v/v)] that provided the best efficiency (recovery plus matrix effect since quantification is carried out against the calibration prepared in methanol). The extraction with dichloromethane-hexane (1:1 v/v) provide recoveries >>100%, especially in *Anguilla anguilla* and *Salmo trutta*. This could be due to an enhance of the analyte response in the presence of other extract components that is more marked in *Salmo trutta* than in *Anguilla anguilla*. The mixture ethyl acetate-acetonitrile provide low recoveries (< 40%) for some PFRs, such as TMPP in the three tested matrices. Then, methanol was selected as the most favourable extraction solvent in both matrices since it allows the best recoveries for soil, *Anguilla anguilla* and *Salmo trutta* (50-121%, 71-117% and 47-123%, respectively).

Next, considering that the sensitivity of the procedure is dependent on the amount of sample extracted, the soil and fish quantities to be taken were investigated by spiking different amounts of sample, i.e. 1 and 5 g. The recoveries were similar for the two amounts in soil and *Salmo trutta*. However, in *Anguilla anguilla* samples, the high lipid content, (8-31%) [28], produced several problems as SPE cartridge clogging that increased variability and analysis time. Furthermore, the injection in the HPLC system of the extracts obtained with 5 g of *Anguilla anguilla* decreased the durability of the analytical column. With soil extracts, the column can be used for at least 250-300 injection whereas with *Anguilla anguilla* extracts (of 5 g samples), after 100 injections peaks start to become broader and split indicating a degraded column performance caused probably by the high lipid content in the extract. Then, the best recoveries (71-117%) and

cleaner extracts were obtained by reducing the amount of sample to 1 g **(Fig. 2)**. Furthermore, 1 g sample injections increase the durability of the analytical column.

During the clean-up step, some cartridges collapsed due to suspended particles in the extracts from soils causing delays in the SPE or requiring the introduction of additional steps (as part of the sample must to be passed for other cartridge). To eliminate suspended solids, different strategies were tested as the use of conventional paper filters, glass microfibre filters or centrifugation. Unfortunately, paper and glass microfibre filters fully retained some PFRs as CDP, TMPP and TEHP. Glass microfiber filter also showed very low recoveries for the rest of compounds (< 30%). Regarding paper filter, recoveries were slightly higher but still unacceptable (< 59%). However, TDBPP was the exception presenting a better recovery in paper filter (114%) than using the centrifuge (50%). (**Fig 3a**). Addition of some mL of an organic solvent improved recoveries but was not appropriate for further SPE. Centrifugation was chosen as previous step to clarify the samples because of its quickness and efficiency (**Fig. 3a**). Best recoveries (59-121%) were obtained by using the centrifuge during 5 min at 3000 rcf.

The seven different types of tested cartridges [STRATA-X polymeric reversed phase of 60, 200 and 500 mg (Phenomenex), OASIS HLB 60 mg (Waters), SUPELCO 60 and 500 mg (Sigma-Aldrich) and ThermoSci C8 200 mg (Thermo Scientific)] gave appropriate recoveries without high differences for most of the PFRs. The most conflictive compound was TCEP that was not well-retained in 60 mg cartridges probably because is the most water soluble compound and then, some breakthrough took place. STRATA-X polymeric reversed phase of 200 mg (Phenomenex) was selected because it provided the best recoveries for all targeted compounds (71-120%) (**Fig. 3b**).

One important step is to remove water of the cartridge before analyte elution. The addition of 250 µL of methanol or to pass air through the cartridges by vacuum were tested. Best results were obtained when cartridges were air-dried for 15 min. Despite the water displacement with methanol could prevent loss of volatile compounds as TEHP, low recoveries obtained suggested that some analytes were dragged by the low volume of methanol used. Another possibility is that this low volume of methanol was insufficient to remove the water from the cartridge.

Finally, four volumes of methanol as elution solvent were tested (5, 8, 15 and 20 mL). The best recoveries (78-117%, data not shown) were obtained with 8 mL. Using 5 mL of methanol, TCEP was totally retained by the SPE cartridges while some PFRs as TEHP and TPP were partly retained. Contrarily, the larger volumes 15 and 20 mL did not improve recoveries being they lower, probably because during the following evaporation step, the longer time needed by these high volumes increased loss by evaporation.

3.2. Optimization of the HPLC-MS/MS

TCIPP was found in procedural and instrumental blank samples indicating background contamination from the injection system and tubing of the HPLC. The trap column placed before the injector distinguished the TCIPP of the sample (peak at 14.9 min in **Fig. S2**) from the TCIPP of the instrument (second peak at 17.0 min in **Fig. S2**).

Although the useful separation of TCIPP from the HPLC equipment, the presence of PFRs in blank samples is a common problem in the analysis of these compounds. PFRs can also come from the glass and plastic material as well as from the solvents used. Despite all precautionary measures to eliminate background contamination of other PFRs (i.e. TDCIPP), there are still low levels of TCIPP (0.5-1 ng g^{-1}) from the HPLC system that are separated by the trap column (**Fig. S2**).

Compounds were eluted between 10 and 25 min, being the lowest and the highest elution times for TCEP and TEHP, respectively (**Fig. S3**). Maximum signal intensity for most PFRs as well as good chromatographic separation for all of them were obtained by reversed-phase chromatography using a methanol/water gradient with 0.1% of formic acid as organic modifier. Selected SRM transitions and the optimal MS/MS parameters for all the PFRs were already optimized [17], and are summarized in **Table S2.** As previously reported [31], the fragmentation of trialkyl- and trichloralkyl phosphates is dominated by three consecutive McLafferty rearrangements. For TCEP, TPP, TCIPP, TPhP, CDP and TMPP, the rearrangements were clearly observed and three transitions were selected, which allows to eliminate false positives because of the co-extraction matrix interferences. For the others, due to their structural characteristics the third McLafferty rearrangement was not so clearly observed and only two transitions were selected.

Calibration curves were linear and the correlation coefficients (R^2) were superior to 0.99 for each compound. For the intra-day assays, RSD values were <8% and for inter-day <11%. The iLODs ranged between 0.1 and 0.6 ng mL^{-1} (0.5 and 3 injected pg), and iLOQs were between 0.4 and 1.9 ng mL^{-1} (2 and 9.5 injected ng) **(Table S3).** These values were similar to those reported when determining PFRs by LC-MS/MS elsewhere [16, 25, 33].

3.3. Method Validation

The optimized methodology is as follows: 1 g of sample was extracted with 10 mL of methanol using an ultrasound system during 15 min and centrifuged for 5 min at 3000 rcf. The extraction was carried out three times, and extracts were combined in a vial. After, samples were evaporated to 1 mL and added to 250 mL volumetric flask with ultra-pure water and passed through a 200 mg STRATA-X cartridge. Once the extract passed

the column, it was air dried under vacuum for 5 min and the analytes were eluted with 8 mL of methanol. Samples were evaporated to dryness, re-dissolved with 250 µL of methanol and sonicated for 2 min.

Quality parameters of the developed method are summarized in **Table 1**. mLOD and mLOQ values were measured injecting by triplicate the extracts obtained from 1 g of spiked soil, *Salmo trutta* or *Anguilla anguilla*. As the instrumental limits, mLOD and mLOQ were defined as the minimum amount of analyte that gave a signal-to-noise ratio of 3 and 10, respectively. In soil, mLOD ranged from 0.06 to 0.20 ng g^{-1} dw and mLOQ from 0.16 to 0.60 ng g^{-1} dw. In biota, mLOD ranged from 0.02 to 0.30 ng g^{-1} ww and mLOQ from 0.04 to 0.86 ng g^{-1} ww.

Recoveries were evaluated at 5 ng g^{-1} of each PFRs (low concentration). Values in soil were 50% for TDBPP, between 59 and 74% for TCEP and TEHP, and >84% for the rest of compounds. All of them with very low RSD values (<12%). In *Salmo trutta*, the lowest recoveries were for TEHP (47%) and TMPP (60%), the rest of compounds were recovered between 88 and 123%. Finally, for *Anguilla anguilla* samples, recoveries were between 71 and 117% for all target compounds. Regarding biota, RSD were <17% in *Salmo trutta* and <23% in *Anguilla anguilla* samples.

An enhancement due to the matrix effect was observed in all matrices for TPP, TCIPP, TMPP and TEHP, and signal suppression for the rest of compounds. The compounds CDP and TEHP were the most affected by the matrix, with 95% of signal suppression and 98% of signal enhancement, respectively. However, this was compensated with the use of the internal standards d_{15}-TDCIPP, d_{15}-TPhP, d_{12}-TCEP, d_{18}-TCIPP.

One recent study about the extraction of PFRs in marine and river fish (including *Salmo trutta*), by ultrasonic extraction and on-line TFC-LC-MS/MS, showed recoveries between

47 and 98 % (RSD values <16%) and mLODs between 0.19 and 3.44 ng g^{-1} of lipid weight [16]. Another study determined PFRs in fish matrix using ultrasonic extraction, SPE and analysis by LC-quadrupole linear ion trap-MS showing recoveries of 46-109% (RSD values <25%), and mLODs of 0.34-51.6 ng g^{-1} of lipid weight [33]. Both studies showed similar quality parameters than the method presented here, with the characteristic differences of the different LC-MS/MS used. To authors' knowledge, there are no published works that report recovery values, mLODs and mLOQs in soil samples.

3.4. Application to real samples

The developed method was applied to determine the presence of the 9 target PFRs in fifteen environmental samples: five soil samples, five *Salmo trutta* and five *Anguilla anguilla* specimens. Each sample was analysed in triplicate. For the correct determination of PFRs in these matrices, calibration samples were included before and after each batch. Furthermore, a quality control was performed by including procedural blanks. The signal on these blanks was subtracted from the environmental samples to avoid false positives. When the concentration in blanks was >50% of the concentration in the environmental sample, this was discarded. **Table S4** shows the range of concentrations detected in soil and biota samples. PFRs were found in all the biota samples in concentrations between 3.3 and 53 ng g^{-1} ww. In these samples, the highest concentration was for TCIPP (53.0 ng g^{-1} ww in *Anguilla anguilla* and 41.2 ng g^{-1} ww in *Salmo trutta*). Concentrations were similar or one order of magnitude lower than those reported by other authors (**Table S4**). As in biota samples, PFRs were ubiquitous in soil samples and were found in concentrations between 13.8 and 89.7 ng g^{-1} dw. The most abundant compounds were TCIPP (14.1-89.7 ng g^{-1} dw) and TDCIPP (16.3-33.5 ng g^{-1} dw). Due to the lack of published data about PFR concentrations in soil, values only can be compared with those

of a previous study determining these compounds in burned and unburned forest soils [23]. PFRs reached total values (sum of all PFRs in the sample) up to 664.4 ng g^{-1}, which are of the same order of magnitude than those obtained in this study.

4. Concluding remarks

In the present work a fast and cost-effective analytical method, based in SLE aided by ultrasound, for the simultaneous determination of PFRs was developed. The best results were obtained using methanol as extractant and SPE as a clean-up step. The developed method uses a low amount of sample (1 g), low volumes of solvent for the SLE (30 mL) and the SPE clean-up step (8 mL). Among the advantages of the method are the low volume of extraction solvent, compared with PLE, and the high sensibility (mLODs in soil and biota ranging from 0.06 to 0.20 ng g^{-1} dw and from 0.02 to 0.30 ng g^{-1} ww, respectively). Some drawbacks include very low concentration of TCIPP in blank samples and the variability of the signal due to the complexity of the matrices used. The method developed here was applied to fifteen real samples consisting in soil and biota (European eel and brown trout). All the studied PFRs were detected in the samples analysed showing the ubiquity of these compounds in the environment.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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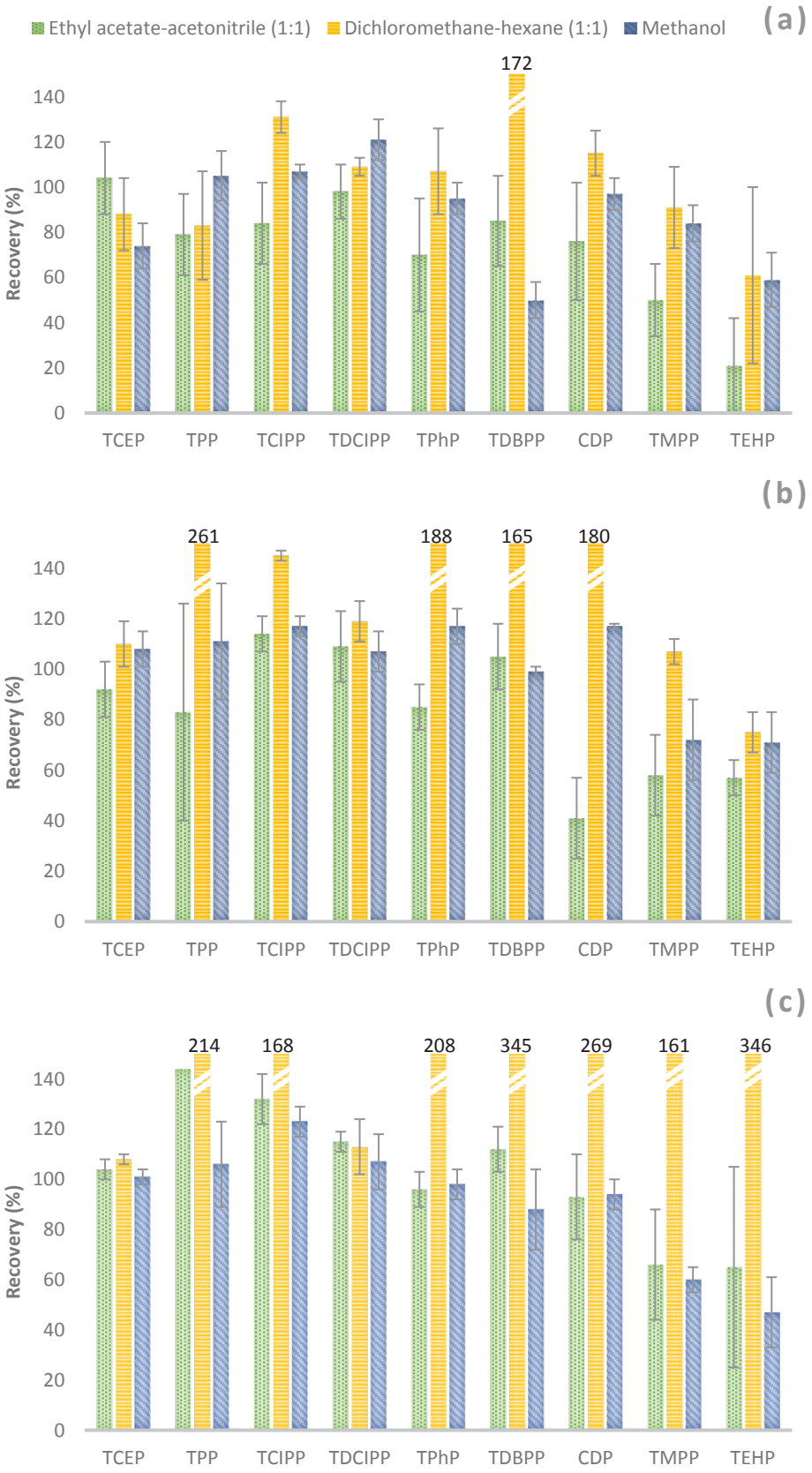

Figure 1. Recoveries and precision (as RSD % error bars) according to the type of extraction solvent for (a) soil, (b) European eel (Anguilla anguilla) and (c) Brown trout (*Salmo trutta*).

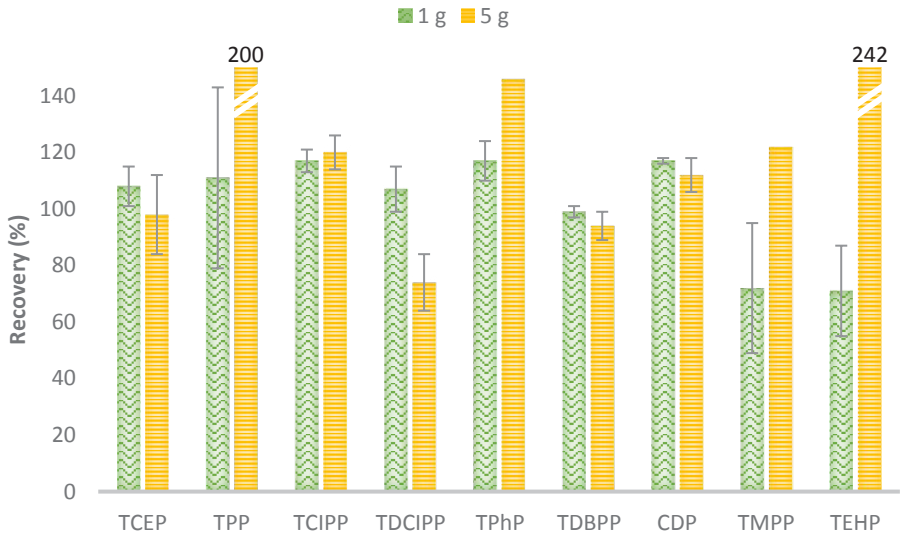

Figure 2. Recoveries and precision (as RSD % error bars) according to the amount of sample (1 and 5 g) for European eel (*Anguilla anguilla*).

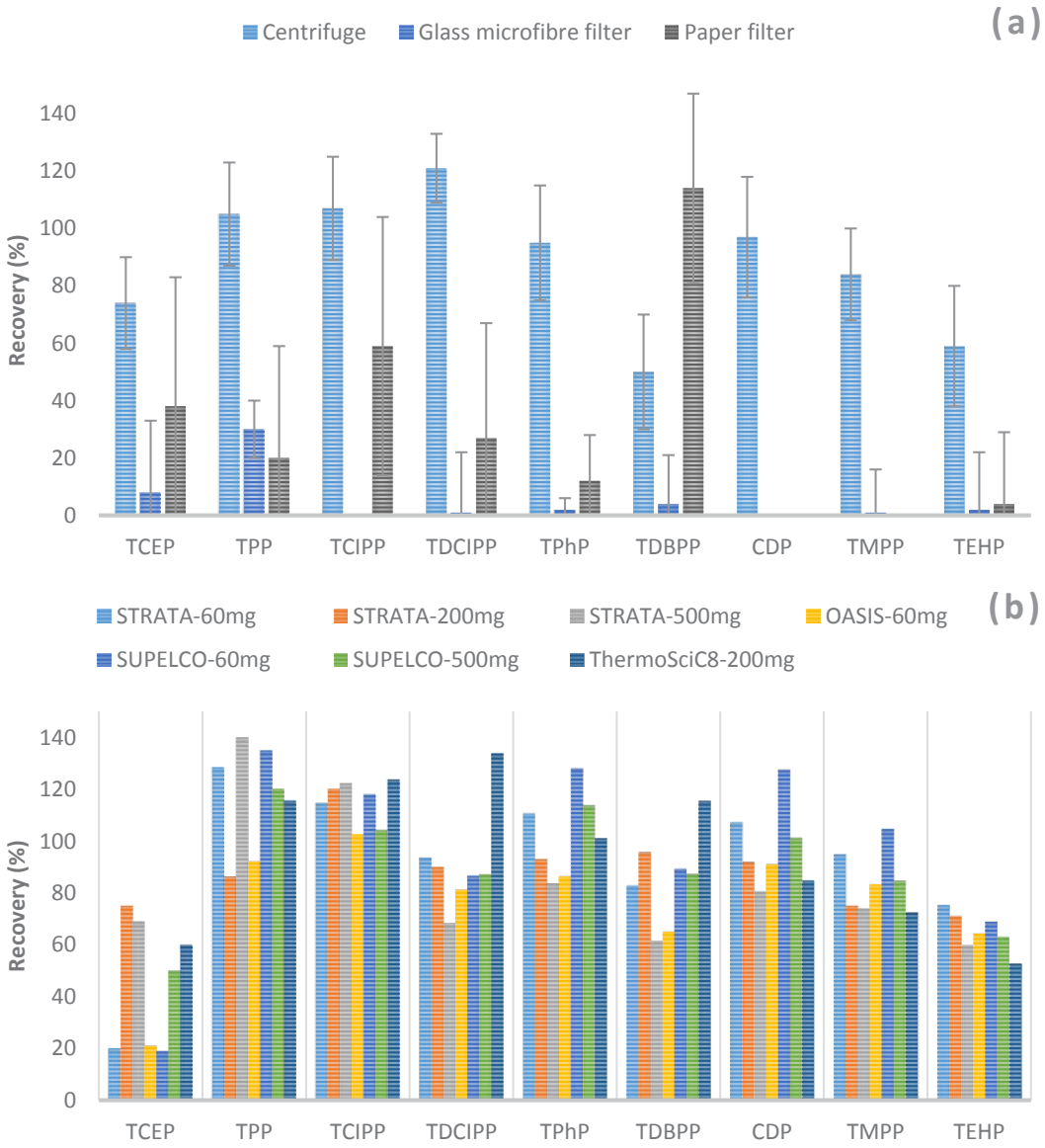

Figure 3. Recoveries and precision (as RSD % error bars) according to (a) the strategy used to eliminate suspended solids and (b) the type of solid-phase cartridge used during the SPE.

Table 1. Method limit of detection (mLOD) and quantification (mLOQ), linearity, matrix effect, recovery and relative standard deviation (RSD in %) of LC-QqQ-MS/MS analysis of selected PFRs in soil, brown trout (*Salmo trutta*) and European eel (*Anguilla anguilla*).

Compound	Matrix effect (%)	Recovery (%)	RSD of the recoveries (%)	mLOD (ng g^{-1})	mLOQ (ng g^{-1})
Soil					
TCEP	-14	74	10	0.20	0.60
TPP	14	105	11	0.08	0.22
TCIPP	1	107	3	0.06	0.20
TDCIPP	-21	121	9	0.06	0.18
TPhP	-7	95	7	0.06	0.16
TDBPP	-5	50	8	0.14	0.44
CDP	-95	97	7	0.20	0.40
TMPP	20	84	8	0.12	0.34
TEHP	79	59	12	0.06	0.20
Salmo trutta					
TCEP	-9	101	3	0.28	0.86
TPP	7	106	17	0.08	0.22
TCIPP	21	123	6	0.10	0.28
TDCIPP	-41	107	11	0.04	0.12
TPhP	-19	98	6	0.04	0.14
TDBPP	-43	88	16	0.12	0.38
CDP	-98	94	6	0.20	0.40
TMPP	25	60	5	0.10	0.30
TEHP	50	47	14	0.02	0.04
Anguilla anguilla					
TCEP	-19	108	7	0.28	0.82
TPP	66	111	23	0.12	0.34
TCIPP	11	117	4	0.08	0.24
TDCIPP	-26	107	8	0.04	0.14
TPhP	-24	117	7	0.06	0.16
TDBPP	-3	99	2	0.30	0.90
CDP	-96	117	1	0.20	0.40
TMPP	28	72	16	0.06	0.18
TEHP	98	71	12	0.10	0.30

SUPPLEMENTARY MATERIAL

Table S1. Compounds selected for this study, acronym, CAS number and formula.

Compound name	Acronym	CAS number	Formula	Internal Standard
Tripropyl phosphate	TPP	513-08-6	$C_9H_{21}O_4P$	d_{15}-TPHP
Tris(2,3-dibromopropyl) phosphate	TDBPP	126-72-7	$C_9H_{15}Br_6O_4P$	d_{15}-TDCIPP
Tris(2-ethylhexyl) phosphate	TEHP	78-42-2	$C_{24}H_{51}O_4P$	d_{15}-TPHP
Tricresyl phosphate	TMPP	1330-78-5	$C_{21}H_{21}O_4P$	d_{15}-TPHP
Triphenyl phosphate	TPhP	115-86-6	$C_{18}H_{15}O_4P$	d_{15}-TPHP
Tris(1,3-dichloro-2-propyl) phosphate	TDCIPP	13674-87-8	$C_9H_{15}Cl_6O_4P$	d_{15}-TDCIPP
Tris(2-chloroethyl) phosphate	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$	d_{12}-TCEP
Tris(2-chloroisopropyl) phosphate	TCIPP	13674-84-5	$C_9H_{18}Cl_3O_4P$	d_{18}-TCIPP
Cresyl diphenyl phosphate	CDP	26444-49-5	$C_{19}H_{17}O_4P$	d_{15}-TPHP
Triphenyl phosphate (D15)	d_{15}-TPHP	1173020-30-8	$C_{18}D_{15}O_4P$	-
Tris(1,3-dichloro-2-propyl) phosphate (D15)	d_{15}-TDCIPP	Not available	$C_9D_{15}Cl_6O_4P$	-
Tris(2-chloroethyl) phosphate (D12)	d_{12}-TCEP	Not available	$C_6D_{12}Cl_3O_4P$	-
Tris(2-chloroisopropyl) phosphate (D18)	d_{18}-TCIPP	Not available	$C_9D_{18}Cl_3O_4P$	-

Table S2. SRM dynamic conditions for the determination of PFRs by UHPLC-QqQ-MS/MS.

Compounds[a]	t_R[b] (min)	Precursor ion for MS_1[c]	MS_1[c]	Frag[d] (V)	CE[e] (V)	Precursor ion for MS_2 and MS_3[c]	MS_2[f]	Frag (V)	CE (V)	MS_3[g]	Frag (V)	CE (V)
d_{12}-TCEP	*10,5*	*299*	*67*	*100*	*20*	*297*	*67*	*100*	*20*	-	-	-
TCEP	10,7	287	99	100	15	285	99	100	15	223	100	10
TPP	15	225	99	84	15	225	141	84	3	81	84	55
d_{18}-TCIPP	*14,8*	*347*	*102*	*100*	*20*	*345*	*102*	*100*	*20*	-	-	-
TCIPP	14,9	329	99	80	15	327	99	80	15	175	80	10
d_{15}-TDCIPP	*16,6*	*448*	*102*	*120*	*15*	*446*	*102*	*120*	*15*	-	-	-
TDCIPP	16,7	433	99	80	15	431	99	80	15	-	-	-
d_{15}-TPhP	*16,7*	*342*	*82*	*120*	*47*	*342*	*160*	*120*	*47*	-	-	-
TPhP	16,8	327	77	117	47	327	152	117	43	215	117	27
TDBPP	17,2	699	99	120	25	697	99	120	25	-	-	-
CDP	17,4	341	91	167	39	341	65	167	67	152	167	43
TMPP	18,4	369	165	192	51	369	165	192	31	91	192	43
TEHP	22,8	435	99	113	7	435	71	113	15	-	-	-

(a) Compounds ordered by retention time; (b) t_R = retention time; (c) MS_1 = selected product ion for quantification; (d) Frag = fragmentor; (e) CE = collision energy; (f) MS_2 = selected product ion for qualification; (g) MS_3 = selected product ion for qualification. Italics mean isotopically labelled internal standard.

Table S3. Instrumental limits of detection and quantification (iLOD and iLOQ) and intra- and inter-day assays (RSD, %) in the analysis of PFRs by UHPLC-QqQ-MS/MS.

Compound	iLOD ($ng\ mL^{-1}$)	iLOQ ($ng\ mL^{-1}$)	Intra-day assay (RSD, %)	Inter-day assay (RSD, %)
TCEP	0.6	1.9	1.1	1.3
TPP	0.1	0.4	7.3	10.8
TCIPP	0.1	0.4	0.6	3.7
TPhP	0.1	0.4	4.3	5.6
TDCIPP	0.1	0.4	1.3	4.9
TDBPP	0.6	1.9	1.5	2.7
CDP	0.6	1.9	6.3	7.1
TMPP	0.1	0.4	4.1	6.0
TEHP	0.1	0.4	3.0	3.9

Table S4. PFR levels in soil and biota samples in the present and other published studies.

Location	No of samples	Reference	Experimental	Range of concentrations (ng g^{-1})
Soil				
Castellón, Spain	28	[23]	Same than in this study but with 5 g of sample	0.1 – 319.8[a]
Valencia, Spain	5	Present study	1 g of sample, SLE assisted by US, methanol as extraction solvent, SPE as clean-up, LC-QqQ-MS/MS analysis	13.8 – 89.7[a]
Biota				
Western Scheldt estuary (the Netherlands)	15	[22]	PLE, DCM/acetone as extraction solvent, SPE clean-up, LC-QqQ-MS/MS analysis	<LOQ – 17[c]
Evrotas (Greece)	4	[24]		34.1 – 55.5[b]
Adige (Italy)	13	[24]	0.5 g of sample, SLE assisted by US, hexane:acetone as extraction solvent, TFC-LS-MS/MS analysis	50.6 – 650[b]
Sava (Slovenia, Croatia, Bosnia and Herzegovina and Serbia)	10	[24]		14.4 – 196[b]
Canada	32	[25]	SLE assisted by US, dichloromethane:hexane as extraction solvent, d-SPE as clean-up, LC-MS/MS analysis	0.14 – 2.2[c]
Norway	8	[26]	PLE, dichloromethane:acetone as extraction solvent, silica gel or hydrid SPE column as clean-up, LC-MS/MS analysis	<LOQ – 537[b]
Valencia (Spain)	10	Present study	1 g of sample, SLE assisted by US, methanol as extraction solvent, SPE as clean-up, LC-QqQ-MS/MS analysis	3.3 – 53.0[c]

[a] ng g^{-1} in dry weight; [b] ng g^{-1} in lipid weight (lw); [c] ng g^{-1} in wet weight (ww).

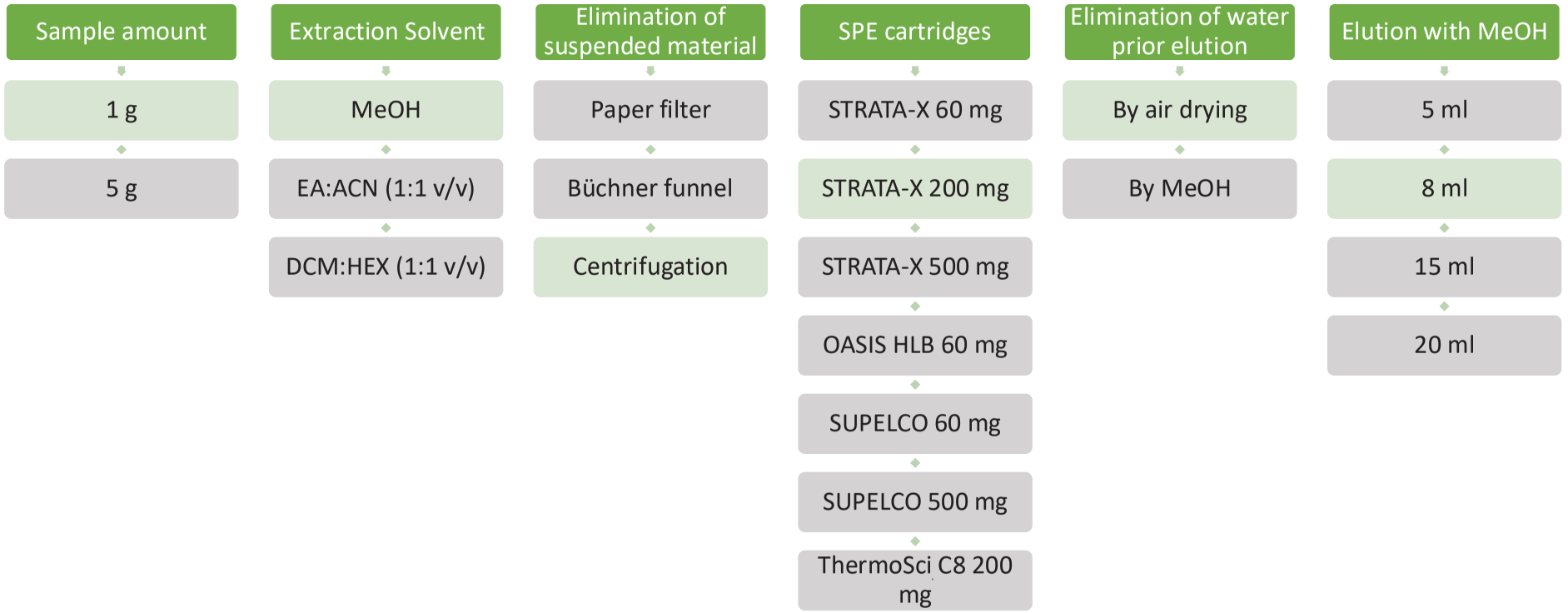

Figure S1. Extraction procedure.

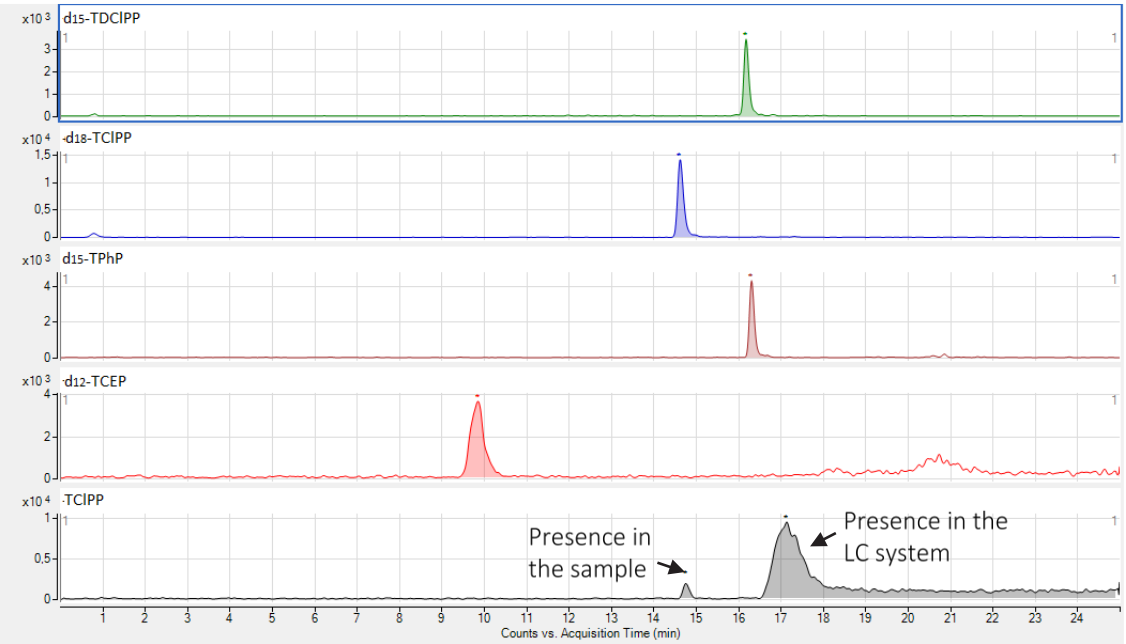

Figure S2. Standard chromatogram of methanol spiked with internal standards (10 ng g^{-1}).

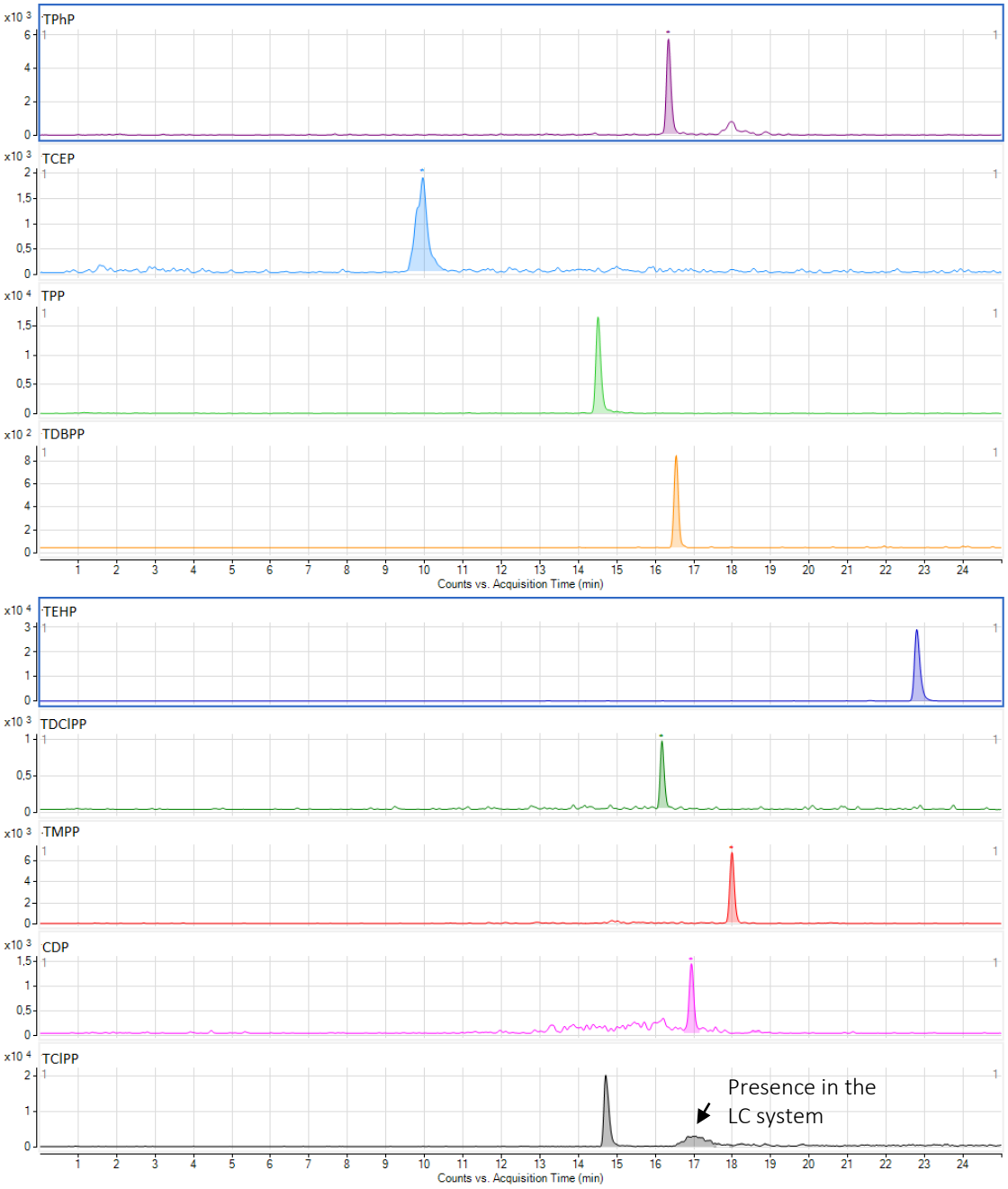

Figure S3. Standard chromatogram of procedural blank of European eel (*Anguilla anguilla*) spiked with internal standards and fortified with PFRs (10 ng g^{-1}).

Sección 3

APLICACIÓN EN ECOSISTEMAS FORESTALES Y ACUÁTICOS

CAPÍTULO 5

Perfluoroalkyl substances in the Ebro and Guadalquivir river basins (Spain).

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María Lorenzo, Julián Campo, Marinel·la Farré, Francisca Pérez, Yolanda Picó, Damià Barceló.

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Perfluoroalkyl substances in the Ebro and Guadalquivir river basins (Spain)

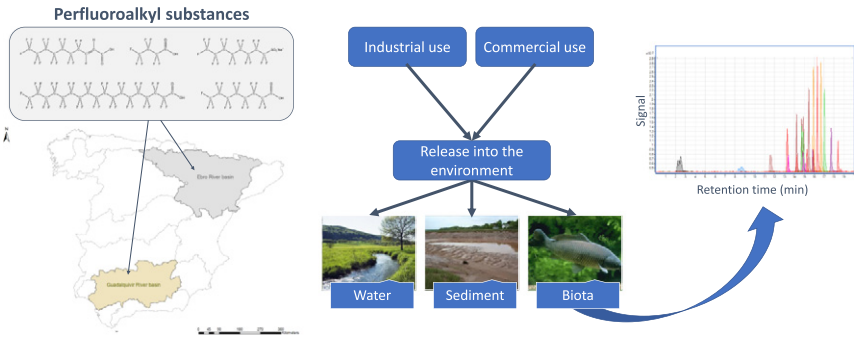
María Lorenzo [a,*], Julián Campo [b,c], Marinella Farré [d], Francisca Pérez [d], Yolanda Picó [a], Damià Barceló [d,e]

[a] Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE-UV, GV, CSIC), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain
[b] Environmental Forensic and Landscape Chemistry Research Group, Desertification Research Centre — CIDE (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana), Spain
[c] Institute for Biodiversity and Ecosystem Dynamics (IBED), Earth Surface Sciences Research Group, Universiteit van Amsterdam, The Netherlands
[d] Department of Environmental Chemistry (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[e] Catalan Institute for Water Research (ICRA), Emili Grahit, 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, E-17003 Girona, Spain

HIGHLIGHTS

- 21 PFASs quantified in surface water, sediment and fish of the two Rivers
- PFBA, PFPeA, PFOA and PFOS occurs frequently in water, sediment and biota.
- PFBA was the predominant compound in sediment and water from both rivers.
- Relationship between high concentrations and sampling points downstream WWTP.
- PFTeDA maximum concentrations may pose acute risk to *Daphnia sp.* and fish.

GRAPHICAL ABSTRACT



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ABSTRACT

Mediterranean rivers are characteristically irregular with changes in flow and located in high population density areas. This affects the concentration of pollutants in the aquatic environments. In this study, the occurrence and sources of 21 perfluoroalkyl substances (PFASs) were determined in water, sediment and biota of the Ebro and Guadalquivir river basins (Spain). In water samples, of 21 analytes screened, 11 were found in Ebro and 9 in Guadalquivir. In both basins, the most frequents were PFBA, PFPeA and PFOA. Maximum concentration was detected for PFBA, up to 251.3 ng L^{-1} in Ebro and 742.9 ng L^{-1} in Guadalquivir. Regarding the sediments, 8 PFASs were detected in the samples from Ebro and 9 in those from Guadalquivir. The PFASs most frequently detected were PFBA, PFPeA, PFOA and PFOS. Maximum concentration in Ebro samples was, in dry weight, for PFOA (32.3 ng g^{-1}) and in Guadalquivir samples for PFBA (63.8 ng g^{-1}). For biota, 12 PFASs were detected in fish from the Ebro River and only one (PFOS) in that from Guadalquivir. In the Ebro basin, the most frequents were PFBA, PFHxA, PFOA, PFBS, PFOS and PFOSA. Maximum concentration in Ebro samples was, in wet weight, for PFHxA with 1280.2 ng g^{-1}, and in Guadalquivir samples for PFOS with 79.8 ng g^{-1}. These compounds were detected in the whole course of the rivers including the upper parts. In some points contamination was

* Corresponding author.
E-mail address: maria.lorenzo@uv.es (M. Lorenzo).

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due to point sources mostly related to human activities (e.g. ski resorts, military camps, urban areas.). However, there are also some areas clearly affected by diffuse sources as atmospheric deposition.

1. Introduction

The Mediterranean climate is a particular variety of subtropical climate. The largest region with these weather conditions is found in coastal areas around the Mediterranean Sea, but also on the coast of California (USA), the central coast of Chile, South Africa and South and Southwest Australia. Mediterranean areas occasionally suffer large rainfalls as well as severe droughts. These irregularities have resulted in significant increases and decreases in river levels, which affect the concentration of pollutants in these aquatic environments. This problem is increased because of the high population density in these areas, translate also in high concentration of industries and intensive agricultural practices. Spanish Mediterranean river basins are then overexploited as consequence of anthropogenic pressures. Some studies have found relevant concentrations of a broad range of pollutants (Campo et al., 2015; Masiá et al., 2013).

Perfluoroalkyl substances (PFASs) are a large group of synthetic amphipathic compounds that present high thermal and chemical stability, being used in several industrial and commercial applications (Buck et al., 2011). Consequently, general concern on PFASs in the environment has increased because of their stability, persistence and bioaccumulative characteristics (Llorca et al., 2012). In recent years, after showing remarkable ubiquity, some PFASs (e. g. perfluorooctane sulfonate – PFOS – and perfluorobutanoic acid—PFOA) have begun to be regulated (Environment Canada, 2010; European Parlament, 2008; UNEP, 2010; USEPA, 2006). The company 3M, the major PFASs producer, voluntary phased out their PFOS production in 2000 (3M, 2002) and introduced a short-chain perfluoroalkyl sulfonate (perfluorobutane sulfonate—PFBS) as substitute compound. PFBS is less toxic but still highly persistent (3M, 2002).

The measurement of PFASs in various environmental compartments give information on different aspects of this contamination (i) PFASs in water indicates the level of these compounds at a given time, (ii) sediment shows accumulation over time and (iii) biota shows bioaccumulation. For the assessment of the aquatic ecosystem pollution, fish is often used as a bioindicator, because they accumulate PFASs in their proteic tissues (Svihlikova et al., 2015). Water inspection includes the conventional treatments at the wastewater treatment plants (WWTPs), as already known with limited efficiency in removal PFASs, so municipal sewage and water effluent are significant sources of these compounds to the aquatic environment (Campo et al., 2014; Gómez-Canela et al., 2012). Moreover, analysis of PFASs in soil and sediment allows an overview of pollution in watersheds, as well as the transference between the different environmental matrices.

Major studies in the Mediterranean area have been made for detection of non-persistent compounds like pharmaceuticals and personal care products (Carmona et al., 2014; Martin et al., 2011; Muñoz et al., 2009) and illicit drugs (Vazquez-Roig et al., 2010, 2012). There are some studies about persistent compounds like pesticides in Mediterranean river basins (Espigares et al., 1997; Masiá et al., 2015a,b). However, little relevant research assesses PFASs in different matrices from Mediterranean areas e.g. sewage sludge (Gómez-Canela et al., 2012; Llorca et al., 2011), wastewater (Arvaniti et al., 2012), coastal water (Sánchez-Avila et al., 2010) and drinking water (Ericson et al., 2009). Mediterranean river basins have been much less studied than other water systems, e.g. PFASs in water from Germany and Spain, including river basins (Llorca et al., 2012), Llobregat River (Campo et al., 2015; Flores et al., 2013), Ebro and Llobregat rivers (Loos et al., 2009), L'Albufera lake (Picó et al., 2012), European rivers including the Guadalquivir River (McLachlan et al., 2007), some sampling points in Catalonian rivers (Sánchez-Avila et al., 2010); only one study about PFASs in biota (invertebrate community) from Iberian river basins (De Castro-Català et al., 2015) and, to our knowledge, no studies were performed in sediment.

In the present study, concentrations of 21 PFASs were quantified in 48 surface waters, 46 sediments and 26 fish samples, which were collected in the Guadalquivir and Ebro rivers that cover almost 1/3 of Spain surface. The objectives of this study were: (i) determine concentrations, profiles patterns of relative concentration and spatial distribution of PFASs; (ii) identify possible PFASs sources and (iii) determine bioconcentration and bioaccumulation in biota.

2. Materials and methods

2.1. Study area and sampling

The Guadalquivir River rises in the mountains of Jaén and ends in the Atlantic Ocean at Sanlúcar de Barrameda after 657 km of travel in a watershed of 57,527 km^2. The main river and several tributaries (Bembézar, Borosa, Cacín, Corbones, Genil, Guadaíra, Guadalmoral, Guadiana menor, Guadalbullón, Guadiamar, Herreros, Magaña, Los Picachos and Yeguas) were sampled. They flow through the region of Andalusia, being the main water source of the zone with more than 7 million inhabitants and passing Córdoba (328,550 inhabitants) and Seville (702,350 inh.) as major cities. On the other hand, the Ebro River runs entirely by Spain, where ranks first among the rivers that rise and flow entirely into the country, both by its length and flow. It rises in the mountains of Cantabria, passes through Logroño (153,400 inh.) and Zaragoza (679,600 inh.) as major cities, and ends in the Mediterranean Sea forming the Ebro delta (Catalonia). The river has a total length of 930 km. Its watershed is the largest in Spain, with an area of 86,100 km^2. The main river and several tributaries (Martín, Algars, Arga, Cinca, Ésera, Gállego, Huerva, Matarranya, Najerillo, Oca, Ribera Salada, Segre and Zadorra) were sampled. The Ebro and Guadalquivir rivers are two of the most important rivers of Spain. They are representative examples of Mediterranean rivers heavily managed, especially in their lower course. Although both are flowing rivers, they have irregular flow and water shortage from soaring demand and climate change.

Due to the importance of these rivers, this study shows a very broad view of the concentration of PFASs, presenting the results of an extensive survey carried out in October 2010. Twenty-four sampling points in each basin were selected (Fig. 1). Water and sediment were collected in each point (except in EBR5 and EBR8 points from the Ebro River where there was no sediment available). References of all sampling points are shown in Supplementary data (Table S1). Water samples (2 L) were collected in clean amber glass bottles, from the middle of the river. Before sample collection, each bottle was thoroughly pre-rinsed with MilliQ water at the laboratory and then, rinsed with sample water prior to actual sample collection. Sediment samples were taken using a Van Veen grab sampler (0.5 L) at the same point that water samples; they were transferred and wrapped into aluminium foil (previously washed with methanol and dried in oven at 100 °C) that was put inside an aluminium box.

Fish samples were taken in both Guadalquivir and Ebro at five selected sites of the river courses using electro-fishing (these points are marked in red in Fig. 1). Due to scarcity of fish in the rivers, it was only possible to base the selection of the species in its abundance, the ease of capture was the most decisive factor. Captured species included Guadiana bogue (*Pseudochondrostoma willkommii*), Andalusian barbel (*Luciobarbus sclateri*), and common carp (*Cyprinus Carpio*) in

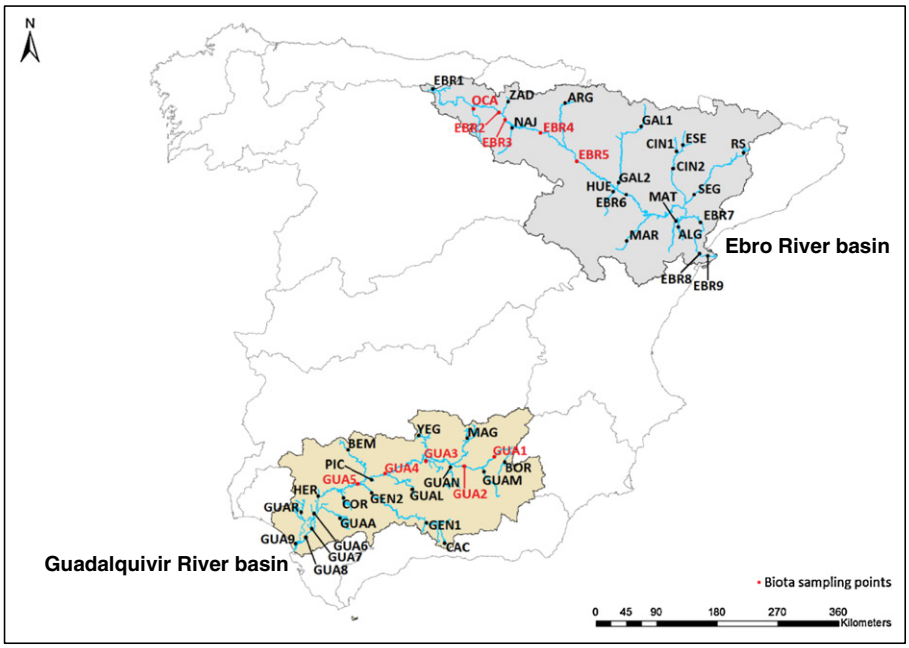

Fig. 1. Location of sampling points.

the Guadalquivir River, and Ebro barbel (*Barbus graellssi*), common carp (*C. carpio*) and European catfish (*Silurus glanis*) in the Ebro River. Each sample consists of at least three units of similar size and age.

All samples were transported in hermetic boxes refrigerated with ice until arrival at the laboratory. Then, water samples were stored at 4 °C within 24 h to avoid any degradation and were pre-treated in the following 5 days. Before the analysis, water samples were vacuum filtered through 1 μm glass fibre filters followed by 0.45 μm nylon membrane filters (VWR, Barcelona, Spain). Sediment and fish samples were frozen, lyophilized (Hetosicc CD4, Birkerod, Denmark), pulverized, thoroughly mixed and then passed through a 2 mm Ø sieve.

2.2. Chemicals and reagents

A total of 21 PFASs have been monitored, among which there are 14 perfluorocarboxylates (PFCAs), 6 perfluorosulfonates (PFSAs) and 1 perfluorosulfonamide (FASA). Some characteristics of target PFASs are provided in Supplementary data (Table S2). In biota samples ipPFNA, PFODA, PFHpS and ipPFNS were not analysed due to the lack of analytical standards necessary for the analysis. Furthermore, internal standards (IS) isotopically labelled with ^{13}C and ^{18}O were used.

Deionized water was from a MilliQ SP Reagent Water System (Millipore, Bedford, MA, USA) and LC-MS grade methanol was purchased from Panreac (Darmstadt, Germany).

2.3. Extraction and analysis

2.3.1. Extraction

Identification and quantification of PFASs was accomplished by use of previously developed methods (Lorenzo et al., 2015). Water samples were pre-concentrated with an off-line solid-phase extraction (SPE) procedure. Sediment samples were extracted with acetic acid and methanol and the extracts were cleaned-up by SPE as water (Campo et al., 2015; Lorenzo et al., 2015). Detailed information of water and sediment extraction procedure is presented in Figs. S1 and S2. For biota, the extraction was made by alkaline digestion and 3–5 fish of the same species were minced and homogenised in a food processor (Oster BPST02-B00, London, UK) to make a representative fish sample (Pérez et al., 2013). Detailed information of fish characteristics (not available for those from Guadalquivir) and the extraction procedure are presented in Table S3 and Fig. S3, respectively.

2.3.2. LC-MS/MS determination

Instrumental analysis were performed using a 1260 Infinity Ultra High-Performance Liquid Chromatograph (UHPLC) combined with a 6410 Triple Quadrupole (QqQ) Mass Spectrometer (MS/MS) of Agilent Technologies (Santa Clara, CA, USA) with electrospray ionisation (ESI) for water and sediment. Data were processed using MassHunter Workstation Software for qualitative and quantitative analysis (A GL Sciences, Tokyo, Japan). Information related to instrumental determination is listed in Tables S4 and S5.

In the case of fish, a turbulent flow chromatograph (TFC) Aria TLX-1 system (Thermo Fisher Scientific, Franklin, MA, USA) consisted of a PAL auto-sampler (CTC Analytics, Zwingen, Switzerland), with two mixing binary pumps (eluting and loading, respectively), and a three-valve (sixport) switching device unit has been used for the analysis of biota. The entire system was controlled via Aria software, version 1.6. The on-line enrichment was achieved using a Cyclone P coupled to a C18 (2.1 × 20 mm, 12 μm particle size from Thermo Fisher Scientific, Franklin, MA, USA). After separation, detection of the selected analytes was accomplished using a triple quadrupole mass spectrometer Thermo Scientific TSQ Vantage (Thermo Fisher Scientific, San Jose, CA). Detailed information related to instrumental determination is presented in Tables S5 and S6.

2.3.3. Validation and quality control

The method quantification limits (Table S7), recoveries (Tables S8-S10), precision and linearity were evaluated for water, sediment and biota samples. In water samples, mean relative recoveries ranging from 55 to 94%, with precision, represented as relative standard deviations (RSDs), between 8 and 18%, using spiked levels at 6 and 60 ng L^{-1}. For sediment samples, recoveries were between 44 and 100% and RSDs were always below 20%. LOQs were 0.01–2.00 ng L^{-1} for water samples and 0.04–8.00 ng g^{-1} for sediment (dry weight: dw). Calibration curves were prepared daily obtaining $R^2 \geq 0.98$. In the case of biota samples, the internal standard mixture was added before extraction and clean-up steps in order to correct loses and potential matrix effects. Recoveries were achieved with spiked samples at 1 and

10 μg L^{-1}. Their values ranged between 16 and 130% with RSDs also below 20% and LOQs from 0.004 to 2.26 ng L^{-1}. A strict quality control was established to avoid false positive and negative.

Before and after each sampling batch (25–30 samples), calibration curves were constructed by injecting standards at different concentrations (1, 2.5, 5, 10, 25, 50, 65, 75 ng L^{-1}). Each 15 samples, one instrumental and one procedural blanks as well as one positive control were analysed to serve as quality control. Deionized water sample was prepared prior to field sampling, and was carried, unopened, with the field monitoring personnel through the monitoring trip.

2.4. Potential adverse effects

ECOSAR™ software was used to estimate toxicity for standard test species because of the lack of PFASs acute toxicity data (Table S11). The QSARs (Quantitative Structure-Activity Relationship) models from ECOSAR estimated toxicity by mathematical relationships between K_{OW} values and the corresponding measured toxicity.

Hazard quotients (HQ) were calculated for the different PFASs. HQs are defined as the ratio of predicted or measured environmental concentration (MEC) and their chronic toxicity, usually expressed as NOEC (Non-Observed Effect Concentration) or PNEC (Predicted Non Effect Concentration) values, referred to three different trophic levels, as recommended by the European Water Framework Directive (WFD). When NOEC values were not available, EC_{50} or LC_{50} values from standard eco-toxicological tests were used after correction by an assessment factor of 1000 according to WFD of Directive 2000/60/EC (European Parlament, 2000):

$$HQ = MEC/PNEC$$

With $PNEC = EC_{50}/1000$ or $LC_{50}/1000$.

In general, HQ > 1 indicates potential risk. However, other adverse properties of chemicals are not included in this kind of risk estimation (e.g. endocrine disruption, bioaccumulation, etc.).

3. Results

Table 1 shows results of water, sediment and biota monitoring (minimum and maximum concentrations, mean levels, and frequency of detection) in both rivers. The cumulative concentration in each

Table 1
Concentration and frequency of occurrence of detected PFASs in water, sediment and biota samples of the Guadalquivir and Ebro rivers.

Matrix	Compound[a,b,c]	Guadalquivir				Ebro			
		Minimum[d,e]	Maximum[d]	Mean[d,f]	Frequency (%)	Minimum[d]	Maximum[d]	Mean[d,f]	Frequency (%)
Water	**PFCAs**								
	PFBA	8.0	742.9	214.3	22 (92)	9.8	251.3	35.2	14 (58)
	PFPeA	0.1	67.8	8.6	15 (63)	0.1	12.5	1.1	10 (42)
	PFHxA	–	–	–	–	9.6	31.4	1.7	2 (8)
	PFHpA	0.4	87.4	4.3	4 (17)	13.7	17.2	2.0	3 (13)
	PFOA	4.1	188.6	11.6	8 (33)	2.0	125.0	7.3	10 (42)
	ipPFNA	–	–	–	–	4.1	5.1	0.4	2 (8)
	PFNA	6.8	116.1	5.1	2 (8)	4.8	7.9	0.5	2 (8)
	PFDA	1.8	13.0	1.0	3 (13)	0.1	6.5	0.7	4 (17)
	PFTeDA	–	–	–	–	6.3	6.3	0.3	1 (4)
	PFSAs								
	PFBS	15.0	228.3	10.1	2 (8)	–	–	–	–
	PFHxS	1.5	88.5	4.1	3 (13)	1.1	5.8	0.5	3 (13)
	PFOS	0.01	42.6	1.8	2 (8)	0.1	27.0	2.2	7 (29)
Sediment	**PFCAs**								
	PFBA	0.9	63.8	3.8	16 (67)	0.6	9.5	3.6	19 (86)
	PFPeA	0.2	0.7	0.2	9 (38)	0.3	27.9	1.6	12 (55)
	PFHpA	1.0	1.0	0.04	1 (4)	0.4	0.6	0.04	2 (9)
	PFOA	0.2	27.1	1.3	8 (33)	0.4	32.3	1.6	6 (27)
	PFDA	0.1	0.7	0.1	6 (25)	0.1	0.4	0.1	4 (18)
	PFUnDA	–	–	–	–	0.5	0.9	0.1	2 (9)
	PFTeDA	0.1	0.1	0.01	1 (4)	–	–	–	–
	PFSAs								
	PFBS	0.5	1.1	0.2	4 (17)	0.5	6.8	0.5	4 (18)
	PFHxS	0.03	0.04	0.01	3 (13)	–	–	–	–
	PFOS	0.04	0.7	0.1	8 (33)	0.01	2.2	0.3	13 (59)
Biota	**PFCAs**								
	PFBA	–	–	–	–	0.1	4.92	0.6	5 (31)
	PFHxA	–	–	–	–	53.5	1280.2	268.4	9 (56)
	PFHpA	–	–	–	–	14.5	14.5	1.8	2 (13)
	PFOA	–	–	–	–	18.8	29.9	13.5	9 (56)
	PFNA	–	–	–	–	15.1	19.9	2.2	2 (13)
	PFDA	–	–	–	–	19.4	19.4	1.2	1 (6)
	PFUnDA	–	–	–	–	16.9	16.9	1.1	1 (6)
	PFSAs								
	PFBS	–	–	–	–	7.1	7.3	4.9	11 (69)
	PFHxS	–	–	–	–	0.01	0.1	0.01	3 (19)
	PFOS	1.4	79.8	29.7	10 (100)	14.9	42.5	17.6	13 (81)
	PFDS	–	–	–	–	19.4	19.4	1.1	1 (6)
	FASA								
	PFOSA	–	–	–	–	11.9	14.3	9.9	13 (81)

[a] PFDoDA, PFTrDA, PFHxDA, PFODA, PFHpS and ipPFNS were not found in any of the samples.
[b] PFUnDA, PFBS and PFOSA were not found either water or sediment.
[c] PFHxA, ipPFNA and PFNA were not found in sediments.
[d] Water in ng L^{-1}, sediment in ng g^{-1} dw, biota in ng g^{-1} ww.
[e] Minimum was the lowest amount quantified.
[f] Mean was calculated with not detected concentrations as zeros.

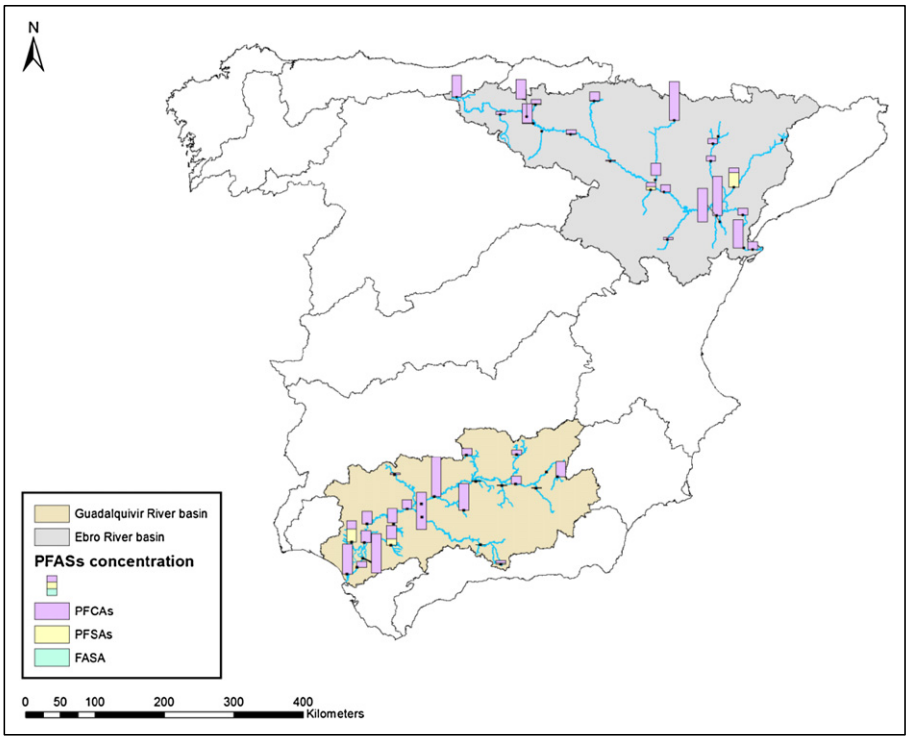

Fig. 2. Cumulated PFASs concentration present in water samples.

sampling point was shown in Fig. 2 (water), Fig. 3 (sediment) and Fig. 4 (biota).

3.1. Occurrence of selected PFASs in water samples

In the Guadalquivir River, except for the first sampling point (GUA1), all water samples analysed were contaminated with at less one PFAS. From the 21 PFAS included in this study, 9 were detected (Table 1). Mean PFCA values were between 1.0 ng L^{-1} (PFDA) and 214.3 ng L^{-1} (PFBA). Mean PFSA values ranged from 1.8 ng L^{-1} (PFOS) up to 10.1 ng L^{-1} (PFBS). The highest concentration found was from PFBA (742.9 ng L^{-1}). The PFASs more frequently detected were the two shortest-chain compounds of the PFCAs family: PFBA (92%) and PFPeA (63%). The rest of PFASs appeared with frequencies below 33% and include according to frequency PFOA > PFHpA > PFDA ≈ PFHxS > PFNA ≈ PFBS and PFOS.

In the Ebro River, of the 21 analytes, 11 were detected in water samples. As in the Guadalquivir River, PFBA was the most detected compound, although the frequency was lower (58%) followed by PFPeA and PFOA (both 42%). Frequencies for the rest of compounds were lower

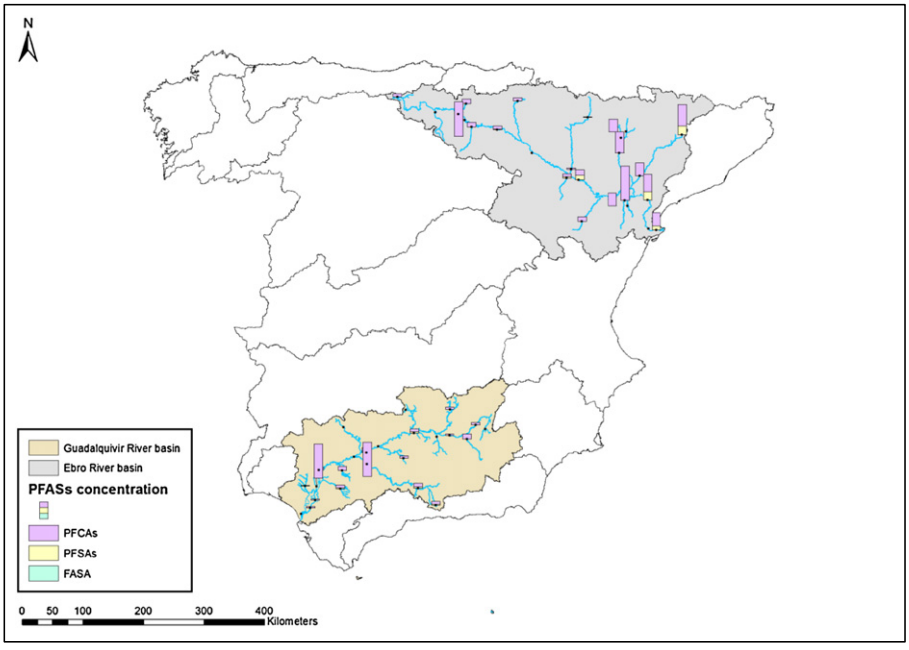

Fig. 3. Cumulated PFASs concentration present in sediment samples.

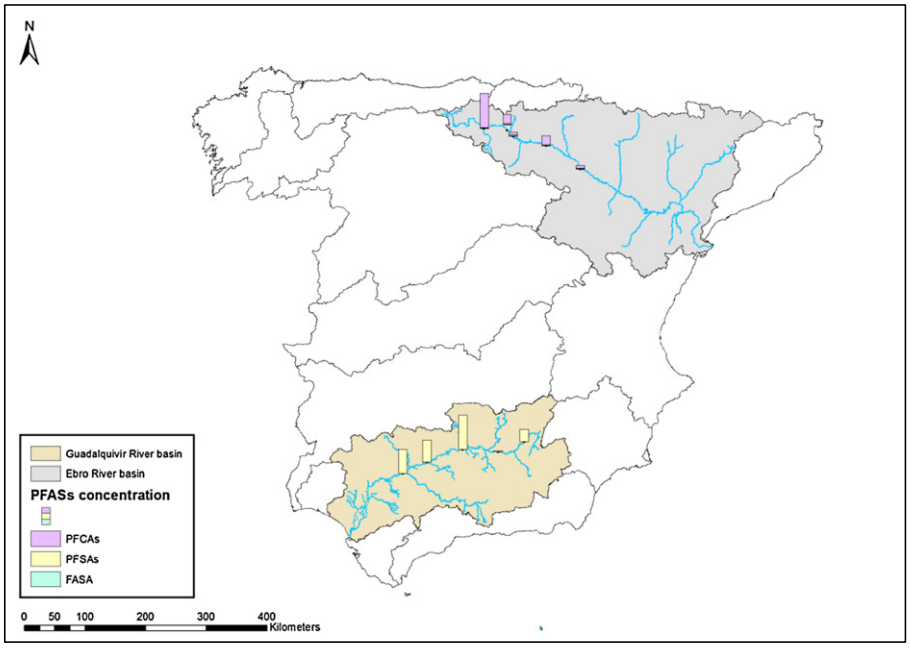

Fig. 4. Cumulated PFASs concentration present in biota (fish) samples.

than 29%. The other PFASs found according to frequency were PFOS > PFDA > PFHpA ≈ PFHxS > PFHxA ≈ ipPFNA ≈ PFNA > PFTeDA. Mean PFCA values ranged from 0.3 ng L^{-1} (PFTeDA) to 35.2 ng L^{-1} (PFBA) and mean PFSAs were from 0.5 ng L^{-1} (PFHxS) up to 2.2 ng L^{-1} (PFOS).

3.2. Occurrence of selected PFASs in sediment samples

In the Guadalquivir River, 19 samples were contaminated with at least one PFAS and 9 different analytes were detected at concentrations over the limits of detection. PFBA was predominant (67%) followed by PFPeA (38%), PFOA and PFOS (both 33%). The rest of compounds – detected with frequencies below 25% – were PFDA > PFBS > PFHxS > PFHpA ≈ PFTeDA. Mean concentration values for PFCAs were from 0.01 ng g^{-1} (PFTeDA) to 3.8 ng g^{-1} (PFBA) and for PFSAs were from 0.01 ng g^{-1} (PFHxS) to 0.2 ng g^{-1} (PFBS). Maximum concentrations were for PFBA (63.8 ng g^{-1}) and PFOA (27.1 ng g^{-1}). The rest of PFASs had maximum concentrations below 1.1 ng g^{-1} (Table 1). High concentrations were found in GUA6 (mainly PFBA) and GEN2 (only PFOA).

Of the 22 sediment samples in the Ebro River, 20 were contaminated with at least one PFAS and 8 of the 21 analytes were detected. PFBA was predominant (86%) followed by PFOS (59%) and PFPeA (55%). The rest of compounds were found in less than 27% of the sampling points and were PFOA > PFDA ≈ PFBS > PFHpA ≈ PFUnDA. Mean values for PFCAs were from 0.04 ng g^{-1} (PFHpA) to 3.6 ng g^{-1} (PFBA) and for PFSAs from 0.3 ng g^{-1} (PFOS) to 0.5 ng g^{-1} (PFBS). Maximum concentrations were for PFOA (32.4 ng g^{-1}) and PFPeA (27.9 ng g^{-1}) (Table 1) both found in EBR3.

3.3. Occurrence of selected PFASs in biota samples

The study in biota was limited by a relative small sample size, which increased the possibility of potential selection biases. This was due to complexity of electro-fishing and because fish are scarce in Mediterranean rivers and this study wants to alter the environment the least possible.

In the Guadalquivir River, the collected fish species included Guadiana bogue (*P. willkommii*), Andalusian barbel (*L. sclateri*), and common carp (*C. Carpio*). Of the 17 compounds analysed in biota, only PFOS was detected but in 100% of samples. The mean concentration for PFOS value was 29.7 ng g^{-1} ww. (Table 1). The highest concentration (79.8 ng g^{-1}) was found in GUA3.

The collected fish species in the Ebro River included Ebro barbel (*B. graellssi*), common carp (*C. carpio*) and European catfish (*S. glanis*), in which 12 PFASs were detected. PFOS and PFOSA were the most abundant compounds (both 81%), followed by PFBS (69%), PFHxA and PFOA (both 56%). While PFBA, abundant in water and sediment samples, was present in 31% of the biota analysed. Mean values for PFCAs ranged from 0.6 ng g^{-1} (PFBA) to 268.4 ng g^{-1} (PFHxA), for PFSAs from 0.01 ng g^{-1} (PFHxS) to 17.6 ng g^{-1} (PFOS) and for FASA (PFOSA) was 9.9 ng g^{-1}. The highest concentration was 1280.2 ng g^{-1} for PFHxA (Table 1).

4. Discussion

4.1. Water samples

Long-chain PFASs were less common in water than short-chain (C ≤ 8), because products containing short-chain compounds are more produced and consumed. In addition, the solubility of long-chain compounds is lower (Onghena et al., 2012). This could explain why long-chain PFCAs (ipPFNA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA and PFODA), PFSAs (ipPFNS and PFDS) and FASA (PFOSA) were not detected in water samples from Guadalquivir River. Very high contamination levels (mainly PFBA) were detected in GUA4 and GEN2, corresponding with sampling points downstream the WWTPs of Córdoba and Écija, respectively. High concentration of PFBA and other PFASs were detected in GUA7 (near an estuary with textile industry), GUAL (olive trees and olive oil factories) and GUAA (near to a military camp and the WWTP of Morón). The last one was the sampling point with the largest number of PFASs detected. The still recent use of film foaming foams used in firefighting in military camps that contain large amounts of PFOA and PFHxA can be a reason as reported by Eschauzier et al. (2013). PFCAs were the predominant family and only two points have presence of PFSAs (GUAR and GUAA). High concentrations were found mainly in the medium and final part of the river course where the most important cities Cordoba and Sevilla are located. This agrees with the results of other authors who also found that short-chain PFASs were predominant in and near urban/industrial areas (Myers et al., 2012). However, the river source also showed some background contamination by PFASs.

Atmospheric deposition of PFBA is a possible explanation to these residuals levels (Eschauzier et al., 2013).

In the Ebro River, the highest concentration was, as in the Guadalquivir River, from PFBA (251.3 ng L^{-1}). Also as in the Guadalquivir, long-chain PFCAs (except ipPFNA and PFTeDA) and PFSAs were not found. The highest concentration (only due to PFBA) was found in GAL1. This point apparently does not belong to a high populated and industrialized area and it is considered a reference site from Gállego catchment. However, this place is surrounded by ski resorts and the high concentration can come from ski waxes that are commonly applied to skis. The sampling was carried out in October 2010, despite not being the most active period of these resorts, the cleaning and preparation of the ski slopes it is performed around this time. Moreover, some of these ski resorts remain active throughout the year. A substantial number of people consume such products in preparation of leisure activities. A study already linked PFASs in the ski waxers serum to exposure from work room aerosols (Freberg et al., 2010). This would be the first evidence of the environmental contamination due to this practice. PFAS cumulative concentrations found in the Ebro River (maximum 251.3 ng L^{-1}) were much lower than those found in the Guadalquivir River (maximum 830.3 ng L^{-1}). As in the Guadalquivir, PFCAs were predominant and only two points have PFSAs (SEG and HUE). The former sampling site is located downstream the city of Lleida and receives the effluent of the WWTP and the latter receives the effluents from many industrial areas around the city of Zaragoza. In contrast to Guadalquivir, spatial distribution is less clear, with high concentrations in the last part of the river but also in the upper part, which may be due because the head of the Ebro, contrary to what happens with many rivers, flows through the Basque Country, a heavily industrialized area. Traditional industrial activities were steel and shipbuilding, mainly due to the rich iron ore resources. Today, the strongest industrial sectors of this area's economy are machine tool, aeronautics and energy. These activities can be a punctual source of PFASs. The low levels in the middle part of the river can be originated from diffuse sources as atmospheric deposition, run-off or infiltration/exfiltration of water. The difference in the range of concentrations between both rivers may be due to the flow difference, while the Ebro is one of the mightiest rivers from Spain (ca. 600 m^3 s^{-1}), the Guadalquivir has a much lower average flow (ca. 164 m^3 s^{-1}) causing a low dilution effect of pollution. Also the presences of a wide range of PFASs in the Ebro River can be related to the industrial activities.

The PFCA concentrations found in Guadalquivir water were also higher than those detected in the Llobregat River by Campo et al. (2015), PFSAs concentrations were lower, although the same compounds were found. Regarding the Ebro River, PFCA concentrations were more similar and PFSAs were lower. FASA (PFOSA) was also not found in the Llobregat River. PFBA was also the main compound in water in some studies, e.g. 93% of frequency in Llobregat River (Campo et al., 2015), 71% in water from different Spanish cities (Llorca et al., 2012), >60% in Tangxun Lake (Zhou et al., 2013) and 52% in Tokyo Bay (Ahrens et al., 2010).

4.2. Sediments

High concentrations from the Guadalquivir River (GUA6 and GEN2) correspond to sampling points downstream the WWTPs of Sevilla and Écija, respectively. Both points are in the middle-final part of the river. The higher co-occurrence of PFOS and PFOA in sediments than in water could indicate the recent changes in production and use of PFASs (as the replacement of these compounds by short-chain ones). However, the distribution of PFAS in the coastal environment is influenced by many factors, such as partitioning behaviour between sediment and water. This behaviour was already reported for WWTP sludges (Campo et al., 2014). It is difficult to correlate the PFASs water concentration with that found in sediments since the adsorption coefficient can only be calculated in equilibrium and rivers are dynamic systems. It could be noted that both locations, particularly GEN2, also present high levels of PFASs in water. However, there are some water hotspot as GUA4, GUA7, GUAL and GUAA that show very low PFAS levels in sediments.

The highest concentration found in the Ebro River (EBR3) was sampled in a point around wine fields. This point is in the area of "La Rioja" one of the most well-known protected designation of origin of the Spanish wines. This area is surrounding by small wine factories. Levels of PFASs were low in the water sample taken at this point. However, the release of PFASs to water can be intermittent depending on the seasonal winery works. Then, the sediments would reflect better the contamination since PFASs remain accumulated. Higher concentrations are clearly observed at the final section of the river (except EBR3 in the head). Those concentrations correlate better to those in water and can be related to points with higher levels of populations. Only in the case of the point RS there is no correlation to the levels in water. This point is representative of a rain-snowmelt fed flow regime (low Mediterraneity), and with no impacts. The only possible PFASs source are the presence in the sampling point and upstream of several water quality and flow monitoring stations. In this sampling point water flow and temperature, suspended sediment and bedload transport are continuously monitoring. The instrumental used can be the source and also can explain why contamination was only detected in sediment. PFAS concentrations in sediments samples from the Ebro River were of the same order of magnitude than those found in the Guadalquivir. However, frequency was higher in the Ebro River.

Until now, only limited data on the presence of PFASs in sediments from the Mediterranean aquatic ecosystem are available; such a large-scale study has never been carried out. PFBA was also the prevalent compound (100% of frequency) in the Llobregat River (Campo et al., 2015). As in the Guadalquivir River, PFBA was found at concentrations comparable to those of PFOA in Lake Michigan (Codling et al., 2014).

4.3. Biota

The most notable observation was the high frequency of PFOS found in fish samples of the two river basins. It was the only PFSA detected in the samples from the Guadalquivir River but was detected in 100% of the samples. The K_{OW} of PFOS does not justify the high bioaccumulation potential found in biota. However, some authors (Jones et al., 2003) pinpoint how the use of K_{OW} is not appropriate to predict bioaccumulation of PFASs because PFOS does not partition into lipids, but instead binds to certain proteins in animals. As a result the use of either water solubility or K_{OW} can misjudged the bioaccumulation of PFOS. The data of the Ebro River showed also high frequency of PFBS, PFOSA, PFHxA and PFOA. In contrast to the Guadalquivir River, the predominant compounds were PFCAs and they were at high concentrations in the head of the river. The pattern of PFASs found in biota does not correlated to that found in water or sediment of any of the rivers. The fish with the highest concentration of PFASs was taken at the point OCA (mainly PFHxA). PFOSA was detected only in biota samples. This compound may appear after metabolization of PFOS by amination or hydrolysis or from precursors accumulated in fish that are biodegraded in PFOSA by fish and in other compounds in the environment (Dimitrov et al., 2004).

The results presented here are in agreement with those previously reported, in which PFOS (100%), PFOSA (75%) and PFBS (67%) were also the compounds found more frequently in fish of the Llobregat River (Campo et al., 2015) and PFBS in fish from the Rhine River (Möller et al., 2010). However, to fully explain all these results more information on the accumulation tendency of PFCAs and PFSAs precursors in biota would be needed.

The concentration found in fish was in general low and due to the general lack of information on the toxicity levels of these compounds, it is very difficult to establish whether levels found in fish can or not be toxic. Then, a classical evaluation that relates toxicity to the PFASs levels in water was carried out through the HQ index. Fortunately, HQ

Table 2
Ranked compounds according to HQ for algae, *Daphnia* sp. and fish in the Guadalquivir and Ebro River basins. Acute toxicity (EC50) values used were modelled with ECOSAR.

Maximum[a]	Rank	Guadalquivir river				Ebro river			
		Compound	HQ algae	HQ *Daphnia* sp.	HQ fish	Compound	HQ algae	HQ *Daphnia* sp.	HQ fish
	1	PFNA	0.02	0.05	0.04	PFTeDA	0.13	1.26	1.57
	2	PFOA	0.01	0.03	0.02	PFOA	0.01	0.02	0.01
	3	PFDA	0.01	0.02	0.02	PFDA	0.00	0.01	0.01
	4	PFHpA	0.00	0.00	0.00	PFNA	0.00	0.00	0.00
	5	PFOS	0.00	0.00	0.00	ipPFNA	0.00	0.00	0.00
	6	PFBA	0.00	0.00	0.00	PFOS	0.00	0.00	0.00
	7	PFHxS	0.00	0.00	0.00	PFBA	0.00	0.00	0.00
	8	PFPeA	0.00	0.00	0.00	PFHpA	0.00	0.00	0.00
	9	PFBS	0.00	0.00	0.00	PFHxA	0.00	0.00	0.00
	10					PFPeA	0.00	0.00	0.00
	11					PFHxS	0.00	0.00	0.00
Mean[b]	1	PFNA	0.00	0.00	0.00	PFTeDA	0.01	0.05	0.07
	2	PFOA	0.00	0.00	0.00	PFOA	0.00	0.00	0.00
	3	PFDA	0.00	0.00	0.00	PFDA	0.00	0.00	0.00
	4	PFHpA	0.00	0.00	0.00	PFNA	0.00	0.00	0.00
	5	PFOS	0.00	0.00	0.00	ipPFNA	0.00	0.00	0.00
	6	PFBA	0.00	0.00	0.00	PFOS	0.00	0.00	0.00
	7	PFHxS	0.00	0.00	0.00	PFBA	0.00	0.00	0.00
	8	PFPeA	0.00	0.00	0.00	PFHpA	0.00	0.00	0.00
	9	PFBS	0.00	0.00	0.00	PFHxA	0.00	0.00	0.00
	10					PFPeA	0.00	0.00	0.00
	11					PFHxS	0.00	0.00	0.00

[a] HQ calculated with the maximum concentration value detected.
[b] HQ calculated with the mean concentration value detected.

were <0.1 for most of the PFASs at the maximum and medium concentrations points for the different tropic levels considered. Only in the Ebro river basin, PFTeDA may pose acute risk to *Daphnia* sp. and fish when considering the maximum concentration value (Table 2).

5. Conclusions

Of the 21 PFASs selected, 11 were found in water, 9 in sediments and 8 in biota being the most common short-chain compounds used as replacing compounds. PFASs can be detected in the Guadalquivir and Ebro rivers in water, sediments and biota showing different patterns. In water and sediments, short-chain perfluoroalkyl acids were the most frequent. However, most frequent compound in fish was PFOS indicating a certain degree of accumulation in sediments and bioaccumulation in biota. Ebro River was of particular concern because PFASs already appear in the headwater and then, they are present at slightly higher or lower concentrations through all the river. Most of the points with high PFASs contamination can be related to anthropogenic impact (urban areas, military activities or ski resorts). Furthermore, low levels in water that had not been in contact with potential contaminant sources, suggests a widespread diffuse contamination from atmospheric deposition, runoff or infiltration/exfiltration processes. PFASs concentrations in the Guadalquivir and the Ebro rivers do not suppose a potential risk to biota, except in the case of PFTeDA.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.07.045.

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Perfluoroalkyl substances in the Ebro and Guadalquivir river basins (Spain)

María Lorenzo[1*], Julián Campo[1, 2, 3], Marinel·la Farré[4], Francisca Pérez[4], Yolanda Picó[1], Damià Barceló[4, 5]

[1] *Food and Environmental Safety Research Group (SAMA-UV), Department of Medicine Preventive, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia Spain*
[2] *Environmental Forensic and Landscape Chemistry Research Group. Desertification Research Centre - CIDE (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana)*
[3] *Institute for Biodiversity and Ecosystem Dynamics (IBED), Earth Surface Sciences Research Group, Universiteit van Amsterdam, The Netherland*
[4] *Department of Environmental Chemistry (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain*
[5] *Catalan Institute for Water Research (ICRA), Emili Grahit, 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, E-17003 Girona, Spain*

[*] Corresponding author Tel. +34 963543092; fax: +34 963544954
E-mail address: maria.lorenzo@uv.es

Table S1. Description of the sampling points

Ebro River

River	Location (UTM)	Samples	Code	Observation	Flow ($m^3 s^{-1}$)	Sediment (% OC)
MARTÍN	Alcaine (X: 693300 Y: 4535853)	W, S	MAR	This is one of the few sites on the right margin of the Ebro and it is also considered a reference site.	0.22	19
ALGARS	Batea (X: 265824 Y: 4554895)	W, S	ALG	---	0	-
ARGA	Echauri (X: 602161 Y: 4740847)	W, S	ARG	Downstream Pamplona WWTP	3.71	44
CINCA	Grado dam (X: 271142 Y: 4667380)	W, S	CIN1	Downstream Grado dam	-	-
	Monzón (X: 264776 Y: 4642241)	W, S	CIN2	Downstream Monzon and an industrial area that produces chlorine compounds	-	-
EBRO	Nestares/Reinosa (X: 405193 Y: 4761644)	W, S	EBR1	5 km downstream river source	1.21	25
	Miranda de Ebro (X: 503672 Y: 4726140)	W, S, B	EBR2	Upper course. The first major city in the mainstream. Large industrial area and inefficient WWTP (high concentration of organic compounds in the river).	-	-
	Haro (X: 513141 Y:4715725)	W, S, B	EBR3	Wine fields around.	-	-
	Mendavia (X: 565335 Y: 4696194)	W, S, B	EBR4	Downstream Logroño WWTP	25.78	12
	El Bocal-Tudela (X: 619147 Y: 4653811)	W, B	EBR5	Downstream Tudela WWTP	-	-
	Pina dam (X: 692418 Y: 4604252)	W, S	EBR6	Downstream Zaragoza WWTP	79.88	43

	Ascó (X: 299521 Y: 4559714)	W, S	EBR7	Low part of the river, downstream the chlorine industrial area of Flix and in the middle of an agricultural area	213.4	50
	Tortosa (X: 294619 Y: 4513636)	W	EBR8	Downstream Tortosa WWTP. Tortosa has both industry and agricultural fields.	130.6	28
	Deltebre (X: 306788 Y: 4509432)	W, S	EBR9	This sampling site is the last before the Ebro reaches the sea. It is in the middle of the Ebro delta surrounded by rice fields.	-	-
ÉSERA	Graus (X: 280915 Y: 4676203)	W, S	ESE	Upstream Barasona Reservoir	-	-
GÁLLEGO	Jabarrella (presa) (X: 714638 Y: 4705571)	W, S	GAL1	Reference site from the Gállego catchment	9.97	12
	Villanueva de Gállego (X: 681725 Y: 4622524)	W, S	GAL2	Big influence form the agricultural fields situated in the Gállego cathment	10.24	32
HUERVA	Zaragoza (Fuente de la Junquera) (X: 673724 Y: 4609044)	W, S	HUE	Inside the city of Zaragoza, just before the Huerva river reaches the Ebro river. Huerva river receives effluents from many industrial areas around Zaragoza. In the past it has been the sampling site with high concentration of alkylphenols.	1.29	46
MATARRANYA	Nonaspe/Fabara (X: 262933 Y: 4564305)	W, S	MAT	---	0.03	18
NAJERILLO	San Asensio (X: 523620 Y: 4703281)	W, S	NAJ	Important agricultural wine area.	3.74	38
OCA	Oña (X: 466118 Y: 4731520)	W, S, B	OCA	Reference site in the upper course	1.38	25
RIBERA SALADA	Inglabaga Monitoring	W, S	RS	---	-	-

	Station (X: 370389 Y: 4658269)					
SEGRE	Torres de Segre (X: 292482 Y: 4601301)	W, S	SEG	Downstream Lleida WWTP	27.45	12
ZADORRA	Villodas (X: 517732 Y: 4742302)	W, S	ZAD	After Vitoria	1.86	21

W: Water; S: Sediment; B: Biota

Guadalquivir River

River	Location (UTM)	Samples	Code	Observation	Flow ($m^3 s^{-1}$)	Sediment (% OC)
BEMBÉZAR	Hornachuelos (X: 279446 Y: 4224770)	W, S	BEM	Good quality	-	-
BOROSA	La Iruela (X: 512435 Y: 4207084)	W, S	BOR	Good quality	-	-
CACÍN	Arenas del Rey (X: 423215 Y: 4086558)	W, S	CAC	Good quality	-	-
CORBONES	Carmona (X: 272990 Y: 4153778)	W, S	COR	SAICA network	1.44	75
GENIL	Loja WWTP (X: 396109 Y: 4116460)	W, S	GEN1	Downstream Loja WWTP	-	-
	Écija (X: 314734 Y: 4161417)	W, S	GEN2	Downstream Écija WWTP	22.18	81
GUADALQUIVIR	Mogon (X: 497027 Y: 4214205)	W, S, B	GUA1	Main stream	-	-
	Puente del Obispo (X: 452771 Y: 4200519)	W, S, B	GUA2	Main stream	16.38	77
	Marmolejo (X: 395434 Y: 4207864)	W, S, B	GUA3	Main stream	44.98	83
	Córdoba	W, S, B	GUA4	Downstream Córdoba WWTP	-	-

	(X: 334794 Y: 4189933)					
	Peñaflor (X: 294339 Y: 4174415)	W, S, B	GUA5	Main stream	40.26	80
	Coria del Río (X: 761545 Y: 4129986)	W, S	GUA6	Estuary and downstream Sevilla WWTP	-	-
	Brazo del Este (X: 759226 Y: 4107247)	W, S	GUA7	Estuary	-	-
	Lebrija (X: 751405 Y: 4094029)	W, S	GUA8	Estuary	-	-
	Sanlúcar de Barrameda (X: 736448 Y: 4084204)	W, S	GUA9	Estuary	-	-
GUADAÍRA	Morón (X: 267816 Y: 4123619)	W, S	GUAA	Downstream military camp and Morón WWTP	-	-
GUADALMORAL	Baena (X: 375522 Y: 4166800)	W, S	GUAL	Agricultural activity: Olive trees. Contaminated site.	-	-
GUADIANA MENOR	Úbeda (X: 481267 Y: 4192450)	W, S	GUAM	SAICA network	-	-
GUADALBULLÓN	Mengíbar (X: 431850 Y: 4199348)	W, S	GUAN	SAICA network	-	-
GUADIAMAR	Alnalcázar (X: 742717 Y: 4130496)	W, S	GUAR	SAICA network	5.69	73
HERREROS	Alcalá del Rio (X: 235090 Y: 4156650)	W, S	HER	Agricultural activity (cereal). Contaminated site	-	-
MAGAÑA	Santa Elena (X: 456338 Y: 4242400)	W, S	MAG	Good quality	-	-
LOS PICACHOS	Fuente Palmera (X: 315008 Y: 4180807)	W, S	PIC	Agricultural activity: Orange trees. Contaminated site	-	-
YEGUAS	Cardeña (X: 384664 Y: 4246754)	W, S	YEG	Contaminated area by purines	-	-

W: Water; S: Sediment; B: Biota

Table S2. PFASs selected in this study, their family, acronym, CAS number and formula.

Family	Compound	Acronym	CAS Nº	Formula
Perfluorocarboxylates		**PFCA**		
	Perfluorobutanoate	PFBA	375-22-4	C_3F_7COOH
	Perfluoropentanoate	PFPeA	2706-90-3	C_4F_9COOH
	Perfluorohexanoate	PFHxA	307-24-4	$C_5F_{11}COOH$
	Perfluoroheptanoate	PFHpA	375-85-9	$C_6F_{13}COOH$
	Perfluorooctanoate	PFOA	335-67-1	$C_7F_{15}COOH$
	Perfluoro-7-methyloctanoate	ipPFNA	Not available	
	Perfluorononanoate	PFNA	375-95-1	$C_8F_{17}COOH$
	Perfluorodecanoate	PFDA	335-76-2	$C_9F_{19}COOH$
	Perfluoroundecanoate	PFUnDA	2058-94-8	$C_{10}F_{21}COOH$
	Perfluorododecanoate	PFDoDA	307-55-1	$C_{11}F_{23}COOH$
	Perfluorotridecanoate	PFTrDA	72629-94-8	$C_{12}F_{25}COOH$
	Perfluorotetradecanoate	PFTeDA	376-06-7	$C_{13}F_{27}COOH$
	Perfluorohexadecanoate	PFHxDA	67905-19-5	$C_{15}F_{31}COOH$
	Perfluorooctadecanoate	PFODA	16517-11-6	$C_{17}F_{35}COOH$
Perfluorosulfonates		**PFSA**		
	Perfluorobutane sulfonate	PFBS	29420-49-3	$C_4F_9SO_2O$
	Perfluorohexane sulfonate	PFHxS	82382-12-5	$C_6F_{13}SO_2O$
	Perfluoroheptane sulfonate	PFHpS		$C_7F_{15}SO_2O$
	Perfluorooctane sulfonate	PFOS	4021-47-0	$C_8F_{17}SO_2O$
	Perfluoro-7-methyloctane sulfonate	ipPFNS	Not available	
	Perfluorodecane sulfonate	PFDS	Not available	$C_{10}F_{21}SO_2O$
Perfluorosulfonamides		**FASA**		
	Perfluorooctane sulfonamide	PFOSA	754-91-6	$C_8F_{17}SO_2NH_2$
Internal standards				
	Perfluoro-n-[1,2,3,4-$^{13}C_4$] butanoate	MPFBA	CASID 39196	C_4HF_7OO
	Perfluoro-n-[1,2-$^{13}C_2$] hexanoate	MPFHxA	CASID 39197	$C_6HF_{11}OO$
	Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoate	MPFOA	CASID 39199	$C_8HF_{15}OO$
	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoate	MPFNA	CASID 39198	$C_9HF_{17}OO$
	Perfluoro-n-[1,2-$^{13}C_2$] decanoate	MPFDA	CASID 39200	$C_{10}HF_{19}OO$

Table S2 (continue). PFASs selected in this study, their family, acronym, CAS number and formula.

Family	Compound	Acronym	CAS Nº	Formula
Internal standards				
	Perfluoro-n-[1,2-$^{13}C_2$] undecanoate	MPFUdA	CASID 39202	$C_{11}HF_{21}OO$
	Perfluoro-1-[$^{18}O_2$] hexanesulfonate	MPFHxS	Not available	$C_6F_{13}SO_2O$
	Perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	MPFOS	Not available	$C_8F_{17}SO_2O$

Table S3. Fish sample characteristics in the Ebro River.

Sampling point	Sample number	Species	Fork length (cm)	Total length (cm)	Weight (g)	Code
OCA	4	*Barbus graellssi*	39.5	41.5	1,244	Ebro_B
	5	*Barbus graellssi*	36.8	38.5	957	
	6	*Barbus graellssi*	30.3	32.5	567	
	18	*Barbus graellssi*	23	25	190	Ebro_G
	19	*Barbus graellssi*	24	26	238	
	20	*Barbus graellssi*	21.5	23.5	162	
	21	*Barbus graellssi*	24.5	26	277	
EBR2	1	*Barbus graellssi*	29	31	387	Ebro_A
	2	*Barbus graellssi*	28	30	443	
	3	*Barbus graellssi*	10	11.5	465	
	15	*Barbus graellssi*	45.5	47	1,638	Ebro_F
	16	*Barbus graellssi*	43.5	45.5	1,577	
	17	*Barbus graellssi*	44.5	46	1,240	
	41	*Cyprinus carpio*	44	46	1,331	Ebro_Ñ
EBR3	10	*Barbus graellssi*	25.5	27.5	238	Ebro_D
	11	*Barbus graellssi*	24.5	26.5	229	
	12	*Barbus graellssi*	23.5	25.5	200	
	13	*Barbus graellssi*	37.5	39.5	694	Ebro_E
	14	*Barbus graellssi*	32.5	34.5	548	
	42	*Barbus graellssi*	38	42	726	
	38	*Cyprinus carpio*	43	45	1,685	Ebro_N
	39	*Cyprinus carpio*	46	50	1,843	
	40	*Cyprinus carpio*	42	46	1,323	
EBR4	32	*Barbus graellssi*	15	16.5	55	Ebro_L
	33	*Barbus graellssi*	18	20	99	
	34	*Barbus graellssi*	20	23	126	
	29	*Barbus graellssi*	33	35	610	Ebro_K
	30	*Barbus graellssi*	41	43	983	
	31	*Barbus graellssi*	31	34	410	
	35	*Cyprinus carpio*	43	48	1,530	Ebro_M
	36	*Cyprinus carpio*	47	50	2,091	
	37	*Cyprinus carpio*	38	41	1,148	
	73	*Silurus glanis*		68	1,992	Ebro_Y
	74	*Silurus glanis*		90	5,840	
	76	*Silurus glanis*		78	2,885	

Table S3 (continue). Fish sample characteristics in the Ebro River.

Sampling point	Sample number	Species	Fork length (cm)	Total length (cm)	Weight (g)	Code
EBR5	7	*Barbus graellssi*	25.5	27.5	356	Ebro_C
	8	*Barbus graellssi*	23.5	25.5	218	
	9	*Barbus graellssi*	20.5	22.5	191	
	27	*Barbus graellssi*	51	53	2,638	Ebro_J
	28	*Barbus graellssi*	50	53.5	2,238	
	22	*Cyprinus carpio*	47	51	2,349	Ebro_H
	23	*Cyprinus carpio*	44.5	50	2,279	
	26	*Cyprinus carpio*	41	45.5	1,938	
	75	*Silurus glanis*		62	1,862	Ebro_X
	76	*Silurus glanis*		78	2,885	

Table S4. Instrumental characteristics used for water and sediment determination

LC CONDITIONS	
Analytical column	Kinetex XB-C18: 50.0 × 4.6 mm, 1.7 µm particle size (Phenomenex, Torrance, USA)
Column temperature	30° C
Volume injected	5 µL
Mobile phase	(A) Water – (B) methanol both with 10 mM Ammonium Formate
Flow rate	0.2 mL min^{-1}
Linear gradient	0 min (30 % B), 0.5 min (30 % B), 12 min (95 % B), 20 min (95 % B), and return to the initial conditions (equilibration time 12 min)
TRIPLE QUADRUPOLE MS/MS CONDITIONS	
Ionization characteristics and source	MS/MS performed in selected reaction monitoring mode (SRM) with electrospray ionization (ESI) in negative mode
Gas temperature	300° C
Gas flow	11 L min^{-1}
Nebulizer	30 psi
Capillary voltage	4000 V
Chamber current	1.27 µA
Scan type	MRM, with MS1 and MS2 at unit resolution and cell acceleration voltage of 7 eV

Table S5. Dynamic MRM conditions for LC-MS/MS determination of PFASs

Target PFAS	t_R[(a)] (min)	Precursor Ion	SRM_1[(b)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2[(e)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2/SRM_1 (%)(%RSD)[(f)]
PFBA	8.0	213	169	66	5				
MPFBA	*8.0*	*217*	*172*	*62*	*0*				
PFPA	8.9	263	219	66	5				
PFBS	9.2	299	99	142	38	80	142	26	15.3 (2.3)
PFHxA	13.3	313	269	71	5	119	71	5	10.6 (3.3)
MPFHxA	*13.3*	*315*	*270*	*71*	*5*	*119*	*71*	*5*	*7.1 (0.8)*
PFHpA	15.4	363	319	76	5	169	76	5	68.5 (9.2)
PFHxS	15.6	399	99	169	37	80	169	29	65.9 (10.8)
MPFHxS	*15.6*	*403*	*103*	*164*	*33*	*84*	*164*	*37*	*23.5 (4.3)*
PFOA	17.2	413	369	87	5	169	87	5	46.7 (1.4)
MPFOA	*17.2*	*417*	*372*	82	*5*	169	82	*13*	22.5 (0.6)
PFHpS	17.3	449	99	179	37	80	179	57	31.9 (8.9)
ipPFNA	19.3	463	419	87	5	169	87	5	27.0 (1.2)
PFNA	19.5	463	419	82	5	219	82	5	13.2 (0.9)
MPFNA	*19.5*	*468*	*423*	*82*	*5*	*223*	*82*	*9*	*17.6 (1.4)*
PFOS	19.9	499	99	190	41	80	190	65	82.2 (3.2)
MPFOS	*19.9*	*503*	*99*	*180*	*41*	*80*	*180*	*61*	*30.0 (1.1)*
PFDA	25.5	513	469	89	5	269	89	13	15.3 (2.2)
MPFDA	*25.5*	*515*	*470*	*92*	*5*	*270*	*92*	*12*	*8.4 (0.4)*
ipPFNS	25.5	549	99	195	45	80	195	73	21.6 (1.6)
PFUnDA	28.1	563	519	104	5	269	104	13	14.1 (0.6)
MPFUnDA	*28.1*	*565*	*520*	*94*	*5*	*269*	*94*	*13*	*4.3 (0.4)*
PFDS	28.2	599	99	80	80	80	80	80	17.6 (1.3)
PFDoDA	32.7	613	569	94	5	269	94	13	9.0 (0.8)
MPFDoDA	*32.7*	*615*	*570*	*112*	*5*				
PFTrDA	33.4	663	619	104	0	169	104	24	8.1 (1.8)
PFTeDA	34.0	713	669	112	5	169	112	25	7.8 (0.2)
PFHxDA	35.2	813	769	114	8	169	114	28	9.6 (1.1)
PFODA	35.8	913	869	134	10	169	128	29	

(a) t_R = retention time; (b) SRM_1 = selected product ion for quantification; (c) Frag = fragmentor; (d) CE = collision energy; (e) SRM_2 = selected product ion for qualification; (f) SRM_2 /SRM_1 (%RSD) = mean values obtained from the matrix-matched calibration curves and relative standard deviation of the ratio. Italics mean isotopically labelled internal standard.

Table S6. Instrumental characteristics used for biota determination

LC CONDITIONS	
Analytical column	Hypersil GOLD PFP (50 × 3) (Thermo Fisher Scientific, Franklin, MA)
Column temperature	20 ºC
Volume injected	20 µL
Mobile phase	(A) NH_4Ac 20 mM in water; (B) NH_4Ac 20 mM in methanol
Flow rate	0.4 mL/min.
Linear gradient	No

Ionization characteristics and source	Electrospray ionization (H-ESI) in negative mode
Sheath gas:	30 UA
Auxiliary gas:	15 UA
Ion Sweep gas:	0.5 UA
Vaporizer temperature	300ºC
Spray voltage	2000 V
Scan type	SRM (0.02 m/z)

Table S7. Method quantification limits for water (ng L^{-1}) and for sediment (ng g^{-1}).

Compound	Water	Sediment
	LOQ (ng L^{-1})	LOQ (ng g^{-1})
PFBA	0.040	0.133
PFPeA	0.040	0.133
PFHxA	0.400	1.333
PFHpA	0.400	0.330
PFOA	0.040	0.133
PFNA	0.400	1.333
ipPFNA	0.400	1.333
PFDA	0.040	0.085
PFUnDA	0.040	0.100
PFDoDA	0.040	0.100
PFTrDA	0.020	0.067
PFTeDA	0.020	0.067
PFHxDA	0.040	0.133
PFODA	0.800	2.667
PFBS	0.020	0.413
PFHxS	0.004	0.013
PFHpS	0.040	0.133
PFOS	0.004	0.013
ipPFNS	0.040	0.133
PFDS	0.004	0.013
PFOSA	0.200	0.667

Table S8. Relative recoveries obtained for spiked water samples at 6 and 60 ng L^{-1}.

PFASs	Internal Standard	Relative Recovery (%)	
		6 ng L^{-1}	60 ng L^{-1}
PFBA	MPFBA	59 ± 11	63 ± 10
PFPeA	MPFBA	55 ± 15	58 ± 11
PFHxA	MPFHxA	58 ± 13	65 ± 8
PFHpA	MPFHxA	58 ± 13	64 ± 13
PFOA	MPFOA	60 ± 18	64 ± 16
PFHpS	MPFHxS	85 ± 12	90 ± 11
PFNA	MPFNA	82 ± 15	86 ± 14
ipPFNA	MPFNA	85 ± 11	87 ± 12
PFDA	MPFDA	80 ± 11	84 ± 10
PFUnDA	MPFUnDA	86 ± 13	91 ± 14
PFDoDA	MPFDoDA	82 ± 11	87 ± 13
PFTrDA	MPFDoDA	85 ± 14	89 ± 14
PFTeDA	MPFDoDA	87 ± 13	92 ± 11
PFHxDA	MPFDoDA	90 ± 15	93 ± 12
PFODA	MPFDoDA	91 ± 12	94 ± 11
ipPFNS	MPFNS	84 ± 9	88 ± 11
PFHxS	MPFHxS	85 ± 10	89 ± 14
PFOS	MPFOS	79 ± 14	83 ± 16
PFDS	MPFOS	78 ± 10	82 ± 12

Table S9. Relative and absolute recoveries obtained for spiked sediment samples at the LOQ levels (0.04-8.00 ng g^{-1}).

PFASs	Internal Standard	Relative recovery (%)	Absolute recovery (%)
PFBA	MPFBA	44 ± 19	62 ± 16
PFPeA	MPFBA	56 ± 17	82 ± 14
PFHxA	MPFHxA	68 ± 15	100 ± 13
PFHpA	MPFHxA	62 ± 16	85 ± 10
PFOA	MPFOA	73 ± 10	98 ± 9
PFNA	MPFNA	74 ± 15	103 ± 8
ipPFNA	MPFNA	70 ± 14	97 ± 7
PFDA	MPFDA	72 ± 13	99 ± 5
PFUnDA	MPFUnDA	59 ± 11	63 ± 10
PFDoDA	MPFDoDA	85 ± 11	87 ± 12
PFTrDA	MPFDoDA	80 ± 11	84 ± 10
PFTeDA	MPFDoDA	84 ± 9	88 ± 11
PFHxDA	MPFDoDA	79 ± 14	83 ± 16
PFODA	MPFDoDA	84 ± 9	88 ± 11
ipPFNS	MPFNS	86 ± 13	91 ± 14
PFBS	MPFBA	58 ± 13	64 ± 13
PFHxS	MPFHxS	80 ± 11	84 ± 10
PFHpS	MPFHxS	79 ± 14	83 ± 16
PFOS	MPFOS	71 ± 12	97 ± 11
PFDS	MPFOS	69 ± 12	98 ± 6

Table S10. Recoveries obtained for spiked biota samples at 1 and 10 μg L^{-1}

PFASs	Internal Standard	1 μg L^{-1}	10 μg L^{-1}
PFBA	MPFBA	29 ± 12	106 ± 11
PFPeA	MPFBA	39 ± 13	96 ± 10
PFHxA	MPFHxA	34 ± 13	101 ± 10
PFHpA	MPFHxA	64 ± 19	79 ± 16
PFOA	MPFOA	31 ± 14	82 ± 11
PFNA	MPFNA	42 ± 15	125 ± 8
ipPFNA	MPFNA	Not available	Not available
PFDA	MPFDA	78 ± 15	103 ± 8
PFUnDA	MPFUnDA	47 ± 12	113 ± 11
PFDoDA	MPFDoDA	37 ± 12	130 ± 11
PFTrDA	MPFDoDA	88 ± 15	86 ± 12
PFTeDA	MPFDoDA	114 ± 11	109 ± 9
PFHxDA	MPFDoDA	16 ± 10	52 ± 13
PFODA	MPFDoDA	Not available	Not available
ipPFNS	MPFNS	Not available	Not available
PFBS	MPFBA	34 ± 11	132 ± 12
PFHxS	MPFHxS	33 ± 9	99 ± 11
PFHpS	MPFHxS	Not available	Not available
PFOS	MPFOS	30 ± 14	135 ± 5
PFDS	MPFOS	26 ± 12	119 ± 6

Table S11. Modelled (ECOSAR) and reported (mean, minimum and maximum) toxicity of selected PFAS in different tropic levels (algae, *Daphnia sp*. and fish)

Family/ Compound	CAS Number	ECOSAR Acute toxicity- EC50 (mg L^{-1})		
		Algae	*Daphnia sp.*	Fish
PFCAs				
PFBA	375-22-4	597.14	760.59	1322.59
PFPeA	2706-90-3	253.58	250.18	408.97
PFHxA	307-24-4	103.82	79.34	127.93
PFHpA	375-85-9	41.43	24.52	35.43
PFOA	335-67-1	16.22	7.44	10.10
ipPFNA	Not available	4.65	1.54	1.93
PFNA	375-95-1	6.26	2.22	2.84
PFDA	335-76-2	2.39	0.66	0.79
PFUnDA	2058-94-8	0.90	0.19	0.22
PFDoDA	307-55-1	0.34	0.06	0.06
PFTrDA	72629-94-8	0.13	0.02	0.02
PFTeDA	376-06-7	0.05	$5\ 10^{-3}$	$4\ 10^{-3}$
PFHxDA	67905-19-5	$6 \cdot 10^{-3}$	$3.74 \cdot 10^{-4}$	$3.09 \cdot 10^{-4}$
PFODA	16517-11-6	$8.37 \cdot 10^{-4}$	$2.98 \cdot 10^{-5}$	$2.18 \cdot 10^{-5}$
PFSAs				
PFBS	29420-49-3	1395.17	2008.25	3597.02
PFHxS	82382-12-5	220.42	190.35	301.32
PFHpS	Not available	85.36	57.10	84.97
PFOS	4021-47-0	32.65	16.92	23.66
ipPFNS	Not available	9.19	3.43	4.44
PFDS	Not available	4.64	1.44	1.78
FASAs				
PFOSA	754-91-6	0.41	0.13	0.16

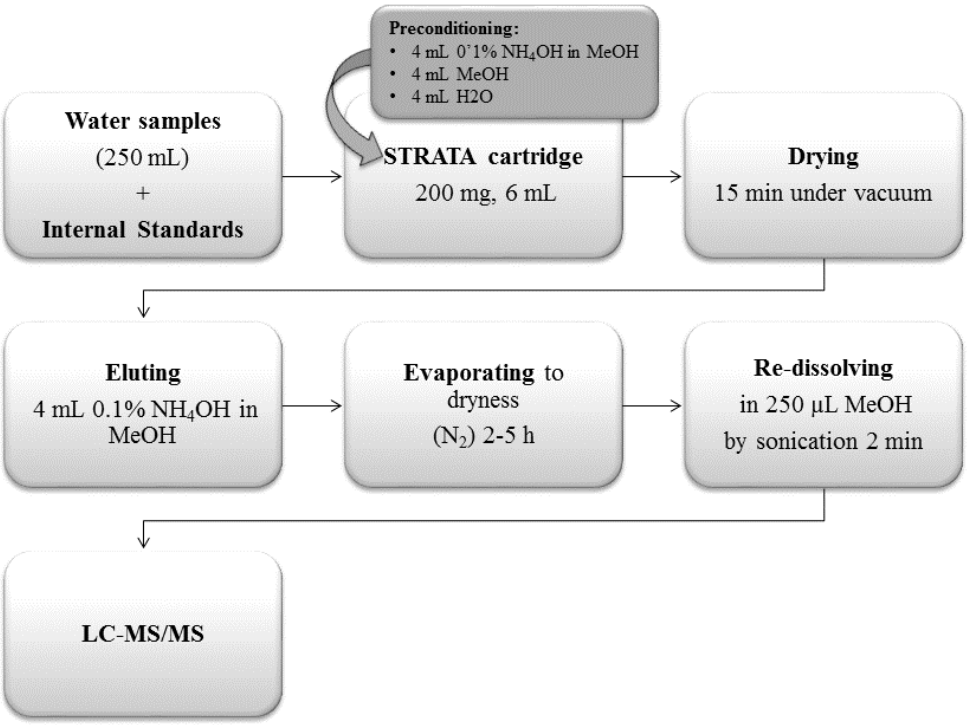

Fig. S1. PFAS extraction procedure for water samples

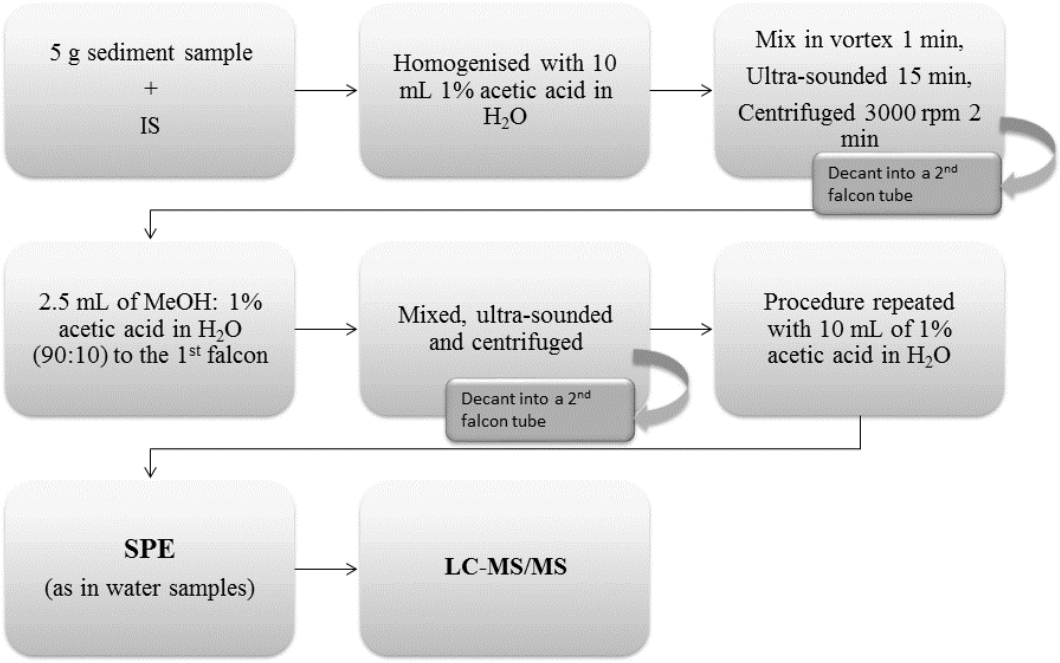

Fig. S2. PFAS extraction procedure for sediment samples

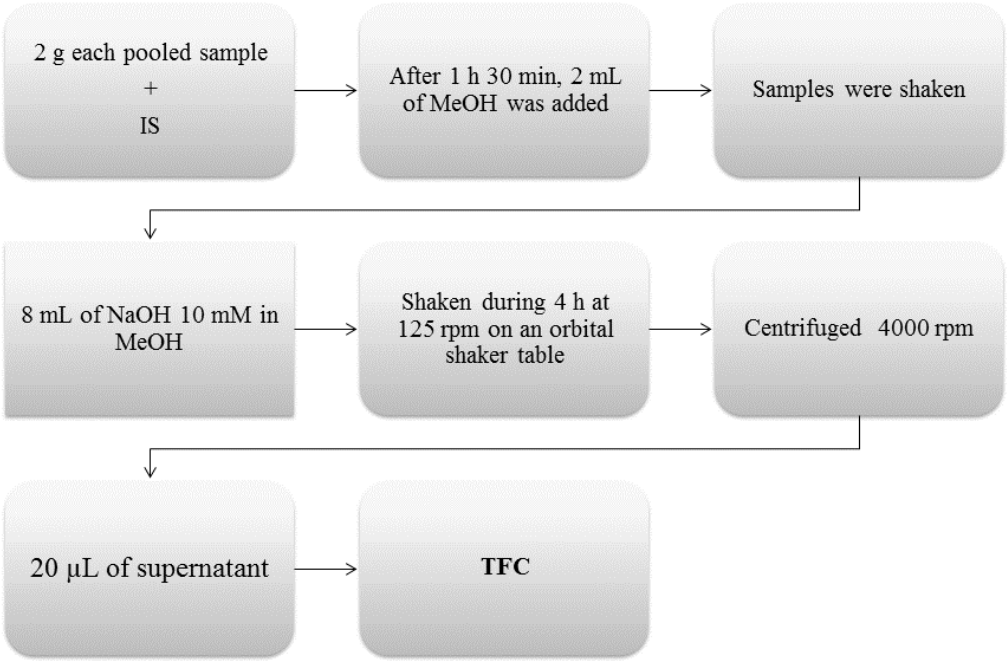

Fig. S3. PFAS extraction procedure for biota samples

CAPÍTULO 6

Analysis of the presence of perfluoroalkyl substances in water, sediment and biota of the Jucar River (E Spain). Sources, partitioning and relationships with water physical characteristics.

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Analysis of the presence of perfluoroalkyl substances in water, sediment and biota of the Jucar River (E Spain). Sources, partitioning and relationships with water physical characteristics

Julian Campo [a,b,*], María Lorenzo [c], Francisca Pérez [d], Yolanda Picó [c], Marinel.la Farré [d], Damià Barceló [d,e]

[a] Environmental Forensic and Landscape Chemistry Research Group. Desertification Research Centre - CIDE (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana), Carretera Moncada - Náquera km 4.5 (Campus IVIA), Moncada, 46113 Valencia, Spain
[b] Earth Surface Science, Institute for Biodiversity and Ecosystems Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands
[c] Food and Environmental Safety Research Group (SAMA–UV), Centro de Investigaciones sobre Desertificación (CIDE, UV–CSIC–GV) and Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, Burjassot, 46100 Valencia, Spain
[d] Department of Environmental Chemistry (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[e] Catalan Institute for Water Research (ICRA), Emili Grahit, 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, E-17003 Girona, Spain

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ABSTRACT

The presence, sources and partitioning of 21 perfluoroalkyl substances (PFASs: C4–C14, C16, C18 carboxylate, C4, C6–C10 sulfonates and C8 sulfonamide) were assessed in water, sediment, and biota of the Jucar River basin (E Spain). Considering the three matrices, perfluoropentanoate (PFPeA) and perfluorooctane sulfonate (PFOS) were the most frequent compounds, being remarkable the high occurrence of short-chain PFASs ($C \leq 8$), which are intended to replace the long-chain ones in several industrial and commercial applications. In general, all samples were contaminated with at least one PFAS, with the exception of three fish samples. Mean concentrations detected in sediments (0.22–11.5 ng g^{-1}) and biota (0.63–274 μg kg^{-1}) samples were higher than those measured in water (0.04–83.1 ng L^{-1}), which might suggest (bio) accumulation.

The occurrence of PFAS is related to urban and industrial discharges (Cuenca city in the upper part of basin, and car's factory, and effluents of the sewage treatment plant (STP) of Alzira, in the lower part). Increasing pollution gradients were found. On the other hand, higher contamination levels were observed after regulation dams of the catchment pointing out their importance in the re-distribution of these contaminants. None of the hazard quotients (HQ) calculated indicate potential risk for the different tropic levels considered (algae, *Daphnia* sp. and fish). PFAS concentrations found in this study can be considered in acceptable levels if compared to existing Regulatory Legislation and, consequently, they do not pose an immediate human health risk.

1. Introduction

Perfluoroalkyl substances (PFASs) present significant thermal and chemical stability being persistent in the environment where they can bio-accumulate and potentially cause adverse effects on humans and wildlife (Campo et al., 2015; Wang et al., 2013; Zareitalabad et al., 2013). Among PFASs, perfluorooctane sulfonate (PFOS) and its synthetic starting material, perfluorooctyl sulfonyl fluoride (POSF), were added in May 2009 to Annex B as new persistent organic pollutants at the Stockholm Convention (United Nations Environment Programme, 2010). European Commission also prohibited the general use of PFOS after June 2008 (European Union Directive, 2006). The water status is controlled according to The European Water Framework Directive (WFD) requirements (European Union Directive, 2000) whose 2012 amendment, proposed Maximum Allowable Concentration for this sulfonate and its salts in inland surface water (European Commission Proposal, 2012). The Directive 2008/105/EC of Environmental Quality Standards (EQS) includes for the first time PFOS as a potential pollutant (European Union Directive, 2008), which is also cited in the Directive amending the WFD and EQS as regards priority substances in the field of water policy (European Union Directive, 2013).

PFASs presence has been reported in various environmental

* Corresponding author at: Earth Surface Science, Institute for Biodiversity and Ecosystems Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.
E-mail address: Julian.Campo@uv.es (J. Campo).

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compartments around the world (Dai et al., 2013; Xu et al., 2014; Zareitalabad et al., 2013), including surface water (Codling et al., 2014; Lu et al., 2015; Llorca et al., 2012; Myers et al., 2012; Onghena et al., 2012), groundwater (Boiteux et al., 2012; Kim et al., 2011; Weiss et al., 2012), sediments (Beskoski et al., 2013; Campo et al., 2015; Picó et al., 2012; Yeung et al., 2013), soils (Kim et al., 2014; Lorenzo et al., 2015a; Milinovic et al., 2015; Zareitalabad et al., 2013), atmosphere (Gawor et al., 2014; Li et al., 2011; Wang et al., 2014), wildlife (Dai et al., 2013; Wang et al., 2013; Xu et al., 2014), and even human body (Liu et al., 2014; Pan et al., 2014; Renzi et al., 2013).

The occurrence and relevant concentrations not only of PFAS (Campo et al., 2015; Llorca et al., 2012; Lorenzo et al., 2015a; Picó et al., 2012) but also of a broad range of other contaminants, such as pesticides, pharmaceuticals, estrogenic compounds, etc. in Mediterranean aquatic environments (Belenguer et al., 2014; González et al., 2012; Masiá et al., 2013) have been reported. Mediterranean climate is characterized by dry (frequently, extremely dry) and very hot summers (Garcia-Ruiz et al., 2013). During this season, river flows are reduced to its minimum with consequent increase in contaminants concentrations, and nowadays this tendency would be increased because of global change (Chirivella Osma et al., 2014; IPCC, 2007).

Accordingly, increased scientific interest has been paid to the relevance of global change impacts on the water quantity and quality. In the Mediterranean region, the SCARCE project made a complete evaluation of the status of four main Spanish Mediterranean River basins (Navarro-Ortega et al., 2012). In the case of Jucar River, the last nationwide report on climate change estimated a 10–25% reduction of the mean annual flow (Belenguer et al., 2014). This lower flow is related to increased concentration of contaminants. According to the evaluation carried out by the Hydrographic Confederation of Jucar, in 2010, of 15 water bodies analysed (artificial channels are not included), 7 did not reach the Good Quality Status for the annual average concentration stablished by the RD 60/2011. Contaminants reported include: chlorpyrifos, bis(2-ethylhexyl) phthalate, 4-*t*-octylphenol and endosulfan.

Despite the big effort of this Office for keeping an updated evaluation of the Jucar River quality, there are a number of other pollutants – as the PFASs – not screened, which knowledge is essential in order to have a general contamination picture of the whole ecosystem including different environmental compartments as water, sediments and biota. This work presents the results of, up to authors' knowledge, the first comprehensive PFAS monitoring survey conducted jointly in these three matrices in the Jucar River, using off-line and on-line extractions and liquid chromatography tandem mass spectrometry, searching also for their possible origin, distribution and partitioning in sediments. In addition, this research pretends to assess the relationships of 21 PFASs of different chemical classes with other water physical parameters as temperature, pH, or sediment suspension that can influence their presence and fate on the environment.

2. Materials and methods

2.1. Description of the study area

The Jucar River with 498 km flows through three different provinces (Cuenca, Albacete, Valencia), being one of the most important rivers in southern Spain with over one million people living in the basin. It is a Mediterranean catchment which source is located at 1700 m a.s.l. in the Iberian System (Montes Universales), and its mouth in the Mediterranean Sea (40 km south of Valencia city) characterized by periods of floods and droughts, in which the water demand can be in excess of water supply.

The watershed of 21578 km^2 is mountainous and well preserved in its upper part, but water quality problems appear in the medium and lower parts where agriculture accounts for nearly 80% of water demand (1394 hm^3 y^{-1} for 200,000 ha of irrigated crops) (Navarro-Ortega et al., 2012). Nowadays, agricultural demand appears to be stabilized or decreasing, whereas urban/industrial demand is forecasted to rise (Estrela et al., 2004; Paredes-Arquiola et al., 2010).

There are a number of reservoirs in the basin (total capacity of 2643 hm^3) for the production of hydropower, river regulation to prevent flooding and for irrigation and human consumption. Groundwater over-extraction has affected the hydrological system, and a marine intrusion of more than 3 km long is reported (Navarro-Ortega et al., 2012). There is also a nuclear power station in the catchment, Cofrentes, which in 2010 generated around 9.5 GV almost 5% of Spanish demand of the year. Beside this, Jucar receives wastewater discharges from more than 20 urban and industrial sewage treatment plants (STPs) including those of large cities as Cuenca and Teruel. In its lower part, after the confluence of Cabriel and Magro Rivers, Jucar River drains to L'Albufera de Valencia, a large coastal lagoon and marshy area where important protected wetlands (Natural Park) and agriculture (mainly rice, citric and other orchards) share the same area. This basin has been selected as European Pilot River Basin for the implementation of the Water Framework Directive (Directive 2000/60/EC) and is of particular interest due to its previously noted characteristics.

2.2. Sampling

The sampling campaign was carried out in October of 2010. This period was selected taken into account the EQS 2008/105/EC: (i) after summer, the river flow is lower allowing an easier sampling (ii) low flow condition (with less dilution) may represent the worst case scenario for exposure from waters, and (iii) coincides with the end of growing season, when the juveniles are large enough to be captured by electrofishing, and after the spawning cycle to not impair fish reproduction. A total of 55 samples were analysed including 15 of water, 15 of sediments, and 25 of biota. Fig. 1 shows the sampling points location (5 at the Cabriel tributary, 2 at the Magro tributary, 8 at the Jucar River). Water and sediment were taken in all sampling points. However, the 25 fish samples (9 different species) were taken in 5 of the locations, of Jucar River, in points JUC1 and JUC2 and from JUC4 to JUC6. The most abundant was the Iberian Gudeon (*Gobio lonzanoi*) present in all points. The Black Bass (*Mycroptero salmoides*) and the Pumpkinseed Sunfish (*Lepomis gibbosus*) are invasive species, and Eel (*Anguila anguila*) is an endangered one (this information is detailed in Tables S1 and S2). Before the analyses, water was vacuum filtered, through 1 μm glass fibre filters followed by 0.45 μm nylon membrane filters (VWR, Barcelona, Spain), and together with sediment samples all were frozen until analyses. Each fish sample (always that possible) was a pool of several individuals of the same species (Table S2), which were chopped and homogenized in a food processor (Oster BPST02-B00, London, UK) to make a representative sample. All containers used during the process were carefully operated to avoid the introduction of contamination.

Physical characteristics of water (temperature, pH, redox potential, conductivity, total dissolved solids, NaCl concentration and dissolved oxygen) were recorded at the sampling sites using a Multiparameter Eutech InstrumentCyberScan PCD 650 (Thermo Fisher Scientific, Basel, Switzerland).

2.3. Chemicals and reagents

A total of 21 PFASs were analysed, including 14 perfluoroalkyl

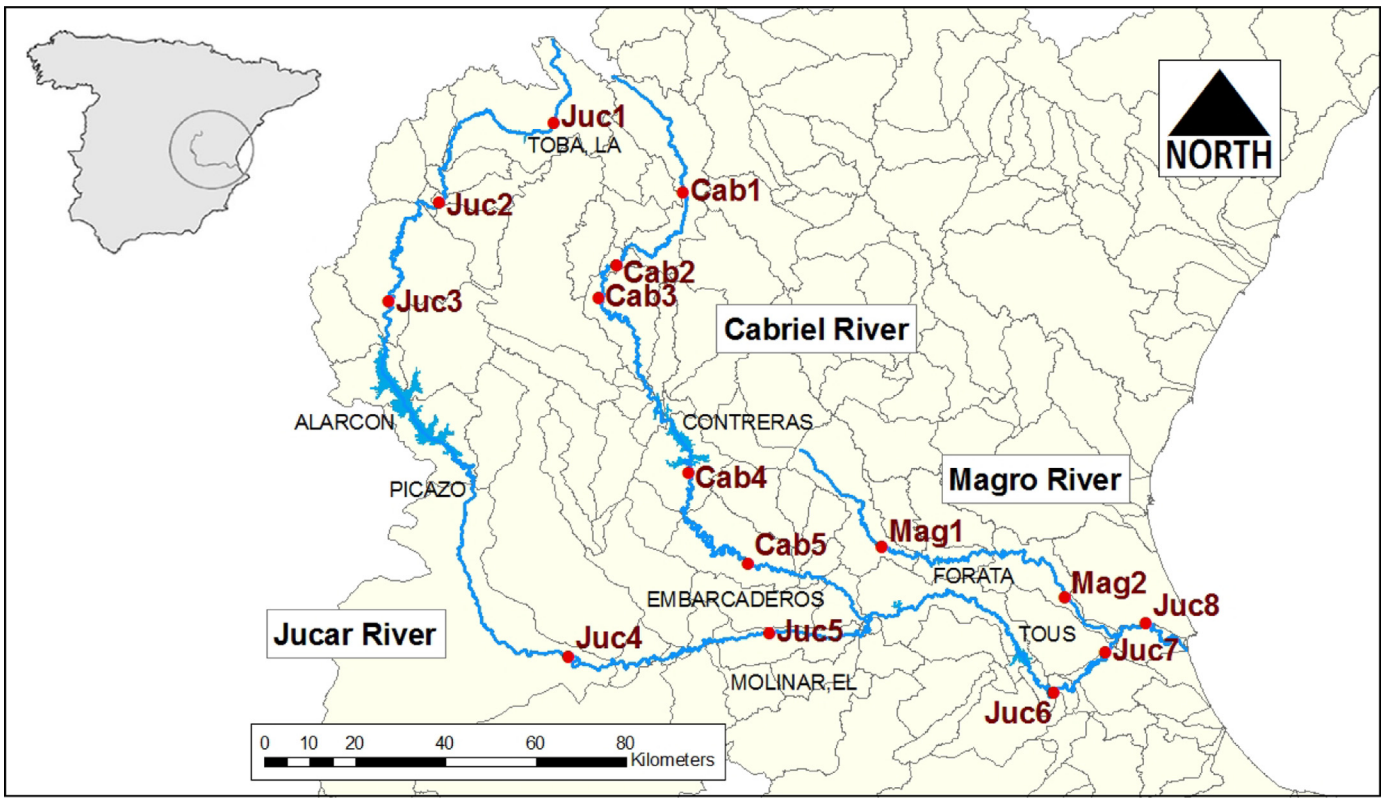

Fig. 1. Location of the sampling points and regulation dams in the Jucar River basin.

carboxylates (PFCAs) C_4–C_{18}, 5 perfluoroalkyl sulfonates (PFSs) C_4, C_6–C_{10} and 1 perfluoroalkyl sulfonamide (PFSA) C_8 (see Table S3 for exhaustive description). Standards were of linear PFAS isomers, except in the case of perfluoro-7-methyloctano sulfonate (i,p-PFNS) and perfluoro-7-methyloctanoate (i,p-PFNA), which are the isopropyl branched isomers. In biota samples i,p-PFNA, PFODA, PFHpS and i,p-PFNS were not analysed due to the lack of analytical standards.

2.4. Sample preparation and instrumental analysis

An off-line solid-phase extraction (SPE) procedure was used for the pre-concentration of water samples (Campo et al., 2014; Picó et al., 2012). Sediments were extracted with acetic acid and methanol and the extracts were cleaned-up using the SPE procedure for water (Campo et al., 2014; Picó et al., 2012) (Figs. S1 and S2 detail the procedures). Biota procedure involved alkaline digestion and direct injection of the extracts into the turbulent flow chromatographic system (Campo et al., 2015; Farré et al., 2012).

The determination in water and sediment extracts was carried out by liquid chromatography tandem mass spectrometry LC-MS/MS using an HP1200 series LC – with an automatic injector, a degasser, a quaternary pump and a column oven – combined with an Agilent 6410 triple quadrupole (QqQ) mass spectrometer, with electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany). Data were processed using MassHunter Workstation Software for qualitative and quantitative (internal standard methodology based on peak areas) analysis (A GL Sciences, Tokyo, Japan). Information related to instrumental determination is listed in Tables S4 and S5. Biota extracts were analysed on a turbulent flow chromatograph Aria TLX-1 system (Thermo Fisher Scientific, Franklin, MA, USA) consisted of a PAL auto-sampler (CTC Analytics, Zwingen, Switzerland), with two mixing binary pumps (eluting and loading, respectively), and a three-valve (six-port) switching device unit. The entire system was controlled via Aria software, version 1.6. Detailed information related to instrumental determination is presented in Table S6.

The analytical methods were carefully validated (detailed information about mean relative recoveries obtained is available in Supplementary information, Tables S7–S9). Limits of quantification (LOQs) were determined by the injection of spiked extracts (n=3) and estimated as the minimum detectable amount of analyte with a signal-to-noise ratio of 10:1. LOQs were 0.01–2.00 ng L^{-1} for water, 0.04–8.00 ng g^{-1} for sediment (dry weight: dw), and 0.02–2.26 μg kg^{-1} for biota samples.

Prior and after each sampling batch (between 25 and 30 samples), calibration curves were constructed daily by injecting standards at different concentrations (1, 2.5, 5, 10, 25, 50, 65, 75 ng L^{-1}) obtaining $R^2 > 0.98$. Each 15 samples, one instrumental and one procedural blanks as well as one positive control were analysed to serve as quality control. In addition, unfiltered deionized water (travel blanks) samples, were prepared at the same time as the river water samples in order to assess any possible contamination due to the sampling campaign. Traces of PFOS were detected, always below the LOQs, in both type of blanks indicating some background contamination from the injection system and tubing of the LC-MS/MS but there was no sample contamination during sampling and analysis. This background contamination can only minimally affect the quantification accuracy and then, it was not taken into account. In the samples with high levels of PFBA and PFPeA, the identification carried out only with one precursor → product ion transition was confirmed by accurate mass using a quadrupole time-of-flight mass spectrometer (QqTOF) (data not shown).

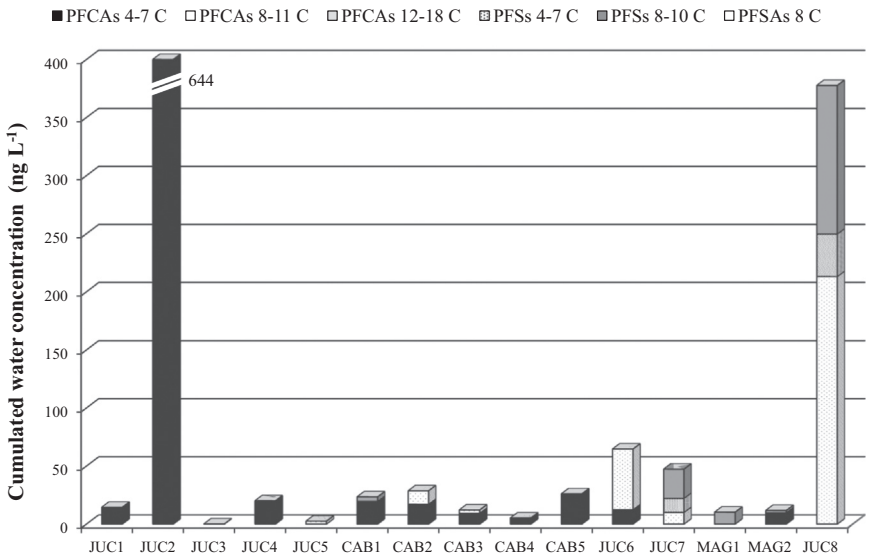

Fig. 2. Cumulated PFCAs, PFSs and PFSAs concentration according to the low (4–7 C) medium (8–11 C) and high (12–18 C) length of the chain present in water samples of Jucar River basin in October 2010. (CAB: Cabriel River: MAG: Magro River; JUC: Jucar River).

3. Results and discussion

3.1. Occurrence of selected PFASs in water samples

From the 21 analytes included in this study, 12 were detected in water samples at frequencies between 7% and 60% (Fig. S3). All the samples had PFASs. In agreement with other studies carried out in other Spanish rivers (Campo et al., 2015; Llorca et al., 2012; Lorenzo et al., 2015b), and in L'Albufera Natural Park wetland (Picó et al., 2012), PFOA (53.3%) showed higher frequency than PFOS (40.0%). Long-chain PFASs ($C \geq 10$) were less common in water samples than short-chain ones (PFBA and PFPeA were present in 60% of the samples). This is related to the low water solubility of long-chain PFASs (Onghena et al., 2012) and to their replacement in the market by short-chain ones – currently present at higher levels in the environment and humans – (Pan et al., 2014; Rahman et al., 2014).

Table 1
Concentration and frequency of occurrence of each PFAS in water samples of the Jucar basin in 2010.

Family/ compound	Water concentration (ng L^{-1})				Frequency (number)	Frequency (%)
	Max	Min	Mean	Mean (no zeros)		
PFCAs[a]	**644**	**0.03**	**7.72**	**18.8**	**9**	**60**
PFBA	644	5.21	49.87	83.1	9	60
PFPeA	2.82	0.08	0.38	0.64	9	60
PFHxA	18.7	1.44	3.82	9.55	6	40
PFHpA	20.1	0.64	2.22	5.55	6	40
PFOA	52.2	0.07	4.36	8.18	8	53
i,p-PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFNA	19.8	0.85	2.31	8.67	4	27
PFDA	213	0.09	14.21	71.1	3	20
PFUdA	0.62	0.62	0.04	0.62	1	7
PFDoA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	0.04	0.04	0.00	0.04	1	7
PFTeDA	0.04	0.03	0.01	0.04	2	13
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSs[a]	**128**	**0.01**	**7.27**	**26.3**	**6**	**40**
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	36.7	12.07	3.25	24.4	2	13
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOS	128	0.01	11.29	28.2	6	40
i,p-PFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs[a]	**n.d.**	**n.d.**	**n.d.**	**n.d**	**n.d.**	**n.d.**
PFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑PFASs	**1.14 10^3**	**21.1**	**91.8**	**240**	**–**	**–**

[a] Concentration given for the different families (bold) are maximum, minimum and mean values (including and excluding zeros) of the compounds, while frequency is the total number of samples with at least one PFAS; n.d.: not detected.

Results are presented in Table 1. Mean PFCA values ranged from 0.04 ng L^{-1} (PFTrDA) up to 83.1 ng L^{-1} (PFBA). Among PFSs only PFHpS and PFOS were detected with mean values of 24.4 ng L^{-1} and 28.2 ng L^{-1}, respectively, while PFSAs were not detected in this study. The highest concentration found was 644 ng L^{-1} of PFBA. Contamination levels in the Jucar River basin are, in general, similar or even lower than those reported elsewhere (Table S10).

Fig. 2 shows the cumulated PFASs concentration in water samples. Low contamination levels were detected in both tributaries, not surpassing 50 ng L^{-1} in any point. On the contrary, high cumulative concentrations were detected the upper part (main contribution was of PFBA), and close to the mouth (main contribution PFOS and PFDA). The high concentration of PFBA is directly related to industrial and urban discharges coming from Cuenca city. In the upper part the contamination was mainly due to short-chain compounds, while in the lower part most important contribution came from long-chain PFASs. High levels of PFOS were also found close to the mouth of other Spanish rivers as the Llobregat (Flores et al., 2013).

There are pollution gradients at the end of the Jucar and Magro Rivers. Between MAG1 and MAG2 there are some industries which years ago spilled their wastewaters directly into the river, and between JUC7 and JUC8 there is a huge car's factory. One important source of PFASs was the STP of Alzira that explain the high concentrations in the lower part of the basin. As it has been demonstrated, these facilities are ineffective eliminating PFASs (Appleman et al., 2014; Castiglioni et al., 2015). Low elimination rates for PFOS (33%) and PFDA ($<20\%$) were calculated in this STP during a sampling carried out also in 2010. This study also showed acceptable removal efficiencies for the Cuenca STP, which can explain the low levels found after it (JUC3).

Other possible PFAS sources were evaluated by mean of the PFHpA/PFOA and PFOS/PFOA ratios (Simcik and Dorweiler, 2005). The PFHpA/PFOA >1 is indicative of atmospheric sources, but the

Table 2
Concentration and frequency of occurrence of each PFAS in sediment samples of the Jucar basin in 2010.

Family/ compound	Sediment concentration (ng g^{-1} dw)				Frequency (number)	Frequency (%)
	Max	Min	Mean	Mean (no zeros)		
PFCAs[a]	**10.7**	**0.15**	**0.66**	**2.55**	**15**	**100**
PFBA	10.7	2.70	5.85	5.85	15	100
PFPeA	6.18	0.40	0.93	1.54	9	60
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	1.06	0.39	0.14	0.69	3	20
PFOA	6.69	0.15	1.32	2.47	8	53
i,p-PFNA	1.97	1.97	0.13	1.97	1	7
PFNA	3.63	3.63	0.24	3.63	1	7
PFDA	1.65	0.37	0.23	0.85	4	27
PFUdA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	4.44	2.40	0.46	3.42	2	13
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSs[a]	29.2	0.05	2.09	4.77	14	93
PFBS	29.2	2.17	10.8	11.5	14	93
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpS	0.58	0.05	0.06	0.22	4	27
PFOS	9.83	0.06	1.71	2.57	10	67
i,p-PFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs[a]	**n.d.**	**n.d.**	**n.d.**	**n.d**	**n.d.**	**n.d.**
PFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑PFASs	**75.9**	**14.3**	**21.8**	**34.7**	**–**	**–**

[a] Concentration given for the different families (bold) are maximum, minimum and mean values (including and excluding zeros) of the compounds, while frequency is the total number of samples with at least one PFAS; dw: dry weight; n.d.: not detected.

< 1 is indicative of non-atmospheric sources associated with urban areas. This ratio could only be calculated for CAB2 (20.6), CAB3 (0.9), CAB4 (7.8) and MAG2 (54.7) and according to these values, main PFAS source in the Jucar River basin could be related to atmospheric deposition (only in CAB3 urban discharges can be likely assumed as responsible of the PFAS contamination). Similarly, the trend from lower to higher PFOS/PFOA ratio appears to correlate with higher urbanization levels (Nguyen et al., 2011). Typical surface waters exhibit PFOS/PFOA < 0.9. The ratio could only be obtained for MAG2 (25.5) and JUC7 (2.5) indicating, in both cases, important input of human settlements, more important in the lower part of the catchment.

Mass loads of individual compounds (g day^{-1}) were only calculated for Cabriel and Jucar Rivers using the course flows at the time of sampling (data not available for Magro River) (Tables S1, S11). In the Cabriel River mass loads ranged between 22.1×10^{-3} (PFOA)–4.47 g day^{-1} (PFHxA); and in the Jucar River 2.21×10^{-3} (PFOS)–204 g day^{-1} (PFBA). Results pinpointed low contamination levels in sampling points close to the Rivers source as well as a great influence of the human impact in the increase of the PFAS concentrations, especially in the main River (JUC2 and JUC8).

In Cabriel River, PFAS concentrations after Contreras dam were higher, while Jucar River presented different contamination pattern before and after Alarcon dam. Clear contributions of PFAS loads from Cabriel to Jucar were observed, and high concentrations were also detected after Tous dam. It seems that these regulation water bodies can play an important role in the re-distribution of these contaminants in the aquatic ecosystem but further research is needed. High concentrations of PFOS at mouth of the river confirms that it still exists in the marketplace (i.e. firefighting foams, treatments for waterproofing or flame-retarding of textiles and leather) and is actively used despite it has been banned by law (European Union Directive, 2006).

The most recent EQS (European Union Directive, 2013) fixed Maximum Allowable Concentration (MAC) for PFOS and its derivative salts of 36,000 ng L^{-1} in inland surface water and of 7200 ng L^{-1} in other types of water. The Annual Average (AA) were 0.65 and 0.13 ng L^{-1} for inland surface and other types of waters, respectively. EQS for biota was 9.1 μg kg^{-1}. Italy recently published EQS for PFOA and short chain PFAS (Decreto Legislativo n. 172, 2015) that additionally to those of PFOS fixed AA of 7000 ng L^{-1} for PFBA, 3000 for PFPeA, 1000 for PFHxA, 3000 for PFBS and 100 for PFOA in inland surface waters and of 1400 ng L^{-1} for PFBA; 600 for PFPeA, 200 for PFHxA, 600 for PFBS and 20 for PFOA in other types of surface waters.

The Provisional Health Advisory (PHA) of the United States Environmental Protection Agency (US EPA, 2009, 2011) set concentrations in drinking water at 400 ng L^{-1} for PFOA and 200 ng L^{-1} for PFOS. On the same line, various states have established drinking water and groundwater guidelines, including the following;.

- Minnesota has established a chronic health risk limit of 300 ng L^{-1} for PFOS and PFOA in drinking water (MDH, 2011) and safety values of 600 ng L^{-1} for PFHxS and PFBS, and of 1000 ng L^{-1} for PFHxA and PFPeA (Minnesota Pollution Control Agency, 2007; US EPA, 2009);
- New Jersey has established a preliminary health-based guidance value of 40 ng L^{-1} for PFOA in drinking water (NJDEP, 2013);
- North Carolina has established an interim MAC (IMAC) of 2000 ng L^{-1} for PFOA in groundwater (NCDENR, 2006);
- In 2010, the North Carolina Secretary's Science Advisory Board (NCSAB) on Toxic Air Pollutants recommended that the IMAC be reduced to 1000 ng L^{-1} based on a review of the toxicological literature and discussions with scientists conducting research on the health effects associated with exposure to PFOA. As of February 2014, the NCSAB's recommendation was still pending review by the North Carolina Division of Water Quality (NCSAB, 2010).

Although a longer study is need to make comparisons reliable (values presented in this research are from a single campaign and those of the Italian legislation are yearly means), it seems that maximum concentrations found for PFOS and PFOA in Jucar river waters are higher than the EQS proposed by Italian government for inland and other surface water.

3.2. Occurrence of selected PFASs in sediment samples

From the 21 analytes screened, 11 were detected in sediment samples, being all of them contaminated with at least one PFAS. Frequencies were quite higher than those observed in water samples, ranging from 7% (i,p-PFNA, PFNA) to 100% (PFBA) (Fig. S3). Other compounds frequently found were PFOS (67%) and PFPeA (60%).

PFASs higher frequencies and mean concentrations in sediments compared to those of water samples (Table 2) pointed out the tendency of these compounds to accumulate. Short-chain compounds presented the highest mean concentrations: 5.85 ng g^{-1} dw of PFBA (maximum 10.7 ng g^{-1} dw), and 11.5 ng g^{-1} dw of PFBS (maximum 29.2 ng g^{-1} dw). PFOA and PFOS showed similar mean (2.47–2.57 ng g^{-1} dw) and maximum concentrations (6.69–9.83 ng–g^{-1} dw) in spite of PFOS is characterized by stronger adsorption into the sediment than PFOA. PFOSA was not detected in any sample. PFAS concentrations detected are in the same range, or even slightly higher, than those reported in other studies (see Table S10).

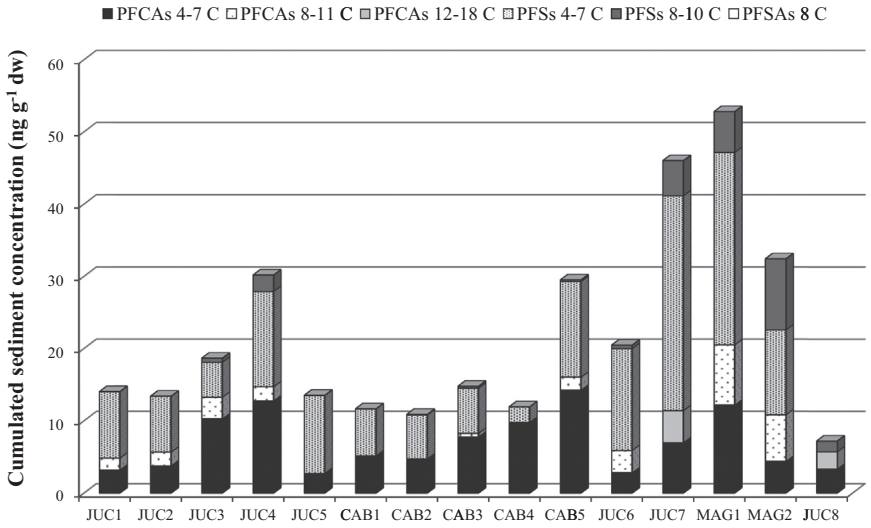

Fig. 3. Cumulated PFCAs, PFSs and PFSAs concentration according to the low (4–7 C) medium (8–11 C) and high (12-18 C) length of the chain present in sediment samples of Jucar River basin in October 2010. (dw: dry weight, CAB: Cabriel River: MAG: Magro River; JUC: Jucar River).

Cumulated concentrations of PFASs in sediments showed a picture different from the waters. The latter showed a predominance of short-chain PFASs in the upper part of the river that progressively evolves towards a predominance of the long-chain ones in the lower part. On the contrary, the short-chain PFASs were detected in sediment samples through the whole catchment (Fig. 3). High contamination levels were found in both tributaries, particularly in Magro River, reaching up 52.8 ng g^{-1} dw in MAG1 (selected as a quality reference point). In Cabriel River, PFASs were accumulated downstream (29.5 ng g^{-1} dw at CAB5) despite a slight decrease observed after Contreras dam (CAB4). Jucar River also revealed an accumulation downstream except in two points, JUC5 and JUC8. The former could be related to sediment precipitation in El Molinar dam but also with changes in its transport during droughts usually registered at JUC4. The latter is difficult to explain especially after the high values observed in previous points (JUC7 and MAG2). Regulation dams in the basin could act as sinks that favour the PFAS accumulation in sediments.

Site-specific, experimental solid-liquid distribution coefficient (K_{Di} in L kg^{-1}) was estimated as $K_{Di}=C_{Si}/C_{Wi}$ [where C_{Si} is the concentration of target compound in sediment at sampling point i (ng kg^{-1} dw), and C_{Wi} is the concentration of target compound in water at sampling point i (ng L^{-1})]. Log K_D calculated for PFASs in this study showed mean values ranging from 1.97 to 5.14 and 1.68 to 2.45 for PFCAs and PFSs, respectively (Table S12). However, as PFAS sorption onto sediment is significantly related to the sediment organic carbon fraction (f_{OC}), distribution coefficients were re-calculated as $K_{OC}=K_D{}^{*}100/f_{oc}$. Log K_{OC} in the Jucar River showed mean values in the range 3.40–6.83, for PFCAs, and 3.37–4.21 for PFSs (Table S12). Levels found in this study are higher than those reported by Ahrens et al. (2010), Campo et al. (2015), Kwadijk et al. (2010), and Picó et al. (2012). Not enough data available to be able to explain the differences. In agreement with these studies, K_D values obtained in the Jucar River basin tend to increase with the perfluorocarbon chain length, in each functional group, with the exception of PFHpA value (log $K_D=0.22^{*}$ number of C+1.93; $R^2=0.79$). It is important to note that these results have to be considered carefully since both coefficients are intended to be evaluated in systems in chemical equilibrium, and a river basin is a dynamic system affected by different factors (e.g. hydrologic, physical, geochemical) and processes (i.e. adsorption/desorption, precipitation, and diffusion) (Ahrens et al., 2010; Kwadijk et al., 2010; Labadie and Chevreuil, 2011).

3.3. Occurrence of selected PFASs in biota samples

In this matrix, 17 compounds were screened and only 5 were detected with frequencies between 4% (PFNA or PFHxS) and 60% (PFOS). The distribution pattern of PFASs is different. For example, PFBA, ubiquitous in sediment and water samples, was not found in any sample (Fig. S3).

Mean concentrations of PFCAs in fish samples were higher than in sediment and water indicating possible bioaccumulation (Table 3). Values ranged from 21.4 μg kg^{-1} of PFHpA to 274 μg kg^{-1} of

Table 3
Concentration and frequency of occurrence of each PFAS in biota samples of the Jucar basin in 2010.

Family/ compound	Biota concentration (μg kg^{-1})				Frequency (number)	Frequency (%)
	Max	Min	Mean	Mean (no zeros)		
PFCAs[a]	**946**	**1.18**	**11.6**	**122**	**13**	**52**
PFBA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFPeA	946	9.84	142	274	13	52
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	111	1.18	6.00	21.4	7	28
PFOA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
i,p-PFNA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFNA	71.5	71.5	2.86	71.5	1	4
PFDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUdA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFSs[a]	**8.13**	**0.56**	**0.33**	**1.39**	**15**	**60**
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	0.63	0.63	0.03	0.63	1	4
PFHpS	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFOS	8.13	0.56	1.29	2.16	15	60
i,p-PFNS	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFSAs[a]	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**n.d.**
PFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑PFASs	**1.14 10^3**	**83.7**	**152**	**369**	**–**	**–**

[a] Concentration given for the different families (bold) are maximum, minimum and mean values (including and excluding zeros) of the compounds, while frequency is the total number of samples with at least one PFAS; n.a.: not analysed; n.d.: not detected.

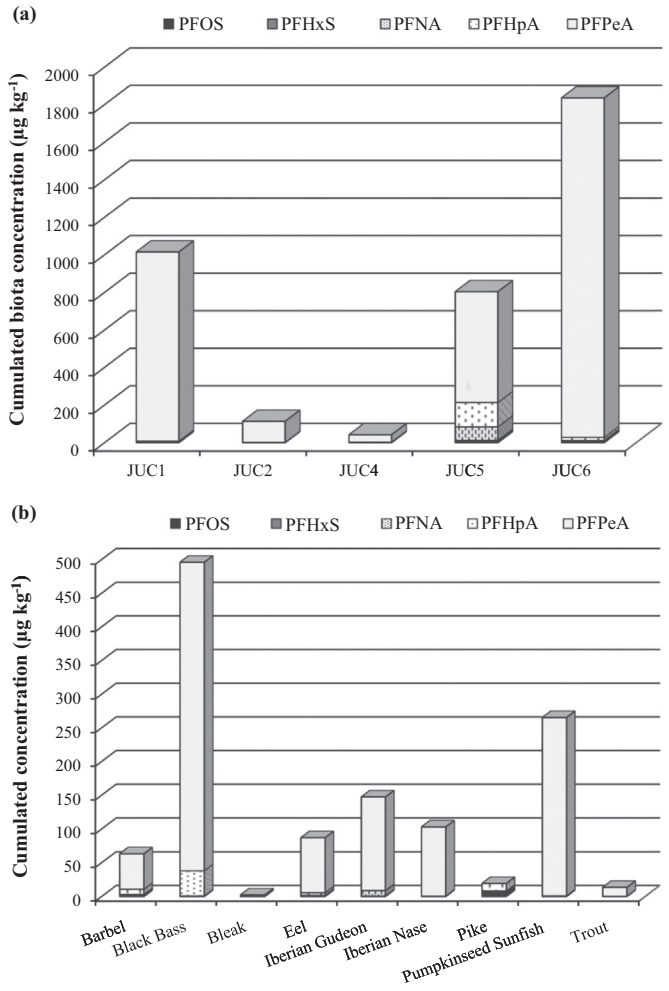

Fig. 4. PFAS concentrations in biota samples of Jucar River basin in October 2010 according to: (a) sampling point (JUC: Jucar); and (b) fish species (Barbel: *Barbus guiraonis*; Black Bass: *Mycroptero salmoides*; Bleak: *Alburnus alburnus*; Eel: *Anguila anguila*; Iberian Gudeon: *Gobio lonzanoi*; Iberian Nase: *Pseudochrondrostoma polylepis*; Picke: *Esox lucius*; Pumpkinseed Sunfish: *Lepomis gibbosus*; Trout: *Salmo trutta*).

PFPeA (maximum value of 946 μg kg^{-1}). Contrarily, PFS mean concentrations were low (up to 2.16 μg kg^{-1} of PFOS). PFAS concentrations were higher than those found in other fish species and areas (Table S10). Nevertheless, similar levels were observed in sardine, hake and red mullet of Catalonia (Domingo et al., 2012), and in different carps (Silver, Common, Crucian), and Tire track eel of Chine (Wang et al., 2013) for PFOS; in eel, Black sea bass, Silver perch and other aquatic wildlife in Georgia, USA for PFNA (Kumar et al., 2009); and in sardine and anchovy, also in Catalonia, Spain for PFHxS (Fernandez-Sanjuan et al., 2010). PFOS concentration found in biota of Jucar River does not represent a risk for it since even the maximum value detected (8.13 μg kg^{-1}) is lower than the EQS of 9.1 μg kg^{-1} established by EU (European Union Directive, 2013).

According to cumulative PFAS concentrations in the different sampling points, highest values were observed at JUC1, but particularly downstream at points JUC5 and JUC6 (Fig. 4a). Biota contamination at this first point is of concern since it is located at the headwaters of the river where water and sediment contaminations were low. High concentration found in the last point (1.84 mg kg^{-1}) can be related to the influence of Cabriel River. Comparing the average values obtained for each species, invasive species as Black Bass, and Pumpkinseed Sunfish presented the highest concentrations. Eel, considered an endangered species, has ∑PFASs of 87 μg kg^{-1}. All species seem to particularly cumulate PFPeA and in a lower proportion PFHpA (Fig. 4b). The limitation of the present study is that even it took into account different species involved a relatively small sample size, increasing the possibility of potential selection biases. Future research with larger and repeated samplings are advised in order to evaluate whether the trends described here are representative of PFASs bioaccumulation in selected fish species.

Experimental bioaccumulation factor (BAF in L kg^{-1}) was determined as: $BAF = C_{BIOTA}/C_W$ where C_{BIOTA} is the concentration of target compound in biota samples (ng kg^{-1}), and C_W is the concentration of target compound in water samples (ng L^{-1}) (Dai et al., 2013; Wang et al., 2013; Xu et al., 2014). Log BAF was calculated for three different zones: (i) upper part: before Alarcon dam (JUC1-2), (ii) medium part: between Alarcon and Embarcaderos dams (JUC4-5), (iii) lower part: between Tous dam and the mouth (JUC6). In the upper part, mean log BAFs ranged from 5.37 (PFOS) to 6.63 (PFPeA), while in the medium and the lower parts were 4.46 (PFNA) and 5.89 (PFPeA) (Table S13). These values were higher than those reported for different fish species in China (0.99–4.44) (Wang et al., 2013; Xu et al., 2014) and in the Llobregat River, Spain (2.36–4.30) (Campo et al., 2015). These differences could be related to different species compared and their different capacities to bio-accumulate PFASs as well as the different environmental conditions and concentrations.

BAF was also calculated for the different species in the three parts of the river established above. However, there were few coincidences of PFAS in fish and water sampling in the same point (even considering the three different sections). Values were mainly obtained for the upper part, I. Gudeon (5.8, $n=2$), I. Nase (5.5, $n=1$) and Trout (5.3, $n=2$). BAFs reported in Table S13 were calculated using average water concentration of each PFAS in the 5 points where fishes were captured. Species showing the highest BAFs (Black Bass and Pumpkin Seed Sunfish) are predators in the top of the trophic web, which could also suggest possible biomagnification. However, species showing the lowest BAFs as trout are also piscivorous in its adult stage, even though when young are fed of invertebrates. Based on this, possible correlations between PFAS concentrations, both mean and maximum values, and trophic level data were explored obtaining no significant coefficients probably because trophic levels are quite similar ranging from 2.5 to 4.1 with most of the them around 3.5 (see Table S14). These results were obtained for a particular sampling, and consequently they should be taken with caution and further studies are needed (increasing the sampling frequency and the number of samples to confirm PFASs trends depicted here).

3.4. Potential adverse effects

Since 1981, the US EPA successfully applied QSARs (Quantitative Structure-Activity Relationship) to predict the aquatic toxicity of industrial chemicals in the absence of experimental toxicity data (Sanderson et al., 2003). Estimations based on mathematical relationships between K_{OW} values and the corresponding measured toxicity can be obtained by means of ECOSAR™ software (Table S15) for any chemical compounds including PFASs. These data were used to calculate hazard quotients (HQ), defined as the ratio between the predicted or measured environmental concentration (MEC) and their chronic toxicity, usually expressed as NOEC (Non-Observed Effect Concentration) or PNEC (Predicted Non Effect Concentration) values, referred to three different trophic levels, as recommended by the WFD. When NOEC values are not available, EC50 or LC50 values from standard eco-toxicological tests can be used after correction by an assessment

Table 4
Ranked compounds according to HQ for algae, *Daphnia* sp. and fish in the Jucar River basin. Acute toxicity (EC50) values used were modelled with ECOSAR.

Rank	Compound	HQ Algae[a]	HQ *Daphnia* sp.[a]	HQ Fish[a]	Rank	Compound	HQ Algae[b]	HQ *Daphnia* sp.[b]	HQ Fish[b]
1	PFDA	0.09	**0.32**	**0.27**	1	PFDA	0.01	0.02	0.02
2	PFNA	3.16×10^{-3}	8.91×10^{-3}	6.98×10^{-3}	2	PFNA	3.70×10^{-4}	1.04×10^{-3}	8.15×10^{-4}
3	PFTeDA	9.15×10^{-4}	8.60×10^{-3}	1.08×10^{-2}	3	PFTeDA	1.06×10^{-4}	1.00×10^{-3}	1.25×10^{-3}
4	PFOS	3.92×10^{-3}	7.57×10^{-3}	5.41×10^{-3}	4	PFOS	3.46×10^{-4}	6.68×10^{-4}	4.77×10^{-4}
5	PFOA	3.22×10^{-3}	7.01×10^{-3}	5.16×10^{-3}	5	PFOA	2.69×10^{-4}	5.86×10^{-4}	4.32×10^{-4}
6	PFUdA	6.83×10^{-4}	3.20×10^{-3}	2.83×10^{-3}	6	PFUdA	4.55×10^{-5}	2.14×10^{-4}	1.89×10^{-4}
7	PFTrDA	3.33×10^{-4}	2.63×10^{-3}	2.63×10^{-3}	7	PFTrDA	2.22×10^{-5}	1.75×10^{-4}	1.75×10^{-4}
8	PFBA	1.08×10^{-3}	8.47×10^{-4}	4.87×10^{-4}	8	PFBA	8.35×10^{-5}	6.56×10^{-5}	3.77×10^{-5}
9	PFHpA	4.86×10^{-4}	8.21×10^{-4}	5.69×10^{-4}	9	PFHpA	5.36×10^{-5}	9.06×10^{-5}	6.27×10^{-5}
10	PFHxA	1.81×10^{-4}	2.36×10^{-4}	1.47×10^{-4}	10	PFHxA	3.68×10^{-5}	4.82×10^{-5}	2.99×10^{-5}
11	PFHxS	1.67×10^{-4}	1.93×10^{-4}	1.22×10^{-4}	11	PFHxS	1.48×10^{-5}	1.71×10^{-5}	1.08×10^{-5}
12	PFPeA	1.11×10^{-5}	1.13×10^{-5}	6.90×10^{-6}	12	PFPeA	1.51×10^{-6}	1.53×10^{-6}	9.38×10^{-7}
13	PFOSA	0.00	0.00	0.00	13	PFOSA	0.00	0.00	0.00
14	PFODA	0.00	0.00	0.00	14	PFODA	0.00	0.00	0.00
15	PFHxDA	0.00	0.00	0.00	15	PFHxDA	0.00	0.00	0.00
16	PFDoA	0.00	0.00	0.00	16	PFDoA	0.00	0.00	0.00
17	PFHpS	0.00	0.00	0.00	17	PFHpS	0.00	0.00	0.00
18	PFDS	0.00	0.00	0.00	18	PFDS	0.00	0.00	0.00
19	PFBS	0.00	0.00	0.00	19	PFBS	0.00	0.00	0.00
20	i,p-PFNS	0.00	0.00	0.00	20	i,p-PFNS	0.00	0.00	0.00
21	i,p-PFNA	0.00	0.00	0.00	21	i,p-PFNA	0.00	0.00	0.00

[a] HQ calculated with the maximum concentration value detected in this study.
[b] HQ calculated with the mean concentration value calculated in this study.

factor of 1000 according to WFD (Directive 2000/60/EC):

$HQ_i = MEC_i / PNEC_i$ with $PNEC_i = EC50_i/1000$ or $LC50_i/1000$

In general, $HQ > 1$ indicate potential risk. According to Table 4, none of the PFFAs may pose acute risk to the three different trophic levels in the Jucar River basin even when considering maximum concentration values. Only PFDA presented $0.1 < HQ < 1$ and then, should have potential risk for *Daphnia* sp. and fish. HQs calculated with mean values did not indicate any potential risk of PFAS contamination for the trophic levels considered. However, it should take into account that other adverse properties of chemicals (e.g. endocrine disruption, bioaccumulation, etc.) are not included in this kind of risk assessment.

3.5. Relationships with water physical characteristics

There are very little or no information on the influence of physical characteristics of water on the distribution of PFASs in environmental matrices (Pan and You, 2010; You et al., 2010). One of the objectives of this study was to establish possible relationships among these compounds and the temperature (°C), pH, redox potential (mV), conductivity (μS), total dissolved solids (TDS in ppm), NaCl concentration (ppm) and dissolved oxygen (mg L^{-1}) of river water.

In water samples, PFSs (4–7 C and 8–10 C) were significantly correlated with pH, mV, conductivity, TDS and NaCl (all relationships were positive but with the pH was negative). The negative correlation between pH and PFASs is difficult to explain since the pH range of the water samples is not enough pronounced to justify changes in solubility/mobility of PFAS in water. This correlation may be due to the existence of discharges with both acid pH and high concentrations of PFAS, such as those of the photovoltaic industry (Ahrens et al. 2010). In sediment samples, PFCAs 12–18 C were positively correlated to conductivity, TDS and NaCl (Table S16). PFAS concentrations determined in biota samples did not showed any relationship with physical characteristics considered.

According to this, short-chain perfluorosulfonates present in Jucar water samples appeared to be dependent on characteristics related to the loss of water quality and salinization (conductivity, TDS and NaCl concentration) reported in the river (Navarro-Ortega et al., 2012). This could be explained by the existence of controlled (STPs) and uncontrolled (undercover industries) discharges that involved both, an increase of PFASs concentrations and a loss of water quality by contamination.

The fact that PFAS contamination in sediment samples presented similar relationships with the physical variables considered reflect the close interaction of these compounds in both matrices, as it was already ascertained with the partition coefficients. The increase of salinity promotes the coagulation of colloids and the precipitation of suspended matter. Wang et al. (2015) suggested that seawater salinity had a significant impact on the partitioning of PFOS and PFOA between the sediment and water in Bohai Bay (China). You et al. (2010) observed that PFOS could be increasingly sorbed by the suspended particles and sediment as the salinity of overlying water increased. According to these authors, these two compounds might be largely scavenged to the sediment with salinity increasing.

Further research is needed to assess the evolution of the basins more vulnerable to the possible effects of global change. To do so, the monitoring of contaminant concentrations has to be completed with related measures (as those presented here) in order to determine quality indexes, which together with the development of health-based concentrations for humans, will allow completing a full risk assessment of human exposure to perfluoroalkyl substances in fragile ecosystems as the Mediterranean ones.

4. Conclusions

Samples of the Jucar River basin were all contaminated with at least one PFAS (except three fish samples), and 12 compounds were detected in water, 11 in sediment and 5 in biota. PFPeA and PFOS were the most predominant analytes confirming the current replacement of long-chain PFASs ($C \geq 10$) by short-chain ones in industrial and commercial applications and their increasing accumulation in environmental matrices. Concentration values measured in the water of both tributaries (Cabriel and Magro Rivers) were low contrary to those found in Jucar River, which presented high levels in its upper part (PFBA) and close to the mouth (PFOS and PFDA). The very high concentration of PFBA (maximum of 644 μg L^{-1}) is directly related to industrial and

urban discharges coming from Cuenca city, and that of PFOS and PFDA to a huge car's factory, and to effluents coming from the STP of Alzira. There are clear PFAS accumulations downstream in the sediments of Cabriel and Jucar Rivers, which suggest that regulation dams in the basin could act as sinks allowing such accumulations.

Higher mean concentrations found in sediments and biota than in water samples suggest PFAS accumulation and bio-magnification (up to 946 $\mu g\ kg^{-1}$ in the case of the PFPeA). According to the bioaccumulation factor, species with highest PFAS contamination (Pumpkinseed sunfish and Black bass) presented the highest coefficients and those with lowest PFAS concentrations the lowest (Trout). Endangered species as Eel presented an intermediate factor. Although these results suggest selective PFASs bioaccumulation in the species considered, they were obtained from a small sample size, and consequently further research is needed to confirm these trends.

None of the hazard quotients calculated with mean PFAS concentrations indicate potential risk for the different tropic levels considered (algae, *Daphnia* sp. and fish). If maximum concentrations are used, only PFDA should have potential risk for *Daphnia* sp. and fish. Nevertheless, these quotients were assessed with modelled acute toxicity levels and have to considered as rough estimates until such levels could be obtained and compared with chronic toxicity ones. PFASs found in Jucar water and sediment samples seemed to be dependent on characteristics related to the marine intrusion (conductivity and NaCl concentration) reported in the river.

Despite PFASs have been accumulating in sediment and biota of the Jucar River catchment, concentrations reported in this study can be considered at acceptable levels compared to existing Regulatory Legislation and, consequently, they do not pose an immediate human health risk. However, monitoring of these compounds in zones under the threat of global change, as the Mediterranean, is required in order to manage and predict their possible effects on ecosystem services according to the European Water Framework Directive, particularly in the development of Special Action Plans in situations of alert and temporary drought in which contaminant concentrations could be increased.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.03.010.

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Supplementary information

Analysis of the presence of perfluoroalkyl substances in water, sediment and biota of the Jucar River (E Spain). Sources, partitioning and relationships with water physical characteristics.

Julian Campo [1, 2*], María Lorenzo [3], Francisca Pérez [4], Yolanda Picó [3], Marinel.la Farré [4], Damià Barceló [4, 5]

[1] Environmental Forensic and Landscape Chemistry Research Group. Desertification Research Centre - CIDE (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana). Carretera Moncada - Náquera km 4.5 (Campus IVIA). 46113 Moncada, Valencia, Spain.

[2] Earth Surface Science, Institute for Biodiversity and Ecosystems Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

[3] Food and Environmental Safety Research Group (SAMA—UV), Centro de Investigaciones sobre Desertificación (CIDE, UV–CSIC–GV) and Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain.

[4] Department of Environmental Chemistry (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain.

[5] Catalan Institute for Water Research (ICRA), Emili Grahit, 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, E-17003 Girona, Spain.

[*] Corresponding author. Tel. +34 963543092; fax: +34 963544954
E-mail address: Julian.Campo@uv.es

Table S1. Description of the sampling points. W: Water; S: Sediment; B: Biota

River	Location (UTM)	Samples	Code	Observation	Flow ($m^3 s^{-1}$)	Sediment (% OC)
CABRIEL	Salvacañete (X: 627162 Y: 4439354)	W, S	CAB1	Headstream reach (drought in summer)	-	1.27
	Pajaroncillo (X: 612534 Y: 4422400)	W, S	CAB2	Near Cañete's spillage	3.01	0.82
	Villar del Humo (X: 614272 Y: 4410987)	W, S	CAB3	Good habitat conditions	-	1.66
	Venta del Moro (X: 628595 Y: 4376586)	W, S	CAB4	Downstream Contreras Dam	7.73	1.19
	Villatoya (X: 642129 Y: 4355758)	W, S	CAB5	Upstream Jucar confluence and Embarcaderos Dam	-	1.68
MAGRO	Requena (X: 667953 Y: 4362542)	W, S	MAG1	Headstream (low impact)	-	1.34
	Carlet (X: 711245 Y: 4348964)	W, S	MAG2	Industrialized area	-	3.69
JUCAR	Huélamo (X: 598583 Y: 4453975)	W, S, B	JUC1	Headstream (low impact)	-	1.09
	Cuenca (X: 573092 Y: 4436231)	W, S, B	JUC2	Upstream Cuenca city's STP	3.66	1.67
	Fresneda de Altarejos (X: 570913 Y: 4414489)	W, S	JUC3	Upstream of Alarcon dam. After Cuenca city's STP	-	2.08
	Quasiermas (X: 601713 Y: 4336028)	W, S, B	JUC4	Drought in several times	2.87	3.53
	Jalance (X: 665927 Y: 4340496)	W, S, B	JUC5	Upstream Embarcaderos dam	2.84	1.77
	Cotes (X: 707741 Y: 4328283)	W, S, B	JUC6	Bypass to the "Royal Jucar Irrigation Chanel"	2.46	2.07
	Alzira (X: 720584 Y: 4336933)	W, S	JUC7	In the Alzira city's spillage	-	2.04
	Sueca (X: 729360 Y: 4343192)	W, S	JUC8	Near to the mouth	4.40	0.84

Table S2. Fish sample characteristics

Sampling point	Sample number	Species	Number of individuals	Total weight [g]
JUC1	33	*Salmo trutta* (big)	3	113.74
	39	*Salmon trutta* (small)	5	25.72
	10, 12, 15	*Gobio lonzano*i (very small, small and big)	12	18.20
			6	12.15
			3	20.48
JUC2	42	*Salmo trutta*	1	396.92
	30	*Gobio lonzanoi*	13	83.42
	36	*Pseudochrondrostoma polylepis*	5	635.20
JUC4	41	*Gobio lonzanoi* (big)	4	94.43
	45	*Gobio lonzanoi* (small)	10	63.10
	1	*Mycroptero salmoides*	6	173.50
JUC5	24	*Barbus guiraonis*	1	35.75
	7	*Lepomis gibbosus*	2	48.08
	4	*Alburnus alburnus*	6	64.50
	57	*Mycroptero salmoides*	5	129.69
	49	*Gobio lonzanoi*	14	47.67
	18	*Anguila anguila*	1	270.20
JUC6	21	*Esox lucius*	1	1275.20
	63	*Mycroptero salmoides*	2	497.43
	60	*Gobio lonzanoi* (small)	7	44.25
	27	*Alburnus alburnus*	16	61.52
	46	*Anguila anguila*	3	227.60
	52	*Barbus guiraonis* (small)	2	838.84
	54	*Lepomis gibbosus*	1	67.25
	55	*Gobio lonzanoi* (big)	4	51.40
	56	*Barbus guiraonis* (big)	1	964.52

Table S3. PFASs selected in this study, their family, acronym, CAS number, formula and use

Family	Compound	Acronym	CAS Nº	Formula	Use
Perfluoro-carboxylates		**PFCA**			
	Perfluorobutanoate	PFBA	375-22-4	C_3F_7COOH	Synthesis precious metals flotation agent
	Perfluoropentanoate	PFPeA	2706-90-3	C_4F_9COOH	Stain- and grease-proof coatings
	Perfluorohexanoate	PFHxA	307-24-4	$C_5F_{11}COOH$	Stain- and grease-proof coatings
	Perfluoroheptanoate	PFHpA	375-85-9	$C_6F_{13}COOH$	
	Perfluorooctanoate	PFOA	335-67-1	$C_7F_{15}COOH$	Teflon, Gore-Tex, emulsifier of fluoropolymers, additive in insecticides, electrical insulator
	Perfluoro-7-methyloctanoate	i,p-PFNA			
	Perfluorononanoate	PFNA	375-95-1	$C_8F_{17}COOH$	Surfactant in emulsion polymerization of PFOA. Denture cleaners, shampoos, carpets, etc.
	Perfluorodecanoate	PFDA	335-76-2	$C_9F_{19}COOH$	
	Perfluoroundecanoate	PFUdA	2058-94-8	$C_{10}F_{21}COOH$	
	Perfluorododecanoate	PFDoA	307-55-1	$C_{11}F_{23}COOH$	
	Perfluorotridecanoate	PFTrDA	72629-94-8	$C_{12}F_{25}COOH$	
	Perfluorotetradecanoate	PFTeDA	376-06-7	$C_{13}F_{27}COOH$	
	Perfluorohexadecanoate	PFHxDA	67905-19-5	$C_{15}F_{31}COOH$	
	Perfluorooctadecanoate	PFODA	16517-11-6	$C_{17}F_{35}COOH$	
Perfluorosulfonates		**PFS**			
	Perfluorobutane sulfonate	PFBS	29420-49-3	$C_4F_9SO_2O$	Replacement of PFOS in 3M's Scotchgard. Metal plating baths, folding carton, etc.
	Perfluorohexane sulfonate	PFHxS	82382-12-5	$C_6F_{13}SO_2O$	Surfactants for paper, films,

Family	Compound	Acronym	CAS Nº	Formula	Use
					photographic plates
	Perfluoroheptane sulfonate	PFHpS		$C_7F_{15}SO_2O$	
	Perfluorooctane sulfonate	PFOS	4021-47-0	$C_8F_{17}SO_2O$	Stain repellent, firefighting foam, impregnation agent for leather, paper, adhesives, metal plating
	Perfluoro-7-methyloctane sulfonate	i,p-PFNS			
	Perfluorodecane sulfonate	PFDS		$C_{10}F_{21}SO_2O$	
Perfluoro sulfonamides		**PFSA**			
	Perfluorooctane sulfonamide	PFOSA	754-91-6	$C_8F_{17}SO_2NH_2$	Formerly used in 3M's Scotchgard
Internal standards					
	Perfluoro-n-[1,2,3,4-$^{13}C_4$] butanoate	MPFBA	CASID 39196	C_4HF_7OO	
	Perfluoro-n-[1,2-$^{13}C_2$] hexanoate	MPFHxA	CASID 39197	$C_6HF_{11}OO$	
	Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoate	MPFOA	CASID 39199	$C_8HF_{15}OO$	
	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoate	MPFNA	CASID 39198	$C_9HF_{17}OO$	
	Perfluoro-n-[1,2-$^{13}C_2$] decanoate	MPFDA	CASID 39200	$C_{10}HF_{19}OO$	
	Perfluoro-n-[1,2-$^{13}C_2$] undecanoate	MPFUdA	CASID 39202	$C_{11}HF_{21}OO$	
	Perfluoro-1-[$^{18}O_2$] hexanesulfonate	MPFHxS		$C_6F_{13}SO_2O$	
	Perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	MPFOS		$C_8F_{17}SO_2O$	

Table S4. Instrumental characteristics used for water and sediment determination

LC CONDITIONS	
Analytical column	Kinetex XB-C18: 50.0 × 4.6 mm, 1.7 µm particle size (Phenomenex, Torrance, USA)
Column temperature	30° C
Volume injected	5 µL
Mobile phase	(A) Water – (B) methanol both with 10 mM Ammonium Formate
Flow rate	0.2 mL min^{-1}
Linear gradient	0 min (30 % B), 0.5 min (30 % B), 12 min (95 % B), 20 min (95 % B), and return to the initial conditions (equilibration time 12 min)
TRIPLE QUADRUPOLE MS/MS CONDITIONS	
Ionization characteristics and source	MS/MS performed in selected reaction monitoring mode (SRM) with electrospray ionization (ESI) in negative mode
Gas temperature	300° C
Gas flow	11 L min^{-1}
Nebulizer	30 psi
Capillary voltage	4000 V
Chamber current	1.27 µA
Scan type	MRM, with MS1 and MS2 at unit resolution and cell acceleration voltage of 7 eV

Table S5. Dynamic MRM conditions for LC-MS/MS determination of PFASs

Target PFAS	t_R [(a)] (min)	Precursor Ion	SRM_1 [(b)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2[(e)]	Frag[(c)] (V)	CE[(d)] (V)	SRM_2/SRM_1 (%)(%RSD)[(f)]
PFBA	8.0	213	169	66	5				
MPFBA	*8.0*	*217*	*172*	*62*	*0*				
PFPA	8.9	263	219	66	5				
PFBS	9.2	299	99	142	38	80	142	26	15.3 (2.3)
PFHxA	13.3	313	269	71	5	119	71	5	10.6 (3.3)
MPFHxA	*13.3*	*315*	*270*	*71*	*5*	*119*	*71*	*5*	*7.1 (0.8)*
PFHpA	15.4	363	319	76	5	169	76	5	68.5 (9.2)
PFHxS	15.6	399	99	169	37	80	169	29	65.9 (10.8)
MPHxS	*15.6*	*403*	*103*	*164*	*33*	*84*	*164*	*37*	*23.5 (4.3)*
PFOA	17.2	413	369	87	5	169	87	5	46.7 (1.4)
MPFOA	*17.2*	*417*	*372*	82	*5*	169	82	*13*	22.5 (0.6)
PFHpS	17.3	449	99	179	37	80	179	57	31.9 (8.9)
i,p-PFNA	19.3	463	419	87	5	169	87	5	27.0 (1.2)
PFNA	19.5	463	419	82	5	219	82	5	13.2 (0.9)
MPFNA	*19.5*	*468*	*423*	*82*	*5*	*223*	*82*	*9*	*17.6 (1.4)*
PFOS	19.9	499	99	190	41	80	190	65	82.2 (3.2)
MPFOS	*19.9*	*503*	*99*	*180*	*41*	*80*	*180*	*61*	*30.0 (1.1)*
PFDA	25.5	513	469	89	5	269	89	13	15.3 (2.2)
MPFDA	*25.5*	*515*	*470*	*92*	*5*	*270*	*92*	*12*	*8.4 (0.4)*
i,p-PFNS	25.5	549	99	195	45	80	195	73	21.6 (1.6)
PFUdA	28.1	563	519	104	5	269	104	13	14.1 (0.6)
MPFUdA	*28.1*	*565*	*520*	*94*	*5*	*269*	*94*	*13*	*4.3 (0.4)*
PFDS	28.2	599	99	80	80	80	80	80	17.6 (1.3)
PFDoA	32.7	613	569	94	5	269	94	13	9.0 (0.8)
MPFDoA	*32.7*	*615*	*570*	*112*	*5*				
PFTrDA	33.4	663	619	104	0	169	104	24	8.1 (1.8)
PFTeDA	34.0	713	669	112	5	169	112	25	7.8 (0.2)
PFHxDA	35.2	813	769	114	8	169	114	28	9.6 (1.1)
PFODA	35.8	913	869	134	10	169	128	29	

(a) t_R = retention time; (b) SRM_1 = selected product ion for quantification; (c) Frag = fragmentor; (d) CE = collision energy; (e) SRM_2 = selected product ion for qualification; (f) SRM_2/SRM_1 (%RSD) = mean values obtained from the matrix-matched calibration curves and relative standard deviation of the ratio. Italics mean isotopically labelled internal standard.

Table S6. Instrumental characteristics used for biota determination

LC CONDITIONS	
On-line enrichment	Cyclone P coupled to a C18 (2.1 × 20 mm, 12 μm) (Thermo Fisher Scientific, Franklin, MA, USA)
Analytical column	Hypersil GOLD PFP (50 × 3) (Thermo Fisher Scientific, Franklin, MA)
Column temperature	20 ºC
Volume injected	20 μL
Mobile phase	(A) NH_4Ac 20 mM in water; (B) NH_4Ac 20 mM in methanol
Flow rate	0.4 mL min^{-1}
Linear gradient	No
TRIPLE QUADRUPOLE MS/MS CONDITIONS*	
Ionization characteristics and source	Electrospray ionization (ESI) in negative mode
Sheath gas:	30 UA
Auxiliary gas:	15 UA
Ion Sweep gas:	0.5 UA
Vaporizer temperature	300ºC
Spray voltage	2000 V
Scan type	SRM (0.02 m/z)

* Thermo Scientific TSQ Vantage (Thermo Fisher Scientific, San Jose, CA).

Table S7. Relative recoveries obtained for spiked water samples at 6 and 60 ng L^{-1}

PFASs	Internal Standard	Relative Recovery (%)	
		6 ng L^{-1}	60 ngL^{-1}
PFBA	MPFBA	59 ± 11	63 ± 10
PFPA	MPFBA	55 ± 15	58 ± 11
PFHxA	MPFHxA	58 ± 13	65 ± 8
PFHpA	MPFHxA	58 ± 13	64 ± 13
PFOA	MPFOA	60 ± 18	64 ± 16
PFHpS	MPFHxS	85 ± 12	90 ± 11
PFNA	MPFNA	82 ± 15	86 ± 14
i,p-PFNA	MPFNA	85 ± 11	87 ± 12
PFDA	MPFDA	80 ± 11	84 ± 10
PFUdA	MPFUdA	86 ± 13	91 ± 14
PFDoA	MPFDoA	82 ± 11	87 ± 13
PFTrDA	MPFDoA	85 ± 14	89 ± 14
PFTeDA	MPFDoA	87 ± 13	92 ± 11
PFHxDA	MPFDoA	90 ± 15	93 ± 12
PFODA	MPFDoA	91 ± 12	94 ± 11
i,p-PFNS	MPFNS	84 ± 9	88 ± 11
PFHxS	MPFHxS	85 ± 10	89 ± 14
PFOS	MPFOS	79 ± 14	83 ± 16
PFDS	MPFOS	78 ± 10	82 ± 12

Table S8. Relative and absolute recoveries obtained for spiked sediment samples at the LOQ levels (0.04-8.00 ng g^{-1})

PFASs	Internal Standard	Relative (%)	Absolute (%)
PFBA	MPFBA	44 ± 19	62 ± 16
PFPA	MPFBA	56 ± 17	82 ± 14
PFHxA	MPFHxA	68 ± 15	100 ± 13
PFHpA	MPFHxA	62 ± 16	85 ± 10
PFOA	MPFOA	73 ± 10	98 ± 9
PFNA	MPFNA	74 ± 15	103 ± 8
i,p-PFNA	MPFNA	70 ± 14	97 ± 7
PFDA	MPFDA	72 ± 13	99 ± 5
PFUdA	MPFUdA	59 ± 11	63 ± 10
PFDoA	MPFDoA	85 ± 11	87 ± 12
PFTrDA	MPFDoA	80 ± 11	84 ± 10
PFTeDA	MPFDoA	84 ± 9	88 ± 11
PFHxDA	MPFDoA	79 ± 14	83 ± 16
PFODA	MPFDoA	84 ± 9	88 ± 11
i,p-PFNS	MPFNS	86 ± 13	91 ± 14
PFBS	MPFBA	58 ± 13	64 ± 13
PFHxS	MPFHxS	80 ± 11	84 ± 10
PFHpS	MPFHxS	79 ± 14	83 ± 16
PFOS	MPFOS	71 ± 12	97 ± 11
PFDS	MPFOS	69 ± 12	98 ± 6

Table S9. Recoveries obtained for spiked biota samples

PFASs	Internal Standard	1µg kg^{-1}	10µg kg^{-1}
PFBA	MPFBA	29 ± 12	106 ± 11
PFPA	MPFBA	39 ± 13	96 ± 10
PFHxA	MPFHxA	34 ± 13	101 ± 10
PFHpA	MPFHxA	64 ± 19	79 ± 16
PFOA	MPFOA	31 ± 14	82 ± 11
PFNA	MPFNA	42 ± 15	125 ± 8
i,p-PFNA	MPFNA	n.a.	n.a.
PFDA	MPFDA	78 ± 15	103 ± 8
PFUdA	MPFUdA	47 ± 12	113 ± 11
PFDoA	MPFDoA	37 ± 12	130 ± 11
PFTrDA	MPFDoA	88 ± 15	86 ± 12
PFTeDA	MPFDoA	114 ± 11	109 ± 9
PFHxDA	MPFDoA	16 ± 10	52 ± 13
PFODA	MPFDoA	n.a.	n.a.
i,p-PFNS	MPFNS	n.a.	n.a.
PFBS	MPFBA	34 ± 11	132 ± 12
PFHxS	MPFHxS	33 ± 9	99 ± 11
PFHpS	MPFHxS	n.a.	n.a.
PFOS	MPFOS	30 ± 14	135 ± 5
PFDS	MPFOS	26 ± 12	119 ± 6

Table S10. Minimum and maximum concentrations of PFAS detected in water (ng L^{-1}), sediment (ng g^{-1}) and biota (ng g^{-1} d.w.) reported in selected rivers

Location	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	PFDS
Water											
America											
Surface river water from USA[1]	5.6-40	2.0-19	0.52-47	0.54-15	5.1-54	0.55-5.7	0.52-2.5	0.41-47	0.3-18	0.4-61	<0.1-0.15
Lake Ontario tributaries, Canada[2]	-	-	-	0.5-4	2-11	0.5-3	0.5-1	-	1-5	4-21	-
Surface river water, Ontario, Canada[3]	-	1.2-270	0.1-25.6	1.2-70.6	5.3-62.4	0.5-17.3	2.8-51.9	2.7-28.6	-	6.1-458	n.d.
Asia											
Rivers from Northern Chine, Chine[4]	-	-	-	<LOD-35	0.43-82	<LOD-4.9	<LOD-5.7	-	<LOD-5.8	<LOD-31	-
Rivers in Marina catchment, Singapore[5]	-	-	-	<0.7-14.4	5.4-38.2	0.9-78.3	0.7-28.2	-	<0.4-73.0	1.3-156.2	-
Haihe River, Tianjin, China[6]	-	-	2-42	0.2-10	0.4-43	0.1-0.3	0.1-0.3	-	-	0.2-1	-
Europe											
Danube River and main tributaries, different countries[7]	-	-	-	0.2-3	0.1-46	0.05-19	-	-	-	0.08-2	-
German surface river water[8]	2.4-23	0.76-9.4	0.23-13	0.23-24	0.16-6.5	0.03	0.19	-	0.06-5.6	0.04-4.6	-
Muga, Fluvia, Besos, Llobregat, Ebro, Catalonia, Spain[9]	-	-	-	-	0.79-9.63	0.06-1.62	-	0.07-0.88	0.03-0.64	1.09-9.56	-
Spanish surface river water[8]	2.4-125	0.76-13	0.23-31	0.23-27	0.16-68	0.03-52	0.19-213	-	0.06-37	0.04-2709	-
Orge River, nearby Paris, France[10]	-	8.9	13.3	4.5	9.4	1.3	1.1	4.4	-	17.4	<0.02
Spanish surface river water[11]	0.8-1.3	n.d.	n.d.	0.9-6.2	0.8-11	n.d.	1.2-1.5	-	7.3-8.6	2.4-171	n.d.
Llobregat River, Spain[12]: 2002-2005					<4.2-130					<1.1-11120	
2005-2007	-	-	-	-	<4.2-1470	-	-	-	-	<1.1-10980	-
2008-2012					4.9-44					20-348	
Water samples in Catalonia, Spain[13]	<0.33-4.4	<0.1-2.5	<0.1-3.6	<0.3-4.4	<0.4-9.6	<0.1-9.6			<0.1-1.6	<0.05-6.6	<LOD
Sediments											
America											
Lake Ontario tributaries, Canada[2]	-	-	-	n.d.-1	2-5	0.2-4	n.d.-3	-	n.d.	20-50	-
Savannah River, Georgia, USA[14]	-	-	n.d.-0.6	n.d.-1.8	n.d.-0.2	n.d.-0.1	-	0.1	n.d.-0.3	0.3-0.8	n.d.
Asia											
Zhujian River, Guangzhou, China[15]		-	-	-	0.09-0.29	-	n.d.-0.11	n.d.-0.35	-	n.d.-0.95	-

Location	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	PFDS
Huangpu River, Shanghai, China[15]	-	-	-	-	0.2-0.64	-	n.d.-<0.09	n.d.-0.1	-	<0.12-0.46	-
Haihe River, Tianjin, China[6]	-	-	0.2-3	<0.1-0.5	1-3.5	<0.1-0.2	0.2-1	-	-	2-9	-
Europe											
Orge River, nearby Paris, France[10]	-	n.d.	0.06	0.03	<0.07	0.05	0.30	<0.05	-	4.3	0.12
Canal of Pančevo, Serbia[16]	n.d.	n.d.	0.07-0.17	<LOQ	0.07-0.13	<LOQ	<LOQ	n.d.	0.17-0.23	0.29-5.7	n.d.-0.29
Biota											
America											
Bluegill, Northern Pike, Black Crappie, Largemouth Bass, lakes in USA[17]	-	-	-	-	-	-	-	-	-	2.29-711	-
Amphipod, Damselfly, Shrimp, Sunfish, Bullhead, Snapping turtle plasma, Ontario, Canada[3]	-	0.1-9.7	0.1-5.4	<0.1-25.1	<0.1-60.4	0.1-65.51	1.2-51.9	-	<0.1-40.2	15.1-2376	0.1-7.2
Sawtooth pen clam, White shrimp, eel, Oyster toadfish, snapper, catfish, Atlantic croaker, Southern kingfish, Southern stingray, Silver perch, Spot, Inshore lizardfish, Tomtate, Sea robin, Black sea bass, Largemouth bass, Atlantic sharp-nose shark, Bonnethead shark[14]*	-	-	<0.1-40	<0.1-0.6	<0.1-2.6	<0.1-23	-	<0.1-2.1	<0.1-3.4	<0.1-318	<0.1-14
Asia											
Surf clam, oyster, Asian periwinkle (soft tissue), crab (eggs, shells, soft tissue), striped mullet, Rockfish (fillet, intestine, liver), mussel, blue mussel, neritid gastropod (soft tissue), Korea[18]	n.d.-5.81	-	n.d.-34.6	n.d.-0.98	n.d.1.46	n.d.-1.27	n.d.-2.08	-	-	0.26-612	n.d.-0.79
Chinese alligators (serum)[19]	0-0.1	0-0.1	-	0-0.03	0-0.1	0.2-18.8	4.7-56.0	0-0.04	0-1.5	6.7-61.8	-
Silver carp, Oriental river prawn, Northern snakehead, Common carp, Tire track eel, Crucian carp, Chine[19]	-	-	<LOQ-1.2	<LOQ-0.7	<LOQ-0.7	0.2-4.5	0.1-8.1	-	-	0.4-7.8	-

Location	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	PFDS
Erythroculter ilishaefor, Aristichthys nobilis, Hyporhamphus intermedius, Hemiculter leucisculus, Hypophthalmichthys molitrix, Coilia ectenes, Cyprinus carpio, Protosalnax hyalocranius, white shrimp, Pelteobagrus fulvidraco, Egrets, Chine[20]	-	-	<LOD-1.5	<LOD-1.5	0.08-3.9	0.08-6.5	0.8-12.0	-	-	2.8-47.5	<LOD-8.7
Europe											
Mussels, Cantabrian Sea, Spain[21]	-	-	-	-	<LOD-0.01	<LOD	-	<LOD	<LOD	0.02-0.06	-
Oyster, sardine, anchovy, zebra mussel, crab, insect larvae, Catalonia, Spain[22]	-	-	-	-	0.14-4.3	0.07-7.5	-	n.d.	0.2-0.8	0.23-144	-
Sardine, tuna, hake, red mullet, cuttlefish, mussel, prawn, Catalonia, Spain[13]	-	-	<0.05-<0.08	<0.05-<0.08	0.04-0.1	0.03-0.51	<0.03-<0.06	-	<0.03-0.04	<0.05-8.32	<0.03-<0.06
Shellfish in France[23]	n.d.	n.d.	n.d.	n.d.	n.d.	0.15	0.04-0.08	n.d.	n.d.	0.01-0.9	n.d.

(1) Appleman et al., 2014; (2) Myers et al., 2012; (3) Solla et al., 2012; (4) Wang et al., 2012; (5) Nguyen et al., 2011; (6) Li et al., 2011; (7) Loos et al., 2010; (8) Llorca et al., 2012; (9) Sánchez-Avila et al., 2010; (10) Labadie and Chevreuil, 2011; (11) Onghena et al., 2012; (12) Flores et al., 2013; (13) Domingo et al., 2012; (14) Kumar et al., (2009); (15) Bao et al., 2010; (16) Beškoski et al., 2013; (17) Xiao et al., 2013; (18) Naile et al., 2010; (19) Wang et al., 2013; (20) Xu et al., 2014; (21) Gomez et al., 2011; (22) Fernandez-Sanjuan et al., 2010; (23) Munschy et al., 2013; d.w.: dry weight; *: wet weight; LOQ: limit of quantification; LOD: limit of detection; n.d.: not detected

Table S11. Mass loads of individual PFASs (mg day^{-1}) calculated from PFAS concentrations and flow in each sampling point

Location	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUdA	PFTrDA	PFHxDA	PFBS	PFHxS	PFOS
JUC1													
JUC2	203706.87	24.03					29.41						2.21
JUC3													
JUC4				4995.19									
JUC5						613.44							
CAB1													
CAB2	3439.87	733.38	4469.20	455.76	22.11	3003.74							
CAB3													
CAB4	3479.61	61.44	1943.51	427.44									
CAB5													
JUC6	2656.80	498.20			11084.17	4208.37							
JUC7													
MAG1													
MAG2													
JUC8							80976.7					13953.51	48652.23

Table S12. Mean log K_D and log K_{OC} values for PFASs obtained from sediment samples of the Jucar basin in 2010, and comparison with values reported in the literature; results are given as mean (min-max, number of observations)

Family / Compound	Jucar River (this study)		L'Albufera Natural Park [1]		Llobregat River, Spain [2]		Orge River, France [3]		The Netherlands [4]	Tokyo Bay, Japan [5]	
	log K_D	log K_{OC}	log K_D	log K_{OC}	log K_D	log K_{OC}	log K_D	log K_{OC}	log K_D	log K_D	log K_{OC}
PFCAs											
PFBA	2.40 (0.70-3.23, n=9)	4.22 (2.48-5.15, n=9)			2.33	4.27					
PFPeA	3.76 (2.44-4.82, n=6)	5.63 (4.53-6.59, n=6)	1.11	2.31	2.40	4.71					
PFHxA			1.18	2.62			0.8	2.1			
PFHpA	1.97 (n=1)	3.40 (n=1)	1.26	2.70			0.8	2.1			
PFOA	3.36 (1.71-4.56, n=5)	5.03 (3.39-6.14, n=5)	1.55	2.98	2.00	4.02			1.83	0.04	1.9
PFNA			2.14	3.56			1.5	2.9	2.89	0.6	2.4
PFDA			2.34	3.74	2.51	4.55	2.4	3.8	2.87	1.8	3.6
PFTeDA	5.14 (n=1)	6.83 (n=1)									
PFSs											
PFBS			1.47	2.79	2.88	4.92			1.42		
PFHxS	1.68 (n=1)	3.37 (n=1)									
PFOS	2.45 (1.07-3.70, n=4)	4.21 (3.14-5.13, n=4)	2.15	3.58	1.30	3.26	2.4	3.7	2.35	2.1	3.8
PFDS			3.17	4.51							

(1) Pico et al., 2012; (2) Campo et al., (2015); (3) Labadie and Chevreuil, 2011; (4) Kwadijk et al., 2010; (5) Ahrens et al., 2010

Table S13. Mean BAF (L kg^{-1}) and log BAF values for PFASs and biota samples captures in the Jucar River in 2010, and comparison with values reported in the literature; results are given as mean (min-max, number of observations)

Family/Compound	BAF	log BAF	BAF (1)	BAF (2)	log BAF (3)	log BAF (4)
PFBA						0.95-3.58
PFPeA	4896371 (771454-12440611, n=3)	6.38				3.53-3.94
PFOA			91±20	21-120	0.99-1.94	2.91
PFNA	28602 (n=1)	4.46	152±21	140-3400	1.69-2.97	
PFDA			175±23	160-9600	1.48-3.75	4.30
PFUdA			270±18	610-28000	2.50-4.17	
PFDoA			380±22	1.1-17	2.89-4.06	
PFOS	236499 (n=1)	5.37	179±25	180-3800	2.23-3.77	3.51-5.02
Species	**BAF**	**log BAF**				
B. guiraonis	42741.5 (251.9-126065.2, n=5)	4.43 (2.40-5.10)				
M. salmoides	422401.5 (5524.6-1135278.5, n=4)	5.02 (3.74-6.06)				
A. alburnus	27463.3 (13268.3-41658.4, n=2)	4.37 (4.12-4.62)				
A. anguila	81221.3 (383.6-195369.9, n=3)	4.19 (2.58-5.29				
G. lonzanoi	121970.6 (164.3-1107798.8, n=13)	4.29 (2.22-6.04)				
P. polylepis	65755.2 (8659.7-122850.7, n=2)	4.51 (3.94-5.09)				
E. lucius	58343.4 (548.7-116138.1, n=2)	3.90 (2.74-5.06)				
L. gibbosus	330315.2 (26199.4-634431.1, n=2)	5.11 (4.42-5.80)				
S. trutta	15721.6 (58.6-35304.2, n=3)	3.46 (1.77-4.55)				

(1) *Daphnia magna* (Dai et al., 2013); (2) silver carp, oriental river prawn, northern snakehead, common carp, tire track eel, crucian carp at Anhui Research Center, China (Wang et al., 2013); (3) fish, zooplankton and phytoplankton at Taihu Lake, China (Xu et al., 2014); (4) *M. salmoides, B. graellsii, C. carpio* in Llobregat River, Spain (Campo et al., 2015)

Table S14. Coefficients of the Pearson's correlations among PFAS concentrations found in fish, both mean and maximum values, and the trophic levels [1]

		Trophic level	
		Mean values	Max. values
PFOS	Pearson Correlation	0.511	0.520
	Sig. (2-tailed)	0.159	0.151
	N	9	9
PFHxS	Pearson Correlation	-0.446	-0.446
	Sig. (2-tailed)	0.229	0.229
	N	9	9
PFNA	Pearson Correlation	-0.074	-0.074
	Sig. (2-tailed)	0.849	0.849
	N	9	9
PFHpA	Pearson Correlation	0.554	0.444
	Sig. (2-tailed)	0.122	0.231
	N	9	9
PFPeA	Pearson Correlation	0.244	0.210
	Sig. (2-tailed)	0.527	0.587
	N	9	9

[1] Trophic levels of studied species: S. trutta (3.4 ± 0.1 se); G. lonzanoi (3.2 ± 0.44 se); M. salmoides (3.8 ± 0.4 se); P. polylepis (2.5 ± 0.24 se); A. anguila (3.6 ±0.3 se); B. guiraonis (3.1 ±0.24 se); A. alburnus (2.7 ± 0.29 se); L. gibbosus (3.3 ±0.1 se); E. Lucius (4.1 ±0.4 se). (FISHBASE database www.fishbase.org)

* Correlation is significant at the 0.05 level (2-tailed).

Table S15. Modelled (ECOSAR) and reported (mean, minimum and maximum) toxicity of selected PFAS in different tropic levels (algae, *Daphnia sp*. and fish)

Family / Compound	CAS Nº	ECOSAR Acute toxicity- EC50 (mg L^{-1})			REPORTED Acute toxicity- EC50 (mg L^{-1})		
		Algae	*Daphnia sp.*	Fish	Algae	*Daphnia sp.*	Fish
PFCA							
PFBA	375-22-4	597.14	760.59	1322.59	262.17 (214.47-320.42) [(1)]	185.15 (183.65-186.43) (b); > 4280.8 (c) [(1)]	273.97 [(1)]
PFPeA	2706-90-3	253.58	250.18	408.97	10.6 [(2)]	1080 [(2)]	1070 [(2)]
PFHxA	307-24-4	103.82	79.34	127.93	90 [(3)]	> 100 [(3)]	> 100 [(3)]
PFHpA	375-85-9	41.43	24.52	35.43	5.21 ± 0.26, 2.40 ± 0.12, 1.42 ± 0.07 [(4)]		
PFOA	335-67-1	16.22	7.44	10.10	748.22 (727.52-769.756) [(1)]	219.87 (209.52-229.81) [(1)]	211.59 (184.68-255.48) [(1)]
i,p-PFNA		4.65	1.54	1.93			
PFNA	375-95-1	6.26	2.22	2.84	481.72 (452.48-512.34) [(1)]	223.22 (201.88-278.91) [(1)]	151.29 (130.41-180.99) [(1)]
PFDA	335-76-2	2.39	0.66	0.79	437.48 (331.07-577.83) [(1)]	174.27 (159.37-188.15) [(1)]	163.48 (142.91-177.36) [(1)]
PFUdA	2058-94-8	0.90	0.19	0.22	318.71 (a) [(1)]	134.25 (d) [(1)]	133.13 (91.95-184.46) [(1)]
PFDoA	307-55-1	0.34	0.06	0.06	241.96 (a) [(1)]	99.48 (93.34-106.85) [(1)]	79.22 (60.18-98.26) [(1)]
PFTrDA	72629-94-8	0.13	0.02	0.02			
PFTeDA	376-06-7	0.05	$5\ 10^{-3}$	$4\ 10^{-3}$			
PFHxDA	67905-19-5	$6\ 10^{-3}$	$3.74\ 10^{-4}$	$3.09\ 10^{-4}$			
PFODA	16517-11-6	$8.37\ 10^{-4}$	$2.98\ 10^{-5}$	$2.18\ 10^{-5}$			
PFS							
PFBS	29420-49-3	1395.17	2008.25	3597.02	5733 [(5)]	2180 [(5)]	1938 [(5)]
PFHxS	82382-12-5	220.42	190.35	301.32			
PFHpS		85.36	57.10	84.97			
PFOS	4021-47-0	32.65	16.92	23.66			
i,p-PFNS		9.19	3.43	4.44			
PFDS		4.64	1.44	1.78	250 [(6)]	2.91 [(6)]	4.8 [(6)]
PFSA							
PFOSA	754-91-6	0.41	0.13	0.16			

(a) Predicted by the relationship found between log EC50 and nC; (b) data for the test without pH adjustment; (c) data for the test with pH adjustment; (d) predicted by the relationship found between log EC50 and nC.

(1) Case studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment. National Institute of Public Health and the Environment (RIVM), Laboratory for Ecological Risk Assessment. Authors: Mojca Kos Durjava, Boris Kolar, Willie Peijnenburg. February 29, 2012; (2) 3M MATERIAL SAFETY DATA SHEET 3M (TM) Novec (TM) 1230 Fire Protection Fluid [FK-5-1-12] 02/01/2005; (3) Environmental Assessment, Duppont Chemical Solutions Enterprise, 2009 http://www.google.es/#sclient=psy-ab&hl=es&tbo=d&rlz=1R2SUNA_esES400&q=PFOA+acute+72+hours+EC50+growth+algae&oq=PFOA+acute+72+hours+EC50+growth+algae&gs_l=serp.3...21906.23312.1.24250.6.6.0.0.0.0.344.1531.0j1j3j2.6.0...0.0...1c.1.ro0rNSdfRgs&psj=1&bav=on.2,or.r_gc.r_pw.&fp=227062ebe4d75b2a&biw=1280&bih=753; (4) Adam Latałaa, Marcin N˛edzia, Piotr Stepnowski. Environmental Toxicology and Pharmacology 28 (2009) 167–171; (5) Existing chemical Hazard Assessesment Report. 2005; (6) Perfluoroalkylated substances. Aquatic Environmental Assessment. 2002

Table S16. Coefficients of the Pearson's correlations among the water physical characteristics and the mean compound concentrations grouped by family and the total mean PFASs

	Water						Sediment						Biota				
	PFCAs 4-7 C	PFCAs 8-11 C	PFCAs 12-18 C	PFSs 4-7 C	PFSs 8-10 C	∑PFASs	PFCAs 4-7 C	PFCAs 8-11 C	PFCAs 12-18 C	PFSs 4-7 C	PFSs 8-10 C	∑PFASs	PFCAs 4-7 C	PFCAs 8-11 C	PFSs 4-7 C	PFSs 8-10 C	∑PFASs
T	-0.07	0.52*	-0.12	0.51*	0.47	0.22	-0.08	0.20	0.51	0.22	0.47	0.29	0.11	-0.20	-0.20	0.05	0.10
pH	-0.13	-0.36	-0.29	-0.61*	-0.52*	-0.34	-0.20	0.28	-0.82**	-0.26	-0.12	-0.26	0.46	0.15	0.15	0.35	0.46
Redox	-0.05	0.46	0.38	0.71**	0.62*	0.23	0.07	-0.35	0.89**	0.24	0.18	0.22	-0.38	-0.06	-0.06	-0.26	-0.38
Conduct.	-0.35	0.42	0.19	0.56*	0.52*	-0.07	-0.13	0.23	0.67**	0.40	0.73**	0.46	-0.12	0.12	0.12	-0.04	-0.11
TDS	-0.36	0.46	0.04	0.57*	0.55*	-0.06	-0.04	0.33	0.58*	0.37	0.72**	0.48	-0.13	0.13	0.13	-0.05	-0.13
NaCl	-0.35	0.42	0.19	0.57*	0.52*	-0.07	-0.13	0.23	0.68**	0.40	0.73**	0.46	-0.12	0.11	0.11	-0.05	-0.11
DO	0.12	-0.44	-0.16	-0.29	-0.32	-0.13	0.56*	0.09	-0.02	0.23	-0.10	0.29	-0.16	-0.19	-0.19	-0.25	-0.16

PFCAs: perfluorocarboxylates; PFSs: perfluorosulfonates; T: temperature; Redox: redox potential; Conduc.: concuctivity; TDS: total dissolved solids; NaCl: NaCl concentration; DO: dissolved oxygen; ** Significant correlation at $p < 0.01$; * Significant correlation at $p < 0.05$.

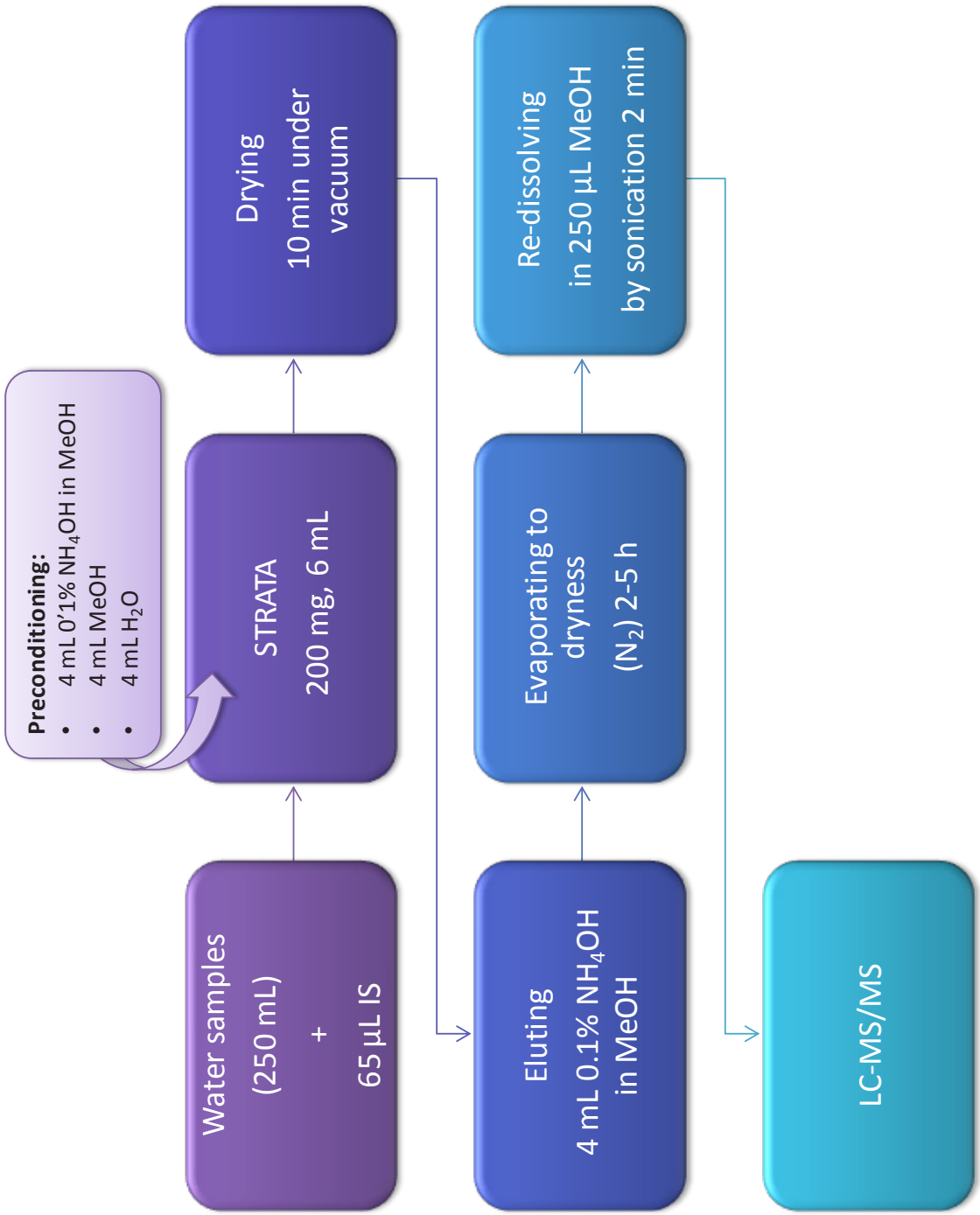

Fig. S1. PFAS extraction procedure for water samples.

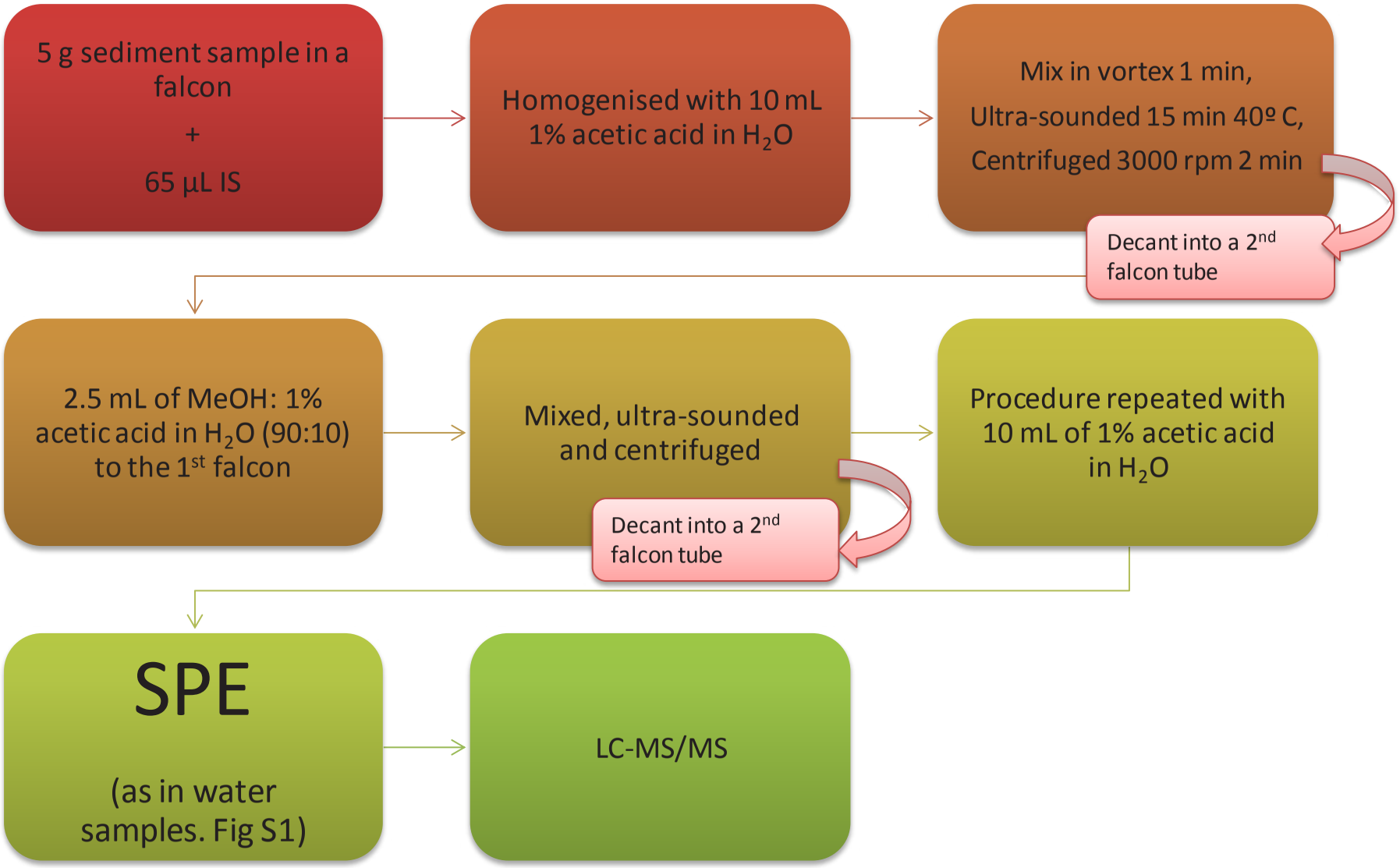

Fig. S2. PFAS extraction procedure for sediment samples.

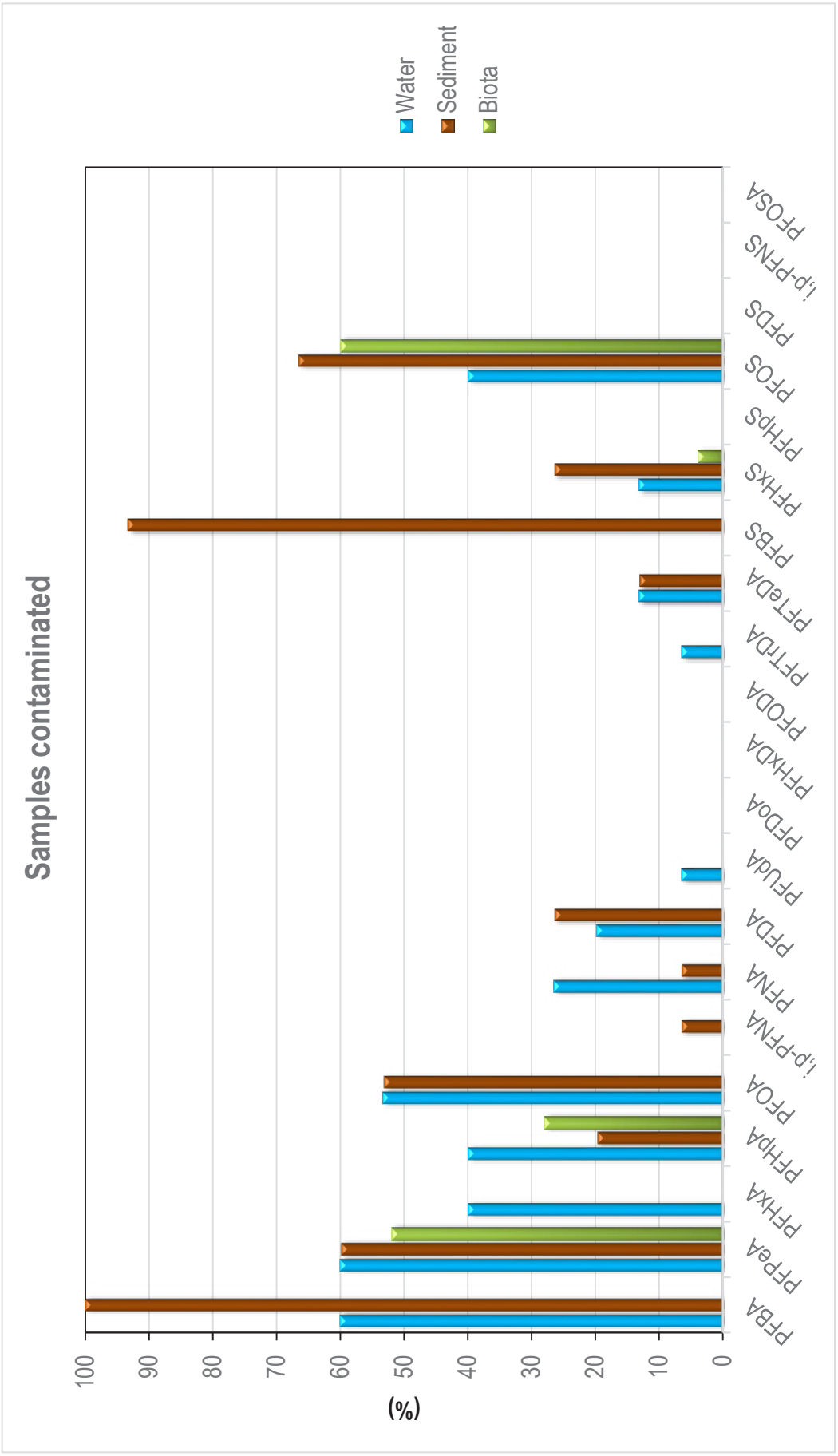

Fig. S3. Frequency (%) of each PFAS in water, sediment and biota samples of Jucar River in 2010.

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CAPÍTULO 7

Occurrence, distribution and behavior of emerging persistent organic pollutants (POPs) in a Mediterranean wetland protected area.

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María Lorenzo, Julián Campo, Yolanda Picó.

Abstract

The analysis of perfluoroalkyl substances (PFASs) and organophosphate flame retardants (PFRs) in the different environmental compartments of a characteristic coastal wetland, as the Albufera Natural Park (Valencia, Spain), is decisive for understanding the transport, accumulation and fate of these pollutants in an area under high anthropogenic pressure. Samples included 13 wastewater treatment plant influents, 13 effluents, 12 surface water, 19 sediment samples and 10 fish individuals from the Albufera Natural Park and the surrounding area. Tris(2-chloroisopropyl) phosphate (TCIPP) and perfluorooctane sulfonate (PFOS) were at the highest concentrations in water. PFOS was also the most detected compound in sediment and fish while perfluorooctanoic acid (PFOA) was in all types of water. Higher levels of target compounds (mainly PFASs) in wastewater effluents compared to influent suggested both, formation from precursors during treatment and poor removal. Mean levels of PFOS in water and fish were higher than the environmental quality standards (EQS) established by the European Union Directive 2013/39/EU. PFRs tend to accumulate in sediment whereas PFASs were mostly in water. The influence of the metropolitan area of Valencia and the industrial belt that surrounds it could explain the significant higher levels reported in the northern part (influenced by the Turia River).

***Keywords**:* Perfluoroalkyl substances (PFASs); organophosphorus flame retardants (PFRs); fish; sediment; environmental samples

1. Introduction

Organophosphate flame retardants (PFRs) and perfluoroalkyl substances (PFASs) are emerging persistent organic pollutants (POPs) widely used in industry as surfactants, plasticizers and anti-foaming agents and as additives in electronics, lubricants, paints, etc. [1, 2]. The concern about the occurrence, transport and fate of these compounds in aquatic ecosystems is raising [3-7]. PFRs and PFASs are (bio)accumulative and could posse adverse effects on humans and wildlife [8-10]. As a result, the European Union banned the manufacture and use of the carcinogenic PFR tris(2-chloroethyl)phosphate (TCEP) [11], which has been replaced progressively by tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) (also classified as carcinogen), and tris(2-chloroisopropyl) phosphate (TCIPP) (considered as possible carcinogen) [11, 12]. Concerning PFASs, perfluorooctane sulfonate (PFOS) (included in the list of the Stockholm Convention) and perfluorooctanoic acid (PFOA) are strongly regulated [13-17]. These emerging POPs were found ubiquitously in the aquatic environment, being present in drinking water [18, 19], wastewater influent and effluent [20, 21], river water [3, 22], fish [23, 24], sediment [22, 25] and seawater [26, 27]. Their continuous release to the environment from point and nonpoint sources such as wastewater treatment plants (WWTP) (point major source) or atmospheric deposition (diffuse minor source) was also documented [28, 29].

Among the different freshwater aquatic ecosystems, wetlands have been recognized at global scale as a driving force for biodiversity conservation and rural socioeconomic improvement [30]. They are very sensitive areas severely threaten by different problems as water pollution, disturbances on the water regime, clogging of marshes, dune system urbanization, industrial pressures, high population density, etc. to the point that recently Davidson [31] estimated that wetland losses in the 20th century were 64-71%, and for some

regions, notably Asia, even higher. Emerging POPs contamination is one of the factors that adversely affect these fragile aquatic ecosystems including their biota. PFAS loads in wetlands have already been determined in the Mediterranean area (the Ebro Delta [23] and the Albufera Natural Park [32]), but also in a Hong Kong wetland [33], in mangrove sediments from India [34], European eels from the Loire Estuary in France [35] and surface waters of Xixi wetland in China [36], establishing the widespread occurrence of these compounds. PFRs have been less studied, and their presence in aquatic environments has been determined in mangrove sediments from the Pearl River Estuary, China [37], and in a restored wetland and lake from Aarhus, Denmark [38]. Furthermore, to our knowledge there are not reports on the co-occurrence of several classes of emerging POPs that could offer a more complete overview of their occurrence and threat to wetland ecosystems.

The purpose of this study was to establish the patterns and concentrations of emerging POPs (PFRs and PFASs) in sediment, fish and water from a typical Mediterranean wetland, the Albufera Natural Park (Valencia, Spain) with an environmental forensics perpective of chemically fingerprinting environmental samples. To this end, this study was focused on the evaluation of the occurrence and environmental fate of 21 PFASs and 9 PFRs in 67 samples (38 water, 19 sediment and 10 fish). Firstly, the identification of contamination sources included the analysis of samples from the two rivers with the largest contribution to the wetland (Turia and Jucar Rivers), and from some major irrigation channels. Samples of influents and effluents from 10 WWTPs, the effluents of which are discharged into the channels in order to allow crop irrigation and mantaintenance of the ecological flow of the lake were also analysed and discussed.

2. Material and methods

2.1 Chemicals and reagents

A total of 21 PFASs, consisted of 14 perfluorocarboxylates (C_4-C_{14}, C_{16} and C_{18}), 1 unsaturated carboxylic acid (C_{10}) and 6 perfluorosulfonates (C_4, C_6-C_{10}), and 9 PFRs, consisted of 5 non-halogen and 4 halogen containing PFRs, have been monitored. Some characteristics of target compounds, including their acronym, CAS number, empirical formula, Log K_{ow} and solubility in water are provided in Supplementary data (**Table S1**). Mass-labelled compounds with ^{2}H, ^{13}C and ^{18}O were used as internal standards (IS). Stock standard and working solutions were prepared in methanol and stored at 4 °C. Methanol was bought from VWR (Radnor, PA, USA) and formic acid from AMRESCO (Solon, OH, USA), all of them with the highest purity grade. Ultra-pure water was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA).

2.2 Study area and sample collection

The Albufera Natural Park has an area of 21120 hectares and it is located just 10 km to the South from Valencia City. The Albufera was declared a Natural Park in 1986, and since 1989 is recognized as "Wetland of International Importance", which is a figure derived from the Ramsar Convention on Wetlands [39]. The park is also part of the Natura 2000 network, it was named Special Protection Area (SPA) in 1990 [40], and Site of Community Importance (SCI) in 2006 [41]. It consists of a highly eutrophic coastal lagoon surrounded mainly by rice fields that occupy the primitive marshland. The Turia River, to the north, the Jucar River, to the South, and a network of irrigation channels bring fresh water to the Albufera system. The sea connection of the Albufera is controlled by artificial channels called “golas” [42]. Water quality is compromised due to the high density of population around the Albufera

Natural Park, the use of agrochemicals in the surrounding fields and the WWTP discharges to alleviate the threat to the park from the general water scarcity of the area [43, 44].

The sampling campaign was carried out during the winter 2016-2017. Twenty-two sampling points and 10 WWTPs were selected. Sixty-seven samples were collected, including 12 surface waters, 13 WWTP influents, 13 WWTP effluents and 19 sediment samples. Ten fish samples, comprising 7 specimens of European eel (*Anguilla anguilla*), 2 flathead grey mullets (*Mugil cephalus*) and one common carp (*Cyprinus carpio*), all of them from the Albufera Natural Park, were obtained from the local Fishermen's Association "El Palmar". European eels are included in the Red List of the International Union for Conservation of Nature (IUCN) and classified as Critically Endangered. At the Albufera Lake, it lives in mud, crevices, and under stones. This is a very long-lived species with a maximum life span of 85 years. Flathead grey mullet is a diurnal feeder, consuming mainly zooplankton, dead plant matter, and detritus. Common carp is often considered a destructive invasive species, being included in the list of the world's 100 worst invasive species created by the IUCN Invasive Species Specialist Group, due to its predilection for the vegetal substrate of the shallow lagoons, as the Albufera Lake, which serves as food for native species. These fish are also an important part of the human diet. Water samples (2 L) from Turia and Jucar Rivers, irrigation channels and the Albufera Lake were collected in clean amber polyethylene terephthalate (PET) bottles. Ten WWTPs were selected: Quart-Benàger (QB), Pinedo I (PI1), Pinedo II (PI2), Port de Catarroja (CAT), Saler (SAL), Palmar (PAL), Perellonet (PER), Perelló-Sueca (PS), Albufera Sud (AS) and Sueca (SU). Due to their importance, samples of two different days were collected for PI1, PI2 and CAT. Selected WWTPs serve predominantly households, with some industrial discharges. Served

municipalities and flow are detailed in **Table S2**. Wastewater samples (24 h composite) were collected using the operational equipment of the WWTPs in a time-proportional manner at 60 min time intervals [45]. Sediment samples from the irrigation channels and the lake were taken using a Van Veen grab sampler, transferred into pre-rinsed aluminum trays and then, wrapped with aluminum foil. Location of WWTPs, sediment and surface water sampling points is shown in **Fig. 1** and their characteristics for sediment and water samples are detailed in **Table S3.**

2.3 Sample preparation, extraction and analysis

The same day of collection, surface water and wastewater samples were filtered with glass microfiber filters (90 mm Ø) and stored at -20˚C until the solid-phase extraction (SPE) with STRATA-X Polymeric Reversed Phase cartridges following the method described elsewhere was performed [46, 47]. Briefly, lyophilized sediments (sieved, 2 mm Ø) and fresh fish (crushed) samples were extracted by ultrasound assisted extraction using methanol followed by the same SPE clean-up than in waters according to the method developed in previous work [48]. Detailed procedure of extraction methods is shown in **Table S4**.

The chromatographic instrument was a 1260 Infinity Ultra-High-Performance Liquid Chromatograph combined with an Agilent 6410 Triple Quadrupole Mass Spectrometer with an electrospray ionization interface (Agilent Technologies, Santa Clara, CA, USA). Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (GL Sciences, Tokyo, Japan). Instrumental characteristics used for PFASs and PFRs determination are detailed in **Table S5** and SRM dynamic conditions for the determination of PFRs and PFASs by UHPLC-QqQ-MS/MS are detailed in **Table S6**.

2.4 Quality assurance and quality control

Procedural blanks were prepared to check for possible contamination from reagents, tubes or equipment. Background contamination of TCIPP was subtracted from concentrations found in blanks. Analytical blanks (methanol) and control samples (fortified with a known concentration of target compounds) were analyzed every 10 injections. Regression coefficients (R^2) of calibration curves (1-75 ng mL^{-1} for PFASs and 1-300 ng mL^{-1} for PFRs) were ≥ 0.998. Limit of detection (LOD) and limit of quantification (LOQs) values were estimated by injecting in triplicate extracts of samples spiked at low concentration (1 and 5 ng g^{-1}) as the amount that provides a height 3 and 10 times higher than the baseline noise. LOQ values for PFRs ranged from 0.02 – 0.4 ng g^{-1} wet weight (ww) for biota, 0.02 – 0.3 ng g^{-1} dry weight (dw) for sediment and 0.3 – 2.5 ng L^{-1} for water (surface and wastewater). For PFASs, LOQ ranged from 0.02 – 2.3 ng g^{-1} wet weight (ww) for biota, 0.2 – 0.9 ng g^{-1} dry weight (dw) for sediment, 0.01 – 2.0 ng L^{-1} for water (surface and wastewater). Recoveries were evaluated using 1 g of sample spiked to obtain a final concentration of 50 ng mL^{-1} of PFRs and 20 ng mL^{-1} of PFASs in the extract. Range of recoveries for PFR in each type of sample were 47 – 123 % for fish, 59 – 121 % for sediment and 94 – 106% for water. For PFASs were 52 – 135% for fish, 45 – 103% for sediment and 55 – 94% for water.

2.5 Statistical analysis

For the parametric analysis, normality of the data was tested using Shapiro-Wilk's test ($n<50$) and homogeneity of variances was tested using Levene's test. For non-parametric analyses, differences in the compound concentrations between Jucar, Turia and Albufera were determined by the tests of Mann-Whitney U (M-W) or Kruskal-Wallis (K-W). In all the cases, results were considered to be statistically significant at $p<0.05$. All statistical analyses were carried out using IBM SPSS Statistics 22®.

3. Results and discussion

Minimum, maximum and mean concentrations as well as frequency of detection for target compounds are shown in **Table 1** (surface water, wastewater influent and effluent) and **Table 2** (fish and sediment). **Table 3** outlined reported levels of PFRs and PFASs in sediment, surface water and fish samples for wetlands in other studies to facilitate comparison with this one. Cumulative concentration in each sampling point is shown in **Fig. 2** (PFRs) and **Fig. 3** (PFASs).

3.1 Presence of emerging POPs in water

In surface waters, from the 9 PFRs included in this study, 6 were detected. Mean PFR values for detected compounds were between 6.4 ng L^{-1} (TCEP) and 70.4 ng L^{-1} (TCIPP). The highest concentration was for TCIPP (330.2 ng L^{-1}) in sampling point 4, at the QB WWTP channel. The PFR most detected in samples was TCIPP (67%), followed by TPhP (58%), TCEP and TPP (50%), TDCIPP (33%) and TMPP (25%). The presence of TPP only in water samples may be due to their high solubility, 827 mg L^{-1} at 25˚C [49]. TCEP was also detected in 100% of water samples of a wetland in Denmark, with mean concentrations (48 and 50 ng L^{-1}), higher than the mean concentration in this study (2.9 ng L^{-1}). To our knowledge, there are no studies about the presence of PFRs in wetlands' water. Mean concentrations reported in rivers around the Bohai Sea (north China) [TCIPP (186 ng L^{-1}), TCEP (80.2 ng L^{-1}) and TDCIPP (4.3 ng L^{-1})] [27] and the Elbe River and its tributaries (Germany) [TCIPP (126 ng L^{-1}), TCEP (81 ng L^{-1}) and TDCIPP (155 ng L^{-1})] [50] were higher than those found in our study. Results are consistent with the physico-chemical properties of PFRs (**Table S1**), the detected ones are highly soluble in water (e.g. TPP) whereas the insoluble were not detected (e.g. CDP and TEHP).

From the 21 PFAS included in this study, 10 were detected in surface water. The PFASs more frequently detected were PFOA (100%), PFOS (92%) and PFPeA (83%). Highest concentrations were found for PFOS (47.8 ng L^{-1}) in sampling point 4 and FOUEA (46.1 ng L^{-1}) in sampling point 1, both in the Turia River. Mean concentrations for detected compounds ranged from 1.0 (PFDA) to 31.6 ng L^{-1} (PFOS) respectively. **Table 3** reports concentrations of PFASs in surface water of wetland environments worldwide. In the Ebro Delta [23], a wetland located also in the east of Spain, about 350 km to the north of that of this study, 6 PFASs –PFPeA, PFOA, PFNA, PFDA, PFUnDA and PFOS– were detected being PFOA the most frequent and PFOS the most abundant. Among the 21 PFASs analyzed in the Mai Po Marshes Nature Reserve in Hong Kong [33], 12 were detected in water samples, being PFOA, PFBS and PFOS the dominant compounds. As in this study, short-chain PFASs predominated in the water samples. In Xixi Wetland [36], an agricultural and rural residential land in China, PFOA, PFHpA and PFNA were detected in water samples and PFOA was the most detected (frequency: 100%) and prominent (maximum concentration: 197.8 ng L^{-1}). The previous study of the Albufera Natural Park [32] included less PFASs than this one (perfluorocarboxylates: C_5-C_{10}, perfluorosulfonates: C_4, C_8 and C_{10}). PFOA and PFOS had the highest frequency of detection (both 100%). Most of PFASs were at lower mean concentrations in this study than in 2012, including PFOA mean levels of 49.5 ng L^{-1} that are much higher than current mean value (9.7 ng L^{-1}). However, PFOS average concentrations found in surface water in this study (31.6 ng L^{-1}) are higher than those from 2012 (14.2 ng L^{-1}), although both of them are high considering the environmental quality standard (EQS) annual average of 0.65 ng L^{-1} set by the European Union Directive 2013/39/EU [51]. PFNA and PFDA were the only long-chain compounds (C≥8) detected in surface water, this could be explained by their lower solubility [52] and replacement by short-chain (C<8) PFASs.

Regarding wastewater, most frequent PFRs in WWTP's influent and effluent were TPhP (100% in both), TDCIPP (92% and 85%) and TCIPP (92% and 77%). The highest concentrations (1543.5 ng L^{-1} in Pinedo I influent and 1908.5 ng L^{-1} in Pinedo II effluent) were found for TCIPP, WWTPs showed lower removal efficiency for chlorinated PFRs (TCIPP, TDCIPP and TCEP) than for non-chlorinated such as TPhP and TMPP. As shown in **Fig. 4**, removal efficiencies ranged from 14 to 66% except for TPP which was only found in effluent samples and TDCIPP that was found in much higher concentrations in effluent samples (-74%). Chlorinated PFRs were also detected in high frequency and concentration in influents and effluents in other studies [20, 53, 54]. Kim et al. [20] analyzed a WWTP in New York State (EEUU) and showed a negative removal efficiency for TCEP, TCIPP and TDCIPP and related it to the presence of precursor compounds and low biotransformation on PFRs.

From the 21 screened PFASs, 13 were found in influent wastewater and 20 in effluent (only PFDS was not detected). PFOA was the most frequent (100%) in both matrices, followed by PFOS with 54% in influent and 92% in effluent. The highest concentrations were for PFBS (101.3 ng L^{-1}) in Pinedo I effluent and for PFOS (63.1 ng L^{-1}) in Perellonet influent. Some PFASs (PFHxA, PFHpA, PFHxS, PFOA, PFHpS, ipPFNS, PFOS and FOUEA) showed higher concentrations in effluent samples than in influents, and some others like PFBS, ipPFNA, PFNA, PFDA, PFUnDA, PFTrDA, PFHxDA and PFODA were only found in effluent samples. The remaining PFASs showed removal efficiencies between 8 and 100%. The fully fluorinated nature of PFASs prevents their aerobic decomposition. Furthermore, there is evidence that an additional source of PFASs in WWTPs is the biotransformation of their precursors to PFASs during activated sludge treatment [55, 56]. The high concentrations of PFASs observed in effluents suggest that WWTP are ineffective removing these compounds [21,

57]. Monitoring data worldwide suggest that WWTPs could be main sources of PFASs and PFRs contamination to surface water and contribute to increase the concentration of these compounds in the Albufera Natural Park environment.

3.2 Presence of emerging POPs in sediment

All the samples were contaminated with at least 4 PFRs and 7 of them were detected at concentrations above the LODs. TCIPP was ubiquitous (100%), followed by TCEP and TPhP (95%), TEHP (89%), TDCIPP (79%), CDP (68%) and TMPP (47%). Mean concentrations for detected compounds were from 2.5 ng g^{-1} dw (TDCIPP) to 53.8 ng g^{-1} dw (TCIPP). Maximum concentration were for TCIPP (246.5 ng g^{-1} dw) in sampling point 3, a horticultural irrigation channel. Our mean values are similar to those detected in the Pearl River Estuary in China [37] for TCEP, TDCIPP and TPhP and slightly higher for TCIPP. Similar or slightly higher concentrations were also found for TCEP, TDCIPP, TCIPP and TPhP in sediments from European [22, 58] and Chinese [59] Rivers. The log Kow values of PFRs vary widely from low (TCEP: 1.44) to very high (TMPP: 6.34), indicating hydrophilic to lipophilic compounds, respectively [49].

Of the 19 sediment samples, 13 were contaminated with at least one PFAS and 7 of the 21 analytes were detected (**Table 2**). PFOS was the predominant (58%). The other PFASs were found in less than 32% of the sampling points and were PFDoDA > PFHxS ≈ PFOA ≈ PFUnDA > PFNA > PFTeDA. Mean values were from 0.01 ng g^{-1} dw (PFTeDA) to 4.9 ng g^{-1} dw (PFOS). Maximum concentration was for PFOS with 21.4 ng g^{-1} dw and was found in the sampling point 9. PFOS was also the prevailing compound in sediment from a Hong Kong wetland [33], the Jucar river [25], and in Korean rivers and lakes [60]. On the contrary, as shown in **Table 3**, PFOA was the predominant compound in sediments from previous study

at the Albufera Natural Park [32], in the Ganges River and Sundarban wetland (India) [34], and in the Ebro Delta [23]. Long-chain PFASs (PFUnDA, PFDoDA and PFTeDA) were detected in sediment but not in water. Long-chain PFASs have high affinity for particles and as suggested by Ahrens et al. [26], surface sediment could act as a sink for them.

3.3 Presence of emerging POPs in fish

Of the 9 PFRs analysed, 5 were found in biota, being TCEP (70%) and TCIPP (50%) the most detected. The other PFRs, detected with frequencies below 20%, were TDCIPP ≈ TPhP > TMPP. Mean concentrations ranged from 0.04 ng g^{-1} ww (TMPP) to 1.9 ng g^{-1} ww (TCIPP). Chlorinated TCEP and TCIPP were detected in all the species. The highest concentration (13.1 ng g^{-1} ww of TCIPP) was found in one sample of *A. anguilla*. The European eel was reported by Belpaire [61, 62] as a suitable species for the screening of toxic substances because this species tends to bioaccumulate contaminants in its muscle tissue as consequence of some specific physiological and ecological features (size, long life span, fat content, feeding, habitat, ecology, distribution, etc.). To our knowledge, there are no studies about the presence of PFRs in fish from wetlands. However, in agreement with our results, TCEP was the most frequently quantifiable compound in Lake Trout from Canadian lakes [63], with levels ranging from <0.07 to 9.8 ng g^{-1} ww. Unlike the PFASs that are extremely stable to degradation, the non-detectable or low PFR concentrations found in this study may be due to abiotic degradation or metabolization by fish as reported for TDCIPP [49]. The concentration in fish can be higher than levels reported here because PFRs are likely to metabolize, and these metabolites are not included in this study [64].

Six out of the 21 PFASs analyzed were found in fish. PFOS was the most detected (60%). The other PFASs, all perfluorocarboxylic acids detected with frequencies below 50%, were

PFDoDA > PFUnDA > ipPFNS > ipPFNA ≈ PFDA. As for PFRSs, the highest concentration (194.5 ng g^{-1} ww for PFOS) was found in eel. PFOS was the most abundant compound in common carp and eel and its mean (30.4 ng g^{-1} ww) concentrations are higher than the EQS value of 9.1 ng g^{-1} established by the European Union Directive 2013/39/EU [51]. Specifically, PFOS was exceeding EQS values in 50% of samples (71.4% of European eels). The presence of PFOS exceeding EQS values in 75% of European eel samples was also reported in the Loire estuary in France [35] (a wetland of similar characteristics to ours). Similarly, in the Ebro Delta, PFOS was the most abundant compound detected in coastal biota, even though upstream in the river PFOA was the main compound (<LOQ – 330 ng g^{-1} ww) [23]. PFOS concentrations recorded in fish samples of Lake Möhne ranged from 4.5 to 150 ng g^{-1}. The highest median PFOS concentrations have been observed in perches (median: 96 ng g^{-1}) and again in eels (77 ng g^{-1}), followed by pikes (37 ng g^{-1}), whitefish (34 ng g^{-1}), and roaches (6.1 ng g^{-1}) [65]. Our results also agree with those of Houde et al. [66] who summarized new biological monitoring information on perfluoroalkyl substances in aquatic ecosystems (post-2005) showing that PFOS is still the predominant PFAS detected (mean concentrations up to 1900 ng g^{-1} ww). PFOS is known for its high bioaccumulative potential, however, this compound is both hydrophobic and lipophobic and it does not follow the typical pattern of partitioning into fatty tissues followed by accumulation, but tends to bind to proteins and therefore is present rather in highly perfused tissues than in lipid tissue [67]. According to Lassen et al. [49] the acids are not very bioaccumulative but precursors such as fluorotelomer alcohols are accumulated and subsequently transformed in the organs of animals to the corresponding acids. Lack of detection of the short-chain PFASs (C<8) in biota could be due to their low bioaccumulation potential. This was in contrast to the concentrations found in waters, where short-chain PFASs were also

detected. Ding and Peijnenburg [68] established that perfluorosulfonic acids with the same number of fluorinated carbons are more bioaccumulative than perfluorocarboxylic acids, which is in agreement with the results obtained in this study for PFOS vs PFOA and ipPFNS vs ipPFNA.

3.4 Spatial incidence and statistical analysis of emerging contaminants

Cumulative concentrations of PFRs and PFASs are shown in **Fig. 2** and **3**, respectively. PFRs tend to accumulate in sediment whereas water accumulate more PFASs. For spatial incidence analysis, sampling points 1-7 were considered influenced by Turia River, 8-15 and 22 by Jucar River, and 16-21 by the Albufera Lake. All the distributions are not normally distributed and consequently non-parametric analyses were carried out. Significant higher concentrations (M-W, $p<0.05$) in the waters influenced by the Turia River than in those of Albufera Lake were found for TPP, TDCIPP, PFDA. Regarding sediment, in all cases concentrations found in the samples of Jucar were similar to those of Albufera and both lower (M-W, $p<0.05$) than those of Turia for TCIPP, TPhP, CDP, TMPP, TEHP, PFUnDA. Higher concentrations found in water and sediment on the north of the studied area could be explained by the high influence of the metropolitan area of Valencia (1.5 million of inhabitants that represent 60% of the total population in the province) and the industrial belt around the city of Valencia. For the WWTPs, concentrations in the influent of north (Turia) and in south (Jucar) do not shown statistically significant differences. Similar results for the effluent with no significant differences between north and south were obtained then, WWTPs of both river contributes similarly to the contamination of the area. This could suggest that, at least in the north part, studied WWTPs are not the only source of these compounds. On the other hand, for the WWTPs of the North, significant differences (M-W,

p<0.05) between influent and effluent were found for PFHpA, PFHxS, PFOA, PFOS while in south WWTPs, significant differences (M-W, p<0.05) between influent and effluent were found for PFOA and PFNA. PFASs have been recurrently reported in the majority of dust samples examined coming from carpet, carpet protectants, textiles and furniture etc. for its excellent waterproofing and oil-resistance performance that could be related to the higher human pressure in the north area [2]. Instead, PFNA is the primary compound followed by PFOA of the commercial product Surflon S-111 (CAS 72968-3-88) used as surfactant for the production of the fluoropolymer polyvinylidene fluoride. PFNA can also form from the biodegradation of 8:2 fluorotelomer alcohol. In any case both compounds can be related with the presence of plastics. This suggests that depending on the more prominent human activities in the area different types of precursors of these compounds reach the WWTPs in the north compared to the south.

4. Conclusions

The results of this study show the presence of PFRs and PFASs in the environmental compartments of the Albufera Natural Park wetland area. WWTPs were identified as an important but not unique point source of these emerging POPs to the environment. High levels of target compounds (mainly PFASs) in wastewater effluents suggest the presence of precursors in water and their poor removal through the treatments. Similar concentrations found in WWTPs in the north (most populated) and south areas as a counterpart of the higher levels of these compounds in water of the north side suggest the existence of additional sources that could be also diffuse. TCIPP and PFOS were at the highest concentrations in all water samples. PFOS was also the most detected compound in sediment and fish samples. Mean levels of PFOS in water and fish were higher than the

annual average established in the EQS. This pointed out global interest of these types of studies and their environmental forensics utility since the analysis of the results could help to identify their sources to the environment.

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Table 1. Concentration and frequency of occurrence of detected PFRs and PFASs in surface water, wastewater influent and effluent samples.

Compounds	Surface water				Influent wastewater				Effluent wastewater			
	Maximum (ng L^{-1})	Minimum (ng L^{-1})	Mean (ng L^{-1})	Frequency (%)	Maximum (ng L^{-1})	Minimum (ng L^{-1})	Mean (ng L^{-1})	Frequency (%)	Maximum (ng L^{-1})	Minimum (ng L^{-1})	Mean (ng L^{-1})	Frequency (%)
Organophosphate flame retardants												
TCEP	39.6	<LOD	6.4	50	153.7	<LOD	22.5	62	81.2	<LOD	19.5	46
TPP	82.0	<LOD	26.6	50	n.d.	n.d.	n.d.	n.d.	30.8	29.5	4.6	15
TCIPP	330.2	<LOD	70.4	67	1543.5	<LOD	676.2	92	1908.5	<LOD	573.6	77
TDCIPP	61.8	<LOD	17.4	33	160.9	<LOD	49.4	92	175.2	<LOD	85.9	85
TPhP	93.8	<LOD	30.5	58	109.0	13.6	58.2	100	67.7	10.4	43.7	100
TMPP	35.4	<LOD	8.8	25	66.0	<LOD	7.6	15	33.9	<LOD	2.6	8
Perfluoroalkyl substances												
PFBA	n.d.	n.d.	n.d.	n.d.	20.9	<LOD	4.8	31	31.6	<LOD	4.4	23
PFPeA	28.4	<LOD	16.1	83	27.0	<LOD	2.1	8	16.6	<LOD	1.3	8
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	101.3	<LOD	16.7	31
PFHxA	18.7	<LOD	4.6	25	23.3	<LOD	1.8	8	18.6	<LOD	3.2	23
PFHpA	n.d.	n.d.	n.d.	n.d.	7.5	<LOD	1.9	31	60.9	<LOD	20.4	62
PFHxS	23.7	<LOD	9.8	58	51.8	<LOD	6.9	38	33.4	<LOD	17.6	92
PFHpS	11.1	<LOD	1.9	17	22.2	<LOD	1.7	8	11.1	<LOD	2.5	23
PFOA	16.0	5.6	9.7	100	5.9	0.04	3.4	100	91.6	21.2	65.3	100
PFOS	47.8	<LOD	31.6	92	63.1	<LOD	11.1	54	58.3	<LOD	34.7	92
ipPFNA	16.1	<LOD	1.4	8	n.d.	n.d.	n.d.	n.d.	19.8	<LOD	7.3	54
PFNA	13.9	<LOD	6.9	50	n.d.	n.d.	n.d.	n.d.	27.8	<LOD	9.6	62
FOUEA	46.1	<LOD	15.3	58	46.0	<LOD	6.7	23	46.1	<LOD	13.3	54
ipPFNS	n.d.	n.d.	n.d.	n.d.	14.2	<LOD	1.1	8	16.0	<LOD	1.2	8
PFDA	12.3	<LOD	1.0	8	n.d.	n.d.	n.d.	n.d.	12.3	<LOD	2.8	31
PFDS	n.d.	n.d.	n.d.	n.d.	12.7	<LOD	1.9	15	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.2	<LOD	2.7	31
PFDoDA	n.d.	n.d.	n.d.	n.d.	41.6	<LOD	3.2	8	10.3	<LOD	1.6	15
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	25.3	<LOD	4.8	31
PFTeDA	n.d.	n.d.	n.d.	n.d.	41.2	<LOD	3.2	8	14.7	<LOD	2.2	15
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.4	11.3	1.8	15
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10.0	10.0	0.8	8

n.d.: not detected; <LOD: below limit of detection.

Table 2. Concentration and frequency of occurrence of detected PFRs and PFASs in fish and sediment samples.

Compounds	**Fish**[a]				**Sediment**[b]			
	Maximum (ng g^{-1})	Minimum (ng g^{-1})	Mean (ng g^{-1})	Frequency (%)	Maximum (ng g^{-1})	Minimum (ng g^{-1})	Mean (ng g^{-1})	Frequency (%)
Organophosphate flame retardants								
TCEP	1.7	<LOD	0.6	70	27.3	<LOD	5.8	95
TCIPP	13.1	<LOD	1.9	50	246.5	0.6	53.8	100
TDCIPP	4.6	<LOD	0.5	20	12.5	<LOD	2.5	79
TPhP	0.4	<LOD	0.1	20	70.9	<LOD	14.1	95
CDP	n.d.	n.d.	n.d.	n.d.	157.6	<LOD	32.5	68
TMPP	0.4	<LOD	0.04	10	16.7	<LOD	3.9	47
TEHP	n.d.	n.d.	n.d.	n.d.	68.9	<LOD	15.8	89
Perfluoroalkyl substances								
PFHxS	n.d.	n.d.	n.d.	n.d.	3.2	<LOD	0.2	16
PFOA	n.d.	n.d.	n.d.	n.d.	3.2	<LOD	0.3	16
PFOS	194.5	<LOD	30.4	60	21.4	<LOD	4.9	58
ipPFNA	2.0	<LOD	0.2	10	n.d.	n.d.	n.d.	n.d.
PFNA	n.d.	n.d.	n.d.	n.d.	0.7	<LOD	0.04	11
ipPFNS	1.1	<LOD	0.2	20	n.d.	n.d.	n.d.	n.d.
PFDA	0.8	<LOD	0.1	10	n.d.	n.d.	n.d.	n.d.
PFUnDA	0.6	<LOD	0.2	40	0.3	<LOD	0.02	16
PFDoDA	25.9	<LOD	3.6	50	2.1	<LOD	0.3	32
PFTeDA	n.d.	n.d.	n.d.	n.d.	0.1	<LOD	0.01	5

<LOD: below limit of detection; n.d. = not detected; [a]ng g^{-1} in wet weight (ww) [b]ng g^{-1} in dry weight (dw).

Table 3. Measured levels of PFRs and PFASs in sediment, surface water and fish samples from wetlands all around the world.

Location	Year	Compounds	Type of sample	No. samples	Range (mean)[a]				Reference
					PFOA	PFOS	TCIPP	TCEP	
Mai Po Marshes Nature Reserve wetland, Hong Kong	2011	PFASs	Sediment	6	(0.1)	(0.2)	n.a.	n.a.	[33]
			Water	12	(7.7)	(6.4)	n.a.	n.a.	
			Fish	21	(0.1)	(2.7 – 8.2)	n.a.	n.a.	
Lake Mohne, Sauerland Area, Germany	2011	PFASs	Fish	44	<LOD – 2.3	4.5 – 150 (6.1 – 96)	n.a.	n.a.	[65]
Aarhus, Denmark	2012	PFRs	Water restored wetland	6	n.a.	n.a.	n.a.	40 – 55 (48)	[38]
			Water lake	6	n.a.	n.a.	n.a.	42 – 55 (50)	
Albufera Natural Park wetland, Valencia, Spain	2012	PFASs	Sediment	12	0.03 – 10.9 (3.2)	0.1 – 4.8 (1.8)	n.a.	n.a.	[32]
			Water	12	0.99 – 120.2 (49.5)	0.9 – 58.1 (14.2)	n.a.	n.a.	
Ganges River and Sundarban mangrove wetland, India	2012	PFASs	Sediment	13	<LOD – 14.09 (8.5)	<LOD	n.a.	n.a.	[34]
Loire estuary, France	2015	PFASs	Fish (*Anguilla anguilla*)	45	n.d.	17.9 – 39	n.a.	n.a.	[35]
Xixi Wetland, Hangzhou, China	2016	PFASs	Water	8	34.6 – 197.8 (76.6)	n.d.	n.a.	n.a.	[36]
Pearl River Estuary, South China	2017	PFRs	Sediment Guangzhou	13	n.a.	n.a.	7.9 – 67.3 (22.5)	2.1 – 26.5 (10.9)	[37]
			Sediment Zhuhai	14	n.a.	n.a.	2.3 – 6.4 (3.4)	1.0 – 11.3 (4.6)	
			Sediment Shenzen	21	n.a.	n.a.	6.1 – 186.3 (29.0)	1.4 – 16.4 (6.6)	
Ebro Delta wetland, Catalonia, Spain	2017	PFASs	Sediment	71	<LOD – 12.0 (0.8 –6.0)	<LOD – 22.6 (0.6 – 2.7)	n.a.	n.a.	[23]
			Water	87	<LOD – 8.7 (0.9 – 1.6)	<LOD – 4.3 (0.4)	n.a.	n.a.	
			Fish	55	<LOD – 330	<LOD – 137	n.a.	n.a.	
Albufera Natural Park wetland, Valencia, Spain	2017	PFASs and PFRs	Sediment	19	<LOD – 3.2 (0.3)	<LOD – 21.4 (4.9)	0.6 – 246.5 (53.8)	<LOD – 27.3 (7.3)	Present study
			Water	12	5.6 – 16.0 (9.7)	<LOD – 47.8 (31.6)	<LOD – 330.2 (70.4)	<LOD – 39.6 (6.4)	
			Fish	10	n.d.	<LOD – 194.5 (30.4)	<LOD – 13.1 (1.9)	<LOD – 1.7 (0.6)	

[a]ng L^{-1} in water, ng g^{-1} dw in sediment, ng g^{-1} ww in biota; <LOD: below limit of detection; n.a. not analysed; n.d. not detected

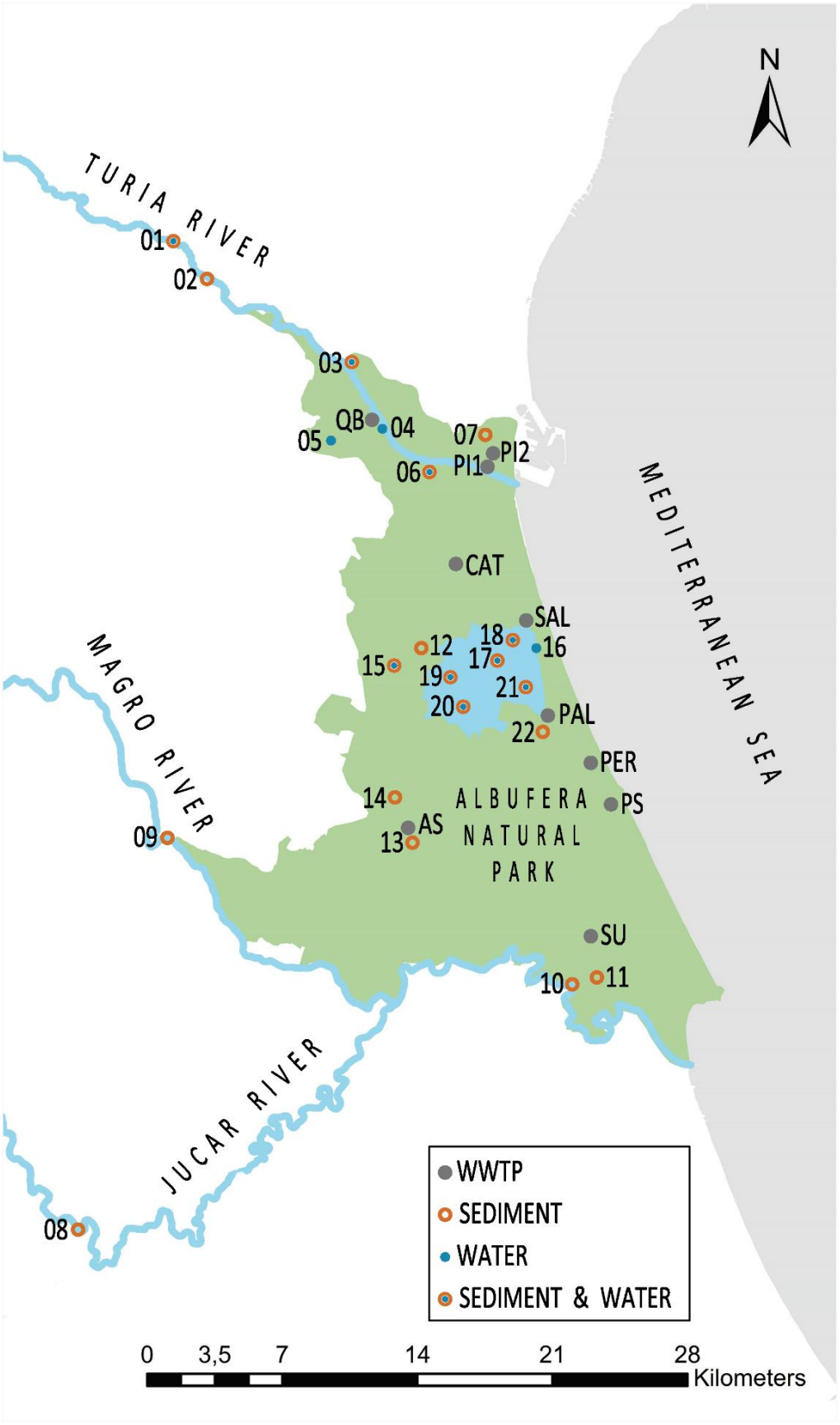

Figure 1. Location of wastewater treatment plants (WWTP) and sampling points of sediment and/or surface water.

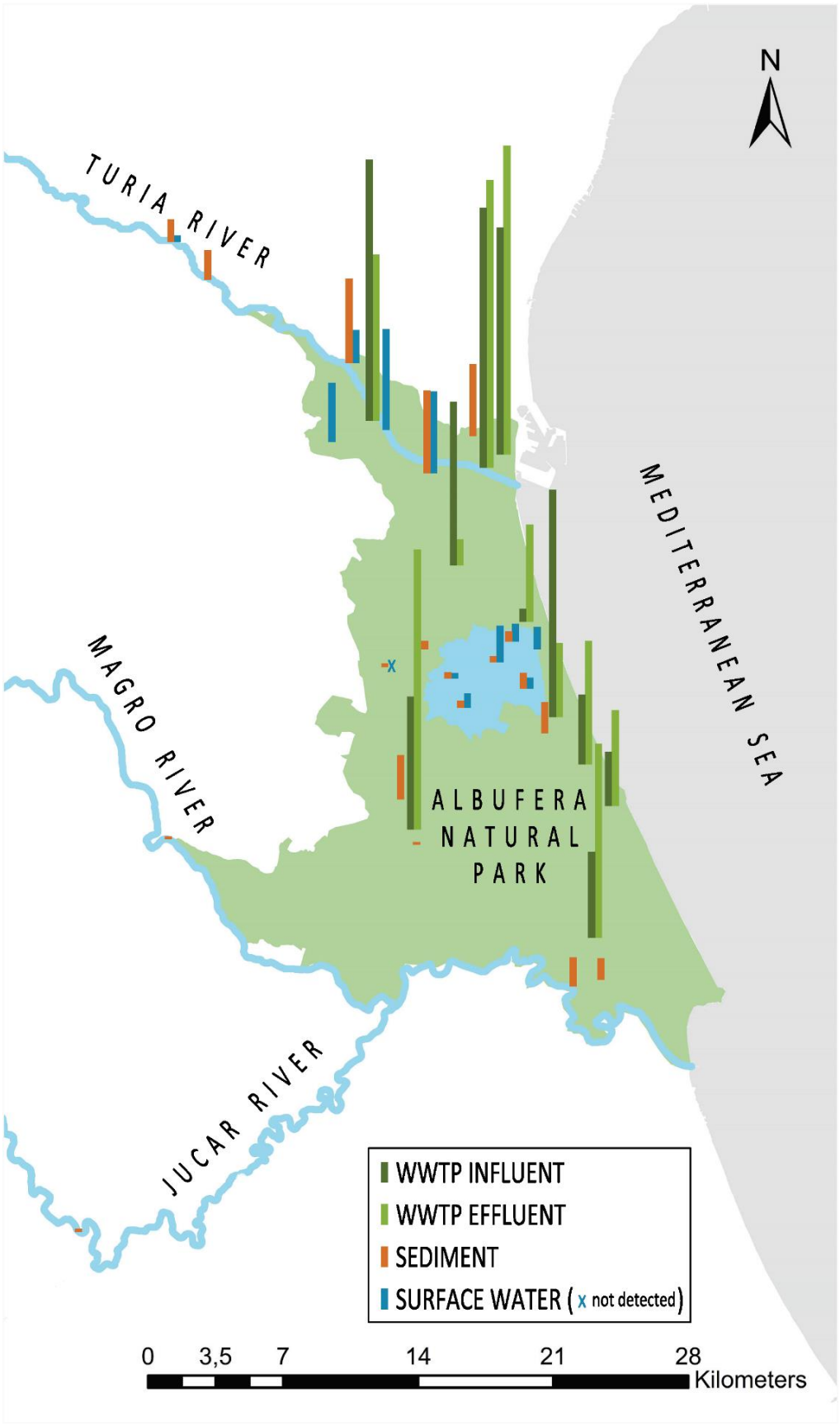

Figure 2. Cumulative concentration of PFR in the sampling points (ng g^{-1} dw in sediment and ng L^{-1} in water) and WWTPs (ng L^{-1}).

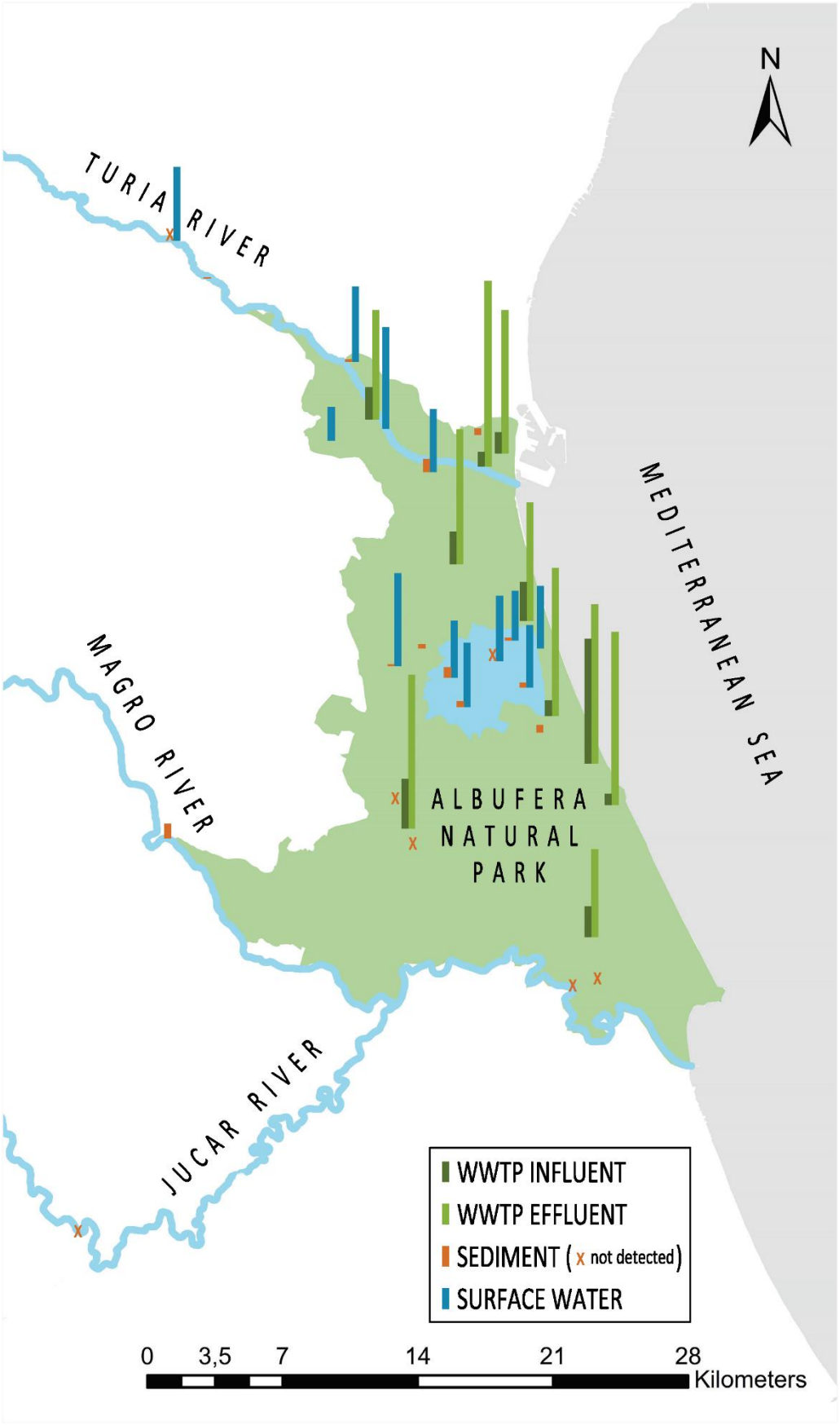

Figure 3. Cumulative concentration of PFASs in the sampling points (ng g^{-1} in sediment and ng L^{-1} in water) and WWTPs (ng L^{-1}).

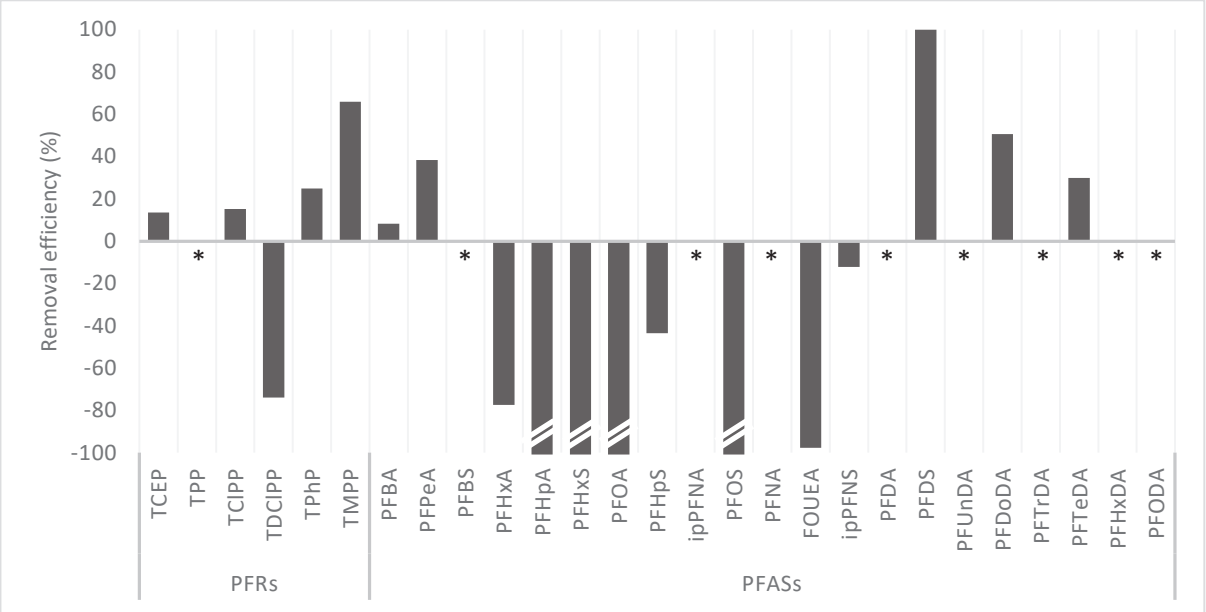

Figure 4. Mean WWTPs removal efficiency (%) of PFRs and PFASs. Compounds with (*) were only found in effluent samples.

SUPPLEMENTARY MATERIAL

Table S1. Target compounds for this study, acronym, CAS number, formula Log K_{ow} and solubility in water.

Compound name	Acronym	CAS number	Formula	Log K_{ow}	Solubility in water (mg L^{-1}) at 25˚C
Organophosphate flame retardants	***PFRs***				
Tripropyl phosphate	TPP	513-08-6	$C_9H_{21}O_4P$	2.67[a]	827[a]
Tris(2,3-dibromopropyl) phosphate	TDBPP	126-72-7	$C_9H_{15}Br_6O_4P$	4.29[b]	Not available
Tris(2-ethylhexyl) phosphate	TEHP	78-42-2	$C_{24}H_{51}O_4P$	4.22[a]	0.6[a]
Tricresyl phosphate	TMPP	1330-78-5	$C_{21}H_{21}O_4P$	6.34[a]	0.02[a]
Triphenyl phosphate	TPhP	115-86-6	$C_{18}H_{15}O_4P$	4.59[a]	1.9[a]
Tris(1,3-dichloro-2-propyl) phosphate	TDCIPP	13674-87-8	$C_9H_{15}Cl_6O_4P$	3.8[a]	1.5[a]
Tris(2-chloroethyl) phosphate	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$	1.44[a]	7000[a]
Tris(2-chloroisopropyl) phosphate	TCIPP	13674-84-5	$C_9H_{18}Cl_3O_4P$	2.59[a]	1600[a]
Cresyl diphenyl phosphate	CDP	26444-49-5	$C_{19}H_{17}O_4P$	4.51[a]	0.2[a]
Internal standards for PFRs					
Triphenyl phosphate (D15)	d_{15}-TPHP	1173020-30-8	$C_{18}D_{15}O_4P$	Not available	Not available
Tris(1,3-dichloro-2-propyl) phosphate (D15)	d_{15}-TDCIPP	Not available	$C_9D_{15}Cl_6O_4P$	Not available	Not available
Tris(2-chloroethyl) phosphate (D12)	d_{12}-TCEP	Not available	$C_6D_{12}Cl_3O_4P$	Not available	Not available
Tris(2-chloroisopropyl) phosphate (D18)	d_{18}-TCIPP	Not available	$C_9D_{18}Cl_3O_4P$	Not available	Not available
Perfluoroalkyl substances	***PFAS***				
Perfluorobutanoate	PFBA	375-22-4	$C_4F_7O_2H$	Not available	447[e]
Perfluoropentanoate	PFPeA	2706-90-3	$C_5F_9O_2H$	Not available	120[e]
Perfluorohexanoate	PFHxA	307-24-4	$C_6F_{11}O_2H$	Not available	29.5[e]

Perfluoroheptanoate	PFHpA	375-85-9	$C_7F_{13}O_2H$	Not available	Not available
Perfluorooctanoate	PFOA	335-67-1	$C_8F_{15}O_2H$	Not available	3400[e]
Perfluoro-7-methyloctanoate	ipPFNA	Not available	$C_9F_{17}O_2H$	Not available	Not available
Perfluorononanoate	PFNA	375-95-1	$C_9F_{17}O_2H$	Not available	Not available
Perfluorodecanoate	PFDA	335-76-2	$C_{10}F_{19}O_2H$	Not available	Not available
Perfluoroundecanoate	PFUnDA	2058-94-8	$C_{11}F_{21}O_2H$	Not available	Not available
Perfluorododecanoate	PFDoDA	307-55-1	$C_{12}F_{23}O_2H$	Not available	Not available
Perfluorotridecanoate	PFTrDA	72629-94-8	$C_{13}F_{25}O_2H$	Not available	Not available
Perfluorotetradecanoate	PFTeDA	376-06-7	$C_{14}F_{27}O_2H$	Not available	Not available
Perfluorohexadecanoate	PFHxDA	67905-19-5	$C_{16}F_{31}O_2H$	Not available	Not available
Perfluorooctadecanoate	PFODA	16517-11-6	$C_{18}F_{35}O_2H$	Not available	Not available
2H-Perfluoro-2-decenoic acid	FOUEA	70887-84-2	$C_{10}F_{16}O_2H_2$	Not available	Not available
Perfluorobutane sulfonate	PFBS	29420-49-3	$C_4F_9SO_3$	Not available	4340[f]
Perfluorohexane sulfonate	PFHxS	82382-12-5	$C_6F_{13}SO_3$	Not available	243.4[f]
Perfluoroheptane sulfonate	PFHpS	Not available	$C_7F_{15}SO_3$	Not available	Not available
Perfluorooctane sulfonate	PFOS	4021-47-0	$C_8F_{17}SO_3$	Not available	680[c]
Perfluoro-7-methyloctane sulfonate	ipPFNS	Not available	$C_9F_{19}SO_3$	Not available	Not available
Perfluorodecane sulfonate	PFDS	Not available	$C_{10}F_{21}SO_3$	Not available	Not available
Internal standards for PFASs					
Perfluoro-n-[1,2,3,4-$^{13}C_4$] butanoate	MPFBA	CASID 39196	$^{13}C_4HF_7O_2$	Not available	Not available
Perfluoro-n-[1,2-$^{13}C_2$] hexanoate	MPFHxA	CASID 39197	$^{13}C_2{}^{12}C_4HF_{11}O_2$	Not available	Not available

Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoate	MPFOA	CASID 39199	$^{13}C_4{}^{12}C_4HF_{15}O_2$	Not available	Not available
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoate	MPFNA	CASID 39198	$^{13}C_5{}^{12}C_4HF_{17}O_2$	Not available	Not available
Perfluoro-n-[1,2-$^{13}C_2$] decanoate	MPFDA	CASID 39200	$^{13}C_2{}^{12}C_8HF_{19}O_2$	Not available	Not available
Perfluoro-n-[1,2-$^{13}C_2$] undecanoate	MPFUnDA	CASID 39202	$^{13}C_2{}^{12}C_9HF_{21}O_2$	Not available	Not available
Perfluoro-n-[1,2-$^{13}C_2$] dodecanoate	MFDoDA	Not available	$^{13}C_2{}^{12}C_{10}HF_{23}O_2$	Not available	Not available
Perfluoro-1-[$^{18}O_2$] hexanesulfonate	MPFHxS	Not available	$C_6F_{13}S^{18}O_2O$	Not available	Not available
Perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	MPFOS	Not available	$^{13}C_4{}^{12}C_4F_{17}SO_2O$	Not available	Not available

[a] I. van der Veen, J. de Boer, Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis, Chemosphere, 88 (2012) 1119-1153; [b] Estimation Programs Interface (EPI) SuiteTM (EPIWEB 4.1) developed by the US Environmental protection Agency's Office of Pollution Prevention and Toxics and Syracuse Research Corporation (SRC); [c] Organisation for Economic Co-operation and Development (OECD), Hazard Assessment of Perfluorooctane Sulfonate (PFOS) and its Salts (2002); [d] U.S. EPA (2005); [e] G. Ding and W. J. G. M. Peijnenburg, Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds, Critical Reviews in Environmental Science and Technology, 43 (2013) 598–678; [f] United Nations Environment Programm, Technical paper on the identification and assessment of alternatives to the use of perfluorooctane sulfonic acid in open applications, Persistent Organic Pollutants Review Committee Eighth meeting (2012) UNEP/POPS/POPRC.8/INF/17.

Table S2. Characteristics of wastewater treatment plants (WWTP).

Sample code	WWTP name	Treated wastewater	Municipalities served	Caudal (m^3 day^{-1})	Type of sample collected	Number of samples collected
QB	Quart-Benàger	40% urban, 60% industrial	Alaquàs, Aldaia, Manises, Mislata, Quart de Poblet, Xirivella	30.318	Influent, effluent	2
PI1	Pinedo I	100% urban	València	94.979	Influent, effluent	4
PI2	Pinedo II	100% urban	València	208.779	Influent, effluent	4
CAT	Port de Catarroja	Not available	Catarroja	35	Influent, effluent	4
SAL	Saler	Not available	València	1.824	Influent, effluent	2
PAL	Palmar	Not available	València	349	Influent, effluent	2
PER	Perellonet	Not available	València	864	Influent, effluent	2
PS	Perelló-Sueca	Not available	Sueca	1.942	Influent, effluent	2
AS	Albufera Sud	Not available	Alginet, Almussafes, Benifaió, Sollana	18.528	Influent, effluent	2
SU	Sueca	Not available	Sueca	12.100	Influent, effluent	2

Table S3. Physical and chemical characteristics for water (W) and sediment (S) sampling points.

Sample name	Type of sample collected	Type of water	Use of land	pH water	EC[a] in water (dS m^{-1})	TDS[b] in water (mg L^{-1})	NaCl in water (mg L^{-1})	DO[c] water (%)	EC extract in sediment (dS m^{-1})	CO_3Ca in sediment (%)	OM[d] in sediment (%)	Silt and clay in sediment (< 0.05 mm) (%)
1	W, S	Turia River	Natural	7.9	1.26	666.8	614.1	90.8	1.58	31.47	1.23	2.33
2	S	Turia River	Natural	-	-	-	-	-	3.10	29.99	3.84	10.93
3	W, S	Irrigation channel	Horticulture	7.7	1.30	690.0	625.9	100.6	1.35	37.87	6.88	14.11
4	W	Irrigation channel	Horticulture	7.3	1.48	784.7	724.2	93.6	-	-	-	-
5	W	Irrigation channel	Horticulture	7.8	1.30	688.4	625.9	91.4	-	-	-	-
6	W, S	Irrigation channel	Horticulture	7.7	1.44	764.8	684.6	83.8	1.49	37.00	4.56	6.58
7	S	Irrigation channel	Horticulture	-	-	-	-	-	3.97	30.51	6.68	14.00
8	S	Jucar River	Natural	-	-	-	-	-	2.05	65.22	1.76	14.56
9	S	Magro River	Natural	-	-	-	-	-	2.10	48.44	0.81	2.86
10	S	Irrigation channel	Citrus	-	-	-	-	-	4.28	39.83	3.75	12.96
11	S	Irrigation channel	Rice	-	-	-	-	-	1.80	36.36	1.70	1.83
12	S	Irrigation channel	Rice	-	-	-	-	-	4.36	42.44	3.63	19.32
13	S	Irrigation channel	Citrus	-	-	-	-	-	1.44	15.80	1.30	14.07
14	S	Irrigation channel	Citrus	-	-	-	-	-	2.69	25.97	2.18	4.17
15	W, S	Irrigation channel	Citrus	8.2	1.78	644.1	873	96.7	1.59	22.12	2.48	10.11
16	W	Pujol Gola	-	7.8	1.53	808.5	725.9	79	-	-	-	-
17	W, S	Albufera lake	Natural	7.9	1.56	790.4	730.6	88.3	5.48	65.24	5.35	35.15
18	W, S	Albufera lake	Natural	8.1	1.60	848.8	777.4	84.3	4.41	54.25	3.88	43.84
19	W, S	Albufera lake	Natural	8.0	1.44	772.8	655.9	86.1	5.06	69.83	6.42	38.48
20	W, S	Albufera lake	Natural	8.4	1.42	763.3	663	97.2	5.00	76.21	7.41	39.16
21	W, S	Albufera lake	Natural	8.2	1.45	771.7	679.2	101.4	4.74	68.94	6.36	14.02
22	S	Irrigation channel	Rice	-	-	-	-	-	5.20	43.60	2.22	3.99

[a] EC: Electrical conductivity; [b] TDS: Total dissolved solids; [c] DO: Dissolved Oxygen; [d] OM: Organic Matter.

Table S4. Extraction methods for the different type of samples analysed.

Type of sample	Pre-treatment of samples	Extraction method	Clean-up
Water (surface water, wastewater influent and effluent)	Glass microfiber filter (90 mm Ø)	-	Precondition of STRATA-X Polymeric Reversed Phase cartridges, pass the sample through cartridges by vacuum, keep vacuum 10-15 min to dry the cartridge, elute target compounds with 8 ml methanol, evaporate to dryness, redissolve with 250 µl methanol, transfer to the LC-MS/MS vial.
Sediment	Lyophilize (48-72 h) and sieve (2-mm Ø)	1 g of sample, 10 ml methanol, vortex 3 min, ultrasound 15 min, centrifuged 5 min, repeat three times, evaporate to 1 ml, add to a 250 ml volumetric flask with ultrapure water	
Fish samples	Crush the whole fish	1 g of sample, 10 ml methanol, vortex 3 min, ultrasound 15 min, centrifuged 5 min, repeat three times, evaporate to 1 ml, add to a 250 ml volumetric flask with ultrapure water	

Table S5. Instrumental characteristics used for PFASs and PFRs determination.

LC CONDITIONS	
Analytical column	Kinetex XB-C18: 50.0 × 4.6 mm, 1.7 µm (Phenomenex, Torrance, USA)
Trap column (only for ***PFRs***)	ZORBAX Eclipse Plus C18: 4.6 x 30 mm, 3.5 µm (Agilent, Santa Clara, USA)
Column temperature	30 °C
Volume injected	5 µL
Mobile phase	***PFASs***: (A) Water – (B) Methanol both with 2.5 mM Ammonium fluoride ***PFRs***: (A) Water – (B) Methanol both with 0.1% Formic Acid
Flow rate	0.2 mL min^{-1}
Linear gradient	***PFASs***: 0 min (30 % B), 0.5 min (30 % B), 12 min (95 % B), 20 min (95 % B), and return to the initial conditions (equilibration time 12 min). ***PFRs***: 0 min (30% B), 0.5 min (30% B), 12 min (95% B), 18 min (98% B) and 25 min (98% B), and return to the initial conditions (equilibration time 15 min).
TRIPLE QUADRUPOLE MS/MS CONDITIONS	
Ionization characteristics and source	MS/MS performed in selected reaction monitoring mode (SRM) with electrospray ionization (ESI) in negative mode for ***PFASs*** and positive for ***PFRs***.
Gas temperature	300° C
Gas flow	11 L min^{-1}
Nebulizer	30 psi
Capillary voltage	4000 V
Chamber current	1.27 µA
Scan type	MRM, with MS1 and MS2 at unit resolution and cell acceleration voltage of 7 eV

Table S6. SRM dynamic conditions for the determination of PFRs and PFASs by UHPLC-QqQ-MS/MS.

Compounds[a]	t_R[b] (min)	Precursor ion for MS_1[c]	MS_1[c]	Frag[d] (V)	CE[e] (V)	Precursor ion for MS_2 and MS_3[c]	MS_2[f]	Frag (V)	CE (V)	MS_3[g]	Frag (V)	CE (V)
PFRs												
d_{12}-TCEP	*10.5*	*299*	*67*	*100*	*20*	*297*	*67*	*100*	*20*	-	-	-
TCEP	10.7	287	99	100	15	285	99	100	15	223	100	10
TPP	15	225	99	84	15	225	141	84	3	81	84	55
d_{18}-TCIPP	*14.8*	*347*	*102*	*100*	*20*	*345*	*102*	*100*	*20*	-	-	-
TCIPP	14.9	329	99	80	15	327	99	80	15	175	80	10
d_{15}-TDCIPP	*16.6*	*448*	*102*	*120*	*15*	*446*	*102*	*120*	*15*	-	-	-
TDCIPP	16.7	433	99	80	15	431	99	80	15	-	-	-
d_{15}-TPhP	*16.7*	*342*	*82*	*120*	*47*	*342*	*160*	*120*	*47*	-	-	-
TPhP	16.8	327	77	117	47	327	152	117	43	215	117	27
TDBPP	17.2	699	99	120	25	697	99	120	25	-	-	-
CDP	17.4	341	91	167	39	341	65	167	67	152	167	43
TMPP	18.4	369	165	192	51	369	165	192	31	91	192	43
TEHP	22.8	435	99	113	7	435	71	113	15	-	-	-
PFASs												
PFBA	8.0	213	169	66	5	-	-	-	-	-	-	-
MPFBA	*8.0*	*217*	*172*	*62*	*0*	-	-	-	-	-	-	-
PFPA	8.9	263	219	66	5	-	-	-	-	-	-	-
PFBS	9.2	299	99	142	38	299	80	142	26	-	-	-
PFHxA	13.3	313	269	71	5	313	119	71	5	-	-	-
MPFHxA	*13.3*	*315*	*270*	*71*	*5*	*315*	*119*	*71*	*5*	-	-	-
PFHpA	15.4	363	319	76	5	363	169	76	5	-	-	-
PFHxS	15.6	399	99	169	37	399	80	169	29	-	-	-
MPHxS	*15.6*	*403*	*103*	*164*	*33*	*403*	*84*	*164*	*37*	-	-	-
PFOA	17.2	413	369	87	5	413	169	87	5	-	-	-
MPFOA	*17.2*	*417*	*372*	82	*5*	*417*	169	82	*13*	-	-	-

PFHpS	17.3	449	99	179	37	449	80	179	57	-	-	-
ipPFNA	19.3	463	419	87	5	463	169	87	5	-	-	-
PFNA	19.5	463	419	82	5	463	219	82	5	-	-	-
MPFNA	*19.5*	*468*	*423*	*82*	*5*	*468*	*223*	*82*	*9*	-	-	-
PFOS	19.9	499	99	190	41	499	80	190	65	-	-	-
MPFOS	*19.9*	*503*	*99*	*180*	*41*	*503*	*80*	*180*	*61*	-	-	-
PFDA	25.5	513	469	89	5	513	269	89	13	-	-	-
MPFDA	*25.5*	*515*	*470*	*92*	*5*	*515*	*270*	*92*	*12*	-	-	-
ipPFNS	25.5	549	99	195	45	549	80	195	73	-	-	-
PFUnDA	28.1	563	519	104	5	563	269	104	13	-	-	-
MPFUnDA	*28.1*	*565*	*520*	*94*	*5*	*565*	*269*	*94*	*13*	-	-	-
PFDS	28.2	599	99	80	80	599	80	80	80	-	-	-
PFDoDA	32.7	613	569	94	5	613	269	94	13	-	-	-
MPFDoDA	*32.7*	*615*	*570*	*112*	*5*	-	-	-	-	-	-	-
PFTrDA	33.4	663	619	104	0	663	169	104	24	-	-	-
PFTeDA	34.0	713	669	112	5	713	169	112	25	-	-	-
PFHxDA	35.2	813	769	114	8	813	169	114	28	-	-	-
PFODA	35.8	913	869	134	10	913	169	128	29	-	-	-

(a) Compounds ordered by retention time; (b) t_R = retention time; (c) MS_1 = selected product ion for quantification; (d) Frag = fragmentor; (e) CE = collision energy; (f) MS_2 = selected product ion for qualification; (g) MS_3 = selected product ion for qualification. Italics mean isotopically labelled internal standard.

CAPÍTULO 8

Emerging contaminants related to the occurrence of forest fires in the Spanish Mediterranean.

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Emerging contaminants related to the occurrence of forest fires in the Spanish Mediterranean

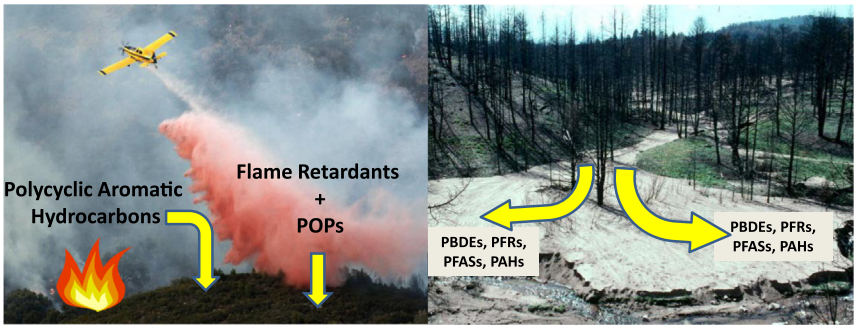
Julian Campo [a,b,*], María Lorenzo [c], Erik L.H. Cammeraat [b], Yolanda Picó [c], Vicente Andreu [a]

[a] Environmental Forensic and Landscape Chemistry Research Group, Desertification Research Centre – CIDE, (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana), Carretera Moncada – Náquera km 4.5 (Campus IVIA), 46113 Moncada, Valencia, Spain
[b] Department of Ecosystem and Landscape Dynamics, Institute for Biodiversity and Ecosystems Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands
[c] Food and Environmental Safety Research Group (SAMA – UV), Desertification Research Centre – CIDE, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

HIGHLIGHTS

- Assessment of PBDEs, PAHs, PFRs and PFASs distribution in burned and unburned hillslopes
- BDE-85 concentrations were highest so no PBDE mixture was present in the fire extinguisher.
- The fire added significant PAH amounts into the soil (1256 ng g^{-1}), mainly in the upper 2 cm.
- PFRs and PFASs were found in both hillslopes with values up to 352 ng g^{-1} and 17 ng g^{-1}, respectively.
- Contaminants transport downslope in the erosion events depends on the compound nature.

GRAPHICAL ABSTRACT



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ABSTRACT

Forest fires can be a source of contamination because, among others, of the use of chemicals to their extinction (flame retardants, FRs), or by the production of Polycyclic Aromatic Hydrocarbons (PAHs) derived from high temperature alteration of organic matter. Up to our knowledge, this study is the first to assess the direct (PAHs 16 on the USA EPA's priority list), and indirect [tri- to hepta- brominated diphenyl ethers (PBDEs), organophosphorus flame retardants (PFRs) and perfluoroalkyl substances (PFASs)] contamination related to forest fires. The abundance and distribution of these contaminants were monitored on two Mediterranean hillslopes, one burned and one unburned, near Azuébar (SE Spain). Samples were taken in the foot, middle, and top of the slope, at two depths, and in two environments (under canopy and bare soil). Sediments were collected from sediment fences after erosive rainfall events. Most of the screened compounds were found in both, burned and control hillslopes, though significant differences were found between both. In burned soil, low concentrations of PBDEs (maximum ΣPBDEs: 7.3 ng g^{-1}), PFRs (664.4 ng g^{-1}) and PFASs (56.4 ng g^{-1}) were detected in relation to PAHs (Σ16 PAHs = 1255.3 ng g^{-1}). No significant influence of the hillslope position was observed for any of the contaminants but differences based on depth and vegetation presence tended to be significant, particularly for the PAHs. After the first erosive event, concentrations of PBDEs and PAHs were higher in sediment than in soil (ΣPBDEs: 17.8 ng g^{-1} and Σ16 PAHs = 3154.2 ng g^{-1}) pointing out the importance of connectivity processes, especially shortly after fire.

* Corresponding author at: Environmental Forensic and Landscape Chemistry Research Group, Desertification Research Centre – CIDE, (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana), Carretera Moncada – Náquera km 4.5 (Campus IVIA), 46113 Moncada, Valencia, Spain.
E-mail address: Julian.Campo@uv.es (J. Campo).

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1. Introduction

According to the International Panel on Climate Change, there is already a high degree of confidence that meteorological conditions associated to climate change will be propitious to increasing extreme events (IPCC, 2014). Impacts on land degradation will be manifested in bigger and more frequent wildfires, and greater water stress. Since 1982, the total area affected by forest fires in Mediterranean countries of the European Union (EU) has declined, and the number of fires tends to stabilize. However, in countries like Spain and Portugal, the trend has been slightly upward in the last 5 years (European Commission, 2015), and forest fires are becoming more frequent (Spano et al., 2014; Turco et al., 2016). Based on Spanish Environment Ministry reports (MAAM 2012, 2014), the mean number of fires in Spain during 2001–2010 was 17,127, burning an average of 113,848 ha of forest surface. In Valencian Community (located in the Mediterranean East of Spain), the average area burned in 1991–2010 was of 8706 ha yr^{-1} but in 1994, 751 fires occurred, one of which burned >25,000 ha. In 2014, there were 92 wildfires in Valencia affecting 1800 ha of woody forest and 116 ha of forest herbaceous.

It is well known that environmental degradation processes are interrelated. One aspect of fire-induced ecosystem degradation that has drawn scientific interest recently is the chemical pollution related to forest fires. This contamination is associated either to the use of chemicals to their extinction known as flame retardants (FRs) (Pepper et al., 2011), or because pyrolysis or incomplete combustion of litter and standing vegetation favours the formation of toxic compounds as polycyclic aromatic hydrocarbons (PAHs) (Yuan et al., 2008). FRs are not only used to extinguish fires but also in the production process of electronics, furniture, clothes and cars (Boyles et al., 2017; Covaci et al., 2003, 2011). Perfluoroalkyl substances (PFASs) are widely used in aqueous film-forming foams (AFFF) but also in industrial and consumer products as protective coatings for textiles and paper, in the production of semiconductors and as polymer additives in herbicide and insecticide formulations and in cosmetics (Hale et al., 2017; Hu et al., 2016). AFFFs, are usually spread over pool fires and they have limited post-fire security and are toxic groundwater contaminants (Hinnant et al., 2017). In general, AFFFs containing PFASs are not expected to be used in the extinction of forest fires. They are usually applied for suppression of combustion in industrial and commercial sites, and particularly in fire training areas (Hu et al., 2016). However, the possible presence and degradation of PFASs in relation to forest fires have been never measured. These compounds are persistent, bio-accumulative and/or toxic to wildlife and humans, as well as potential endocrine disruptors (Campo et al., 2016; Duan et al., 2015; Eulaers et al., 2014; Segev et al., 2009). Consequently, penta-BDEs, octa-BDEs, perfluorooctane sulfonate (PFOS) and perfluorooctyl sulfonyl fluoride (POSF) have been included as Persistent Organic Pollutants in the Stockholm Convention, and PAHs are candidates for their inclusion (UNEP, 2010). Additionally, in the EU to comply with the Water Framework Directive (WFD), Directive 2013/39/EU set the Environmental Quality Standards in the field of water policy for Priority Substances and other pollutants, including PAHs, which must be controlled to progressively reduce discharges and losses.

Brominated flame retardants (BFRs) account for a large group of FRs used for firefighting. Polybrominated diphenyl ethers (PBDEs) are the second highest production group of BFRs, and their presence has been reported in different environmental matrixes as water (Wang et al., 2017; Ricklund et al., 2010); sediment (Ross et al., 2009); soil (Akortia et al., 2017) and biota (Eulaers et al., 2014; Boyles et al., 2017). In soils and sediments, photolytic degradation and debromination are possible (Segev et al., 2009; Lee and He, 2010). Debromination can be caused by microbial activity and can be harmful for the environment because lower brominated PBDEs are considered to be more toxic (Rodenburg et al., 2014). The sources of PBDEs contamination are leaching from a wide range of plastics, electronic equipment and textiles (Akortia et al., 2017; Covaci et al., 2011) or their incineration and subsequent long-range transport in air. de Wit et al. (2006) reported their presence in living organisms and air of the Arctic. BDE-47, −99 and −209 are also present in sewage sludge (de Wit, 2002; Wu et al. 2017). The production and use of the most common PBDEs, penta- octa- and deca-BDE, is nowadays highly restricted in the USA and EU (Kemmlein et al., 2009). Despite PBDEs have been used for fire extinguishing (Alaee et al., 2003; Pepper et al., 2011) there are not published studies on their occurrence in relation to forest fires.

Since the ban on some BFRs, phosphorus flame retardants (PFRs) have been proposed as an alternative (van der Veen and de Boer, 2012). PFRs are widely used as plasticizers and anti-foaming agents in a variety of industries including plastics, furniture, textile, electronics, construction, vehicles and petroleum industries (Wei et al., 2015). Only few reports on possible PFR adverse effects have been published (Araki et al., 2014; Dishaw et al., 2011; Farhat et al., 2014; Kojima et al., 2013; Sun et al., 2016; Wang et al., 2015). Most of the recent research priorities have focused on the occurrence of PFRs in house dust, indoor and outdoor air, surface and ground waters, but data about their presence in soil and sediment after a forest fire are not available.

A small number of studies have already looked into PAH production in forest fires (Kim et al., 2003; Campo et al., 2011; Vergnoux et al., 2011; Choi, 2014). These studies have generally found a significant increase in soil PAH levels after fire, but, not so much to reach harmful levels. However, the increase could still affect local ecosystems (Pizarro-Tobías et al., 2015). In a fire, PAHs are formed by incomplete combustion of litter and standing vegetation (Kim et al., 2003), but they are also probably produced from soil organic matter (SOM). During fire only the upper few centimetres of soil could reach temperatures above 200 °C, at which formation of char and possibly PAHs starts (González-Pérez et al., 2004; Certini, 2005). Incorporation of partly burned organic material into the soil increases both its SOM (Campo et al., 2008) and PAH concentrations (Choi, 2014),

The produced PAHs are either volatilized or retained within the organic material from which they are formed. The volatilized PAHs may also become associated with organic material, as they easily adsorb onto litter, vegetation or floating ash particles (Kim et al., 2003; Choi, 2014). These organic materials are deposited on the surface forming a PAH-rich fire-litter layer (Johnsen and Karlson, 2007) and can become incorporated in the soil, increasing soil PAH levels. PAHs can also enter the soil by moving downwards in gaseous form, or by leaching from the fire-litter layer (Vergnoux et al., 2011; Choi, 2014). Several researchers have noted that most of the PAHs added with fire are removed from a location because the fire-litter, rich in both SOM and PAHs, is eroded downslope (Smith et al., 2011; Zheng et al., 2012; Luo et al., 2013). This eroded material would be transported to the streams as researched by Vila-Escalé et al. (2007), who stated that PAHs were more correlated with organic suspended substances than with total suspended substances.

The main of this study is to establish the impact of a high severity fire in a hillslope of Azuébar, Castellón (Spain) on the occurrence of PAHs and emerging POPs in soil and sediment from the burned area. Samples from coupled slopes (burned and unburned) were analysed and compared for PAHs, PBDEs, PFASs and PFRs concentrations. Furthermore, the specific objectives of the study are to (i) determine whether the fire occurred, in 2014, in Azuébar added significant amounts of PAHs and emerging POPs to the soil, (ii) establish possible relationships between concentrations and position on the hillslope, soil depth and presence of vegetation, and (iii) study the transport of the contaminants downslope. To our knowledge, this is the first report on the presence of flame retardants in burned soils.

2. Material and methods

2.1. Study site and sampling

This work was carried out in the municipality of Azuébar, Natural Park of Sierra de Espadán, in the Province of Castellón, Spain (Fig. 1).

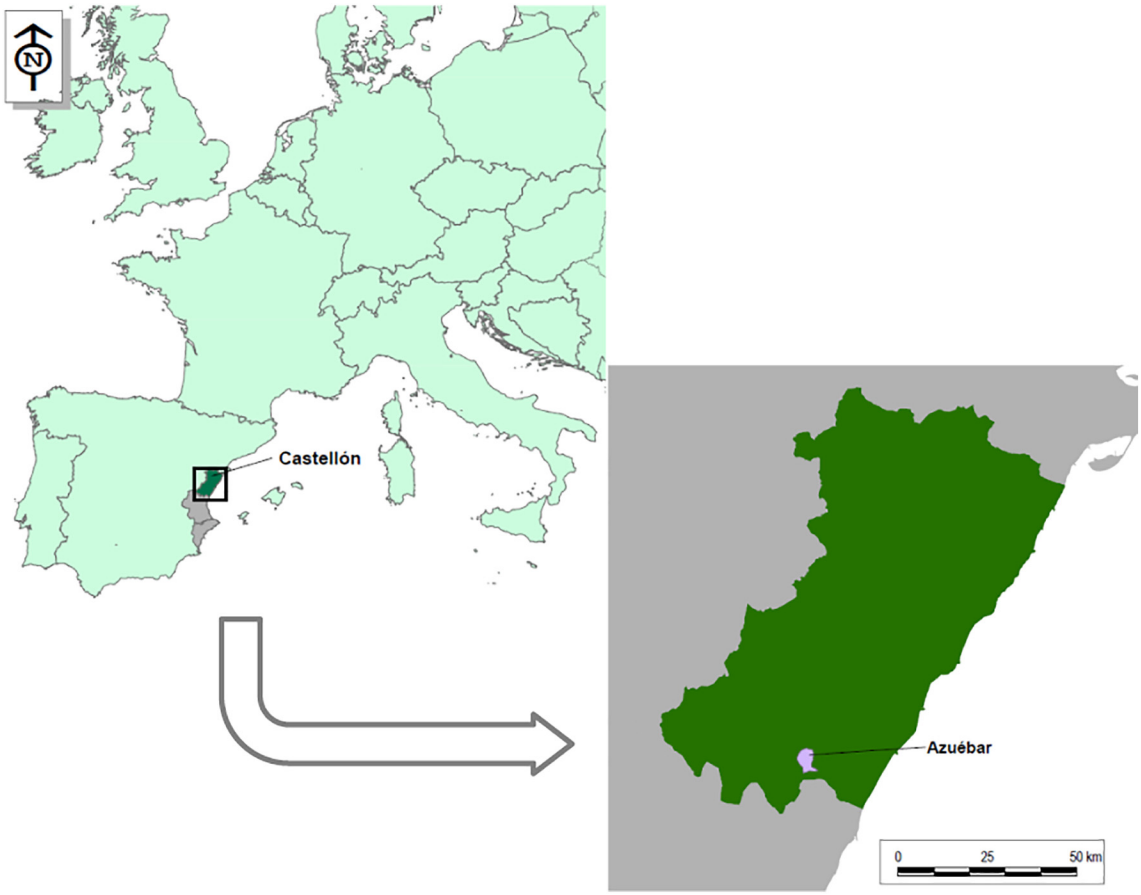

Fig. 1. Location of the study area. Coupled hillslopes in the municipality of Azuébar, Castellón (Spain), were used (Burned: 39°50′45.11″N, 0°22′20.52″W; Control: 39°51′08.7″N, 0°22′17.6″W).

Coupled hillslopes (burned: BU, and control: CO) belonging to the coastal foothills of the Iberian Mountain System were selected (BU: 39°50′45.11″N, 0°22′20.52″W; CO: 39°51′08.7″N, 0°22′17.6″W). Both slopes are located on forested concave hillsides, with ENE aspect, 25–28° of slope and an altitude around 370 m a.s.l. (more information is available in Supplementary information, SI, Fig. S1).

The climate of the area is meso-Mediterranean (mean annual precipitation of 450 mm), with a maximum precipitation in autumn (70 mm in October) and a second, but less rainy period in spring (45 mm in May). According to a close climatological station (Sot de Ferrer, 4.5 km from the hillslopes), the dry period (June–September), in which usually forest fires occur, presents a maximum temperature of 28 °C. The mean annual temperature is 16 °C (minimum of 5 °C approx.). Vegetation cover is characterized by a Mediterranean shrubland developed after recurrent wildfires in the zone. Soils are classified as Luvisol Chromic Skeletic type according to the FAO (2006), developed on Triassic dolomite, which show variable depth, always <50 cm thick, with 40% gravel content and clay-loam texture (Fig. S2).

Last wildfire on Azuébar municipality occurred on 28 August 2014 and burned 10.59 ha of forested area, according to the Emergency Coordination Centre of the Generalitat Valenciana. Eight air assets, as well as six emergency brigades participated in its extinction. About 70% of the affected area was in high- or moderate-high burn severity classes according to field burn indexes (Bento-Gonçalves et al., 2012). This municipality was affected by several wildfires during last years, registering up to five intentioned fires between 2012 and 2014.

Coupled Mediterranean hillslopes were sampled (19/09/2014) following a connectivity design according to which the top of the hillslope is the eroding zone, the middle part is the transport site and the foot's slope is the depositional zone (Bracken et al., 2015). Soil was taken in two environments (under canopy soil: UC; inter-plants or bare soil: BS), and in two depths (TS: 0–2 cm and SS: 2–5 cm). Samples from each location, environment and depth (BU: $n = 12$; CO: $n = 12$; Table S1) were transported in sealed plastic containers, and dried at room temperature. All samples were dried at air temperature and sieved to <2 mm. Sediments were collected from four sediment fences constructed at the foot of the burned slope (Fig. S3). Sediment was negligible in the control hillslope, and samples could not be collected.

2.2. Sample extraction

The analysis for PBDEs was done simultaneously with the one for PAHs and the same with PFASs and PFRs analysis. Due to isomerism a total of 209 PBDE congeners is possible, but in this research only the abundance of some tetra-, penta-, hexa-, hepta-BDE congeners is assessed (Table S2). All soil and sediment samples were analysed for their concentrations of the 16 PAHs on the EPA's priority list, PFRs and PFASs listed in Table S2.

2.2.1. PBDEs and PAHs

Extraction of the PBDEs and PAHs from the samples was done by pressurized liquid extraction (PLE) using an ASE (Dionex ASE 200, Sunnywale, CA, USA). Sample (2 g) were placed into stain steel extraction cell (6 cm) and sea sand (particle size 30–50 mesh, Fisher Chemicals) was loaded after the sample to avoid any void spaces. Both, PBDE and PAH internal standards (50 μL of each), were added before extraction. The PBDE internal standard contained three PBDEs (BDE-47, BDE-99 and BDE-153, which are tetra-, penta- and hexa-BDE congeners) ^{13}C labelled and dissolved in toluene. The PAH internal standard was a standard solution of the EPA's 16 PAHs, also labelled, in toluene (120 ng ml^{-1}). Two blank samples were prepared with only sand and internal standards to assess background noise and pollution during

the analysis process. The PLE was done using acetone/hexane (1:1) as solvent and with the following setting: pressure 1500 psi, temperature 100 °C, preheat time 5 min, heat time 5 min, static extraction time 10 min, flush 90%, purge time 120 s and 2 cycles.

The resulting extracts were concentrated to 0.5 ml by heating them in a sand bath and slowly evaporating with a Vigreux column. The extracts were cleaned up through a column packed with 1 cm of folded glass wool at the bottom, 6.75 cm hydrated Al_2O_3 (11% water) and 1 cm granulated Na_2SO_4, and eluted with 25 ml hexane. This method was successful in making most extracts clear and colourless. After the clean-up, the extracts were concentrated again with a Vigreux column to 1.0 ml. All extracts were stored dark, until analysis, to prevent degeneration of the compounds. Finally, 100 μl of the extracts were pipetted into a vial suitable for GC–MS.

2.2.2. PFRs and PFASs

PFRs and PFASs were extracted by ultrasound solid-liquid extraction (US-SLE) using methanol as described in Lorenzo et al. (2015). Briefly, 5 g of sample was weighted into 50 ml polypropylene tubes. PFRs and PFASs isotopically labelled standards were spiked to the samples. Then, 10 ml of methanol were added to each tube and they were vortexed for 3 min, sonicated for 15 min and centrifuged for 5 min (950 rcf). The process was repeated three times. Finally, the sample was cleaned up by solid-phase extraction (SPE). This was performed by passing the samples through Phenomenex Strata™ C-18 cartridges and the compounds were eluted with methanol. The samples were evaporated to dryness under nitrogen stream, re-dissolved in 250 μl of methanol and transferred to a vial suitable for LC-MS/MS.

2.3. Determinations

2.3.1. PBDEs and PAHs

The determination was carried out with a Thermo-Quest Trace GC 2000 gas chromatograph (Thermo Fischer Milan, 176 Italy). Separation was performed using a fused silica column (J&W, 60 m × 0.32 mm i.d.) coated with DB-5 (film thickness 0.50 μm) and helium as carrier gas. Injection volume was 2 μl and injection temperature 60 °C (cold on-column injection), with a temperature programme reaching up to 320 °C. For details about the GC method see Table S3. The column was coupled to a Finnigan Trace mass spectrometer (MS) with the following operating conditions: 70 eV ionization potential of the electron impact source, 250 °C ion source temperature, and data acquisition in Selected Ion Mode (SIM). MS was set to start acquiring at 6 min.

Target compounds were identified using Xcalibur Software by interpretation of the mass spectra considering their retention times, and/or by comparison with literature data (Korytár et al. 2005). Quantitation of identified compound was done with internal standard methodology based on peak areas.

2.3.2. PFRs and PFASs

The analysis was carried out in a 1260 Infinity ultra-high-performance liquid chromatograph combined with a 6410 triple quadrupole mass spectrometer of Agilent Technologies (Santa Clara, CA, USA) with electrospray ionization (ESI). Compounds were separated with a Kinetex C_{18} (50 × 2.1 mm, 1.7 μm) from Phenomenex (Torrance, CA, EEUU). For PFRs, ZORBAX Eclipse Plus C_{18} was selected as trap column in order to control background contamination. The mobile phases consisted of (A) water and (B) methanol, both containing 2.5 mM ammonium fluoride for PFASs and 0.1% formic acid for PFRs. The flow rate was kept at 0.2 ml min^{-1} throughout the run, and the sample volume injected was 5 μl. Analysis was performed in negative ion mode for PFASs and in positive ion mode for PFRs (instrumental characteristics are described in Table S4). Data acquisition was carried out in selected reaction monitoring (SRM) to identify and quantify using two precursor and product ion transitions (except for PFBA and PFPeA that gave only one transition), retention times, and the ratio of intensities between the two product ions. Data were processed using MassHunter Workstation Software for qualitative and quantitative analysis (GL Sciences, Tokyo, Japan).

2.4. Quality assurance/quality control

The analytical methods were carefully validated. The LOD was calculated as the mass of analyte required to produce a signal-to-noise ratio (S/N) of 3:1, where the noise is calculated as the standard deviation of the background signal. The LOQ was also established as the concentration whose S/N was 10:1. Method LOQ values were in the range from 0.3 to 0.8 ng g^{-1} dry weight (d.w.) for PBDEs, 0.2 to 2.8 ng g^{-1} d.w. for PAHs, 0.3 to 2.5 ng g^{-1} for PFRs and 0.83 to 3.33 ng g^{-1} for PFASs.

Calibration curves were prepared daily obtaining $R^2 > 0.98$. Prior and after the batch (28 samples), calibration curves were constructed by injecting standards at different concentrations (2–20 ng ml^{-1} for PBDEs and PAHs, 1–75 ng ml^{-1} for PFASs and 1–300 ng ml^{-1} for PFRs). Fig. S4 shows a chromatogram resulting from one of the external standard measurements. One instrumental and one procedural blanks as well as one spiked sample were analysed at the beginning and at the end of each batch to serve as quality control.

2.5. Statistical analyses

For the parametric analysis, normality of the data was tested using Shapiro-Wilk's test ($n < 50$) and homogeneity of variances was tested using Levene's test. Differences in the compound concentrations between treatments (BU, CO), hillslope positions (eroding, transport and deposition), environments (UC and BS) and depths (TS and SS) were established through analyses of variance with a General Linear Model (GLM), and confirmed *a posteriori* with the tests of Tukey's or *t*'student. Interaction of studied variables: treatment (T), position (P), environment (E) and depth (D) were also analysed. For non-parametric analysis, differences were determined by the tests of Mann–Whitney U (M-W) or Kruskal-Wallis (K-W). In all the cases, results were considered to be statistically significant at $p < 0.05$. The effect of soil organic carbon content on the contaminant distributions was quantified by calculating correlation relationships (Pearson's r) between these variables. All statistical analyses were carried out using IBM SPSS Statistics 22®.

3. Results

3.1. Soil

The hepta- and hexa-BDEs 153, 154 and 183 were not found in any of the soil samples. As shown in Table S5, concentrations of the detected tri- to penta-BDEs were in general low (the highest value was for BDE-85: 5.6 ng g^{-1} d.w.). PBDEs were observed in both, BU and CO soils with sums of the PBDE values ranging from 0.5 ng g^{-1} d.w. (CO) to 7.3 ng g^{-1} d.w. (BU) (Fig. 2). The most frequent compound was BDE-47, which was found in all the burned samples and in most of the control ones. On the other hand, BDE-85 was only detected in the BU samples.

Concentration values of the individual compounds were not normally distributed, while their sum actually was it. According to the M-W test, only the BDE-85 values showed significant differences between BU and CO samples. None of the variables, slope position, vegetation and depth, have a significant influence on PBDEs levels individually, however decreasing trends were observed from transport, to erosion and finally to deposition zones. Similarly, in TS compared to SS, and in UC compared to BS. BU soils presented higher concentrations of the ΣPBDEs than CO ones ($p < 0.05$). Significant differences for the ΣPBDEs were not found based on the position, vegetation or depth. Interactions between these variables were not significant according to a GLM.

In general, all PAHs were found in BU and CO soils (Table S6). Only Acy and DahA are more frequent in BU soils. Concentrations ranged

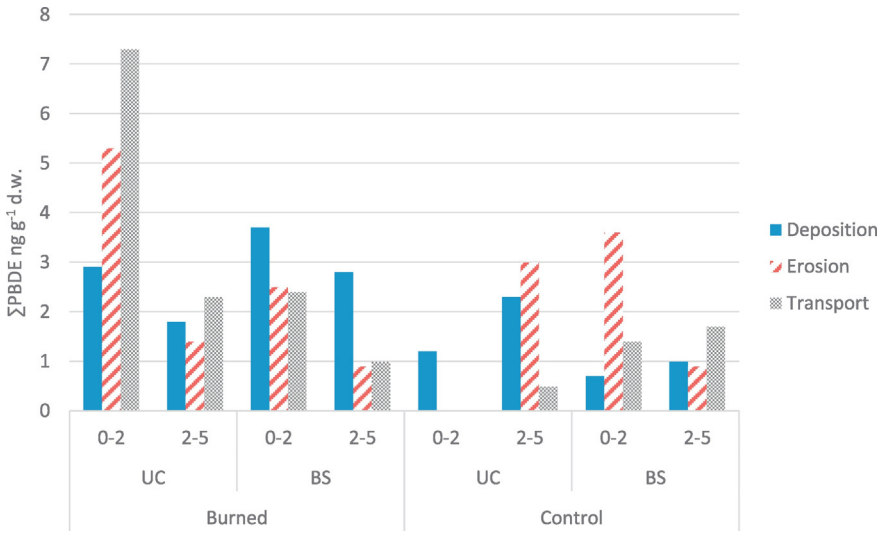

Fig. 2. Cumulated PBDE concentrations in the hillslope positions based on the different variables (Treatment: Burned, Control; Vegetation: UC: Under Canopy, BS: Bare Soil; Depth: TS: 0–2 cm, SS:2–5 cm).

from <LOQ (Acy) to 803 ng g^{-1} d.w. (Nap). Despite the large variation of compounds as Nap and Phe, no clear outliers were found. Fig. 3 shows the total concentration of 16 PAHs in soil samples, reaching up to 1255.3 ng g^{-1} d.w. in one of the burned hillslope TS samples. The proportion of light PAHs (2 and 3 aromatic rings) was higher in BU than in CO (Fig. 4a), indicating that these were the most produced by the fire. The heavy PAHs (4, 5 and 6 rings) were dominant in CO. The tendency in BU is actually dominated by just two light PAHs, Nap (2 rings) and Phe (3 rings).

Light PAHs and the ∑ 16 PAH were not normally distributed whereas heavy PAHs followed a normal distribution. According to the M-W test, the BU slope had significantly higher light and ∑ 16 PAH concentrations than the CO slope ($p < 0.05$). None of the other factors considered had a significant influence on their values. On the contrary, significant differences were obtained for the heavy PAHs in relation to treatment (BU > CO, $p < 0.05$) and depth (TS > SS, $p < 0.05$). For both, parametric and non-parametric tests, no significant differences associated to the slope position were found. Based on a GLM, double and triple interactions of the considered variables did not have a significant influence on the occurrence and concentration of PAHs in the area of study.

Regarding PFRs, TCEP, TPP, TDBPP and CDP were not detected in any soil sample. As shown in Table S7, TMPP was only found in one BU soil at low concentration (0.3 ng g^{-1} d.w.). TDClPP, TPhP and TEHP were at low concentration in both BU and CO soils (from 0.2 to 13.1 ng g^{-1} d.w.). As for the rest of compounds, TClPP, TnBP and TBEP were at high concentrations in BU and CO, being 319.8 ng g^{-1} d.w. the highest value found for TnBP. Concentration values for PFRs were not normally distributed for any variable (depth, vegetation, slope position and treatment). Double and triple interactions of the studied variables did not have any significant influence on the occurrence of PFRs. However, ∑ PFR concentration tended to be higher in CO than in BU soils (Fig. 5).

Some perfluorocarboxylates, short-chained (PFPeA, PFHxA and PFHpA) and long-chained (PFUnDA, PFTeDA and PFHxDA), and perfluorosulfonates (PFBS and ipPFNS) were not found in any sample. PFBA, PFDA and PFTrDA were only observed in BU soils (values ranging between 0.1 and 1.2 ng g^{-1} d.w.). The higher value was detected for FOUEA (14.0 ng g^{-1} d.w.). All PFASs concentrations can be found in Table S8. ∑ PFAS was normally distributed and based on the t'student test, UC showed significantly higher concentration than BS ($p < 0.05$,

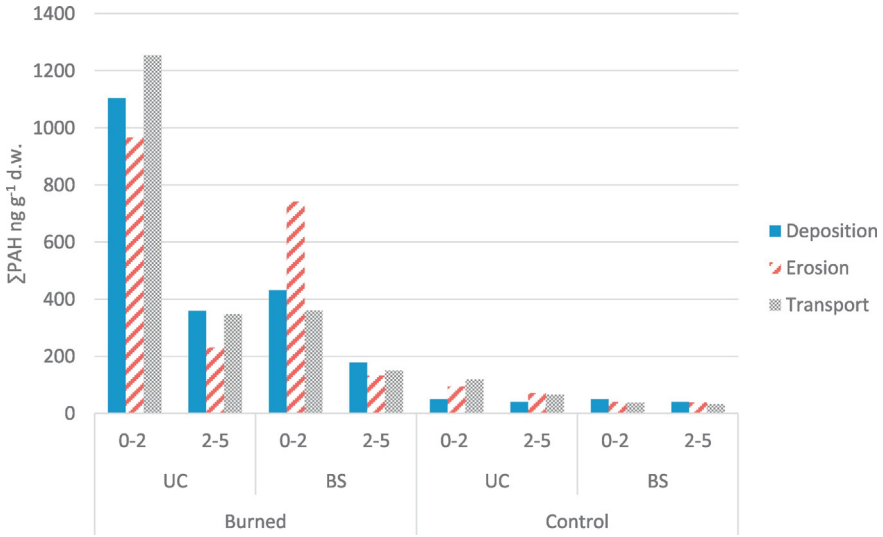

Fig. 3. Cumulated PAH concentrations in the hillslope positions based on the different variables (Treatment: Burned, Control; Vegetation: UC: Under Canopy, BS: Bare Soil; Depth: TS: 0–2 cm, SS:2–5 cm).

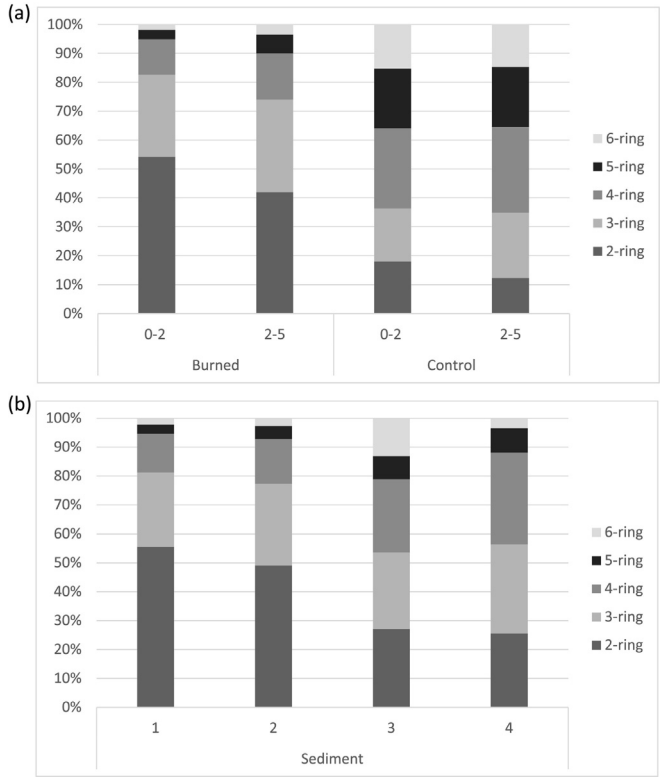

Fig. 4. Contribution of light (2–3 aromatic rings) and heavy (4–6 aromatic rings) PAHs in the: (a) burned and control soils (at different depths) and (b) sediment.

Fig. 6). The other variables considered (treatment, position or depth) did not present any significant influence individually as for double and triple interactions.

3.2. Sediment

Four erosive rain events were considered in this study. Based on the data of the closest pluviometer (Sot de Ferrer: 4.5 km), these were registered in 29/11/2014, 23/3/2015, 15–16/6/2015 and 2/11/2015, and produced 12.7, 143.6, 12.6 and 62.2 kg of sediment, respectively. These events did not produce any sediment in the CO hillslope.

As it was expected, BDE-153, −154 and −183 were not detected in the sediment samples (Table S5). Concentrations of the tri- to penta-BDEs were also low, but BDE-85 presented the highest value detected (11.4 ng g^{-1} d.w.). Only the BDE-47 was observed in all the sediment samples. The sum of the PBDE values was high in the sediment of the first erosive event (17.8 ng g^{-1} d.w., Fig. S5), being higher than in soils (7.3 ng g^{-1} d.w.). These concentrations decreased up to one order of magnitude in sediments of the following events.

Similarly, the PAH concentrations in the sediments from the first event ($\sum$16 PAH = 3154.2 ng g^{-1} d.w., Fig. S6) were higher than those found in soil ($\sum$16 PAH = 1255.3 ng g^{-1} d.w.). Concentrations also decreased in the sediments of the following events, but the main reduction (one order of magnitude) was observed in the samples of the third rainfall. The second rainfall event transported about ten times more sediment than the first and third events. The distribution of PAHs in the first sediment was very similar to that observed in the soil samples from the BU hillslope (Fig. 4b). Over time, however, the dominance of Nap and Phe decreased (Table S6).

As it was expected TCEP, TPP, TDBPP and CDP were not observed in the soil or sediment. On the other hand, TBEP, TMPP, TPhP found in soil samples were not present in sediment. TDClPP and TEHP were only detected in sediment samples from the second event (23/03/2015) that was the most erosive one. TnBP showed the highest concentration (26.5 ng g^{-1} d.w.) and frequency. Conversely to other contaminants, the $\sum$PFR in the first event was not higher than the values in soil samples (Fig. S7) but it decreased at the end of the studied period.

The perfluorocarboxylates, PFPeA, PFHxA, PFHpA, PFNA, PFUnDA, PFTeDA, PFHxDA and PFODA, and the perfluorosulfonates PFBS, ipPFNS and PFDS were not found in any soil or sediment sample. The PFBA, ipPFNA, PFNA, PFDoDA, PFODA, PFDS, PFHpS were present in soil but in sediment. The cumulated PFAS concentration was one order of magnitude higher in soil (maximum $\sum$PFAS = 16.9 ng g^{-1} d.w.) than in sediment ($\sum$PFAS = 4.2 ng g^{-1} d.w., Fig. S8).

4. Discussion

According to the results, some of the detected PBDEs were abundant on both, BU and CO (Table S5). It was hypothesised that PBDEs would be preferentially found on the BU hillslope, because they were probably used for the forest fire extinction, in comparison to CO where there has not been a fire for at least 20 years. The occurrence of PBDEs on the CO might be related to atmospheric transport and deposition as stated by Eljarrat et al. (2008). These authors found concentrations of PBDEs

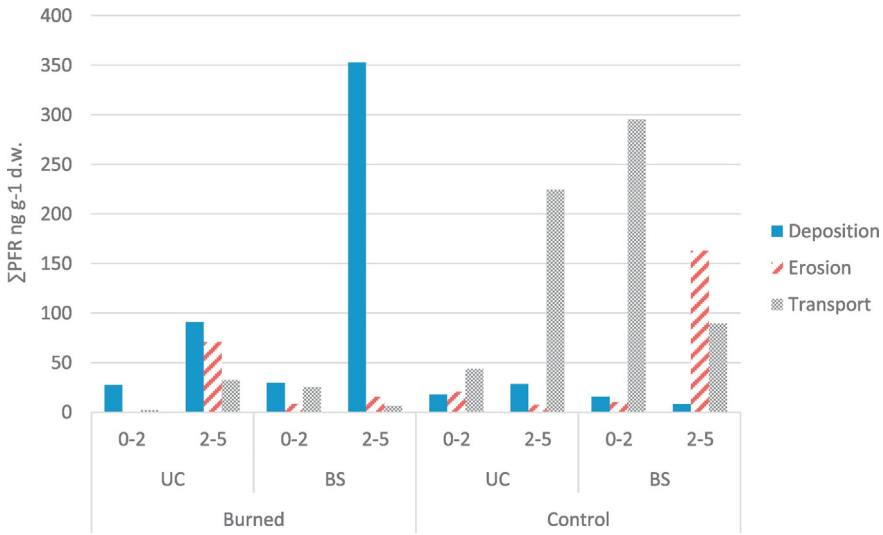

Fig. 5. Cumulated PFR concentrations in the hillslope positions based on the different variables (Treatment: Burned, Control; Vegetation: UC: Under Canopy, BS: Bare Soil; Depth: TS: 0–2 cm, SS:2–5 cm).

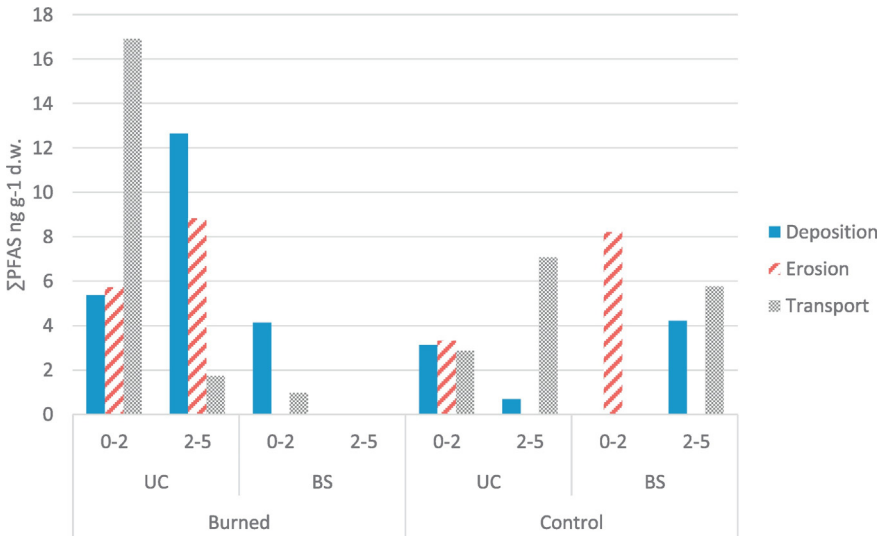

Fig. 6. Cumulated PFAS concentrations in the hillslope positions based on the different variables (Treatment: Burned, Control; Vegetation: UC: Under Canopy, BS: Bare Soil; Depth: TS: 0–2 cm, SS:2–5 cm).

up to 0.84 ng g^{-1} in reference soils used to study the effect of PBDEs-containing sewage sludge on agricultural soils, which was attributed to atmospheric deposition. In a similar research, Matscheko et al. (2002) used reference (non-treated) and sewage-sludge amended soils from three agricultural research stations in Sweden, and from two privately owned farms, and analysed them for BDE-47, −66, −99, −100, −153, −154 and −183. Concentrations in the reference soils from the agricultural research stations and two farm varied from 29 to 95 ng kg^{-1} d.w. and 110 and 190 ng kg^{-1} d.w. for ∑PBDE, respectively. Atmospheric deposition was also the main source of BDE-47, −99, −100, −153 and −154 in soil samples collected along a latitudinal transect through the UK and Norway, at remote/rural woodland and grassland sites, by Hassanin et al. (2004) who found concentrations in such samples ranging from 65 to 12,000 ng kg^{-1} d.w.

Significantly higher concentrations of BDE-85 were detected in BU compared to CO (Table S5). Values of BDE-85, which is a penta-BDE, were also the highest of all PBDEs with a maximum of 5.6 ng g^{-1} in soil, and 11.4 ng g^{-1} in sediments. This might implicate that BDE-85 could have been used in the fire extinguisher. However, according to La Guardia et al. (2006) BDE-85 accounts for only 2–3% of the most widely produced penta-BDE mixtures. The largest contributors to these mixtures are BDE-47, −99, −100, −153 and −154 (La Guardia et al., 2006). These PBDEs were not found or were detected at low concentrations on the BU samples, probably degraded by high temperatures. There is a paucity of data in the literature related to BDE-85 occurrence in soils and sediment but, when measured, the levels found are often in the same range: 0.12–57 ng g^{-1} d.w. in sewage sludge (Kupper et al., 2008; Davis et al., 2012; Lee et al., 2014; Venkatesan and Halden, 2014) and 0.016–0.151 ng g^{-1} d.w. in sediment (Hlouskova et al., 2014). These previous BDE patterns should be taken into account with some caution because most of the available data on BDE congener patterns are from sediments and sewage sludge. Then, these patterns are little comparable to those obtained in soil before and after burning. Although ∑PBDEs was higher in BU than in CO, it is questionable whether a penta-BDE mixture was present in the fire extinguisher applied to the studied soil. As previously stated, the position on the slope did not have a significant influence on the distribution of PBDEs along the hillslope. However, in BU, two different trends were observed. In UC, PBDE values increased from erosion/deposition to transport sites, while in BS these tended to augment from erosion/transport to deposition depicting different connectivity tendencies in relation to the presence/absence of vegetation (Fig. 2).

Concentrations of BDE-85 tended to be higher at the TS than at the SS of BU (Fig. 2), in relation to the absence of rainfall in the period between fire occurrence and sample collection. This fact reduced the possibility of infiltration, and therefore of vertical distribution, and explain the very high concentration measured in the sediment sample of the first erosive event (Table S5). Vegetation did not significantly influence the distribution of PBDEs in the soil. However, the values of BDE-85 tended to be higher in UC than in BS. In Mediterranean areas, vegetation operates as an obstacle for erosion (acting as a sink) causing the accumulation of eroded soil (Urgeghe and Baptista, 2015). This eroded soil might transport contaminants, as proved the high concentrations found in this study, and can be the source of the PBDEs detected in under canopy sites. Beside this, a research conducted by Huang et al. (2011) proved that vegetation uptakes lower brominated PBDEs in a contaminated soil. When this vegetation is burned part of the contaminants that are not volatilized could be deposited on the soil and probably increased their concentration on these sites.

It is important to highlight the difficulty to ascertain the actual contamination of soils with PBDEs in relation to forest fires. PBDE concentrations in soils have been mainly investigated near electronic waste recycling sites and on agricultural fields where sewage sludge is applied as a fertilizer. For instance, at an electronic waste recycling site in Accra (Ghana) concentrations of ∑13 PBDEs ranged from 15.6 to 96.8 ng g^{-1} d.w. and BDE-28 was the dominant congener followed by −209 and −47 (Akortia et al., 2017). In Spain, BDE-209 has been widely used and high concentrations have been reported in sewage sludge and soils amended with sludge (*i.e.* Eljarrat et al., 2004, 2005, 2008; Gorga et al., 2013). Unfortunately, this congener was not analysed in this study and its levels cannot be compared with those reported elsewhere.

The fire in Azuébar indeed added significant amounts of PAHs to the soil (BU > CO, Table S6). The Σ16 PAHs reported here for BU are between 133.5 and 1255.3 ng g^{-1} d.w. (Fig. 3), which are in agreement with the ones reported by Choi (2014). This author sampled pine bark, litter and soils from a burned site in Pohang, South Korea and obtained ∑16 PAH concentrations of 5920 ng g^{-1}, 1540 ng g^{-1}, and 133 ng g^{-1}, respectively. In fact, PAH concentrations in BU are between those Choi obtained for soil and litter samples (considering the average of both soil depths since Choi's soil samples were taken at 0–5 cm). PAHs distribution in Azuébar's soil and Korea's litter are similar, suggesting that soils of this research could have incorporated significant quantities of burned organic material.

Similar results were also presented by Kim et al. (2003) who found a range of 150–1600 ng g^{-1} in soils after several forest fires in the eastern coastal region of Korea. However, concentrations found in the present study are higher than the findings of Vergnoux et al. (2011) in repeatedly burned sites of the South of France (Σ14 PAHs = 77–157 ng g^{-1} d.w.) and the values reported by Pizarro-Tobías et al. (2015) after controlled fires in the Parque Natural de los Montes de Málaga (Spain) (400 ng g^{-1} for Σ15 PAHs). PAH patterns in burned soils are remarkably constant across studies and very similar to the one described here (Fig. 4a). In relation to Σ16 PAH levels detected in CO (33.8–120.2 ng g^{-1} d.w., Table S6), they are in the same order of magnitude than the values obtained by Pizarro-Tobías et al. (2015): average 58 ng g^{-1}, Kim et al. (2003): 49 ng g^{-1}, and Choi (2014): 26 ng g^{-1}.

According to the results, the position did not have a significant influence on the distribution of PAHs along the hillslope. However, a trend to accumulate PAHs in the middle of the hillslope was observed in both treatments (in BU the Σ16 PAHs tended to increase from erosion to deposition and from this one to transport, while in CO from deposition to erosion and from erosion to transport). Despite significant differences were not found between TS and SS, for either the individual compounds or their sum, there must be some downwards movement during or shortly after fire, because PAH levels increased at both depths when BU and CO were compared (Fig. 3). No rain fell in the study area between fire and sampling, and therefore, gaseous movement could be responsible for this movement.

Despite partly burned vegetation is an important source of PAHs to the soil (Choi, 2014), there were not significant differences based on the presence/absence of vegetation. Loose PAHs that move downwards could be retained where vegetation was present (again sink effect). Soil in these vegetation patches had higher SOM levels already before fire, causing SOM and PAHs to increase together when the burned organic material is incorporated into the soil, though it is argued that the PAH production from SOM might be quite small (Kim et al., 2003).

The high PAH concentrations in the sediment of the first erosive rainfall suggest that erosion of burned organic material on the surface is an important process for PAHs transport since their profile in the sediments is similar to the one of burned soils. A sign of degradation and volatilisation may be responsible of the increasing dominance of heavy PAHs in the sediments over time. In the burned soil, the light, degradable PAHs probably declined in the months after fire as stated by Choi (2014). This would also lead to a lower contribution of light PAHs in the sediments (Fig. 4b). Accordingly, it is possible to state that the amount of PAHs moved downslope is thus not only dependent on the time since fire, but also on the intensity of the rainfall event.

Similar to PBDEs, it was hypothesised that PFRs would be mostly found on the BU hillslope, as consequence of their possible use as fire extinguisher, in comparison with CO hillslope. High potential for long-range atmospheric transport and persistence of PFRs would be responsible for their presence in the CO hillslope (Mihajlović et al., 2011; Wei et al., 2015).

For our knowledge, there are limited data about PFRs presence in sediment and soil and no data at all in these matrices related to forest fires. Other studies showed the presence of TCEP and TClPP in sediments of two lakes from the Lazio Region in Italy (between <MQL and 0.5 ng g^{-1} and between <MQL and 0.3 ng g^{-1}, respectively) and in sediments collected from the banks of the Tiber river and the Liri river, (TClPP 0.4–32 ng g^{-1} and TCEP <MQL–15 ng g^{-1}) (Cavaliere et al., 2016). TEHP and TPhP were also found at high concentration (2.1–290 ng g^{-1}) in sediment samples from the Spanish rivers Arga, Nalón and Besòs (Catalonia) and, as in this study, TClPP was detected in most of the sediment samples with concentrations ranging from 13 to 365 ng g^{-1} (Cristale et al., 2013). TBnP, TBEP, TCEP, TClPP and TDClPP were highly found as the main components in 28 sediment samples collected from three districts and the central part of Taihu Lake (China), with concentrations between 3.38 and 14.25 ng g^{-1} (Cao et al., 2012). These authors suggested as possible sources, the industrial wastewater discharge and domestic sewage.

Despite PFAS have been usually found in firefighting facilities, they have been never screened in an area affected by forest fires. Studies about PFAS concentrations in firefighting training facility sites showed high levels of PFOS, between 21 and 8520 ng g^{-1} (Filipovic et al., 2015; Hale et al., 2017; Houtz et al., 2013; Kupryianchyk et al., 2016). These concentrations are far away from those found in this study which were between 0.1 and 1.7 ng g^{-1} d.w., suggesting that PFASs were not present in the materials used to extinguish the forest fire. Presence and movement of volatile PFAS precursors in the atmosphere is frequently suggested as the principal distribution pathways of these compounds in the environment (Bossi et al., 2016; Lai et al., 2016; Strynar et al., 2012). Although discharges from wastewater facilities have also been commonly cited as PFAS sources (Campo et al., 2014; Hu et al., 2016) A global survey showed PFAS concentrations similar to those found in this research, with total PFCAs ranging from 0.03 to 14.3 ng g^{-1} and PFSAs from <LOQ-3.3 ng g^{-1} (Rankin et al., 2016).

There is scarce knowledge about sorption of PFASs in soil and their distribution in the mineral and organic phases. Some factors as the type of mineral and the charge are crucial for PFAS sorption in the environment, affecting their leaching, transport and bioavailability (Hellsing et al., 2016). These authors showed that the sorbed PFASs could easily be desorbed by gentle rinsing with water, and so by rainfall the more water-soluble PFASs can be released and leach into groundwater or be taken up by plants, explaining to a certain point the lower concentrations found in sediment with regard to soil in this study and the higher concentrations of PFAS in UC than in BS.

Despite the increased PAH values in the burned hillslope, concentrations were not harmful. In Spain, there is no legislation regulating the admissible levels of these contaminants in soil. In the Netherlands, for instance, standards for soil quality give a maximum value of 1500 ng g^{-1} for 10 specific PAHs (Nap, Ant, Phe, Flu, BaA, Chr, BaP, BghiP, BkF and Ind) if soil is to be used in a residential area, but pollution is considered dangerous at 40000 ng g^{-1} (RIVM, n.d.) The highest levels measured in this study for these 10 PAHs were found in a burned soil sample located in the transport zone (UC, TS) and in the first sediment sample, with 1100 and 2800 ng g^{-1}. These are far below the dangerous limit. For individual PAHs, Kalf et al. (1995) estimated maximum permissible concentrations (MPC) for soil, which should protect 95% of the species in an ecosystem. Only the MPC value for naphthalene proposed by Kalf et al., 1995 (140 ng g^{-1}) was exceeded in Azuébar; however, levels at which species are acutely affected are probably much higher than this MPC. It can be stated that at some parts of the slope, PAH levels could be possibly harmful to humans and animals if they are exposed for long periods of time.

Some processes, however, might increase pollution hazard after fire. Firstly, the results show that erosion processes can concentrate PBDEs and PAHs locally where sediments are deposited. Secondly, PAHs could accumulate in the soil with recurrent fires. Light PAHs can be produced by fire in such amounts that their levels remain elevated for years despite their degradability (Vergnoux et al., 2011). Heavy PAHs, even though produced in small amounts, can also remain in the soil for long periods of time (Duan et al., 2015). The persistence of heavy PAHs could explain their relatively high levels in the control soils, which may be a remnant of a fire occurred in the past. Although fire frequency was not found to be a very good predictor of PAH levels (Vergnoux et al., 2011), the possibility of PAHs accumulating with frequent fires cannot yet be ruled out.

Table S9 shows the relationships between soil organic carbon (SOC) and the sum of the concentrations of the different families of contaminants in soil samples. Altogether and in CO, only PAHs presented a significant correlation with SOC. In BU, both PAHs and PBDEs were significantly correlated to SOC content. These correlations between SOC and PAH levels supports the influence of vegetation patches in the distribution of these compounds. At both slopes, more SOC is

associated with more PAHs. This is in line with the image of high-PAH organic material being added to the soil during fire.

It should be noted that the sampling group used in this research was relatively low. Additional research on soil composition and microbial behaviour is required to assess the effects of the contaminants. A next step for research should be to find out if the contamination in soil and sediment actually affects the capability of ecosystems to regenerate from fire. The low number of samples limits the conclusions that can be drawn about the compounds distribution at the hillslope scale, the depth effect and the vegetation influence. Additional research with more sampling points and more replicates per treatment would help to create a deeper understanding of the mechanisms involved in POPs contamination related to fires impact.

5. Conclusions

The fire in Azuébar actually added significant amounts of PAHs to the burned soil. Mainly, the upper soil layer was affected, and vegetation patches generally tended to have higher concentrations. Besides depth and canopy presence, soil organic carbon was important in the occurrence and distribution of PAHs. PBDE values were relatively low on the burned slope and most of the concentrations found can probably be related to atmospheric deposition, because these contaminants were also found on the control hillslope. PBDEs seemed to be more abundant in the top layer of the soil. It is questionable whether a PBDE mixture was applied to the burned hillslope due to the fact that BDE-85 values were highest, and the penta-BDE mixture contains twenty times more BDE-99, which showed low concentrations here, than other compounds. PFRs and PFASs concentrations in this study were similar to those found in others not related to fires, suggesting that these compounds were not used in this forest fire. In the case of PFASs, significant differences have been found between under canopy and bare soils. Further studies about the presence of vegetation and bioremediation should be done.

There was no clear pattern for the distribution of screened contaminants over the different slope positions, though higher concentrations tended to be found on the transport and deposition levels. In comparison to the soil, the sediments transported downslope contained significant amounts of PBDEs and PAHs pointing out the importance of connectivity processes. The contaminants enrichment in the first post-fire sediments was related to movement of burned organic material.

Concentrations of the different compounds seem to be low, and therefore the contamination from a fire like the studied here is unlikely to directly harm ecosystems. However, PAHs might accumulate in the soil with recurrent fires, and substantial inputs to other ecosystem compartments may happen with the first rains after fire. Erosion processes can concentrate contaminants on the lower parts of the hillslope, or in the valley, leading to bioaccumulation and potentially hazardous values in higher trophic levels.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.06.005.

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Supplementary information

Emerging contaminants related to the occurrence of forest fires in the Spanish Mediterranean.

Julian Campo [1, 2*], María Lorenzo [3], Erik L. H. Cammeraat [2], Yolanda Picó [3], Vicente Andreu [1]

[1] *Environmental Forensic and Landscape Chemistry Research Group. Desertification Research Centre - CIDE (Spanish Council for Scientific Research, University of Valencia, Generalitat Valenciana). Carretera Moncada - Náquera km 4.5 (Campus IVIA). 46113 Moncada, Valencia, Spain.*

[2] *Department of Ecosystem and Landscape Dynamics, Institute for Biodiversity and Ecosystems Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.*

[3] *Food and Environmental Safety Research Group (SAMA – UV), Desertification Research Centre - CIDE and Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain.*

[*] Corresponding author. Tel. +34 963424217; fax: +34 963424160
E-mail address: Julian.Campo@uv.es

Table S1. Samples description

Sample	Treatment	Position	Depth	Vegetation	Comment
1	Burned	Erosion	0-2	UC	-
2	Burned	Erosion	2-5	UC	Triplicate
3	Burned	Erosion	0-2	BS	-
4	Burned	Erosion	2-5	BS	-
5	Burned	Transport	0-2	UC	-
6	Burned	Transport	2-5	UC	-
7	Burned	Transport	0-2	BS	-
8	Burned	Transport	2-5	BS	-
9	Burned	Deposition	0-2	UC	-
10	Burned	Deposition	2-5	UC	-
11	Burned	Deposition	0-2	BS	-
12	Burned	Deposition	2-5	BS	-
13	Burned	Sediment 1			14/11/2014
14	Burned	Sediment 2			15/3/2015
15	Burned	Sediment 3			15-16/6/2015
16	Burned	Sediment 4			15/11/2015
17	Control	Erosion	0-2	UC	-
18	Control	Erosion	2-5	UC	-
19	Control	Erosion	0-2	BS	-
20	Control	Erosion	2-5	BS	-
21	Control	Transport	0-2	UC	-
22	Control	Transport	2-5	UC	-
23	Control	Transport	0-2	BS	-
24	Control	Transport	2-5	BS	-
25	Control	Deposition	0-2	UC	-
26	Control	Deposition	2-5	UC	-
27	Control	Deposition	0-2	BS	-
28	Control	Deposition	2-5	BS	-

Table S2. Compounds selected for this study, their family, acronym, CAS number, formula

Family	Compound	Acronym	CAS Nº	Formula
Polybrominated diphenyl ethers		**PBDEs**		
Tri-BDE	2,4,4'-tri-bromodiphenyl ether	BDE-28	41318-75-6	$C_{12}H_7Br_3O$
Tetra-BDE	2,2′,4,4′-tetra-bromodiphenyl ether	BDE-47	5436-43-1	$C_{12}H_6Br_4O$
Penta-BDE	2,2′,3,4,4′-penta-bromodiphenyl ether	BDE-85	182346-21-0	$C_{12}H_5Br_5O$
	2,2′,4,4′,5-penta-bromodiphenyl ether	BDE-99	60348-60-9	$C_{12}H_5Br_5O$
	2,2′,4,4′,6-penta-bromodiphenyl ether	BDE-100	189084-64-8	$C_{12}H_5Br_5O$
Hexa-BDE	2,2′,4,4′,5,5′-hexa-bromodiphenyl ether	BDE-153	32536-52-0	$C_{12}H_2Br_6O$
	2,2′,4,4′,5,6′-hexa-bromodiphenyl ether	BDE-154	207122-15-4	$C_{12}H_4Br_6O$
Hepta-BDE	2,2′,3,4,4′,5′,6-hepta-bromodiphenyl ether	BDE-183	189084-67-1	$C_{12}H_3Br_7O$
Polycyclic Aromatic Hydrocarbons		**PAHs**		
2-rings	Naphthalene	Nap	91-20-3	$C_{10}H_8$
3-rings	Acenaphthylene	Acy	208-96-8	$C_{12}H_8$
	Acenaphthene	Ace	83-32-9	$C_{12}H_{10}$
	Fluorene	Flu	86-73-7	$C_{13}H_{10}$
	Phenanthrene	Phe	85-01-8	$C_{14}H_{10}$
	Anthracene	Ant	120-12-7	$C_{14}H_{10}$
4-rings	Fluoranthene	Flt	206-44-0	$C_{16}H_{10}$
	Pyrene	Pyr	129-00-0	$C_{16}H_{10}$
	Benzo[a]anthracene	BaA	56-55-3	$C_{18}H_{12}$
	Chrysene	Chr	218-01-9	$C_{18}H_{12}$
5-rings	Benzo[*b*]fluoranthene	BbF	205-99-2	$C_{20}H_{12}$
	Benzo[*k*]fluoranthene	BkF	207-08-9	$C_{20}H_{12}$
	Benzo[*a*]pyrene	BaP	50-32-8	$C_{20}H_{12}$
	Dibenz[*a,h*]anthracene	DahA	53-70-3	$C_{22}H_{14}$
6-rings	Indeno[*1,2,3-cd*]pyrene	Ind	193-39-5	$C_{22}H_{12}$
	Benzo[ghi]perylene	BghiP	191-24-2	$C_{22}H_{12}$
Perfluorocarboxylates		**PFCA**		
	Perfluorobutanoate	PFBA	375-22-4	$C_4F_7O_2H$
	Perfluoropentanoate	PFPeA	2706-90-3	$C_5F_9O_2H$
	Perfluorohexanoate	PFHxA	307-24-4	$C_6F_{11}O_2H$
	Perfluoroheptanoate	PFHpA	375-85-9	$C_7F_{13}O_2H$
	Perfluorooctanoate	PFOA	335-67-1	$C_8F_{15}O_2H$
	Perfluoro-7-methyloctanoate	ipPFNA		
	Perfluorononanoate	PFNA	375-95-1	$C_9F_{17}O_2H$
	Perfluorodecanoate	PFDA	335-76-2	$C_{10}F_{19}O_2H$

Family	Compound	Acronym	CAS Nº	Formula
	2H-Perfluoro-2-decanoate	FOUEA	70887-84-2	$C_{10}F_{16}O_2H_2$
	Perfluoroundecanoate	PFUnDA	2058-94-8	$C_{11}F_{21}O_2H$
	Perfluorododecanoate	PFDoDA	307-55-1	$C_{12}F_{23}O_2H$
	Perfluorotridecanoate	PFTrDA	72629-94-8	$C_{13}F_{25}O_2H$
	Perfluorotetradecanoate	PFTeDA	376-06-7	$C_{14}F_{27}O_2H$
	Perfluorohexadecanoate	PFHxDA	67905-19-5	$C_{16}F_{31}O_2H$
	Perfluorooctadecanoate	PFODA	16517-11-6	$C_{18}F_{35}O_2H$
Perfluorosulfonates		**PFS**		
	Perfluorobutane sulfonate	PFBS	29420-49-3	$C_4F_9SO_3$
	Perfluorohexane sulfonate	PFHxS	82382-12-5	$C_6F_{13}SO_3$
	Perfluoroheptane sulfonate	PFHpS		$C_7F_{15}SO_3$
	Perfluorooctane sulfonate	PFOS	4021-47-0	$C_8F_{17}SO_3$
	Perfluoro-7-methyloctane sulfonate	ipPFNS		
	Perfluorodecane sulfonate	PFDS		$C_{10}F_{21}SO_3$
Perfluorosulfonamides		**PFSA**		
	Perfluorooctane sulfonamide	PFOSA	754-91-6	$C_8F_{17}SO_2NH_2$
Phosphorus flame retardants		**PFRs**		
	Tripropylphosphate	TPP	513-08-6	$C_9H_{21}O_4P$
	Tris(2,3-dibromopropyl)phosphate	TDBPP	126-72-7	$C_9H_{15}Br_6O_4P$
	Tris(2-ethylhexyl)phosphate	TEHP	78-42-2	$C_{24}H_{51}O_4P$
	Tricresylphosphate	TMPP	1330-78-5	$C_{21}H_{21}O_4P$
	Triphenylphosphate	TPhP	115-86-6	$C_{18}H_{15}O_4P$
	Tris(1,3-dichloro-2-propyl)phosphate	TDClPP	13674-87-8	$C_9H_{15}Cl_6O_4P$
	Tris (2-chloroethyl) phosphate	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$
	Tris(2-chloroisopropyl)phosphate	TClPP	13674-84-5	$C_9H_{18}Cl_3O_4P$
	Cresyldiphenylphosphate	CDP	26444-49-5	$C_{19}H_{17}O_4P$
	Tris(2-butoxyethyl)phosphate	TBEP	78-51-3	$C_{18}H_{39}O_7P$
	Tributylphosphate	TBnP	126-73-8	$C_{12}H_{27}O_4P$

Table S3. GC temperature programme

Parameter	Setting
Initial temperature	60 °C
Initial time	2 minutes
Rate 1	75 °C / minute
Final temperature	110 °C
Rate 2	12 °C / minute
Final temperature	150 °C
Rate 3	6 °C / minute
Final temperature	320 °C
Hold time	30 minutes
Total time	64 minutes

Table S4. Instrumental characteristics used for PFRs and PFASs determination

LC CONDITIONS	
Analytical column	Kinetex XB-C18: 50.0 × 4.6 mm, 1.7 µm particle size (Phenomenex, Torrance, USA)
Column temperature	30° C
Volume injected	5 µL
Flow rate	0.2 mL min^{-1}
Linear gradient	PFASs: 0 min (30 % B), 0.5 min (30 % B), 12 min (95 % B), 20 min (95 % B), and return to the initial conditions (equilibration time 12 min) PFRs: 0 min (30% B), 0.5 min (30% B), 12 min (95% B), 18 min (98% B) and 25 min (98% B) and return to the initial conditions.
TRIPLE QUADRUPOLE MS/MS CONDITIONS	
Ionization characteristics and source	MS/MS performed in selected reaction monitoring mode (SRM) with electrospray ionization (ESI) in negative mode
Gas temperature	300° C
Gas flow	11 L min^{-1}
Nebulizer	30 psi
Capillary voltage	4000 V
Chamber current	1.27 µA
Scan type	MRM, with MS1 and MS2 at unit resolution and cell acceleration voltage of 7 eV

Table S5. Concentrations of BDEs in samples in ng g^{-1} d.w., where UC = under canopy soil, BS = bare soil, NF = not found, <LOQ = below limit of quantification. The variables of the samples are included in the table.

Sample	Treatment	Position	Depth	Vegetation	BDE-28	BDE-47	BDE-85	BDE-99	BDE-100	Sum BDEs
1	Burned	Erosion	0-2	UC	NF	0.7	4.6	<LOQ	<LOQ	5.3
2	Burned	Erosion	2-5	UC	NF	1.4	NF	<LOQ	NF	1.4
3	Burned	Erosion	0-2	BS	NF	0.9	1.0	<LOQ	0.6	2.5
4	Burned	Erosion	2-5	BS	NF	0.9	NF	<LOQ	NF	0.9
5	Burned	Transport	0-2	UC	NF	1.1	5.6	0.6	<LOQ	7.3
6	Burned	Transport	2-5	UC	NF	1.0	1.3	<LOQ	<LOQ	2.3
7	Burned	Transport	0-2	BS	NF	1.2	0.7	<LOQ	0.5	2.4
8	Burned	Transport	2-5	BS	NF	1.0	<LOQ	<LOQ	<LOQ	1.0
9	Burned	Deposition	0-2	UC	NF	1.0	1.5	<LOQ	0.4	2.9
10	Burned	Deposition	2-5	UC	0.3	0.9	<LOQ	<LOQ	0.6	1.8
11	Burned	Deposition	0-2	BS	2.1	0.8	0.8	<LOQ	NF	3.7
12	Burned	Deposition	2-5	BS	2.1	0.7	<LOQ	<LOQ	NF	2.8
13	Burned	Sediment 1			2.8	2.2	11.4	0.6	0.8	17.8
14	Burned	Sediment 2			NF	0.7	2.3	<LOQ	NF	3.0
15	Burned	Sediment 3			NF	1.3	1.7	0.7	0.8	4.5
16	Burned	Sediment 4			0.5	1.0	<LOQ	<LOQ	0.4	1.9
17	Control	Erosion	0-2	UC	NF	<LOQ	NF	NF	NF	0.0
18	Control	Erosion	2-5	UC	NF	1.2	NF	1.0	0.8	3.0
19	Control	Erosion	0-2	BS	0.6	1.3	NF	1.0	0.7	3.6
20	Control	Erosion	2-5	BS	0.9	<LOQ	NF	<LOQ	<LOQ	0.9
21	Control	Transport	0-2	UC	NF	<LOQ	NF	NF	NF	0.0
22	Control	Transport	2-5	UC	<LOQ	<LOQ	NF	0.5	<LOQ	0.5
23	Control	Transport	0-2	BS	0.5	0.9	NF	NF	<LOQ	1.4
24	Control	Transport	2-5	BS	1.0	0.7	NF	NF	<LOQ	1.7
25	Control	Deposition	0-2	UC	0.3	0.9	NF	<LOQ	<LOQ	1.2
26	Control	Deposition	2-5	UC	1.2	1.1	NF	<LOQ	<LOQ	2.3
27	Control	Deposition	0-2	BS	<LOQ	0.7	NF	<LOQ	<LOQ	0.7
28	Control	Deposition	2-5	BS	1.0	<LOQ	<LOQ	<LOQ	<LOQ	1.0

Table S6. Concentrations of PAHs in samples in ng g^{-1} d.w., where UC = under canopy soil, BS = bare soil, fOC = organic carbon fraction, NF = not found and <LOQ = Below limit of quantification. Compound acronyms are explained in Table S2. The variables of the samples are included in the table.

Sam	Treat	Posit	Depth	Veg	fOC	Nap	Acy	Ace	Flu	Phe	Ant	Flt	Pyr	BaA	Chr	BbF	BkF	BaP	Ind	DahA	BghiP	Sum PAHs
1	BU	Erosio	0-2	UC	0.14	547.0	30.5	9.7	37.2	159.0	13.6	43.2	34.4	11.6	26.9	15.9	5.2	10.7	8.5	2.9	10.9	967.3
2	BU	Erosio	2-5	UC	0.08	77.6	7.9	5.1	12.8	45.2	4.7	18.0	15.4	5.2	9.6	8.3	2.8	7.2	4.7	0.8	5.5	230.8
3	BU	Erosio	0-2	BS	0.08	327.0	20.6	7.8	28.5	167.0	15.5	51.0	37.4	13.5	23.2	16.6	4.4	9.8	8.2	2.4	10.1	743.0
4	BU	Erosio	2-5	BS	0.06	33.1	3.9	3.2	7.2	30.2	2.5	10.6	8.6	3.5	5.9	7.5	2.3	6.5	3.6	<LOQ	4.9	133.5
5	BU	Trans	0-2	UC	0.13	803.0	26.9	12.0	41.0	198.0	21.0	50.2	30.7	8.9	21.3	13.7	4.1	6.6	5.7	2.4	9.8	1255.3
6	BU	Trans	2-5	UC	0.07	173.0	8.5	4.8	17.2	71.2	6.8	18.7	15.4	0.5	9.1	6.3	2.6	5.6	3.3	<LOQ	5.6	348.6
7	BU	Trans	0-2	BS	0.05	127.0	8.6	4.0	12.1	102.0	6.4	29.5	23.1	7.0	13.4	10.1	2.9	4.2	4.3	1.1	6.1	361.8
8	BU	Trans	2-5	BS	0.05	45.1	7.6	4.3	7.2	35.6	3.3	11.6	9.7	3.0	5.4	5.1	1.5	5.4	2.8	<LOQ	3.5	151.1
9	BU	Depo	0-2	UC	0.11	631.0	23.6	12.1	36.5	214.0	27.2	54.0	27.9	9.0	26.5	14.5	4.2	7.0	6.3	2.2	7.9	1103.9
10	BU	Depo	2-5	UC	0.06	184.0	7.1	6.1	11.5	73.7	5.4	17.7	11.0	4.8	11.5	8.4	2.6	4.6	4.4	1.1	5.0	358.9
11	BU	Depo	0-2	BS	0.07	202.0	8.3	4.8	15.5	108.0	9.8	24.1	17.9	5.9	4.6	10.7	3.3	4.6	6.0	<LOQ	6.0	431.5
12	BU	Depo	2-5	BS	0.05	74.7	2.6	2.8	5.7	41.6	3.3	12.1	8.2	2.6	6.6	6.1	1.9	3.9	2.7	<LOQ	3.2	178.0
13	BU	Sed 1			0.22	1751	84	20.5	119.2	498.2	88.5	166.7	95.5	45.3	115.2	51.2	16.7	26.4	26	5.8	44	3154.2
14	BU	Sed 2			0.11	522	23.8	7.2	33	207.8	27.9	62.4	41.5	16.7	44.7	25.7	7.4	10.4	10.9	3.3	17.7	1062.4
15	BU	Sed 3			0.12	72.9	4.3	3.5	4.6	51.4	7.5	22.3	21.2	6.7	17.7	16	4.6	NF	7.4	0.9	27.8	268.8
16	BU	Sed 4			0.08	50.6	5.2	2.8	3.7	40.1	9.4	20	19.2	6.5	17.2	13	3.6	NF	6.9	<LOQ	NF	198.2
17	CO	Erosio	0-2	UC	0.09	7.0	0.6	2.3	2.3	14.7	0.9	9.5	6.2	2.1	9.3	14.4	3.2	6.8	8.8	<LOQ	7.1	95.2
18	CO	Erosio	2-5	UC	0.05	4.5	0.2	2.9	1.9	8.4	0.6	7.4	5.8	2.5	8.2	10.5	3.0	4.7	5.1	<LOQ	6.6	72.3
19	CO	Erosio	0-2	BS	0.03	6.5	<LOQ	1.6	2.0	6.2	0.4	3.9	3.7	1.2	3.8	4.6	1.6	NF	2.4	<LOQ	3.7	41.6
20	CO	Erosio	2-5	BS	0.02	3.9	<LOQ	2.0	3.2	6.9	0.3	3.3	2.9	1.0	2.8	3.7	1.1	3.4	2.2	<LOQ	2.8	39.5
21	CO	Trans	0-2	UC	0.17	43.3	<LOQ	2.2	1.8	10.1	0.9	7.7	5.4	2.2	10.5	13.7	2.6	5.0	8.2	<LOQ	6.6	120.2
22	CO	Trans	2-5	UC	0.07	4.6	<LOQ	1.6	2.7	9.6	0.5	6.5	5.6	2.5	7.2	8.8	2.6	5.4	4.9	<LOQ	4.9	67.4
23	CO	Trans	0-2	BS	0.03	4.8	<LOQ	1.3	1.4	5.8	0.3	3.2	0.2	0.9	6.2	6.3	1.9	NF	2.7	<LOQ	3.8	38.8
24	CO	Trans	2-5	BS	0.03	8.6	<LOQ	1.5	1.5	4.3	0.3	1.8	1.9	0.4	3.2	3.4	1.1	<LOQ	1.4	<LOQ	4.4	33.8
25	CO	Depo	0-2	UC	0.15	4.4	<LOQ	1.2	1.1	5.7	0.3	4.4	3.9	2.0	7.1	5.9	1.9	3.5	3.9	<LOQ	4.2	49.5
26	CO	Depo	2-5	UC	0.08	10.4	<LOQ	1.3	2.2	5.3	<LOQ	3.3	2.9	1.2	5.3	4.5	1.4	<LOQ	2.4	<LOQ	<LOQ	40.2
27	CO	Depo	0-2	BS	0.07	4.7	<LOQ	1.4	1.2	6.1	0.8	4.4	4.1	1.9	6.0	7.6	2.3	NF	3.5	<LOQ	5.3	49.3
28	CO	Depo	2-5	BS	0.06	4.1	0.7	1.3	1.0	5.0	1.3	3.0	2.7	1.8	3.7	5.1	1.8	<LOQ	2.2	0.3	6.3	40.3

Table S7. Concentrations of PFRs in samples in ng g^{-1} d.w., where UC = under canopy soil, BS = bare soil, NF = not found and <LOQ = Below limit of quantification. Compound acronyms are explained in Table S2. The variables of the samples are included in the table.

Sample	Treatment	Position	Depth	Vegetation	TClPP	TDClPP	TPhP	TnBP	TBEP	TMPP	TEHP	Sum PFRs
1	Burned	Erosion	0-2	UC	NF	NF	NF	NF	NF	NF	NF	0.0
2	Burned	Erosion	2-5	UC	NF	NF	NF	NF	59.3	NF	11.8	71.1
3	Burned	Erosion	0-2	BS	8.9	NF	NF	NF	NF	NF	NF	8.9
4	Burned	Erosion	2-5	BS	15.7	NF	NF	NF	NF	NF	NF	15.7
5	Burned	Transport	0-2	UC	2.4	NF	NF	NF	NF	NF	NF	2.4
6	Burned	Transport	2-5	UC	NF	0.9	2.4	29.2	NF	0.3	<LOQ	32.8
7	Burned	Transport	0-2	BS	NF	0.8	13.1	11.7	NF	NF	NF	25.7
8	Burned	Transport	2-5	BS	6.7	NF	NF	NF	NF	NF	NF	6.7
9	Burned	Deposition	0-2	UC	9.0	NF	NF	18.8	NF	NF	NF	27.8
10	Burned	Deposition	2-5	UC	NF	NF	NF	90.7	NF	NF	NF	90.7
11	Burned	Deposition	0-2	BS	7.4	NF	NF	22.3	NF	NF	0.2	29.9
12	Burned	Deposition	2-5	BS	7.0	NF	NF	319.8	26.0	NF	NF	352.8
13	Burned	Sediment 1			NF	NF	NF	26.5	NF	NF	NF	26.5
14	Burned	Sediment 2			NF	4.0	NF	6.3	NF	NF	0.5	10.9
15	Burned	Sediment 3			11.1	NF	NF	15.7	NF	NF	NF	26.8
16	Burned	Sediment 4			0.1	NF	NF	NF	NF	NF	NF	0.1
17	Control	Erosion	0-2	UC	21.1	NF	NF	NF	NF	NF	NF	21.1
18	Control	Erosion	2-5	UC	7.4	NF	NF	NF	NF	NF	NF	7.4
19	Control	Erosion	0-2	BS	6.9	NF	NF	3.8	NF	NF	<LOQ	10.7
20	Control	Erosion	2-5	BS	11.1	NF	NF	103.2	48.7	NF	NF	163.0
21	Control	Transport	0-2	UC	5.2	NF	11.0	26.9	NF	NF	0.9	44.1
22	Control	Transport	2-5	UC	8.3	1.3	NF	NF	214.9	NF	NF	224.5
23	Control	Transport	0-2	BS	16.2	NF	NF	130.8	148.5	NF	NF	295.5
24	Control	Transport	2-5	BS	21.4	NF	NF	NF	68.4	NF	NF	89.8
25	Control	Deposition	0-2	UC	17.8	NF	NF	NF	NF	NF	0.2	18.0
26	Control	Deposition	2-5	UC	NF	0.2	NF	27.6	NF	NF	0.8	28.5
27	Control	Deposition	0-2	BS	9.5	NF	1.8	4.5	NF	NF	NF	15.8
28	Control	Deposition	2-5	BS	NF	0.7	2.1	5.5	NF	NF	0.2	8.5

Table S8. Concentrations of PFASs in samples in ng g^{-1} d.w., where UC = under canopy soil, BS = bare soil, NF = not found and <LOQ = Below limit of quantification. Compound acronyms are explained in Table S2. The variables of the samples are included in the table.

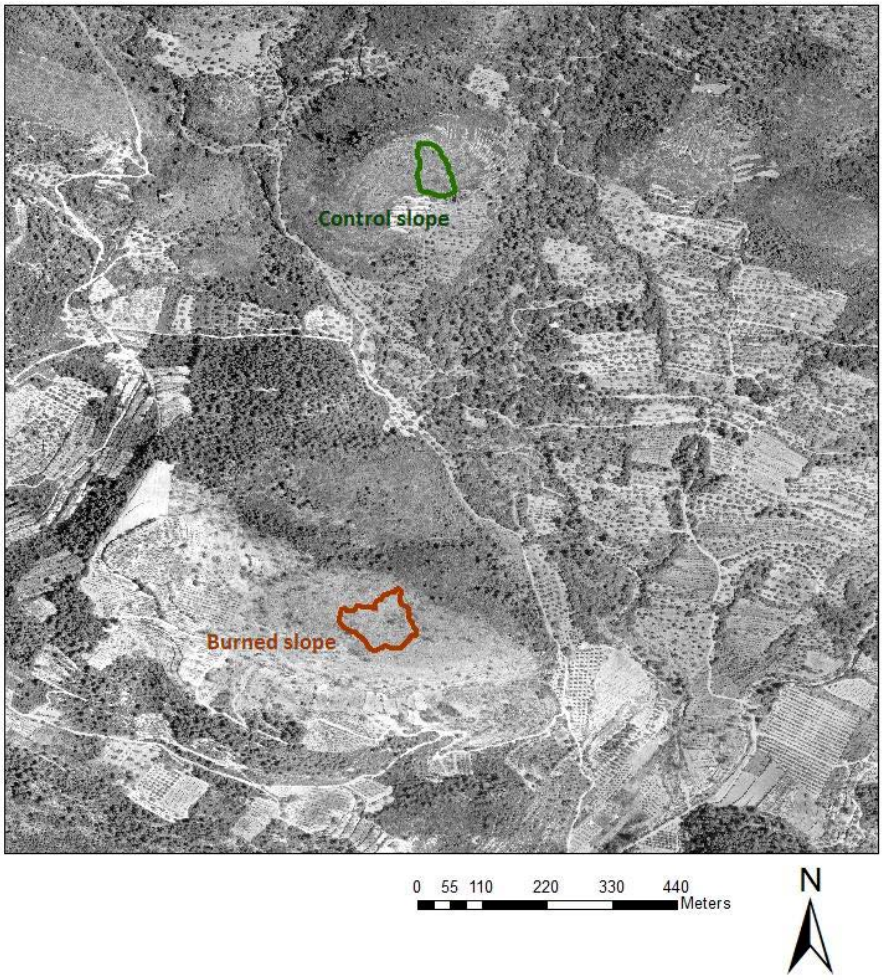
Sample	Treatment	Position	Depth	Veget	PFBA	PFHxS	PFHpS	PFOA	ipPFNA	PFOS	PFNA	FOUEA	PFDA	PFDS	PFDoDA	PFTrDA	PFODA	Sum PFASs
1	Burned	Erosion	0-2	UC	NF	3.1	2.7	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	5.7
2	Burned	Erosion	2-5	UC	NF	NF	NF	1.8	2.1	1.7	2.9	NF	NF	NF	0.3	NF	NF	8.8
3	Burned	Erosion	0-2	BS	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
4	Burned	Erosion	2-5	BS	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
5	Burned	Transport	0-2	UC	NF	NF	NF	NF	NF	NF	NF	14.0	NF	3.0	NF	NF	NF	16.9
6	Burned	Transport	2-5	UC	1.2	NF	NF	0.4	<LOQ	0.1	NF	NF	0.1	0.01	NF	NF	NF	1.7
7	Burned	Transport	0-2	BS	NF	NF	NF	0.8	NF	0.2	NF	<LOQ	NF	NF	NF	<LOQ	NF	1.0
8	Burned	Transport	2-5	BS	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	0
9	Burned	Deposition	0-2	UC	NF	NF	NF	4.2	<LOQ	0.8	NF	0.1	NF	NF	0.3	NF	NF	5.4
10	Burned	Deposition	2-5	UC	NF	NF	NF	2.4	NF	0.8	8.3	0.1	NF	0.2	0.9	NF	NF	12.7
11	Burned	Deposition	0-2	BS	NF	0.04	NF	1.4	NF	0.6	NF	0.03	1.9	0.1	NF	0.1	NF	4.1
12	Burned	Deposition	2-5	BS	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	0.0
13	Burned	Sediment 1			NF	NF	NF	0.9	NF	0.1	NF	0.1	3.2	NF	NF	<LOQ	NF	4.2
14	Burned	Sediment 2			NF	0.04	NF	NF	NF	0.3	NF	0.02	3.2	NF	NF	0.1	NF	3.6
15	Burned	Sediment 3			NF	NF	<LOQ	2.3	NF	0.4	NF	0.1	NF	NF	NF	NF	NF	2.8
16	Burned	Sediment 4			NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
17	Control	Erosion	0-2	UC	NF	0.2	NF	NF	NF	NF	NF	NF	NF	0.1	NF	NF	3.1	3.3
18	Control	Erosion	2-5	UC	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
19	Control	Erosion	0-2	BS	NF	0.1	NF	3.4	1.5	1.2	2.0	NF	NF	NF	NF	NF	NF	8.2
20	Control	Erosion	2-5	BS	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
21	Control	Transport	0-2	UC	NF	0.2	0.2	1.2	<LOQ	1.2	<LOQ	0.1	NF	NF	0.1	NF	NF	2.9
22	Control	Transport	2-5	UC	NF	NF	NF	4.1	NF	1.5	1.5	0.1	NF	NF	NF	NF	NF	7.1
23	Control	Transport	0-2	BS	NF	NF	NF	NF	NF	NF	NF	0	NF	NF	NF	NF	NF	0.0
24	Control	Transport	2-5	BS	NF	NF	NF	NF	NF	NF	NF	5.8	NF	NF	NF	NF	NF	5.8
25	Control	Deposition	0-2	UC	NF	NF	NF	2.2	NF	0.9	NF	0.03	NF	NF	NF	NF	NF	3.1
26	Control	Deposition	2-5	UC	NF	NF	NF	NF	NF	0.7	NF	NF	NF	NF	NF	NF	NF	0.7
27	Control	Deposition	0-2	BS	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	0
28	Control	Deposition	2-5	BS	NF	NF	NF	3.9	NF	0.2	NF	0.1	NF	NF	NF	NF	NF	4.2

Table S9. Pearson's correlation coefficients among cumulated contaminant concentrations and the soil organic carbon (SOC) content.

		ΣBDEs	ΣPAHs	ΣPFRs	ΣPFASs	SOC
BU and CO	ΣBDEs	1	0.749**	0.050	-0.073	0.257
	ΣPAHs		1	-0.013	-0.074	0.474*
	ΣPFRs			1	-0.013	-0.172
	ΣPFASs				1	-0.110
	SOC					1
BU	ΣBDEs	1	0.806**	0.074	0.316	0.853**
	ΣPAHs		1	-0.168	0.123	0.937**
	ΣPFRs			1	0.251	-0.209
	ΣPFASs				1	0.353
	SOC					1
CO	ΣBDEs	1	-0.483	-0.211	-0.226	-0.387
	ΣPAHs		1	-0.059	-0.217	0.904**
	ΣPFRs			1	-0.128	-0.157
	ΣPFASs				1	-0.261
	SOC					1

** Significant correlation at p<0.01; * Significant correlation at p<0.05

(a)



(b)

Fig. S1. (a) Scheme of the coupled hillslopes. (b) Left: Burned hillslope (BU). Right: Control hillslope (CO).

Fig. S2. Studied soil (Luvisol Chromic Skeletic according to the FAO UNESCO, 2006).

Fig. S3. Sediment fences constructed at the foot of the burned slope.

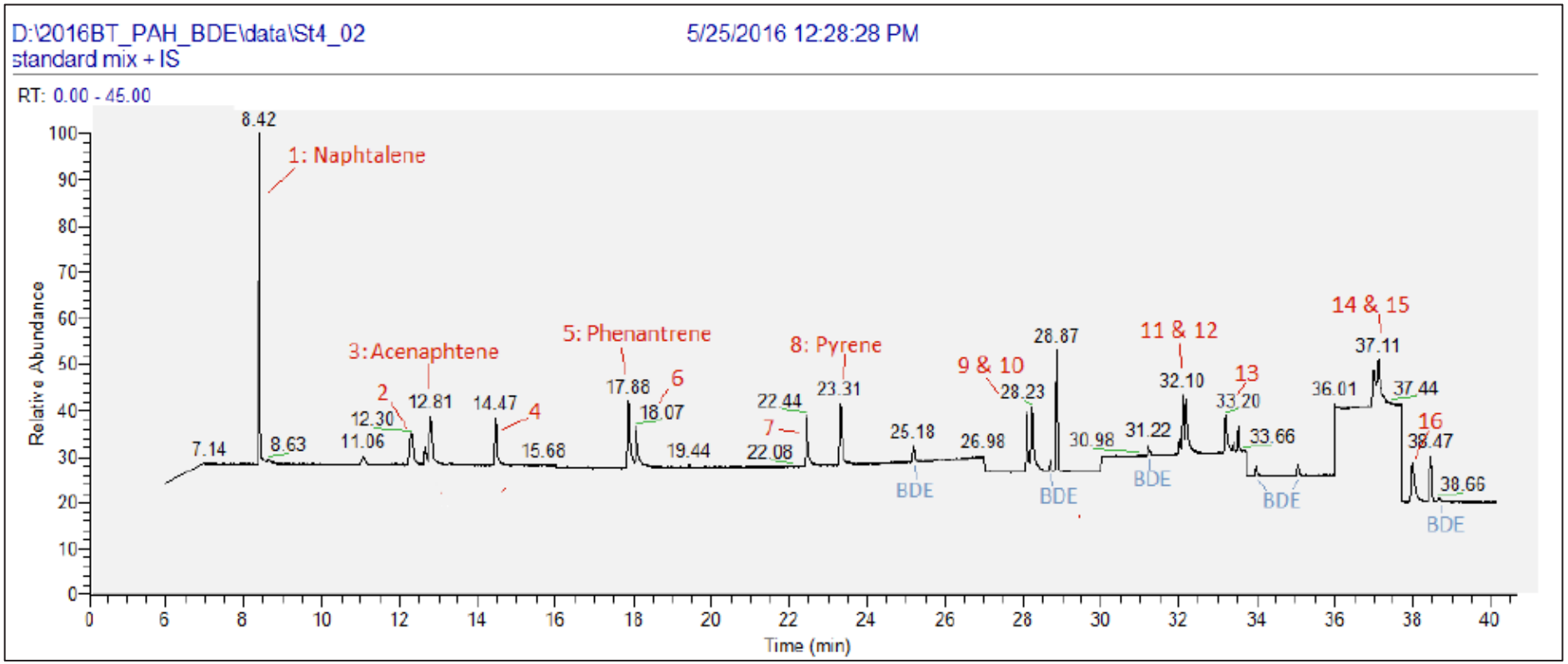


Fig. S4. Chromatogram of one of the external standard (20 ng ml^{-1}). PAHs: 1: Nap, 2: Acy, 3: Ace, 4: Flu, 5: Phe, 6: Ant, 7: Flt, 8: Pyr, 9:BaA, 10: Chr, 11:BbF, 12:BkF, 13: BaP, 14: DahA, 15: Ind, 16: BghiP; BDEs (from left to right): BDE-28, BDE-47, BDE-100, BDE-154, BDE-153, BDE-183.

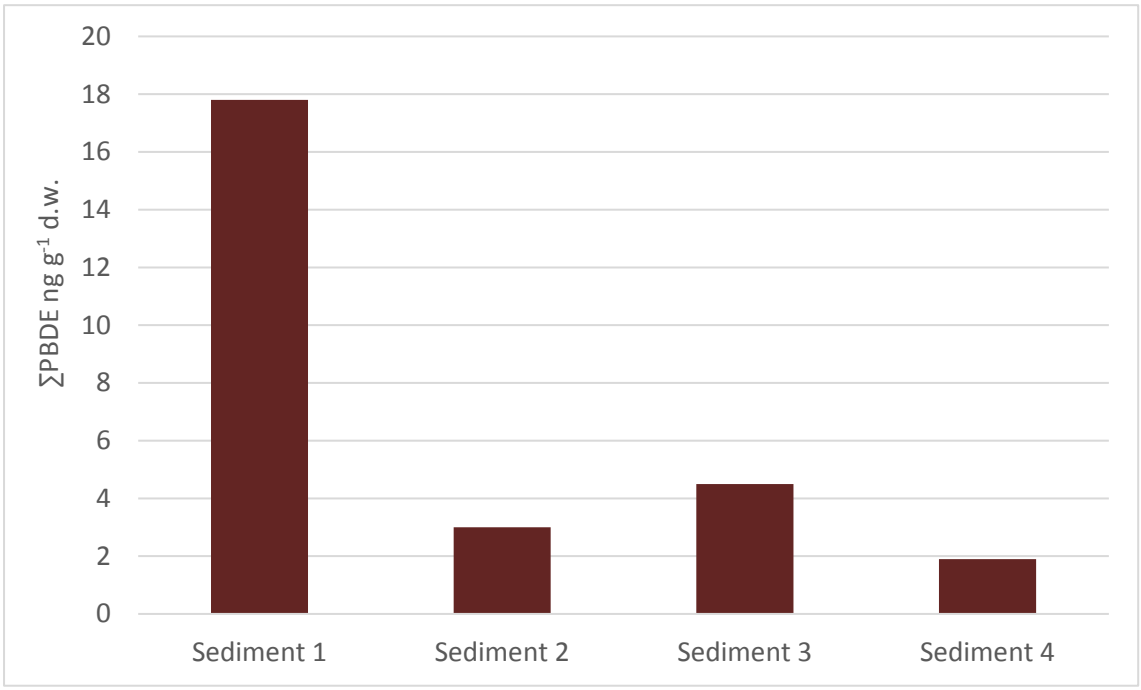


Fig. S5. Cumulated PBDE concentrations in the sediments

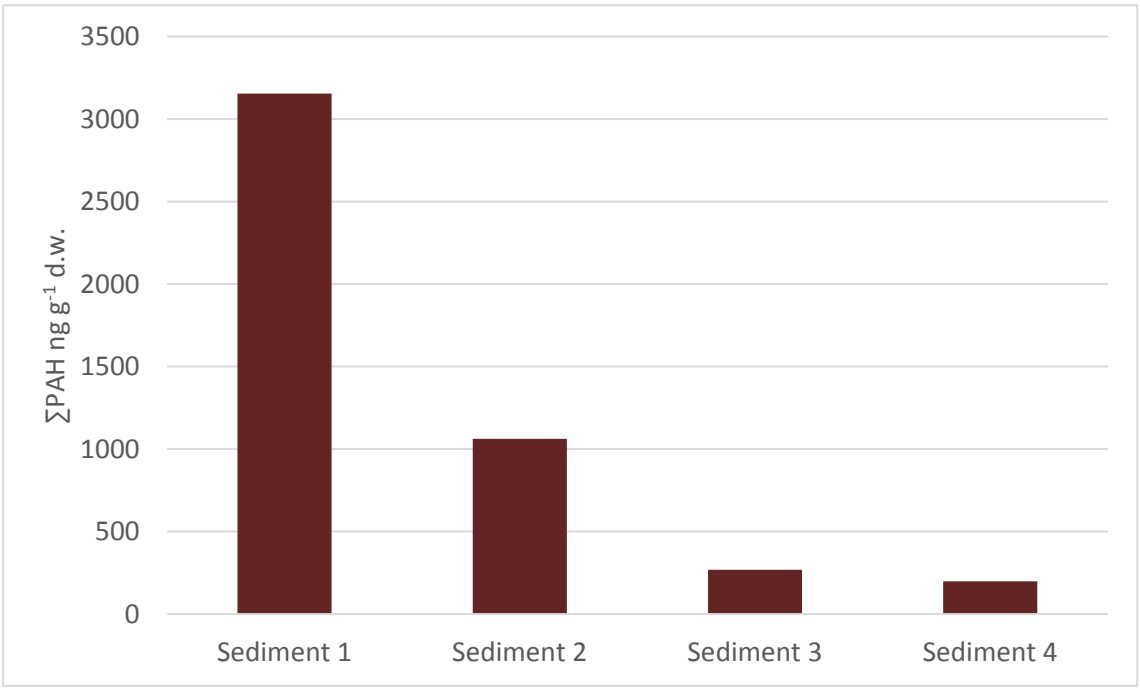


Fig. S6. Cumulated PAH concentrations in the sediments

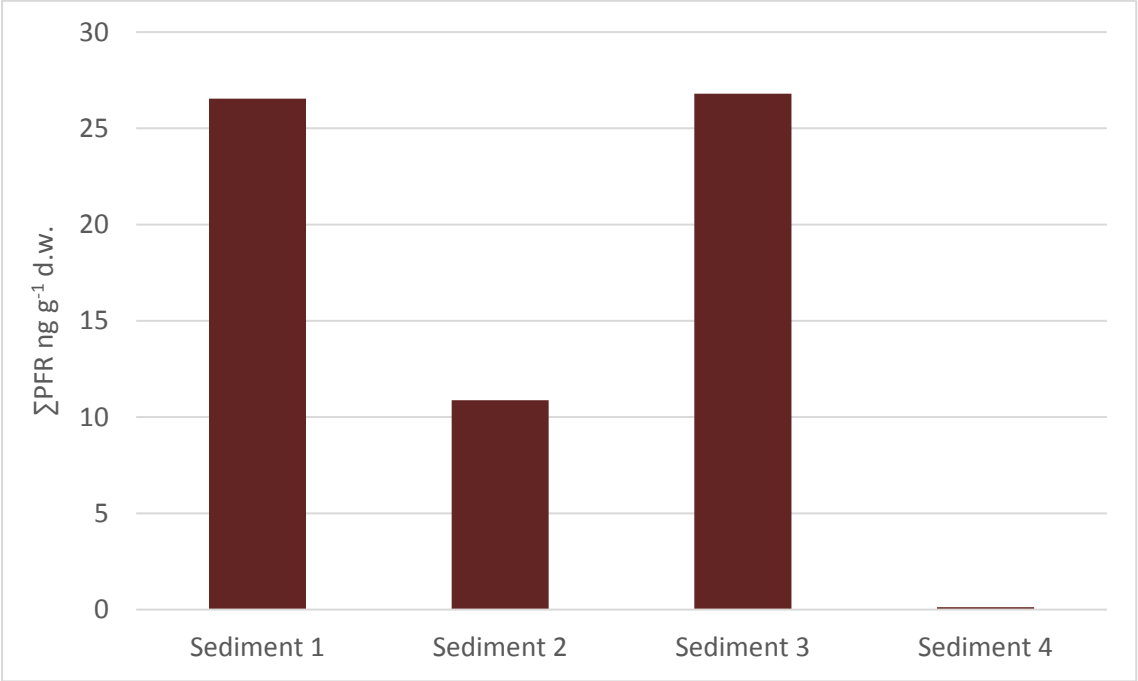


Fig. S7. Cumulated PFR concentrations in the sediments

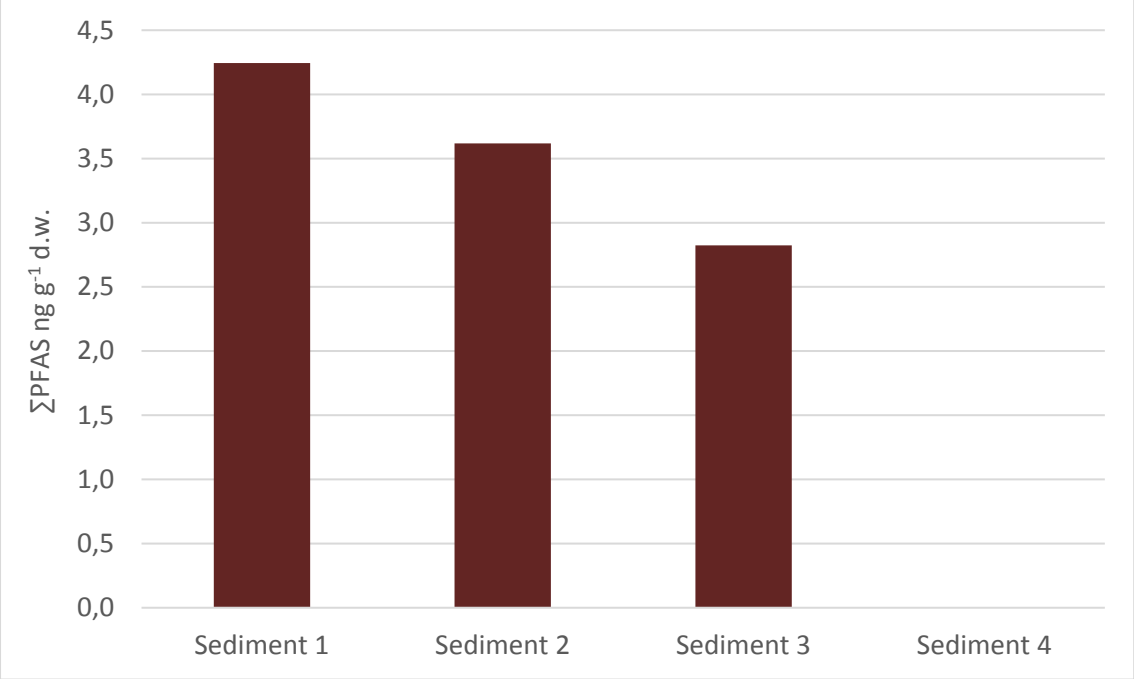


Fig. S8. Cumulated PFAS concentrations in the sediments

Sección 4

APLICACIÓN A LA EXPOSICIÓN EN HUMANOS

CAPÍTULO 9

Perfluoroalkyl substances in Breast milk, infant formula and baby food from Valencian Community (Spain).

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Perfluoroalkyl substances in Breast milk, infant formula and baby food from Valencian Community (Spain)

María Lorenzo[a,*], Marinella Farré[b], Cristina Blasco[a], Matthias Onghena[a], Yolanda Picó[a], Damià Barceló[b,c]

[a] Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE-UV, GV, CSIC), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100, Burjassot, Valencia, Spain
[b] Department of Environmental Chemistry (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Catalan Institute for Water Research (ICRA), Emili Grahit, 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, E-17003 Girona, Spain

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ABSTRACT

Environmental and human exposures to perfluoroalkyl substances (PFASs) are of emerging concern since they are persistent and bioaccumulative. The present study reports PFASs levels in human milk, infant formulas and baby food (dry cereals and pots) from the Valencian Community (Spain) in order to evaluate the infant exposure to these substances through the diet. The results show that perfluorobutanoic acid (PFBA) and perfluorooctanoic acid (PFOA) were in all the samples of the four selected matrices (except PFOA in one sample of dry cereal baby food). Perfluoroheptanoic acid (PFHpA) and perfluorodecanoic acid (PFDA) were also detected in 70% of the breast milk samples. In infant formulas, PFDA and perfluorooctanesulfonate (PFOS) were detected in 75% and 69%, respectively. In dry cereals baby food, PFBA was in 100% of the samples while PFOA and PFOS in 92%. In baby food pots, PFDA was also detected in 83% of the samples. Estimated daily intakes (EDIs) of PFOA (maximum 32.2 ng kg^{-1} day^{-1}) and PFOS (9.0 ng kg^{-1} day^{-1}) are lower than tolerable daily intakes (TDIs) established for PFOA (1500 ng kg^{-1} day^{-1}) and PFOS (150 ng kg^{-1} day^{-1}).

1. Introduction

Perfluoroalkyl substances (PFASs) are a group of chemicals that include the perfluoroalkyl sulfonates, perfluoroalkyl carboxylates, perfluorosulfonamides and perfluorinated telomere alcohols as well as their derivatives (Ericson et al., 2008). Since the 1950s, PFASs have been manufactured for a wide range of consumer applications, such as coating in textiles, carpets and food packaging and to a lesser extend in industrial applications as antistatic additives because their unique properties as repellents of water and oil. They are also precursors of certain fluoropolymers such as polytetrafluoroethylene (PFTE). The strong carbon-fluorine bonds make them resistant to chemical and biological degradations (Onghena et al., 2012).

The reported global distribution and high environmental persistence of PFASs (Blum et al., 2015) together with the adverse health effects detected in laboratory animals (Bull et al., 2014), generate increasing concern about these compounds. From a regulatory point of view, PFASs fulfil the criteria to be considered as "persistent organic pollutants" (POPs). Indeed, PFOS, its salts and sulfonyl fluoride were classified as such in 2009 (UNEP, 2010). A recent study concluded that there was a significant decrease of cord blood immune globulin E with high maternal PFOA levels among female infants. However, there were no significant associations among maternal PFOS and PFOA levels and infant allergies or infectious diseases at age of 18 months (Okada et al., 2012). The mechanisms involved in thyroid homeostasis are complex and PFASs interact at several levels with this endocrine system. Consequently, higher concentrations of PFOA and PFOS in serum were associated with current thyroid disease in the U.S. general adult population (Melzer et al., 2010). The decrease of the thyroid hormone levels in serum of rats and monkeys after PFASs exposure (Lau et al., 2007) was also reported. These changes may affect foetal and neonatal development but more studies are needed to establish clinical significances (Wang et al., 2013; Webster et al., 2014). Therefore, a high scientific concern arises about how PFASs can influence human health at neonatal and early postnatal stages of life.

Several studies have suggested that diet is the primarily route of exposure to PFASs for the human being including newborns (Calafat et al., 2007; Picó et al., 2011). The first food normally given to an

* Corresponding author.
E-mail address: maria.lorenzo@uv.es (M. Lorenzo).

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infant is breast milk. The World Health Organization (WHO) recognizes breast milk as the ideal food for the healthy growth and development of infants (WHO, 2015); breastfeeding is also an integral part of the reproductive process with important implications for the health and affectivity of both mother and child. As a counterpart, it could also be a potential excretion route for mothers that may contain toxic compounds due to the mother's exposure. During the last years, few studies have assessed the levels of PFASs in human breast milk (Al-sheyab et al., 2015; Antignac et al., 2013; Barbarossa et al., 2013; Croes et al., 2012; Fujii et al., 2012; Kadar et al., 2011; Lankova et al., 2013; Liu et al., 2010; Llorca et al., 2010; Motas Guzmàn et al., 2016; Sundström et al., 2011; Tao et al., 2008) reporting concentrations in wide range of scales from pg mL^{-1} to $\mu g\,L^{-1}$.

However, with changing lifestyles and the availability of commercially prepared formulae, these formula have become a popular alternative to be taken into account in the dietary studies as potential source of contaminants. The introduction of complementary foods by about 6 months is another potential source of contamination. Cereals are generally the first foods that are introduced into the infant's diet followed by fruits, meat, vegetables and fish. There is scarce information related to baby food and infant formulas. To our knowledge, there are only three previous studies on these types of food. One of them analyses 6 PFASs in 3 brands of commercial milk infant formulas and 2 brands of cereal baby food from Barcelona city (Llorca et al., 2010), the second determines 6 PFASs in infant formula and dairy milk from the USA (Tao et al., 2008), and the other determines 18 PFASs in infant formula from the Czech Republic (Lankova et al., 2013).

The aim of the present study is to evaluate infant exposure to 20 PFASs (perfluoroalkyl sulfonates and carboxylates) in 51 samples of different types of baby food (breast milk, infant formulas, dry cereals and baby food pots). The results of the occurrence combined with the dietary intake of these products attain a preliminary evaluation of the exposure through the estimated daily intake (EDI). The present study contributes new information being the first time that breast milk from the Valencian Community in Spain has been studied. Furthermore, the study covers cereal-based baby food, proteic baby-food and baby food based on fruit and vegetables that has been to the moment scarcely studied.

2. Materials and methods

2.1. Sample collection and preparation

Individual breast milk samples from 10 women from the province of Valencia (Spain) were taken in 2012. All women were healthy (body mass index between 18 and 25), of similar age, physic constitution and primiparous. Characteristics of the volunteer mothers are shown in Table 1. Informed consent was obtained of all volunteer mothers according to the rules of the local ethics committee. After signing the informed consent, the mothers were asked to complete a questionnaire about some topics and habits related to her breastfeeding and baby food until infants are two years old. The women collected their milk at home using their own breast – milk pump previously tested for blank values (data not shown). In the sampling, the breast was completely emptied because milk composition is not homogeneous during one feeding. Aliquots of 25–30 mL were stored at −20 °C into the pre-washed and tested 50 mL polypropylene (PP) tubes.

Table 1
Characteristics of the volunteer mothers (n = 10).

Characteristics	Mean ± SD
Mother age (year)	32 ± 2.36
Mother weight (kg)	64 ± 10.10
Mother height (cm)	167 ± 3.13
Time postpartum (month)	18 ± 5.79
Number of children in family	1 ± 0.00
Total mother breastfeeding duration (month)	18 ± 5.79
Present breastfeeding duration (week)	74 ± 5.66
Exclusive breastfeeding duration (month)	6 ± 0.84
Lactation frequency (feeds/24 h)	3 ± 1.64
Fish consume frequency (times/week)	2 ± 1.21

A wide range of industrial baby foods and infant formulas from retail – store, pharmacies and supermarket were included in this study. Industrial baby foods and infant formulas were supplied in different packaging (involving plastic, sealed containers, pouches, glass jars, boxes, tubs, aluminium bags, etc.). Detailed sample composition is given in Table 2. The wide range of infant formulas included in this study covers all the baby age segments for this kind of industrial food. The composition of these infant formulas depends on the age of the infant and the different brands, among other factors. Composition of carbohydrates ranges from 4.6% in dry cereals baby food to 18% in fruits and vegetables pots, for proteins from 0.6% in fruits and vegetables pots to 4.3% in pots considered as rich in proteins (e.g. meat) and for fats from 0.1% in fruits and vegetables pots to 5.9% in first and follow-on powdered infant formulas. Infant formulas were in powder (16 samples: 5 first, 9 follow on and 2 toddler formulas). Analysed formulas included milk, soy protein based, partially hydrolysed and extensively hydrolysed ("hypoallergenic") formulas.

Among the variety of dry-cereal baby food on the market, the samples for this study (n = 13) were chosen as representatives of the reality as possible (5 and 8 cereals, with honey, biscuits, honey and biscuits, without gluten, etc.). They comprised (i) simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids; (ii) cereals added with high protein food which are or have to be reconstituted with water or other protein-free liquid and (iii) rusks and biscuits which are to be used either directly or after pulverisation, with the addition of water, milk or other suitable liquid.

The baby food pots (*n = 12*) can be distinguished into (i) proteics, in which the only or main ingredient is meat, poultry, fish, offal or other traditional source of protein constituting not less than 8% of the total product and (ii) non-proteics if the main ingredients are fruit or vegetables.

2.2. Chemicals and standards

A detailed list of chemicals and standards used in this study is given in the Supporting information (Section I). PFASs were from two groups: perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs). Several isotopically labelled internal standards were used to ensure analytical quality of the results.

2.3. Sample extraction

Sample pre-treatment and extraction procedure was based on an alkaline digestion according to a protocol described elsewhere (Llorca et al., 2010). Briefly, solid samples (2 g) or liquid samples (15 mL), were transferred into a 50 mL PP tube. Then 2 mL of deionized water (only for solid samples) were added and shaken. Sample homogenates were fortified with the surrogate internal standards at 25 ng mL^{-1} (see Section I of the Supporting information) and digested with 8 mL of NaOH (10 mM in methanol) during 3 h at room temperature on an orbital shaker. After the orbital digestion, the samples were centrifuged during 15 min at 1810*g* and 3 mL of supernatant was taken, diluted with 27 mL of deionized water in a 50 mL PP tube and vortexed during 5 min. SPE was performed using Strata-X 33 μm Polymeric Reversed Phase 60 mg cartridges preconditioned with 5 mL of methanol and 5 mL of deionized water. Then,

Table 2
Detailed sample composition (%) of breast milk, infant formula and baby food (dry cereals and pots).

Composition	Breast milk (*n* = 10)	Powdered infants formulas			Dry cereals baby food (*n* = 13)	Baby food pots	
		First (*n* = 5)	Follow on (*n* = 9)	Toddler (n = 2)		Proteics (*n* = 6)	Fruit and vegetables (*n* = 6)
Carbohydrates	7.0	9.7–10.7	11.6–12.0	7.5–10.2	11.6–15.6	7.4–9.5	14.0–18.0
Proteins	1.9	2.1–2.3	1.8–2.1	2.2–2.7	0.7–1.1	4.1–4.3	0.6–.5
Fat	3.0	5.2–5.9	3.8–5.9	2.9–3	0.3–0.5	2.3–2.8	0.1–0.4

pre-treated samples (30 mL) were loaded onto the cartridge. After that, the cartridge was washed with 5 mL of deionized water and dried under vacuum at room temperature. The elution was carried out with 5 mL of 0.1% (v/v) ammonium hydroxide in methanol, and dried completely under vacuum for 15 min. The 15 mL PP tubes were reduced to dryness under a gentle stream of nitrogen. The extracts were reconstituted with 150 μL of methanol. All the samples were extracted in triplicate.

Possible contamination by PFASs —the Achilles heel of this determination— was avoided by a strict quality control. All the instrumental used in extraction and chemical analysis were pre-washed with PFASs-free solvents. All reagents and solvents used in the sample preparation and instrumental analysis were checked for possible contamination by UHPLC–MS/MS. A preliminary screening of the samples was performed applying the method in order to select those that does not contain the target compounds. The latter were used to validate the method and to prepared blank and quality control samples to avoid false positive or negative samples.

2.4. Instrumental analysis

The analysis was performed by a HP1200 series LC chromatographic instrument —with an automatic injector, a degasser, a quaternary pump and a column oven— combined with an Agilent 6410 triple quadrupole (QqQ) mass spectrometer, equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany). Data were processed using a MassHunter Workstation Software for qualitative and quantitative analysis (GL Sciences, Tokyo, Japan).

Separation was accomplished on a Kinetex 1.7 V XB – C18 analytical column of 50 × 2.1 mm and 5 μm particle diameter from Phenomenex. The mobile phases, elution gradient, ionization and fragmentation optimized settings, etc. are detailed in text and Table S1 of the Supporting information.

2.5. Validation and quality control

Validation of the method include determination of linearity (with a range of 2–750 ng L^{-1} and $R^2 > 0.99$), intra-assay precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). All the matrices tested were validated (Table 3). The validation protocol followed is detailed in the Supporting information (section III) and outlined in Table 3 footnotes. There were not apparent differences between the results in the different matrices, other than those derived of the different amount of sample taken for extraction.

3. Results

3.1. Levels and profiles of PFASs in human milk, infant formula and other baby food

Table 4 shows PFASs detected in breast milk, infant formulas and baby food (dry cereals and pots) as well as minimum, maximum, average and median concentrations and the frequency of detection. For detailed results, see Supporting information Table S2 for breast milk, Table S3 for powdered formula, Table S4 for dry cereals and Table S5 for baby food pots. Fig. 1 presents contamination profiles of PFASs for each type of samples.

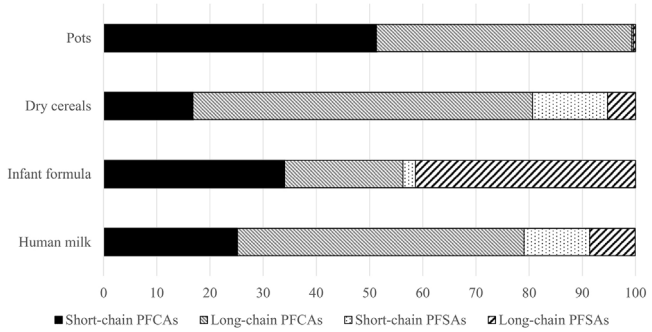

Fig. 1. Contribution of long and short-chain PFCAs and PFSAs (%) to the total concentrations in baby food. (*) Where PFCAs are perfluoroalkyl carboxylates (PFBA, PFPA, PFHxA, PFHpA, PFOA, ipPFNA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFHxDA) and PFSAs are perfluoroalkyl sulfonates (PFBS, PFHxS, PFHpS, PFOS and PFDS) and short-chain include compounds with less than 8 carbons and long-chain with equal or more than 8 carbons in the structure.

3.2. Exposure levels of PFASs for breast-fed infants

In order to evaluate possible risks to infant health associated to PFASs intake, their estimated daily intake (EDI) was calculated for their first two years, according to:

$$\mathrm{EDI} = \frac{\mathrm{Concentration}^{a} \cdot \mathrm{Consumption}^{b}}{\mathrm{Body\ weight}^{c}}$$

And was expressed in ng kg^{-1} per day, where *(a)* was the concentration of PFASs in baby food, *(b)* was the daily average consumption of human milk, infant formula or baby food (g) and *(c)* was the body weight of the child.

Infant daily consumption can vary depending on many factors. **Table S6** fixes estimated daily consumption of baby food according to dietary reference intake (DRI) developed by Institute of Medicine Food and Nutrition Board (2005). Based on infant tables of weights of the Centres for Disease Control and Prevention, these are set on: 1 month = 4.4 kg, 6 months = 8 kg, 12 months = 10 kg, 18 months = 12 kg and 24 months = 13 kg.

The results of EDI for breast milk samples, infant formula, cereals baby food and pots during the first two years of the infants are shown in Table 5. Levels were well-below the tolerable daily intake (TDI) established by European Food Safety Authority (EFSA) (2008) that were 1500 ng kg^{-1} day^{-1} for PFOA and 150 ng kg^{-1} day^{-1} for PFOS. To calculate these EDIs, non-detected PFASs were taken as zero. The mean EDI for the sum of all PFASs to the infants age is shown in Fig. 2a and b considering that they are feeding according DRIs and infant formula instead of breast milk.

Table 3
Validation parameters for the determination of PFASs in human milk, infant formula and baby food.

PFASs	LOD[a] ($ng\,L^{-1}$)	LOQ[b] ($ng\,L^{-1}$)	Recovery[c] (n = 5)		
			at LOQ	at 25 $ng\,L^{-1}$	at 75 $ng\,L^{-1}$
Breast milk and infant formula (reconstituted)					
PFBA	0.6	2.0	81 ± 11	81 ± 11	90 ± 9
PFPeA	0.6	2.0	84 ± 20	98 ± 8	81 ± 8
PFBS	0.6	2.0	76 ± 21	99 ± 3	91 ± 8
PFHxA	0.3	1.0	97 ± 18	80 ± 3	81 ± 9
PFHpA	0.3	1.0	101 ± 13	88 ± 3	90 ± 14
PFHxS	0.6	2.0	101 ± 18	81 ± 3	98 ± 7
PFOA	0.2	0.5	111 ± 19	81 ± 3	96 ± 3
PFHpS	0.6	2.0	101 ± 11	81 ± 6	79 ± 7
ipPFNA	0.1	0.5	70 ± 11	81 ± 10	89 ± 8
PFNA	0.1	0.5	88 ± 17	90 ± 18	80 ± 12
PFOS	0.1	0.5	120 ± 19	102 ± 13	112 ± 5
ipPFNS	0.1	0.5	98 ± 14	89 ± 5	107 ± 3
PFDA	0.1	0.5	81 ± 14	84 ± 3	90 ± 2
PFDS	0.1	0.5	71 ± 11	98 ± 7	91 ± 1
PFUnDA	0.1	0.5	81 ± 17	100 ± 2	91 ± 3
PFDoDA	0.1	0.5	88 ± 17	81 ± 8	89± 9
PFTrDA	0.1	0.5	79 ± 15	91 ± 11	97 ± 4
PFTeDA	0.1	0.5	89 ± 11	108 ± 3	98 ± 4
PFHxDA	0.1	0.5	77 ± 19	81 ± 8	92 ± 16
PFODA	0.1	0.5	99 ± 20	117 ± 20	108 ± 15

PFASs	LOD[a] ($ng\,g^{-1}$)	LOQ[b] ($ng\,g^{-1}$)	Recovery[c] (n = 5)		
			at LOQ	at 25 $ng\,kg^{-1}$	at 75 $ng\,kg^{-1}$
Dry cereals baby food					
PFBA	4.5	15	75 ± 12	78 ± 10	80 ± 9
PFPeA	4.5	15	76 ± 20	75 ± 16	78 ± 12
PFBS	4.5	15	87 ± 15	89 ± 12	90 ± 11
PFHxA	2.25	7.5	92 ± 19	95 ± 10	97 ± 9
PFHpA	2.25	7.5	96 ± 14	95 ± 11	95 ± 11
PFHxS	4.5	15	95 ± 14	94 ± 10	97 ± 9
PFOA	1.5	3.75	97 ± 13	96 ± 9	95 ± 8
PFHpS	4.5	15	90 ± 12	92 ± 10	91 ± 10
ipPFNA	0.75	3.75	75 ± 11	77 ± 10	80 ± 9
PFNA	0.75	3.75	80 ± 15	83 ± 13	82 ± 14
PFOS	0.75	3.75	105 ± 15	110 ± 19	109 ± 12
ipPFNS	0.75	3.75	80 ± 14	84 ± 11	85 ± 8
PFDA	0.75	3.75	89 ± 13	88 ± 10	90 ± 9
PFDS	0.75	3.75	82 ± 14	85 ± 12	87 ± 10
PFUnDA	0.75	3.75	87 ± 16	87 ± 11	86 ± 10
PFDoDA	0.75	3.75	86 ± 15	88 ± 10	89 ± 9
PFTrDA	0.75	3.75	82 ± 14	84 ± 11	85 ± 8
PFTeDA	0.75	3.75	80 ± 13	83 ± 10	84 ± 9
PFHxDA	0.75	3.75	81 ± 17	85 ± 13	90 ± 10
PFODA	0.75	3.75	79 ± 15	78 ± 13	77 ± 12

PFASs	LOD[a] ($ng\,g^{-1}$)	LOQ[b] ($ng\,g^{-1}$)	Recovery[c] (n = 5)		
			at LOQ	at 25 $ng\,kg^{-1}$	at 75 $ng\,kg^{-1}$
Baby food pots (meat based)					
PFBA	4.5	15	72 ± 14	76 ± 13	76 ± 9
PFPeA	4.5	15	75 ± 17	76 ± 15	80 ± 10
PFBS	4.5	15	86 ± 13	85 ± 12	88 ± 10
PFHxA	2.25	7.5	91 ± 15	93 ± 12	94 ± 8
PFHpA	2.25	7.5	90 ± 17	93 ± 10	96 ± 9
PFHxS	4.5	15	90 ± 16	94 ± 11	95 ± 9
PFOA	1.5	3.75	91 ± 15	92 ± 11	99 ± 8
PFHpS	4.5	15	85 ± 15	89 ± 13	92 ± 9
ipPFNA	0.75	3.75	80 ± 14	84 ± 12	90 ± 10
PFNA	0.75	3.75	80 ± 13	83 ± 10	89 ± 11
PFOS	0.75	3.75	99 ± 12	105 ± 10	99 ± 9
ipPFNS	0.75	3.75	83 ± 16	85 ± 14	91 ± 10
PFDA	0.75	3.75	82 ± 15	85 ± 13	88 ± 9
PFDS	0.75	3.75	81 ± 14	84 ± 13	86 ± 8
PFUnDA	0.75	3.75	85 ± 16	86 ± 12	85 ± 9
PFDoDA	0.75	3.75	80 ± 14	84 ± 10	87 ± 10
PFTrDA	0.75	3.75	78 ± 15	80 ± 11	83 ± 10
PFTeDA	0.75	3.75	77 ± 15	75 ± 12	80 ± 11
PFHxDA	0.75	3.75	79 ± 16	71 ± 14	83 ± 9
PFODA	0.75	3.75	77 ± 17	79 ± 12	82 ± 10

[a] Calculated as 3 times the signal-to-noise ratio.
[b] Estimated as the lowest concentration that provides RSDs <20% and recoveries >80%.
[c] Recoveries (accuracy) and RSD (intra-day precision) were calculated extracting milk samples spiking at different concentrations in five different days.

Table 4
Average (A), median (M), minimum (min) and maximum (max) concentrations and frequency (freq) of PFASs for breast milk, infant formula and baby food.

PFASs	Breast milk *(n = 10)*					Infant formulas[c] *(n = 16)*					Dry cereals baby food[c] *(n = 13)*					Baby food pots *(n = 12)*				
	A[a]	M[a]	Min[a]	Max[a]	F (%)	A[b]	M[b]	Min[b]	Max[b]	F (%)	A[b]	M[b]	Min[b]	Max[b]	F (%)	A[b]	M[b]	Min[b]	Max[b]	F (%)
PFBA	50	35	6	155	100	165	107.5	14	496	100	276	225.5	1.4	968	100	519	62	17	5013	100
PFPeA	–	–	–	–	–	210	3356	3356	3356	6	–	–	–	–	–	250	2997	2997	2997	8
PFBS	2	8.5	8	9	20	39	160.5	25	280	25	148	148	117	179	15	6	38	20	56	17
PFHxA	6	60	60	60	10	194	794.5	284	1236	25	134	129	66	207	23	–	–	–	–	–
PFHpA	98	67	11	700	70	492	193.5	12	2127	63	100	89.5	50	17	31	19	222	222	222	8
PFHxS	–	–	–	–	–	34	201	143	201	19	265	201	165	429	23	–	–	–	–	–
PFOA	177	57.5	12	980	100	415	128	27	2490	100	179	122	59	557	92	216	190	151	356	100
PFHpS	74	210	70	455	30	–	–	–	–	–	519	159	35	1563	38	11	68.5	66	71	17
ipPFNA	14	139	139	139	10	30	243	150	3360	13	1417	548.5	70	5102	31	75	306	252	336	25
PFNA	4	20	2	21	30	29	53	17	195	31	164	163.5	162	165	15	76	171	165	224	42
PFOS	50	47	5	246	60	61	65	17	563	69	1321	47.5	23	9795	92	19	111	36	186	17
PFDA	43	4	1.4	306	70	155	211	41	398	75	203	249	26	269	69	247	281	252	387	83
PFDS	2	25	25	25	10	1228	192	65	9645	31	71	71	71	71	8	7	40.5	33	48	17
PFUnDA	88	49.5	18	37	60	42	39	26	189	56	1505	121	32	5744	31	68	196.5	191	227	33
PFDoDA	–	–	–	–	–	18	108	57	117	19	218	209	59	386	23	28	167.5	167	168	17
PFTrDA	–	–	–	–	–	4	69	69	69	6	18	18	18	18	8	13	156	156	156	8
PFHxDA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	37	220	219	221	17

[a] Concentration in ng L^{-1}.
[b] Concentration in ng kg^{-1}.
[c] Concentration in powdered product (not reconstituted).

Table 5
Infant Estimated Daily Intake (EDI) of PFASs for breast milk, infant formula, dry cereals and pots (ng kg^{-1} day^{-1}).

	Breast milk					Infant formula					Dry cereals					Pots				
Age (months)	1	6	12	18	24	1	6	12	18	24	1	6	12	18	24	1	6	12	18	24
Baby weight (kg)	4.4	8	10	12	13	4.4	8	10	12	13	4.4	8	10	12	13	4.4	8	10	12	13
Consumption (mL or g)	800	600	300	300	0	0	25	50	25	0	0	30	75	100	150	0	0	250	200	175
PFBA	9.1	3.7	1.5	1.2	–	–	0.5	0.8	0.3	–	–	1.0	2.1	2.3	3.2	–	–	13.0	8.7	7.0
PFPA	–	–	–	–	–	–	0.7	1.0	0.4	–	–	–	–	–	–	–	–	6.2	4.2	3.4
PFBS	0.3	0.1	–	–	–	–	0.1	0.2	0.1	–	–	0.1	0.2	0.2	0.3	–	–	0.2	0.1	0.1
PFHxA	0.6	–	0.2	0.2	–	–	0.6	1.0	0.4	–	–	0.1	0.2	0.3	0.4	–	–	–	–	–
PFHpA	17.8	7.3	2.9	2.4	–	–	1.5	2.5	1.0	–	–	0.1	0.2	0.3	0.4	–	–	0.5	0.3	0.2
PFHxS	–	–	–	–	–	–	0.1	0.2	0.1	–	–	0.2	0.5	0.5	0.7	–	–	–	–	–
PFOA	32.2	13.3	5.3	4.4	–	–	1.3	2.1	0.9	–	–	0.6	1.2	1.4	1.9	–	–	5.4	3.6	2.9
PFHpS	13.4	5.5	2.2	1.8	–	–	–	–	–	–	–	0.7	1.5	1.7	2.3	–	–	0.3	0.2	0.2
ipPFNA	2.5	1.0	0.4	0.3	–	–	0.1	0.2	0.1	–	–	1.6	3.3	3.6	5.0	–	–	1.9	1.2	1.0
PFNA	0.8	0.3	0.1	0.1	–	–	0.1	0.1	0.1	–	–	0.1	0.2	0.2	0.3	–	–	1.9	1.3	1.0
PFOS	9.0	3.7	1.5	1.2	–	–	0.2	0.3	0.1	–	–	0.4	0.7	0.8	1.2	–	–	0.5	0.3	0.2
PFDA	7.1	2.9	1.2	1.0	–	–	–	0.8	0.3	–	–	–	–	–	–	–	–	6.2	4.1	3.3
PFDS	0.5	0.2	0.1	0.1	–	–	0.5	6.1	2.6	–	–	0.5	1.1	1.2	1.6	–	–	0.2	0.1	0.1
PFUnDA	15.9	6.6	2.6	2.2	–	–	3.8	0.2	0.1	–	–	–	–	–	0.1	–	–	1.7	1.1	0.9
PFDoDA	–	–	–	–	–	–	0.1	0.1	–	–	–	1.7	3.5	3.9	5.3	–	–	0.7	0.5	0.4
PFTrDA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.2	0.2
PFHxDA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.9	0.6	0.5
$\sum$ PFASs	109.1	44.8	18.1	15.2	–	–	9.7	15.6	6.5	–	–	7.5	15.1	16.7	23.2	–	–	39.7	26.5	21.4

4. Discussion

4.1. Levels of PFASs in human milk, infant formula and baby food

Of the 20 PFASs included in this study, 17 were detected in breast milk, infant formula and baby food. Only ipPFNS, PFTeDA and PFODA were not detected in any of the samples. PFBA and PFOA were detected in all samples (except in dry cereals with 92% of samples containing PFOA). PFDA was also ubiquitous with a frequency of occurrence between 69 and 83%. PFOS was more irregular, with a frequency ranging from 17 to 92% depending on the matrix. Considering average concentrations, in each matrix predominates different compounds. In breast milk PFHpA and PFOA. In infant formulas PFPeA, PFHxA, PFHpA, PFOA and PFDA and PFDS. The higher levels and frequency of the perfluoroalkyl acids in comparison to perfluoroalkyl sulfonates was already reported (Llorca et al., 2012). PFNA, PFUnDA and PFOS were predominant in dry cereals instead PFBA and PFPeA are the most important PFASs in baby food pots. The origin of these compounds in the case of infant formulas, dry cereals and food pots can be the production chain since many parts and pieces of the different equipment were made of any perfluoroalkylated materials. In general, concentration values were lower in breast milk. The highest averages were in infant formulas and dry cereals.

For breast milk, PFBA and PFOA were in all the samples, followed by PFHpA and PFDA both detected in 70% of the samples and PFOS and PFUnDA both in 60%. The concentrations of PFBA ranged between 6 and 155 ng L^{-1} and from PFOA between 12 and 980 ng L^{-1}. PFOA involve 29% of the total PFASs concentration followed by PFHpA with 16% and PFUnDA with 14%, while PFOS and PFBA both represent 8%. Considering the media value that takes into account frequency and concentration the relevant PFASs in breast milk (median > 1) were PFBA, PFHpA, PFOA, PFOS, PFDA and PFUnDA. In contrast to other POPs that accumulate in the fatty tissues, PFASs circulate in the blood, mainly bound to proteins, and accumulate in high protein content tissues, such as liver and kidneys of living organisms. Their occurrence in breast milk can be explained because the most abundant proteins in human

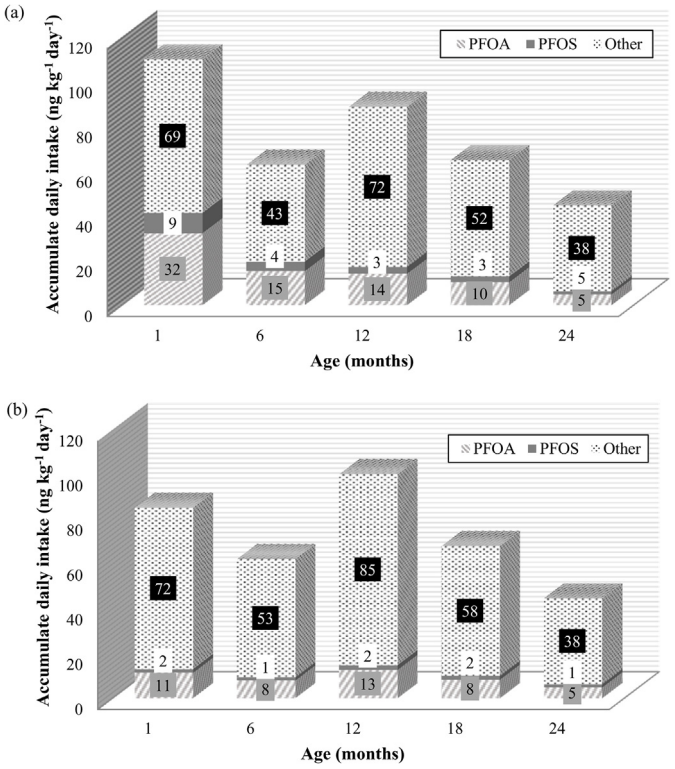

Fig. 2. Accumulate infant daily intake of PFASs considering that infant are feeding according DRIs (a) feeding with breast milk and (b) feeding with infant formula.

milk are casein, α-lactalbumin, lactoferrin, secretory immunoglobulin IgA, lysozyme, and serum albumin. The scientific evidence pointed out that this last protein may not be synthesized by the mammary gland. Instead, it could be transferred from maternal circulation, and this can be the vehicle of the PFASs transfer to human milk (Lönnerdal et al. in press). High levels of these short carbon-chain PFCAs in breast milk observed in the present study population may reflect recent use pattern of these PFASs that are replacing long-chain ones in the industry. Our results are also consistent with previous findings of Kang et al. (2016) who already noted the emergence of short-chain PFASs in breast milk and reported higher concentrations of these compounds. Kang et al. (2016) also related the presence of PFHpA and PFOA to cooking utensils (mostly frying pans) and the presence of PFHpA and PFOS to the use of personal care products. In our study, the high levels and frequency of PFBA can be explained because it is a breakdown product of stain and grease proof coatings in food packaging, couches, and carpets and are in agreement with several studies that described widespread environmental contamination with these short-chain PFASs in the environment of the studied area (Campo et al., 2016; Lorenzo et al., 2016; Llorca et al., 2012).

The pattern of PFASs in human milk depends on the serum concentration, which in turn depends on exposure, accumulation and half-lives of the compounds. As already suggested Kang et al. (2016) short chain PFASs reflect recent exposures because their half-lives in human plasma are only few days (e.g. 3 days for PFBA). Contrarily, half-lives of long-chain PFASs are longer (e.g. PFOA half-life in human serum/plasma was estimated to be 2.3–8.5 yr).

There were not apparent correlations between PFASs concentration in breast milk and characteristics of the volunteer mothers showed in Table 1 (mother age and weight, breastfeeding duration and frequency, etc.) in agreement with previous works that reported deeper study of the possible relation between diet and PFAS concentrations in human milk. This can be because of the moderate size of the sample, in any case, and due to the lack of apparent differences, no statistical test was applied.

In the infant formulas, as in the breast milk, PFBA and PFOA were detected in 100% of the analysed samples, followed by PFDA (75%) and PFOS (69%). The concentrations of PFBA ranges from 14 to 496 ng kg^{-1}, PFOA from 27 to 2490 ng kg^{-1} (dry weight). However, the highest average concentration (1228 ng kg^{-1}) correspond to PFDS because even through it was detected in only 5 samples, it was at very high concentrations. The median values shows PFBA, PFHpA, PFOA, PFOS, PFDA and PFUnDA as in the human milk. The sources of PFASs in cow milk could be the same as in human milk plus contamination through the food chain and packaging material that could justify the high concentrations of PFDS. The concentrations of PFASs in infant formula are calculated in the powder formula not in the reconstituted product. Infant formulas and other baby foods were purchased in retail stores, pharmacies and supermarkets. They were from multinational corporations, then, the different ingredients were not necessarily from Spain.

For dry cereals, PFBA was detected in 100% of the samples at concentrations from 1.4 to 968 ng kg^{-1} while PFOS and PFOA were detected in 92% and PFDA in 69% up to 9795, 557 and 260 ng kg^{-1}, respectively. Median values shows that more relevant PFASs are PFBA, PFHpA, PFOA, PFOS and PFDS. The difficulty to know the origin of these foods has already been pointed out for the case of cereals (D'Hollander et al., 2015).

Finally, for baby food pots, PFBA and PFOA were detected in 100% of analysed samples with concentrations up to 5013 ng kg^{-1}, fol-lowed by PFDA (83%) up to 387 ng kg^{-1}. However, PFOS was detected only in 17% of the samples. In this matrix, three compounds almost represent the whole contamination profile: PFBA (49%), PFOA (21%) and PFDA (20%).

The concentrations found in the breast milk, formula and cereal baby food analyzed in this study were, in general terms, of the same order of magnitude as those reported in other dietary studies of perfluoroalkyl substances in baby foods (Table 6). PFOS and PFOA were under 200 ng L^{-1}, in line with levels reported in previous studies performed in other developed areas (Antignac et al., 2013; Barbarossa et al., 2013; Croes et al., 2012; Völkel et al., 2008). Only one study covering human milk and infant formula from Barcelona in 2009 (Llorca et al., 2010) showed higher values of PFOS (28–865 ng L^{-1}) than those of this study but very similar for PFOA (12–908 ng L^{-1}). Differences between both studies may be due to the greater industrialization of the Barcelona area compared to Valencia or to the different data of sample collection.

In powdered formulas, PFOA concentrations were in a range of 27–2490 ng kg^{-1}, for PFDA 41–398 ng kg^{-1} and for PFOS 17–224 ng kg^{-1}, in agreement with previously reported study (Llorca et al., 2010). There are no more data available for comparison. For dry cereals, average concentrations of PFOA, PFNA, PFDA and PFDS (179, 164, 203 and 71 ng kg^{-1}) are similar in other studies in Spain (Llorca et al., 2010), while average of PFOS and ipPFNA (1321 and 1417 ng kg^{-1} respectively) are higher. In baby food pots, PFOA and PFOS concentrations ranged from 151 to 356 ng kg^{-1} and 36–186 ng kg^{-1}, respectively, similar to other data reported (Llorca et al., 2010).

The profile of PFASs in the different samples (Fig. 1) shows that PFCAs are more relevant than PFSAs in all the studied matrices. Infant formulae have the most important contribution of PFSAs (mostly due to PFOS and PFDS). The distribution patterns of PFASs in breast milk observed in this study are consistent with those previously reported (Kadar et al., 2011; Liu et al., 2010; Llorca et al., 2010). Long chain PFCAs are the most prevalent in breast milk and cereals whereas short-chain are the most relevant in infant formulae and pots.

Table 6
Measured PFASs levels in breast milk and baby food samples in present study and other published data (range or median).

Reference	Year	Country	No. samples	Type of sample	PFOA (ng L^{-1})
Völkel et al. (2008)	2009	Germany	307	Breast milk	15–289
Llorca et al. (2010)	2010	Spain	20	Breast milk	15–907
			2	Cereals baby food	166–438[a]
			3	Infant formulas	374–723[a]
Kadar et al. (2011)	2011	France	30	Breast milk	18–102
Fujii et al. (2012)	2011	Japan	30	Breast milk	89
		Korea	30	Breast milk	62
		China	30	Breast milk	51
		Japan	5	Infant formulas	22.5–35.8
		China	4	Infant formulas	29.9–35.4
Croes et al. (2012)	2012	Belgium	40	Breast milk	80
Barbarossa et al. (2013)	2013	Italy	37	Breast milk	24–241
Antignac et al. (2013)	2013	France	48	Breast milk	50–224
Lankova et al. (2013)	2013	Czech Republic	50	Breast milk	12–128
Motas Guzmàn et al. (2016)	2014	Spain	67	Breast milk	<LOQ-211
Present study	2013	Spain	10	Breast milk	12–980
			16	Infant formulas	27–2490[a]
			13	Cereals baby food	59–557[a]
			12	Baby food pots	151–356[a]

[a] PFOA in ng kg.

4.2. Estimated daily intake of infants

According to the EFSA guideline, TDI for PFOS was 150 ng kg^{-1} and 1500 ng kg^{-1} for PFOA, these data could be used like a reference about a total daily intake of PFASs. In Fig. 2a the daily intake of PFASs in 1 month infants, is 126 ng kg^{-1} (34.7 of PFOA, 9.7 of PFOS and 81.8 of the rest of PFASs). The EDIs are far away from the TDIs established by EFSA for PFOS and PFOA. However, there are other congeners present in the samples that can present a quite different toxicity range. Furthermore, the TDI established by the EFSA were provisional until more data to perform a more accurate risk assessment would be available

The more milk consumption decreases, the more daily intakes of PFASs decreases. For 6 month infants, daily intake of PFASs was 94.8 ng kg^{-1} per day (18.5 of PFOA, 12.2 of PFOS and 64.1 of the rest of PFASs). In 1-year infants was 72.1 ng kg^{-1} per day (10.8 of PFOA, 8.4 of PFOS and 52.8 of the rest of PFASs). For 18 moth infants was 50.8 ng kg^{-1} per day (8.1 of PFOA, 6.8 of PFOS and 35.9 of the rest of PFASs). Finally, for 2-year infants was 30.4 ng kg^{-1} per day (5.1 of PFOA, 4.9 of PFOS and 22.3 of the rest of PFASs). These results demonstrated the role of lactation (mother's lactation or infant formulas fed) as a source of PFASs for new-borns.

In Fig. 2b, the daily intake of PFASs for new-borns and children (up to 2 years old) feeding with infant formulas instead of mother milk is illustrated. The results are similar showing that artificial lactation does not present any advantage regarding the levels of these contaminants in the diet. Intakes are in general higher for child that are feeding with infant formulas except during the first six month of life, in which the intake is slightly higher due to the high PFOA content of mothers milk. This fact was already reported in other studies (Antignac et al., 2013; Barbarossa et al., 2013; Llorca et al., 2010). The transfer of PFASs from blood to the breast milk during breastfeeding was confirmed by correlations between measured values in these two media was already proved (Fromme et al., 2010). The breast milk samples taken in this study were all from primiparous mothers. Barbarrosa et al. (Barbarossa et al., 2013) took into account the primipara/multipara status of study's milk donors pointing out that the mean concentration and frequency were higher for both the monitored analytes in milk provided by women nursing for the first time. This can explain the high levels of PFOA found in this study as well as confirm the suggestion of a potentially higher exposure to PFASs for first-born infants.

In certain cases, drinking water can be a source of exposure to PFASs as important as the dietary intakes of these pollutants (Ericson et al., 2008; Llorca et al., 2012). In this study, intakes of PFASs from drinking water samples have not been included and should be carried out further studies in order to increase the knowledge of the role of drinking water in infant exposure to PFASs. However, previous studies already noted that the contribution of drinking water to the total exposure of these compounds is commonly negligible (Noorlander et al., 2011). Only in the cases where freshwater is directly used for the preparation of drinking water or when local sources contain high PFOS and PFOA concentrations drinking water may become an important route of exposure to these compounds. In the Valencian Community, fresh water is never directly used for drinking water due to water scarcity in the area and local sources (previously analyzed) do not present high levels of these compounds (Llorca et al., 2012).

Summarizing, this is the first study that investigates PFASs levels in human breast milk, infant formula and baby food in the Valencian Community. The results show a widespread distribution of PFBA in 100% of analyzed samples of breast milk and other baby food. Although previous studies are very punctual with a scarce number of samples, our results are within the range of concentration reported. The dietary intake of PFOS and PFOA varies significantly depending on the infant diet, but in general, the low concentrations and corresponding low intakes of these compounds indicated a low risk for this group of population. However, the EFSA has only established provisional TDI for PFOS and PFOA modifiable and expandable to other PFASs as more studies become available. Owing to the lack of available objective information of risk assessment associated to exposure to other PFASs, more research is needed for both adult population and infant population. Given these outcomes, studies will be required in order to increase the knowledge on the importance of infant exposure to PFASs and identify potential influencing factors, especially considering that breast milk, or in its defect, starting milk formulas represents almost the entire diet for children during the first 4–6 months of life.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.enmm.2016.09.001

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SUPPORTING INFORMATION

Perfluoroalkyl Substances in Breast Milk, Infant Formula and Baby Food from Valencian Community (Spain)

María Lorenzo, Marinel.la Farré, Cristina Blasco, Matthias Onghena, Yolanda Picó and Damià Barceló

I. Materials and Methods

a. Chemicals and standards

Standards: Perfluorobutanoic acid (PFBA) [MW: 214; purity: > 98 %], perfluoropentanoic acid (PFPeA) [264; > 98 %], perfluorohexanoic acid (PFHxA) [314; > 98 %], perfluoroheptanoic acid (PFHpA) [364; > 98 %], perfluorooctanoic acid (PFOA) [414; > 99 %], perfluorononanoic acid (PFNA) [464; > 99 %], perfluoro-7-methyloctanoic acid (ipPFNA) [464], perfluorodecanoic acid (PFDA) [514; > 99 %], perfluoroundecanoic acid (PFUnDA) [564; > 98%], perfluorododecanoic acid (PFDoDA) [614; > 98%], perfluorotridecanoic acid (PFTrDA) [664; > 98%], perfluorotetradecanoic acid (PFTeDA) [714; > 98%], perfluorohexadecanoic acid (PFHxDA) [814; > 98%], perfluorooctadecanoic acid (PFODA) [914; > 98%], potassium perfluorobutanesulfonate (PFBS) [338.19], potassium perfluorohexanesulfonate (PFHxS) [422.10], Sodium perfluoroheptanesulfonate (PFHpS) [MW: 472.10] sodium perfluorooctanesulfonate (PFOS) [522.11], sodium perfluoro-7-methyloctanesulfonate (ipPFNS) [572.12; > 98 %], sodium perfluorodecanesulfonate (PFDS) [622.13; > 99 %] were purchased from Wellington Laboratories Inc. Canada.

Internal standards: sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octane sulfonate ($^{13}C_4$-PFOS) [MW: 526.08; purity: > 99 %], sodium perfluoro-1- hexane [$^{18}O_5$] sulfonate ($^{18}O_5$-PFHxS) [426.09; > 99 %] and surrogates perfluoro-n-[1,2,3,4-$^{13}C_4$] butanoic acid ($^{13}C_4$-PFBA) [222; > 99 %],perfluoro-n-[1.2-$^{13}C_2$] hexanoic acid ($^{13}C_2$-PFHxA) [318; > 99 %], perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid ($^{13}C_4$-PFOA) [418; > 99 %], perfluoro-n-[1,2,3,4,5 -$^{13}C_5$] nonanoic acid ($^{13}C_5$-PFNA) [469; > 99 %], perfluoro-n-[1,2-$^{13}C_2$] decanoic acid ($^{13}C_2$-PFDA) [516; > 99 %], perfluoro-n-[1,2-$^{13}C_2$] undecanoic acid ($^{13}C_2$-PFUdA) [566; > 99 %] and perfluoro-n-[1,2-$^{13}C_2$] dodecanoic acid ($^{13}C_2$-PFDoA) [616; > 99 %] were also purchased from Wellington Laboratories Inc.

Methanol (MeOH) were of HPLC grade and they were from Merck (Darmstadt. Germany). Deionized water (< 18 MΩ cm) was from a Mili-Q SP Reagent Water System (Millipore, Bedford, MA. USA).

Ammonium hydroxide (25 % in water) were purchased from Merck (Darmstadt, Germany), ammonium formate (HCO_2NH_4, MW: 63.06; ≥97) was obtained from Sigma-Aldrich, (Steinheim, Germany), sodium hydroxide base (NaOH, MW: 39.997; >97 %) was from Merck (Darmstadt, Germany). Strata-X cartridges of 200 mg (6 mL) from Phenomenex (Torrance, CA, USA).

II. Instrumental analysis

The mobile phase consisted of ammonium formate 10 mM in (A) ultra-pure water and (B) methanol. The flowrate was 0.5 mL min^{-1} programmed with the following elution gradient conditions: 10–80% B over 5 min. then 80–90% B over other 5 min followed by an isocratic hold at 90% B for 8 min. At 18 min B was returned to 10% in 2 min. The total run time for each injection was 20 min. The sample volume injected was 20 µL.

Ionization and fragmentation settings were optimized by direct injection of PFASs standard solutions. MS/MS was performed in the selected reaction monitoring (SRM) mode using electrospray ionization source (ESI) in negative mode. For each compound, two characteristic fragments of the protonated molecule $[M+H]^-$ were monitored, the first and most abundant one was used for quantification, while the second one was used as a qualifier. Collision energy and cone voltage were optimized for each PFAS (Table S1). Nitrogen was used as collision, nebulizing and desolvation gas. The ESI conditions were: capillary voltage 4000 V, nebulizer 30 psi, source temperature 300 °C and gas flow 11 L min^{-1}. In order to maximize sensitivity, dynamic MRM was used, with MS_1 and MS_2 at unit resolution and cell acceleration voltage of 10 eV for all the compounds.

Table S1. Molecular weight, retention time, main transition, internal standard (IS), declustering potential (fragmentor) and collision energy of selected PFASs.

Compound	Molecular weight	Retention time (min)	Main transition	Internal standard	Fragmentor (V)	Collision Energy (V)
PFBA	214.03	1.595	213 > 169*	MPFBA	66	5
PFPeA	264.04	4.598	263 > 219*	MPFBA	66	5
PFBS	338.19	7.478	299 > 80*	MPFBA	142	38
			299 > 99	MPFBA		
PFHxA	314.05	9.569	313 > 219	MPFHxA	71	5
			313 > 169*	MPFHxA		
PFHxS	422.09	11.142	399 > 99	MPFHxS	169	37
			399 > 80*	MPFHxS		29
PFHpA	364.06	11.971	363 > 319	MPFHxA	76	5
			363 > 169*	MPFHxA		5
PFHpS	472.10	12.713	449 > 99	MPFHxS	179	37
			449 > 80*	MPFHxS		57
PFOA	414.07	13.025	413 > 369	MPFOA	87	5
			413 > 169*	MPFOA		
PFOS	538.22	13.704	499 > 99	MPFOS	190	41
			499 > 80*	MPFOS		65
ipPFNA	464.08	13.893	463 > 219	MPFNA	87	5
			463 > 169*	MPFNA		
PFNA	464.08	13.893	463 > 219*	MPFNA	82	5
			463 > 169	MPFNA		
ipPFNS		14.530	549 > 99	MPFNA	195	45
			549 > 80*	MPFNA		73
PFDA	514.08	14.656	513 > 469	MPFDA	89	5
			513 > 269*	MPFDA		13
PFDS	622.00	15.025	599 > 99	MPFOS	80	80
			599 > 80*	MPFOS		80
PFUnDA	564.09	15.358	563 > 269	MPFUnDA	104	5
			563 > 519	MPFUnDA		13
PFDoDA	614.10	16.329	613 > 269	MPFDoDA	94	5
			613 > 569	MPFDoDA		13
PFTrDA	664.10	16.701	663 > 169	MPFDoDA	104	0
			663 > 619	MPFDoDA		24
PFTeDA	714.11	17.094	713 > 669	MPFDoDA	112	5
			713 > 169*	MPFDoDA		25
PFHxDA	814.13	18.046	813 > 769	MPFDoDA	114	8
			813 > 169*	MPFDoDA		28
PFODA	914.15	18.066	913 > 169*	MPFDoDA	134	10

* m/z quantification transitions

III. Validation and quality control

Linearity

The linearity was evaluated in matrix-matched standards to avoid any matrix effect. An aliquot of the mixture of all labelled compounds was diluted to obtain 8 calibration points (internal standards were at 25 ng mL^{-1}). The calibrate solutions were analysed by LC-MS/MS. The peak ratio of the analyte versus the corresponding internal standard was plotted against their respective concentration. Linear regression using least-squares estimation was employed to calculate the linear equation of the calibration curves.

Limits of detection and quantification

The LOQ was determined by spiking the samples with 10 µL of the reference mixture of PFASs at three different concentration levels (50, 150, and 200 ng mL^{-1}, equal to 0.5, 1.5 and 2 ng L^{-1} in liquid samples and to 2.5, 7.5 and 10 ng kg^{-1} in powdered samples) and 10 µL of the mixture of all labelled compounds. The solutions were added with a GC syringe taken care to uniformly spread them on the sample. The lowest concentration with a precision ≤ 20 % RSD and an accuracy > 80 % of the nominal value defined the lowest quantifiable concentration. The LOD was defined for the concentration giving a signal-to-noise ratio (S/N) > 3.

Recoveries and RSDs

Recoveries were determined by spiking the twenty selected compounds in blank breast milk, infant milk formula and baby food as described in the previous section. Five replicates of each type of sample spiked at three levels of concentration were performed (LOQ, 25 and 75 ng L^{-1}). Recoveries for spiked samples were in the range of 70 – 110 % and for RSD was always below 21 % (**Table 3**). Therefore, the applicability of the method for PFASs in milk and baby food was demonstrated. Figure SI1 show a chromatogram of spiked (25 ng mL^{-1}) breast milk with the 20 studied PFASs.

Quality control

The linearity was analysed twice at the beginning and end of each sample batch. Procedural blanks tests were injected after 10 samples to check any potential contamination during the extraction of the samples, followed by the injection of extracts of samples spiked at the LOQ level to avoid either false positives or negatives. Then, methanol was analysed twice for monitoring the instrumental background.

Table S2. PFASs concentration (ng L^{-1}) in breast milk (n = 10).

PFASs	Breast milk									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
PFBA	119	6	39	10	31	109	110	10	155	14
PFPeA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFBS	n.d.	8	n.d.	n.d.	9	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxA	60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	67	58	11	n.d.	21	83	700	371
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	15	12	47	32	32	80	195	68	980	310
PFHpS	n.d.	n.d.	n.d.	n.d.	455	n.d.	n.d.	70	210	n.d.
ipPFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	139
PFNA	n.d.	2	n.d.	n.d.	n.d.	n.d.	21	20	n.d.	n.d.
PFOS	246	12	68	5	n.d.	n.d.	n.d.	26	140	n.d.
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	4	<LOQ	3	3	70	n.d.	1.4	n.d.	306	n.d.
PFDS	25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	18	n.d.	n.d.	43	30	n.d.	56	n.d.	360	370
PFDoDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

<LOQ: lower than limit of quantification; n.d.: not detected

Table S3. PFASs concentration (ng kg^{-1}) in powdered formulas for infants (n = 16).

PFASs	FIRST FORMULA (0 – 6 months)					FOLLOW ON FORMULA (> 6 months)									TODDLER FORMULA (> 12 months)	
	1	2	3	4	5	1	2	3	4	5	6	7	8	9	1	2
PFBA	109	45	23	432	116	88	61	295	71	286	34	106	496	14	205	262
PFPeA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3356	n.d.	n.d.	n.d.	n.d.
PFBS	n.d.	n.d.	n.d.	52	n.d.	n.d.	269	n.d.	280	25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxA	n.d.	353	284	n.d.	n.d.	n.d.	1236	n.d.	1236	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	141	992	n.d.	23	n.d.	93	2127	n.d.	2036	2075	n.d.	120	246	n.d.	n.d.	12
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	201	143	201	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	191	77	180	65	27	81	2490	68	2473	30	175	173	281	201	40	83
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ipPFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	150	n.d.	n.d.	n.d.	n.d.	336	n.d.	n.d.	n.d.	n.d.
PFNA	17	n.d.	n.d.	n.d.	n.d.	n.d.	53	36	n.d.	n.d.	n.d.	n.d.	195	164	n.d.	n.d.
PFOS	224	n.d.	188	69	108	n.d.	56	113	56	n.d.	65	26	59	n.d.	n.d.	17
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	86	n.d.	n.d.	107	n.d.	41	113	143	177	398	245	251	290	332	296	n.d.
PFDS	n.d.	n.d.	n.d.	96	n.d.	n.d.	9645	n.d.	9645	n.d.	n.d.	65	192	n.d.	n.d.	n.d.
PFUnDA	n.d.	26	119	n.d.	n.d.	50	26	39	33	n.d.	188	n.d.	n.d.	189	n.d.	32
PFDoDA	n.d.	n.d.	117	57	108	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	69	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S4. PFASs concentration (ng kg^{-1}) in dry cereals baby food.

PFASs	Dry cereals baby food												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
PFBA	761	323	128	327	206	968	37	1.4	24	171	245	20	373
PFPeA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	117	n.d.	n.d.	n.d.	179	n.d.	n.d.
PFHxA	207	n.d.	n.d.	n.d.	n.d.	129	n.d.	n.d.	n.d.	66	n.d.	n.d.	n.d.
PFHpA	116	63	n.d.	50	170	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxS	165	n.d.	n.d.	n.d.	429	201	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	84	89	59	87	73	68	155	n.d.	174	231	379	192	557
PFHpS	n.d.	n.d.	n.d.	117	159	1563	35	n.d.	719	n.d.	n.d.	n.d.	n.d.
ipPFNA	n.d.	n.d.	n.d.	159	70	n.d.	n.d.	5102	n.d.	n.d.	338	n.d.	n.d.
PFNA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	162	n.d.	165	n.d.	n.d.	n.d.	n.d.
PFOS	370	47	207	23	39	n.d.	36	98	47	264	80	48	41
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	195	197	114	n.d.	26	n.d.	254	n.d.	263	259	n.d.	249	269
PFDS	n.d.	n.d.	n.d.	71	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	n.d.	54	n.d.	n.d.	32	n.d.	188	5744	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	n.d.	n.d.	n.d.	n.d.	59	n.d.	n.d.	n.d.	n.d.	n.d.	209	386	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected

Table S5. PFASs concentration (ng kg^{-1}) in baby food pots.

PFASs	Baby food pots											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
PFBA	378	61	26	5013	338	39	26	157	65	63	49	17
PFPeA	n.d.	n.d.	n.d.	2997	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	56	20	n.d.	n.d.
PFHxA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHpA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	222	n.d.	n.d.	n.d.
PFHxS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	183	192	165	154	203	188	165	356	332	313	151	194
PFHpS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	71	66	n.d.	n.d.	n.d.
ipPFNA	n.d.	306	n.d.	336	n.d.	n.d.	n.d.	252	n.d.	n.d.	n.d.	n.d.
PFNA	n.d.	n.d.	n.d.	224	n.d.	n.d.	n.d.	n.d.	165	183	171	171
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	36	186	n.d.	n.d.	n.d.
ipPFNS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDA	294	276	281	363	281	252	281	293	n.d.	n.d.	255	387
PFDS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	48	n.d.	n.d.	n.d.	33
PFUnDA	192	n.d.	n.d.	227	201	n.d.	n.d.	n.d.	n.d.	n.d.	191	n.d.
PFDoDA	n.d.	167	n.d.	n.d.	168	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFTrDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	156
PFTeDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFHxDA	n.d.	n.d.	n.d.	221	219	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S6. Estimated daily consumption of baby food for infants.

Baby food	Age (months)				
	0 – 6	**6 – 12**	**12 – 18**	**18 – 24**	**24 – 36**
Breast milk (mL)	800	600	300	300	-
Infant formula (g)	-	25	50	25	-
Cereal baby food (g)	-	30	75	100	150
Baby food pots (g)	-	-	250	200	175

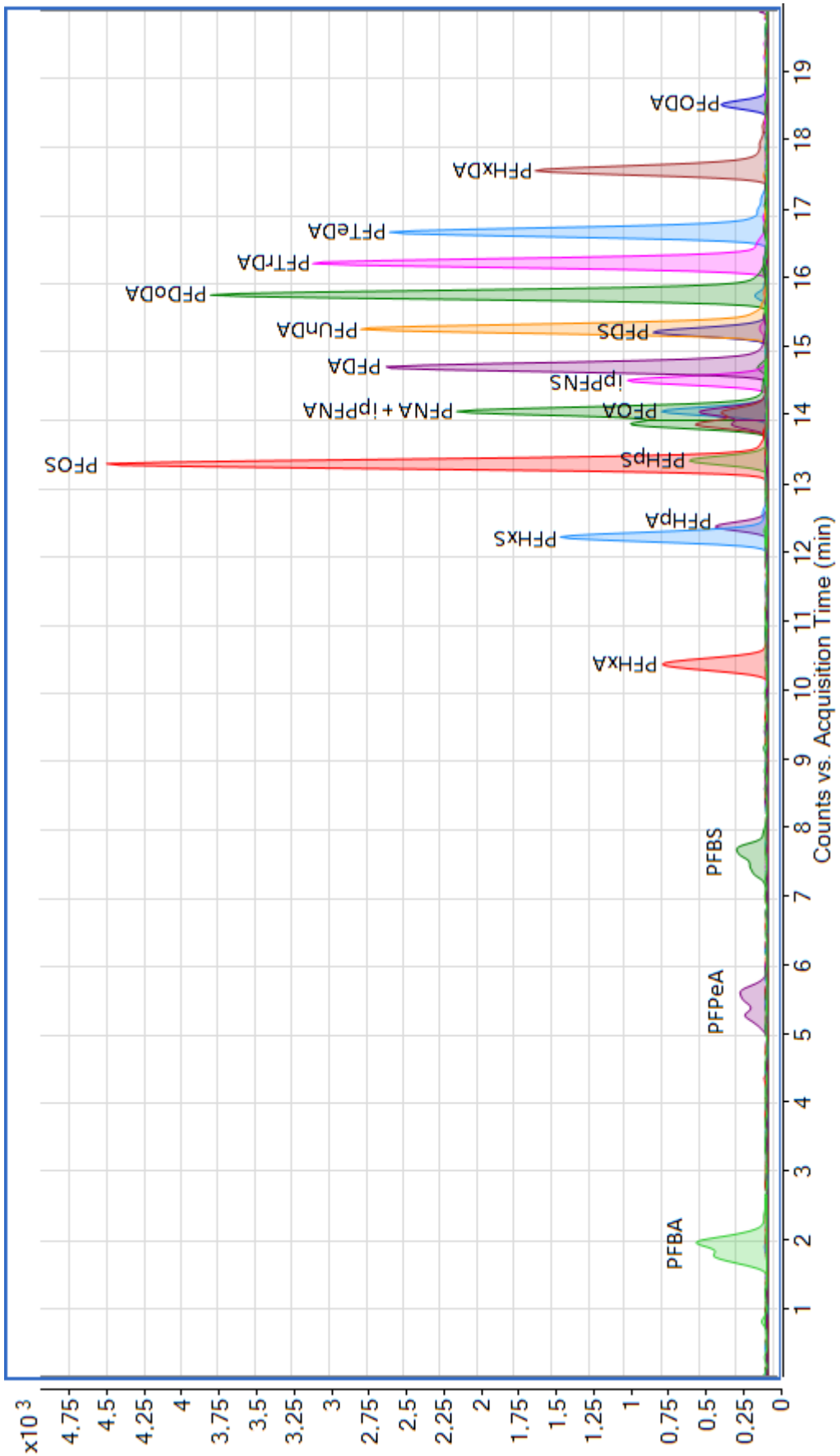

Figure S1. Standard chromatogram of spiked breast milk (25 ng mL^{-1}) with PFASs.

CAPÍTULO 10

Polydimethylsiloxane brooch as a personal passive air sampler for semi-volatile organic compounds.

Parte de este capítulo ha sido enviado a la revista *Chemosphere* y firmado por los autores:

Joseph O. Okeme, Maria Lorenzo, Linh V. Nguyen, Suman Dhal, Yolanda Picó, Victoria H. Arrandale, Miriam L. Diamond.

Abstract

Exposure assessment conducted using a personal sampler includes the contribution of human activities to exposure that is neglected when using a stationary air sampler. This study characterized and evaluated the polydimethylsiloxane (PDMS) brooch as a passive air sampler for measuring concentrations of two groups of semi-volatile organic compounds (SVOCs), namely phthalates and organophosphate esters (OPEs), indoors in promixity to the breathing zone.

Uptake rates of the PDMS brooch were calibrated against a personal low volume active air sampler (PLV-AS) co-deployed on each of five study participants working for 8 hours daily for four days in offices. Sampling rates measured here ranged from 0.41 ± 0.33 to 1.33 ± 0.34 m^3 day^{-1} dm^{-2} with an average value of 0.86 ± 0.29 m^3 day^{-1} dm^{-2}. A higher sampling efficiency was observed for gas- than particle-phase compounds.

Personal air concentrations were measured for three study participants who used the PDMS brooches continuously for seven days. Values derived based on the sampling rates measured here ranged from 1540 to 4010 ng m^{-3} for $\sum_5$ phthalates and 507 to 1310 ng m^{-3} for $\sum_5$ OPEs. These concentrations resulted in an estimated inhalation exposure of ~ 20 to 40 µg day^{-1} for $\sum_5$ phthalates and 4 to 11 µg day^{-1} for $\sum_5$ OPEs.

This study demonstrated that the PDMS brooch can be used to assess inhalation exposure when worn for at least 24 hours indoors for compounds present in ng m^{-3} to µg m^{-3} range such as the phthalates and OPEs tested here.

1 Introduction

Inhalation can be an important pathway for exposure to semi-volatile organic contaminants (SVOCs) (Schreder et al., 2016; Merkel et al., 2017). Inhalation exposure in micro-environments is commonly assessed by measuring air concentrations at a central, stationary location. However, stationary air measurements may not reflect personal exposure because they exclude the influence of personal activities on exposure. Personal activities create a personal cloud of contaminants in close proximity to one's body, leading to increased exposure. This phenomenon has been called the personal cloud or the "Pig Pen" effect (Wallace 1991; Rodes et al., 1991; Allen et al., 2007).

Representative personal inhalation exposure can be captured using personal low volume active air samplers (PLV-AAS). An advantage of using active samplers is their ability to provide reliable measurements because they run at known and adjustable flow rates. But active samplers are expensive, obtrusive and inconvenient to use because they require power, maintenance, training, frequent calibration and they are noisy. Personal passive air samplers (PPAS) could be useful alternatives to personal active samplers because they work by diffusion and deposition and as such do not have the logistic disadvantages of active samplers (Shoeib and Harner, 2002). PPAS have disadvantages as well. PPAS likely require long deployment times to achieve analytical detection and they need to be calibrated against active air samplers to obtain their passive sampling rates that vary according to circumstance. Passive air samplers (PAS) can also be calibrated using performance reference or depuration compounds, but this method is well studied for only gas-phase compounds in outdoor deployments. However, studies have shown that PASs collect compounds in both gas- and particle- phases (Melymuk et al. 2011; Bohlin et al., 2014, Saini et al., 2015).

PPAS have been well characterized for measuring volatile organic compounds (VOCs) (e.g., Palmes et al., 1976; Zabiegala et al., 2011), whereas little progress has been made in using them to measure SVOCs (Bohlin, 2010). VOCs are easier to measure than SVOCs because VOCs are often more abundant in air and they exist only in the gas-phase due to their high vapour pressure range of 10 to 10^4 Pa (Bidleman, 1988). In contrast, SVOCs are usually present in trace amounts in air and they partition to both gas and particle phases, owing to their low vapour pressure values, which range from 10^{-9} to 10 Pa (Bidleman, 1988).

Examples of PPAS that have been introduced for sampling SVOCs include the mini-polyurethane foam (min-PUF) (Bohlin et al., 2010), the polydimethylsiloxane or silicone rubber wristbands (O'Connell et al., 2014; Hammel et al., 2016), and the miniature bird-borne PAS (Sorais et al., 2017). The mini-PUF was used to assess occupational exposure to elevated levels of polycyclic aromatic hydrocarbons (PAHs) (Bohlin et al. 2010). That study calibrated a stationary mini-PUF as a proxy for mini-PUF as a PPAS. No other study has tested the mini-PUF to date to the best of our knowledge. The PDMS wristband has been used to qualitatively assess exposure of roofers to PAHs and as a surrogate for assessing the internal dose of

organophosphate esters (OPEs) in the general population (O'Connell et al., 2014; Hammel et al., 2016). The miniature bird-borne PAS has been used to study the exposure of gulls to flame retardants during foraging and other activities. The PDMS wristband and miniature bird-borne PAS have not been calibrated as PPAS. Therefore, all three PPAS have yet to be used to estimate volumetric air concentrations that are useful for quantitatively assessing non-dietary exposure pathways such as inhalation. Previous work calibrated PDMS as a stationary sampler and found it to have a high uptake rate and uptake capacity for gas-phase compounds (Okeme et al., 2016b; Okeme and Yang et al., submitted).

This study aimed to characterize the PDMS brooch as a PPAS as proof-of-concept to improve estimates of inhalation exposure in a non-industrial indoor environment to SVOCs, specifically phthalates and organophosphate esters (OPEs) that are examples of SVOCs used as plasticizers and flame retardants, respectively, in many indoor materials. We chose PDMS because it is light in weight, easily used, collects many compounds, and has low blank levels (Okeme et al., 2016b). In this paper, we report the sampling rates of the PDMS brooch as PPAS and the personal air concentrations and estimates of exposure obtained from the use of the PDMS brooch.

2 Methods

2.1 Calibration study of PDMS brooch

The purpose of the calibration study described here was to measure sampling rates for converting the mass of compounds collected by the PDMS brooch in a field study into volumetric air concentrations and then inhalation exposure. The calibration study was conducted in January 2016 in two offices located in downtown Toronto, Canada. Five participants wore personal low volume active samplers (PLV-AAS) and PDMS brooch PPAS for 8 working hours daily for four days while using workstations in their offices. Individual target compounds, CAS numbers and monitored ions are listed in Table S1.

The PDMS brooch consisted of a PDMS strip (length 9 cm × breath 5.5 cm × thickness 0.1 cm; Specialty Silicone Products, Inc., Ballston Spa, NY) stapled to an aluminum housing having a similar surface area to the PDMS. The exposed PDMS surface area was 50 cm^2. The size was

based on the maximum surface area that would not interfere with a participant's activities. The aluminum housing acted as a barrier to prevent contact between the PDMS and clothing, to minimize cross contamination. The PDMS brooch was designed specifically to collect airborne SVOCs, in contrast to the wristband sampler (e.g., O'Connell et al., 2014), which may capture multiple routes of exposure as a result of contact with skin and surfaces.

A PLV-AAS was used as a reference device for calibrating the PDMS brooch. The PLV-AAS consisted of a pump (Aircheck sampler, model 224-PCXR8, SKC INC, Eighty-Four, PA) connected to a sorbent tube containing PUF and styrene divinylbenzene copolymer (PUF/XAD/PUF) sandwich (ORBO 49P (OVS) Supelpak; Sigma Aldrich). The sandwich collected a combination of gas- and particle- phase compounds indistinguishably. The PLV-AAS ran at a flow rate of 0.4-0.5 L/min (NIOSH Method 5600) to collect a total of 192-240 L (0.192 - 0.240 m^3) of air each 8-hour sampling period

On day 1 of the calibration study, each study participant wore four PDMS brooches in the breathing zone (upper chest) along with a PLV-AAS, with the inlet of the PLV-AAS also positioned in the breathing zone. At the end of the work shift, one PDMS brooch and the PLV-AAS sorbent tube were harvested for each participant. The remaining three brooches were stored in a stainless-steel case and worn again on the following day along with a new PLV-AAS sorbent tube. This procedure was repeated until the last PDMS brooch and PLV-AAS samples were harvested on day 4. Duplicate samples were not tested here because of the limited space of a participant's upper chest.

2.2 Field deployment of the calibrated PDMS brooch

A seven-day field experiment was designed to use the PDMS brooch calibrated in this work to measure personal air concentrations. The participants were three office workers who used workstations during office hours. The participants and locations of the field study were different from those of the calibration study and all three participants worked and lived in different buildings from one another. They wore PDMS brooches in their breathing zone indoors for seven consecutive days except when they were sleeping or bathing, at which time the PDMS brooches were kept on bedside tables, bed stands or bathroom countertops. Only PDMS brooches were worn in this part of the study (Figure 1), no PLV-AAS were deployed. Participants

were not asked to log their activities as the factors associated with exposure were not the focus in this proof-of-concept study.

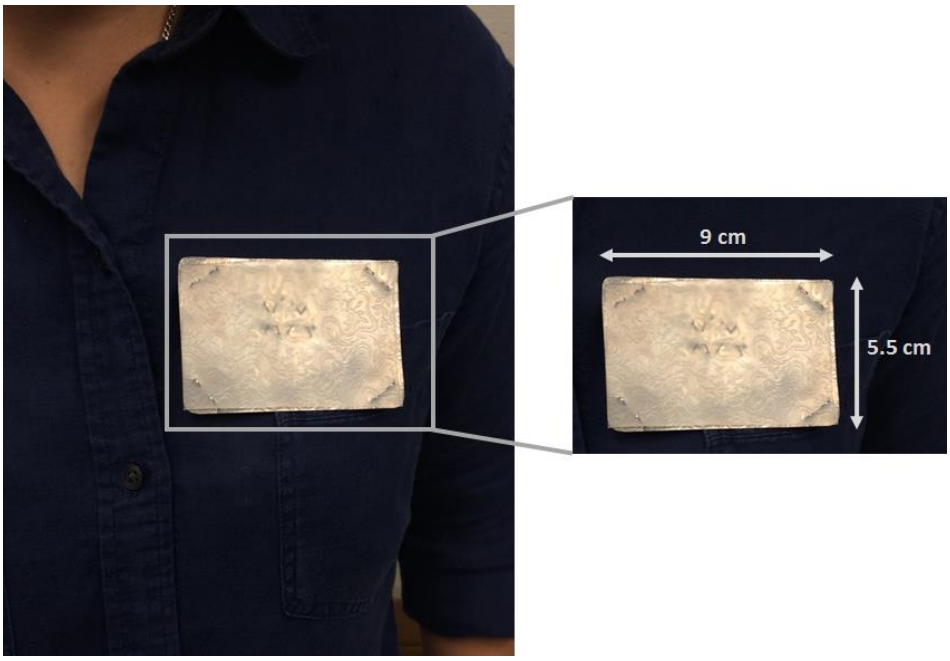

Figure 1. The PDMS brooch tested in this study worn in the breathing zone (upper chest area) of a study participant. The PDMS strip rests on aluminum support.

2.3 Field blanks and sample storage

Five PDMS brooches and five PLV-AAS field blanks were collected during the calibration study. For the field deployment, one PDMS brooch field blank was collected for each participant throughout the one-week sampling. Field blanks and samples were treated equally except that field blanks were exposed for ~1 minute. All PDMS brooch and PLV-AAS samples and their respective field blanks were retrieved into air tight glass jars and stored at -18 °C pending extraction and analysis.

2.4 Extraction and instrumental analysis

Prior to extraction, the LV-AAS and PDMS samples and their respective field blanks were spiked with the following deuterated and mass labelled surrogate standards: DEP-d4, DnBP-d4

and DEHP-d4 for phthalates and TEP-d15, TBP-d27, TCEP-d12, TDCPP-d15, TPrP-d21 and mTPhP for OPEs (AccuStandards Inc, New Haven, USA). Spiked samples were extracted by shaking and soaking in acetonitrile for PDMS (Okeme et al., 2016) and by sonication in hexane, dichloromethane and acetone (2:1:1, v/v) for the LV-AAS, Fluoranthene d-10 and Mirex (AccuStandards Inc) were added as internal standards for phthalates and OPEs, respectively, to the concentrated extracts prior to gas chromatography mass spectrometer (GC-MS) analysis. See Table S1 for the full list of target compounds and surrogate standards and S2 for details on extraction of PLV-AAS samples. Extracts were analyzed on Agilent 6890N/5973 or 5975 gas chromatograph/inert mass selective detector (GC-MSD) system using a negative chemical ionization (ENCI) source for TDCPP and an electron impact ionization (EI) source for phthalates and all other OPEs measured here. GC-MSD results were blank and recovery corrected as appropriate (Saini et al., 2015; Okeme et al., 2016b). See S1 for details on analytical method and quality assurance and quality control.

3 Results and discussion

Results presented and discussed here are those of diethyl phthalate (DEP), di isobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), benzyl butyl phthalate (BzBP), di (2-ethylhexyl) phthalate (DEHP), and tris (1-chloro-2-propyl) phosphate (TCPP-1) for the calibration study, whereas all these compounds plus tris (2-chloroethyl) phosphate (TCEP), tris (1,3-dichloro-2-propyl) phosphate (TDCPP), and two other isomers of tris (1-chloro-2-propyl) phosphate (TCPP-2 and TCPP-3) are discussed for the field study. Data for the compounds presented here met the quality control and quality assurance (QA/QC) criteria including those for blank and recovery corrections (Saini et al., 2015,2016; Okeme et al., 2016) and had detection frequency > 80%. See the SI for details on QA/QC.

3.1 Concentrations measured using PLV-AAS

Average concentrations ± standard deviations measured using PLV-AAs ranged from 2450 ± 400 to 2870 ± 500 ng/m^3 for $\sum_5$ phthalates and 490 ± 50 to 1,180 ± 620 ng/m^3 for TCPP-1. Daily personal air concentrations of the five participants varied over four days by 6 to 27% for all

compounds, except DnBP for one participant, and DEHP and TCPP-1 for three participants for which concentrations varied by 32 to 64%. This higher variability may be due to changes in concentrations of total suspended particles particularly for compounds such as DEHP and TCPP-1 with high propensity for particle phase distribution or daily activity variation amongst study participants. Details of PLV-AAS results are provided in Table S3.

3.2 PDMS brooch sampling rates

To obtain the sampling rate (Rs, m^3 day^{-1} or m^3 $hour^{-1}$) of each compound, its mass collected by the PDMS brooch was divided by its corresponding PLV-AAS-derived air concentration to obtain its equivalent air volume. The equivalent air volume, *Veq* (m^3), was plotted against the brooch deployment time (Eq. 1, Shoeib and Harner, 2002) to generate an uptake curve:

$$(dM_{PAS} / C_{Air}) = Rs\ \Delta t \qquad \text{Eq. 1}$$

where C_{Air} (ng m^{-3} or µg m^{-3}) is air concentration from the PLV-AAS, M_{PAS} is the mass of the target compound on the brooch (ng or µg) and Δt (hours or days) is PAS deployment time.

The slope of the curve represented the Rs of the compound. Figures 2 and 3 show uptake curves and values of Rs (m^3 $hour^{-1}$), respectively. The uptake of all compounds was linear, which is consistent with air-side controlled uptake (Shoeib and Harner, 2002) and the large uptake capacity of PDMS for SVOCs (Okeme et al., 2016a). Rs values ranged from 0.20 ± 0.16 (TCPP-1) to 0.66 ± 0.17 m^3 day^{-1} (DiBP). Rs varied by a range 17 to ≤ 26% for DEP, DiBP and DnBP and by ~ 80% for DEHP and TCPP-1 between participants, and by 42 to 74% within participants for all measured compounds. The high variability of Rs observed within and between participants reflects, in part, the variability in the air concentrations of DEHP and TCPP-1 in each participant's breathing zone.

When uptake is air-side controlled, values of Rs are not expected to vary significantly between individual gas-phase compounds (Shoeib and Harner, 2002). Rs variability may result, in part, when a PAS collects gas- and particle-phase compounds with different efficiencies (Bohlin et al., 2014; Harner 2014), which may explain the lower Rs measured for DEHP and TCPP relative

to the rest of the compounds. DEHP and TCPP are known to have high particle-phase fractions, (e.g., Okeme and Yang et al., submitted) whereas DEP, DiBP and DnBP are predominantly gas-phase compounds (e.g., Saini et al., 2015). These gas-particle characterizations are based on filter versus sorbent detection during active sampling, as mentioned. Particle sampling efficiency was not investigated here.

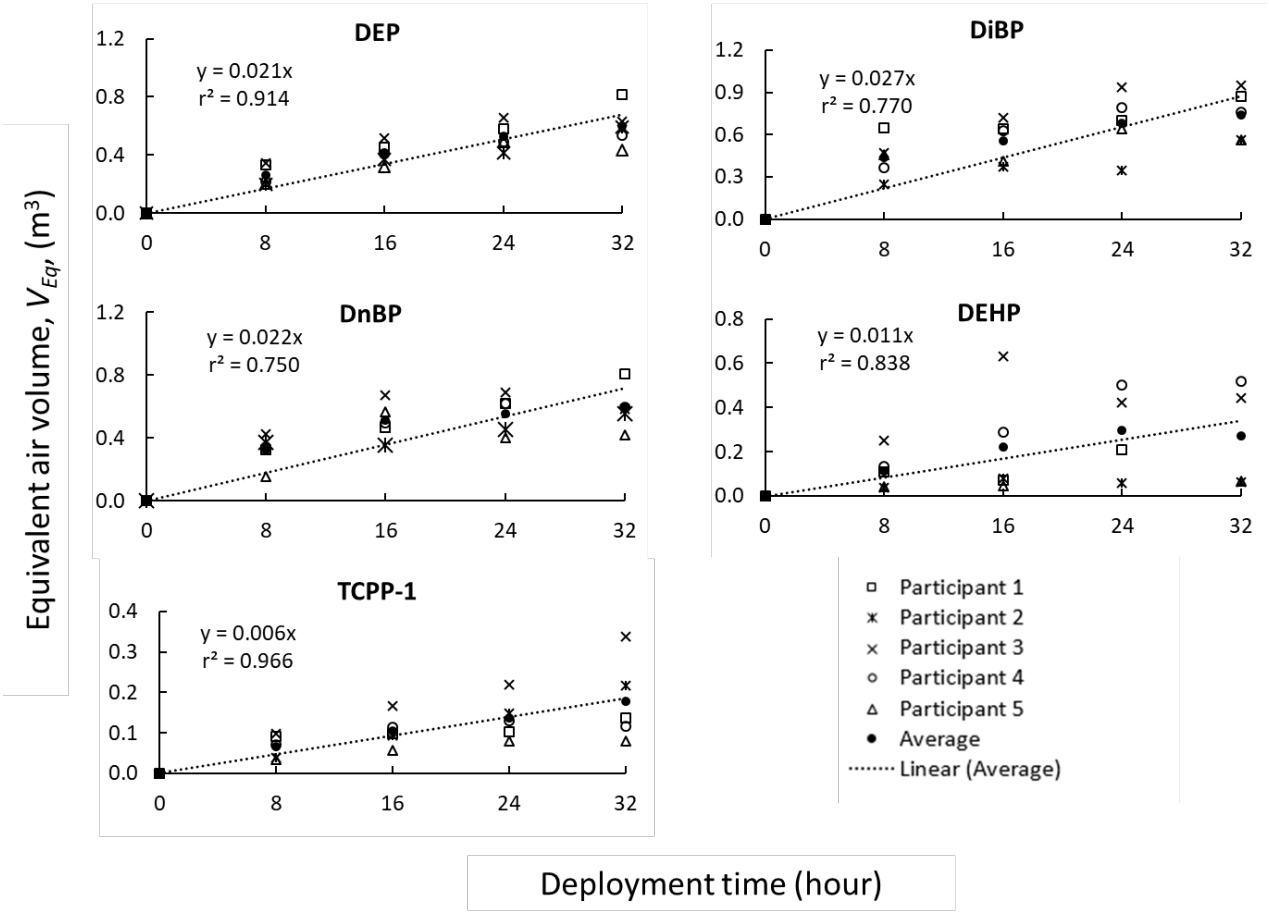

Figure 2. Uptake curves of compounds collected by PDMS brooches worn by five participants in offices for 8 hours daily for 4 days. The PDMS brooches were co-deployed with personal low volume active samplers (PLV-AAS) throughout the sampling duration.

The average Rs measured here was 0.43 ± 0.14 m^3 day^{-1} and the Rs value normalized to surface area, Rs', has a value of 0.86 ± 0.29 m^3 day^{-1} dm^{-2}. This Rs' is comparable to the value of 0.84 ± 0.40 m^3 day^{-1} dm^{-2} (Okeme et al., 2016b) and approximately half the value of 1.5 ± 1.1 m^3 day^{-1} dm^{-2} (Okeme and Yang et al., submitted) reported for a stationary PDMS tested in two

different locations. Higher Rs' would be expected for a PPAS compared to stationary PAS because personal activities should increase air circulation around the individual and reduce the thickness of the air-side boundary layer. The comparable or lower Rs' measured here for the PDMS brooch and the stationary PDMS in the literature was probably due to the nature of office work during which participants sat at their desk for most of the sampling period. We believe that air flow that influences the uptake rate was reduced to the exposed single surface of the brooch since it was worn on the chest. In contrast, the stationary samplers of Okeme et al. (2016b) and Okeme and Yang (submitted) had no barrier to air circulation. Okeme and Yang et al. (submitted) found that Rs' varied amongst indoor locations for reasons that could not be explained, but show the influence of local conditions on uptake rates.

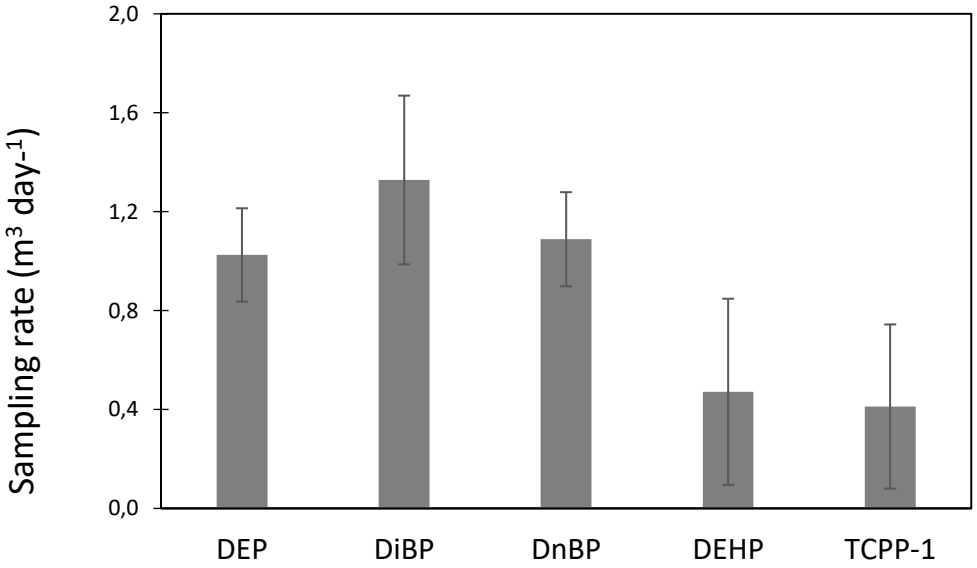

Figure 3. Sampling rates of compounds measured using PDMS brooches worn for 8 hours daily for 4 days by five study participants. Error bars denote standard deviations.

3.3 Brooch derived personal air concentration and exposure estimate

A generic sampling rate of 0.43 m^3 day^{-1} measured in the calibration study was applied to the mass of compounds sampled using PDMS brooches to derive personal air concentrations and estimates of inhalation exposure of the three participants involved in the field study (Table 1). Phthalates were approximately three times more abundant than OPEs, with concentrations ranging from 1210 to 2650 ng m^{-3} for $\sum_5$ phthalates and 264 to 663 ng m^{-3} for $\sum_5$ OPEs. Personal

sampling data for phthalates were not available in the literature for comparison. The average concentration of ∑TCPP-1 + TDCPP + TCEP of 334 ng m^{-3} measured here was comparable to the value of 426 ng/m^3 measured for the same three OPEs using PLV-AAS in a US study (Schreder et al., 2016). TCPP measured here accounted for > 90% OPEs which is consistent with other studies (van der Veen and de Boer, 2012), and may support the hypothesis that the use of TCPP as a flame retardant has increased following the decline in using TCEP and TDCPP, which have been designated as carcinogens (Schreder et al., 2016).

Inhalation exposure was estimated for the three participants in the field study using standard inhalation rate of 16 m^3 day^{-1} for adults (EPA 2011). Estimates were 19400 to 42400 ng day^{-1} for $\sum_5$ phthalates and 4070 to 10600 ng day^{-1} for $\sum_5$ OPEs. Median exposure estimates of 4140, 518 and 292 for TCPP-1, TCEP and TDCPP, respectively, were comparable to the median values of 4540, 186 and 102 (within the uncertainty of Rs) estimated for the same OPEs by Schreder et al. (2016) using a PLV-AAS results. The US study of Schreder et al. (2016) showed that inhalation exposure was significantly higher than dust ingestion exposure.

Table 1. PDMS brooch-derived personal air concentration and inhalation exposure for adults estimated in this study using inhalation rate of 16 m^3 day^{-1}.

	Personal air concentrations (ng m^{-3})				**Inhalation exposure (µg day^{-1})**			
Compounds	Participant 1	Participant 2	Participant 3	Median	Participant 1	Participant 2	Participant 3	Median
DEHP	465	379	1550	465	7.4	6.1	25	7.4
DiBP	227	460	423	423	3.6	7.4	6.8	6.8
DEP	283	432	275	283	4.5	6.9	4.4	4.5
DnBP	219	207	374	219	3.5	3.3	6.0	3.5
BzBP	17	10	20	17	0.27	0.16	0.32	0.27
∑5 Phthalates	1211	1480	2640	1410	19	24	42	23
TCPP-1	378	283	115	283	6.1	4.5	1.8	4.5
TCPP-2	150	125	44	125	2.4	2.0	0.70	2.0
TCPP-3	93	101	27	93	1.5	1.6	0.43	1.5
TCEP	27	36	34	34	0.43	0.58	0.54	0.54
TDCPP	17	4	34	17	0.26	0.61	0.55	0.26
∑5 OPEs	663	550	254	552	12	8.8	4.1	8.8

Future work should calibrate the PDMS brooch to capture overall exposure including the time people spend at home and other locations, to assess variability of uptake rates depending on activity during the day, and to more fully compare the PDMS brooch side-by-side with PLV-AAS to evaluate passively derived air concentrations.

4 Conclusions

This study evaluated the PDMS brooch as proof-of-concept for passively measuring personal air concentrations and inhalation exposure of selected phthalates and OPEs indoors. The average sampling rate of 0.86 ± 0.29 m^3 day^{-1} dm^{-2} measured in this study was comparable or lower than values measured for the stationary PDMS-PAS. A higher sampling rate was anticipated based on the supposition that participants would be active, leading to a higher diffusion rate relative to a stationary sampler. This unanticipated finding was, in part, probably because study participants performed limited activity associated with office work. The sampling rate of the PDMS brooch of 0.86 ± 0.29 m^3 day^{-1} dm^{-2} (0.43 ± 0.14 m^3 day^{-1}) is sufficient to capture personal exposure to abundant phthalates and OPEs when worn for 8 hours daily for three to four days or approximately 24 hours in total.

Compared to gas-phase compounds, sampling rates were approximately 50% lower for DEHP and TCPP, which reside predominately in the particle phase. This finding suggests that gas-particle partitioning could contribute to variability in sampling rates between compound groups.

The PDMS brooch-derived air concentrations varied amongst participants in the field study, suggesting differences in activity pattern and exposure situations. Personal air concentrations were in the ng m^{-3} to µg m^{-3} range for phthalates and OPEs, resulting in estimated exposures of ~ 20 to 40 µg day^{-1} for $\sum_5$ phthalates and 4 to 11 µg day^{-1} for $\sum_5$ OPEs. Similar or lower levels of OPEs measured using a PLV-AAS in a U.S study were estimated to result in inhalation exposure significantly higher than dust ingestion exposure.

This study demonstrates that the PDMS brooch can be used to assess indoor inhalation exposure over a 24 hours period for compounds present in ng m^{-3} to µg m^{-3} range such as the phthalates and OPEs included here.

Acknowledgments

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Abstract

Exposure assessment conducted using a personal sampler includes the contribution of human activities to exposure that is neglected when using a stationary air sampler. This study characterized and evaluated the polydimethylsiloxane (PDMS) brooch as a passive air sampler for measuring concentrations of two groups of semi-volatile organic compounds (SVOCs), namely phthalates and organophosphate esters (OPEs), indoors in promixity to the breathing zone.

Uptake rates of the PDMS brooch were calibrated against a personal low volume active air sampler (PLV-AS) co-deployed on each of five study participants working for 8 hours daily for four days in offices. Sampling rates measured here ranged from 0.41 ± 0.33 to 1.33 ± 0.34 m^3 day^{-1} dm^{-2} with an average value of 0.86 ± 0.29 m^3 day^{-1} dm^{-2}. A higher sampling efficiency was observed for gas- than particle-phase compounds.

Personal air concentrations were measured for three study participants who used the PDMS brooches continuously for seven days. Values derived based on the sampling rates measured here ranged from 1540 to 4010 ng m^{-3} for $\sum_5$ phthalates and 507 to 1310 ng m^{-3} for $\sum_5$ OPEs. These concentrations resulted in an estimated inhalation exposure of ~ 20 to 40 µg day^{-1} for $\sum_5$ phthalates and 4 to 11 µg day^{-1} for $\sum_5$ OPEs.

This study demonstrated that the PDMS brooch can be used to assess inhalation exposure when worn for at least 24 hours indoors for compounds present in ng m^{-3} to µg m^{-3} range such as the phthalates and OPEs tested here.

1 Introduction

Inhalation can be an important pathway for exposure to semi-volatile organic contaminants (SVOCs) (Schreder et al., 2016; Merkel et al., 2017). Inhalation exposure in micro-environments is commonly assessed by measuring air concentrations at a central, stationary location. However, stationary air measurements may not reflect personal exposure because they exclude the influence of personal activities on exposure. Personal activities create a personal cloud of contaminants in close proximity to one's body, leading to increased exposure. This phenomenon has been called the personal cloud or the "Pig Pen" effect (Wallace 1991; Rodes et al., 1991; Allen et al., 2007).

SUPPLEMENTARY MATERIAL

S1 Details of target and surrogate compounds and their monitored ions

Table S1. Full names, CAS numbers, and molecular weights (g mol^{-1}) of target phthalates and organophosphate esters (OPEs) investigated in this study.

Acronym	Full name	CAS number	Molecular weight
Phthalate Esters			
DEP	Diethyl phthalate	84-66-2	222
DEP-d4	Diethyl phthalate-3,4,5,6-d4	93952-12-6	226
DiBP	Diisobutyl phthalate	84-69-5	278
DnBP	Di-n-butyl phthalate	84-74-2	278
DnBP-d4	Dibutyl phthalate-3,4,5,6-d4	93952-11-5	282
BzBP	Benzylbutyl phthalate	85-68-7	312
DEHP	Bis(2-ethylhexyl) phthalate	117-81-7	390
DEHP-d4	Bis(2-ethylhexyl)phthalate-3,4,5,6-d4	93951-87-2	394
DiNP	Diisononyl phthalate	68515-48-0	418
Organophosphate esters (OPEs)			
EHDPP	2-Ethylhexyl diphenyl phosphate	1241-94-7	362.40
T2iPPP	Tris (2-isopropyl phenyl) phosphate	64532-95-2	452.52
TBP	Tributyl phosphate	126-73-8	266.31
TBP-d27	Tributyl-d27 phosphate	61196-26-7	293.48
TCEP	Tris(2-chloroethyl) phosphate	115-96-8	285.5
TCEP-d12	Tris(2-chloroethyl) phosphate-d12	1276500-47-0	297.5
TCPP-1 (TClPP)[a]	Tris (2-chloroisopropyl) phosphate	13674-84-5	327.57
TCPP-2[a]	Bis (2-chloro-1-methyl (2-chlorophyl) phosphate	76025-08-6	327.57
TCPP-3[a]	Bis (2-chloropropyl) (2-Chloro-1-methylethyl) phosphate	76649-15-5	327.57
TDCPP (TDClPP)	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8	431
TDCPP-d15	Tris(1,3-dichloro-2-propyl-d5) phosphate	1447569-77-8	446
TEHP	Tris(2-ethylhexyl) phosphate	78-42-2	434.63
TEP-d15	Triethyl-d15 phosphate	135942-11-9	197
TmCP	Tri-m-cresyl phosphate	563-04-2	368.36
ToCP	Tri-o-cresyl phosphate	78-30-8	368.36
TpCP	Tri-p-cresyl phosphate	78-32-0	368.37
TPhP	Triphenyl phosphate	115-86-6	326.28
MTPhP	Triphenyl phosphate-d15	1173020-30-8	341.38
TPrP	Tripropyl phosphate	513-08-6	224.2
TPrP-d21	Tripropyl phosphate-d21	Not available	245.2
Internal standards			
Fluoranthene d-10	Fluoranthene d-10	93951-69-0	212.31

Table S2. Monitored quantifier and qualifier ions for surrogate* and native phthalates and organophosphate esters (OPEs).

Compounds	Quantifier	Qualifier
Phthalates		
DEP	212	
DEP-d4	153	181
DiBP	149	150, 57
DnBP	223	167, 205, 149
DnBP-d4	153	
BzBP	206	91, 149
DEHP	279	167, 149
DEHP-d4	153	
DiNP	149	167, 293
Organophosphate esters (OPEs)		
EHDPP	251	250
T2iPPP	118	452
TBP	99	
TBP-d27	103	167, 231
TCEP	261	263
TCEP-d12	249	251, 205
TCP (O, M, P)	368	367
TCPP	99	125
TDCPP	317	319
TDCPP-d15	328.8	326.9
TDMPP	410	193
TEHP	99	113
TEP-d15	167	103, 135
TPhP	326	325
MTPhP	344	343,342
TPrP	141	99
TPrP-d21	103	151,131

*The letter d indicates deuterated.

S2 Pre-cleaning and extraction of personal low volume active air samples (PLV-AAS) and PDMS brooch samples

The polyurethane foam and styrene-divinylbenzene copolymer resin (PUF/XAD/PUF) used in the PLV-AAS was purchased precleaned. Post deployment PUF/XAD/PUF samples were transferred into 40 ml vials, spiked with surrogate standards (Table S1) and sonicated for 20 mins in 20 ml of a mixture of hexane, dichloromethane and acetone (2:1:1, v/v). The extracts were transferred to pre-baked and pre-cleaned TurboVap concentration tubes. The process was repeated twice by adding 10 ml of a mixture of hexane, dichloromethane and acetone (2:1:1, v/v) each time. The extracts were concentrated to 0.5 ml, transferred to gas chromatography vials, blown down to near dryness under a gentle stream of Nitrogen, and reconstituted to approximately 0.5 mL in isooctane before gas chromatography mass spectrometry (GC-MS) analysis. PDMS brooch personal passive air samples (PPAS) were pre-cleaned and extracted as described by Okeme et al., 2016.

S3 Quality assurance and quality control (QA/QC)

All PLV-AAS and PDMS brooch samples were spiked with deuterated (d) surrogate standards DEP-d4, DnBP-d4 and DEHP-d4 for phthalates and TEP-d15, TBP-d27, TCEP-d12, TDCPP-d15, TPrP-d21 and mass labeled MTPhP for OPEs (AccuStandard, New Haven, USA and Wellington Laboratories, ON, Canada). Recoveries of surrogate standard ranged from 70 to 120% for all compounds. Results were recovery corrected for individual target compounds.

The extraction method for the PLV-AAS was evaluated by analyzing PUF/XAD/PUF samples spiked with surrogate and native compounds (Figure S1). Replicate PLV-AAS were repeatability within 10% relative standard deviation (RSD) for all compounds, except for TCPP-3 for which had an RSD of 16%. Recoveries of all surrogate and native compounds ranged from 68 to 120%, except for dTCEP and mTPhP with higher values of 129 and 150%.

Beside the extraction method for the LV-AAS evaluated and used here, analytical methods used in this study were those that have been previously validated (Saini et al., 2015, 2016; Okeme et al., 2016b). Limits of detection (LOD) and quantitation (LOQ) corresponded to signal to noise ratios of 3 and 10, respectively for phthalates and OPEs (Saini et al., 2015, 2016; Okeme et al., 2016). Measurements with blank levels of ≤ 5 % were not blank corrected, those with blank levels between > 5% and ≤ 35 % were blank corrected, whereas those with and blank levels exceeding 35 % were not reported. Blank levels were

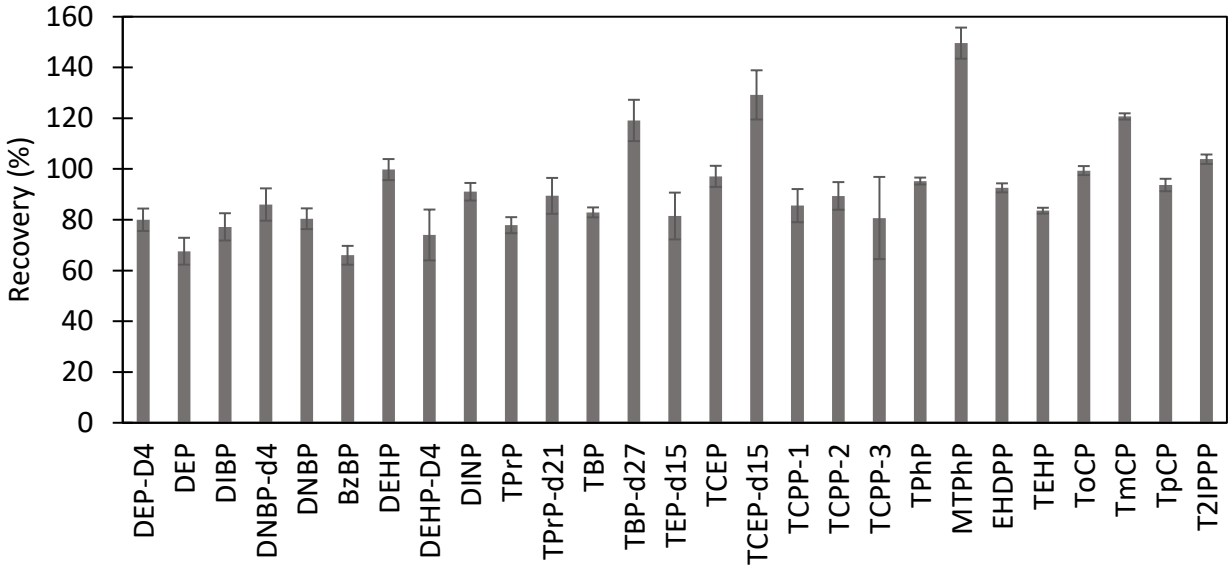

Figure S1: Recovery of native and surrogate phthalates and organophosphate esters spiked into samples to evaluate efficiency of sonication for extracting PLV-AAS co-deployed with PDMS brooch in the calibration study.

S4 Personal air concentrations and sampling rates measured in the PDMS brooch calibration study

Table S3. Personal air concentrations (ng m^{-3}) of phthalates and TCPP-1 measured for five study participants using personal low volume active air samplers (PLV-AAS) co-deployed with PDMS brooch passive air samplers (PPAS) for eight hours daily during a four-day calibration study conducted indoors.

Compounds	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5
DEP	217±11	281±14	194±3	264±11	272±27
DiBP	153±23	236±12	323±15	217±12	208±27
DnBP	69±9	140±32	140±23	119±7	95±8
DEHP	2010±17	2230±18	1820±15	2190±44	1910±25
TCPP-1	1180±52	769±27	493±11	515±63	495±15

Table S4. Individual sampling rates (Rs, m^3 day^{-1}) and correlation coefficient (r^2), average Rs, relative standard deviation (RSD) of phthalates and TCPP-1 measured for five participants who wore PDMS brooch passive air samplers (PPAS) co-deployed with personal low volume active air samplers (PLV-AAS) for eight

hours daily during a four-day calibration study conducted indoors.

	Participant 1		Participant 2		Participant 3		Participant 4		Participant 5		Average Rs	RSD
Compounds	Rs	r^2	Rs	r^2	Rs	r^2	Rs	r^2	Rs	r^2		
DEP	0.62	0.95	0.46	0.96	0.59	0.78	0.46	0.89	0.41	0.82	0.51	18
DiBP	0.75	0.55	0.43	0.85	0.85	0.84	0.7	0.81	0.55	0.54	0.66	26
DnBP	0.63	0.95	0.47	0.70	0.62	0.46	0.56	0.74	0.42	0.50	0.54	17
DEHP	0.19	0.76	0.06	0.46	0.43	0.36	0.43	0.96	0.06	0.68	0.23	80
TCPP-1	0.11	0.66	0.47	0.36	0.24	0.98	0.12	0.64	0.07	0.89	0.20	80

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Sección 5

RESUMEN

1. Desarrollo de metodología analítica

1.1 Contaminación de fondo

La contaminación de fondo es uno de los principales desafíos para la mayoría de laboratorios que realizan análisis de concentraciones traza o ultratraza de sustancias perfluoroalquiladas (PFAS) y retardantes de llama fosforados (PFR) y bromados (BFR) ya que las fuentes de contaminación, así como las técnicas y pautas para reducirla, no están bien establecidas. Estos contaminantes orgánicos persistentes emergentes (ePOP) no solo están presentes de forma habitual en el aire y polvo de los ambientes interiores, sino que también son ampliamente utilizados en equipos y material de laboratorio.

Las estrategias utilizadas en esta tesis para minimizar o evitar la contaminación durante la preparación de las muestras y posterior determinación del contenido en ePOP han sido:

- Evitar, en la medida de lo posible, el uso de cualquier material de caucho y plástico, excepto polipropileno.
- Limpiar el polipropileno previamente con distintos solventes polares y apolares.
- Minimizar el contacto entre las superficies del material y las muestras durante su manejo mediante la reducción de los pasos de purificación, extracción y evaporación.
- Calentar el vidrio a 450 °C y enjuagarlo con un disolvente orgánico.
- Llevar a cabo controles estrictos para garantizar que el material y los reactivos están libres de contaminación.
- Sustituir algunas partes de los instrumentos de análisis que contenían fluoropolímeros.

A pesar de todas las precauciones, se encontraron niveles muy bajos de contaminación en los blancos para ciertos compuestos como el sulfonato de perfluorooctano (PFOS) y el fosfato de tris(2-cloroisopropilo) (TCIPP). La contaminación en los blancos fue insignificante en comparación con las concentraciones cuantificadas de PFOS en las muestras. Sin embargo, en el caso de TCIPP, fue necesaria la instalación de una columna trampa antes del inyector del cromatógrafo líquido de alta eficacia (HPLC) para distinguir los niveles de la muestra respecto a los niveles que provenían del equipo o las fases móviles.

1.2 Métodos de extracción

1.2.1 Agua

La preconcentración y extracción de los PFR y PFAS presentes en las muestras de agua superficial y residual, se realizó mediante la extracción en fase sólida (SPE) *offline*. Se seleccionaron los cartuchos con sorbente polimérico de fase reversa Strata-X 33 μm (200 mg) por proporcionar una fuerte retención de las distintas clases de compuestos. Este sorbente se basa en tres mecanismos de retención: unión π-π, enlace de hidrógeno (interacción dipolo-dipolo) e interacción hidrofóbica. Los cartuchos se colocaron en un colector de vacío de 12 puertos donde se acondicionaron con 6 ml de metanol-diclorometano (1:1, v/v), 6 ml de metanol y 6 ml de agua. Las muestras (250 ml) se pasaron por los cartuchos mediante la aplicación de vacío a un flujo de 10 ml min^{-1} y después, los cartuchos se secaron durante 15 min dejando pasar aire al no romper el vacío. La elución se realizó a gravedad con 8 ml de metanol para el análisis de PFAS y de 10 ml de metanol-diclorometano (1:1, v/v) en el caso de los PFR. Cuando se trató de eluciones combinadas de PFAS y PFR, se realizó con 8 ml de metanol. Las muestras se evaporaron a sequedad en un concentrador de muestras mediante corriente de nitrógeno y los extractos se redisolvieron en 1 ml de metanol y se pasaron a viales ámbar de vidrio de 2 ml de capacidad con insertos de polipropileno para evitar la adsorción de los analitos al vidrio de los viales.

Como paso previo a la extracción, las muestras de aguas residuales se filtraron mediante filtros de microfibra de vidrio de 90 mm de diámetro y 0,45 μm de poro. Las muestras de agua superficial no requirieron este paso debido a la baja presencia de materia en suspensión.

1.2.2 Suelo y sedimento

Se optimizaron, validaron y compararon cuatro métodos de extracción de PFAS en suelo y sedimento basados en la extracción sólido-líquido (SLE) asistida por ultrasonidos (USE) o agitación mecánica. La SLE-USE se aplicó con una solución acuosa de metanol o de ácido acético y metanol (Higgins *et al.*, 2005), o bien mediante una digestión alcalina con hidróxido de sodio en metanol. El método basado en la agitación mecánica consistió en la formación de un par-iónico con tetrabutilamonio hidrogenosulfato (TBAS) a pH 10. La fase final de los cuatro métodos de extracción optimizados incluyó la reducción del volumen de la muestra mediante la evaporación bajo corriente de nitrógeno y la adición de un volumen elevado de agua ultrapura (100-250 ml) para finalmente purificar los extractos mediante una SPE *offline*. Este paso eliminó ácidos, sales y otros compuestos presentes en la matriz que potencialmente podían causar supresión o aumento

de la señal durante el análisis de PFAS. La SPE se llevó a cabo pasando los extractos a través de cartuchos Strata-X 33 μm (200 mg). Los cartuchos se acondicionaron con 4 ml de hidróxido de amonio al 0,1 % en metanol, 4 ml de metanol y 4 ml de agua ultrapura. Seguidamente, se pasaron las muestras mediante vacío y a continuación se dejaron secar los cartuchos durante 15 min. Los analitos retenidos se eluyeron a gravedad con 4 ml de hidróxido de amonio al 0,1 % en metanol y se recuperaron en tubos de polipropileno de 15 ml. Los tubos se evaporaron a sequedad mediante una corriente de nitrógeno, se redisolvieron en 0,25 ml de metanol, se sonicaron y fueron transferidos a viales para su posterior análisis por cromatografía líquida de alta eficacia acoplada a un espectrómetro de masas en tándem (HPLC-MS/MS). De los cuatro métodos testados, los que presentaron mejores recuperaciones, precisión y límites de cuantificación (LOQ) fueron la extracción con metanol (34-109 %, <25 % y 0,01-6,00 ng g^{-1}) y con metanol acidificado (44-125 %, <25 % y 0,06-8,00 ng g^{-1}). Además, fueron los únicos métodos que extrajeron todas las PFAS analizadas (Tablas 1 y 2, Capítulo 2).

En cuanto a la determinación de PFR, se desarrolló un procedimiento basado en la SLE-USE para extraer muestras de suelo. El rendimiento de la SLE es dependiente de las propiedades del disolvente seleccionado, lo que determina la selectividad y fiabilidad del método. Por tanto, se probaron diferentes tipos de disolventes, incluyendo metanol, acetonitrilo, acetato de etilo, diclorometano, hexano y varias mezclas de ellos. El metanol se seleccionó como el disolvente de extracción más favorable ya que permitió obtener las mejores recuperaciones (50-121 %) (Tabla 1, Capítulo 4). Durante el paso de purificación mediante SPE, algunos cartuchos se colapsaron debido a las partículas en suspensión presentes en el extracto, causando retrasos en la SPE que requirieron la introducción de etapas adicionales. Para eliminar los sólidos suspendidos, se probaron diferentes estrategias como el uso de filtros convencionales de papel, filtros de microfibra de vidrio o la centrifugación. Desafortunadamente, ambos tipos de filtros retuvieron completamente algunos PFR. El filtro de microfibra de vidrio, además, mostró una recuperación muy baja para el resto de compuestos (<30 %). La centrifugación durante 5 min a 3000 rcf se eligió como paso previo para eliminar los sólidos en suspensión de las muestras debido a su rapidez y eficiencia, presentando recuperaciones entre el 59 y el 121 % (Fig. 3a, Capítulo 4). Se probaron siete cartuchos de SPE: STRATA-X (60, 200 y 500 mg), OASIS HLB (60 mg), Super-Select HLB (60 y 500 mg) y ThermoSci C8 (200 mg). El compuesto más conflictivo fue el fosfato de tris(2-cloroetilo) (TCEP), que no se retuvo adecuadamente en los cartuchos de 60 mg, probablemente por ser el compuesto más soluble en agua. Los cartuchos poliméricos de fase reversa STRATA-X de 200 mg proporcionaron las mejores recuperaciones para todos los compuestos (71-120 %) (Fig. 3b, Capítulo 4). Un paso importante de la SPE es el de eliminar el agua del cartucho antes de la

elución del analito. Se probó la adición de 0,25 ml de metanol o el secado al vacío durante 15 min. Al igual que en el método para las PFAS, se obtuvieron mejores resultados cuando se utilizó la segunda opción. Finalmente, para la elución de los PFR, se probaron cuatro volúmenes de metanol (5, 8, 15 y 20 ml). Las mejores recuperaciones (78-117 %) se obtuvieron con 8 ml.

La extracción de BFR, concretamente de polibromodifenil éteres (PBDE), e hidrocarburos aromáticos policíclicos (PAH) se llevó a cabo mediante extracción líquida presurizada (PLE). Las muestras (2 g) fueron colocadas en celdas de extracción de acero inoxidable (6 cm) junto con arena de mar (tamaño de partícula 30-50 *mesh*) para evitar espacios vacíos. La PLE se realizó utilizando acetona-hexano (1:1, v/v) como disolvente y con los siguientes parámetros: 1500 psi de presión, 100 °C, 2 ciclos estáticos, tiempo de precalentamiento 5 min, tiempo de calentamiento 5 min, tiempo de extracción estática 10 min, *flush* 90 % y tiempo de purga 120 segundos. Los extractos resultantes se concentraron a 0,5 ml calentándolos en un baño de arena y evaporando lentamente con una columna *Vigreux*. Los extractos se purificaron pasándolos a través de una columna que contenía 1 cm de lana de vidrio plegada en el fondo de la columna, 6,75 cm de Al_2O_3 hidratado y 1 cm de Na_2SO_4 granulado. La columna fue eluida con 25 ml de hexano. Después de la limpieza, los extractos se concentraron nuevamente con una columna *Vigreux* hasta 1 ml. Finalmente, alícuotas de 100 µl de los extractos fueron pasadas a viales adecuados para el análisis por cromatografía de gases acoplada a un espectrómetro de masas (GC-MS).

1.2.3 Peces

El mismo método para la extracción de PFR de suelo (**apartado 1.1.2**) fue optimizado y validado en muestras de biota (peces). En dichas muestras, se hizo hincapié en la cantidad utilizada. El alto contenido de lípidos de algunas especies como la anguila (*Anguilla anguilla*), con valores entre el 8 y el 31 %, obstruyó en determinadas ocasiones los cartuchos de SPE, aumentando la variabilidad de los resultados y el tiempo de análisis. Además, la introducción en el HPLC de los extractos obtenidos con 5 g de anguila, disminuyó la durabilidad de la columna analítica. Tras 100 inyecciones de extractos procedentes de 5 g de muestra de anguilla, los picos comenzaron a ser más anchos y divididos, indicando un deterioro del rendimiento de la columna causado probablemente por la acumulación de lípidos. Por tanto, los extractos más limpios y que proporcionaron las mejores recuperaciones (71-117 %) se obtuvieron al reducir la cantidad de muestra a 1 g (Fig. 2, Capítulo 4). Además, estos extractos, al tener un menor contenido en lípidos, aumentaron la durabilidad de la columna analítica.

En los Capítulos 5 y 6, la extracción de PFAS se efectuó en colaboración con el Instituto de Diagnóstico Ambiental y Estudios del Agua – IDAEA (CSIC) de Barcelona. El método de extracción desarrollado en los laboratorios de esa institución consistió en una digestión alcalina con hidróxido de sodio en metanol asistida por agitación mecánica en un agitador orbital. Tras centrifugar la muestra, se tomaron 0,02 ml de sobrenadante y se inyectaron de forma directa en un sistema de purificación *online* basado en la cromatografía de flujo turbulento (TFC). En los Capítulos 4 y 7, los análisis se realizaron en los laboratorios de la Universitat de València y las muestras de biota fueron extraídas y purificadas utilizando el mismo método desarrollado para PFR descrito en el **apartado 1.2.2** y optimizado para biota en el párrafo anterior.

1.2.4 Leche y alimentos infantiles

Las muestras sólidas (2 g) o líquidas (15 ml) fueron extraídas mediante una digestión alcalina con hidróxido de sodio en metanol asistida por agitación mecánica en un agitador orbital. La purificación posterior de las muestras fue llevada a cabo mediante SPE utilizando cartuchos poliméricos de fase reversa Strata-X 33 μm (60 mg) preacondicionados con metanol y agua ultrapura. Tras pasar las muestras por los cartuchos, éstos se lavaron con agua y se secaron a vacío. La elución se realizó con hidróxido de amonio al 0,1 % en metanol. Los extractos fueron recogidos en tubos de polipropileno de 15 ml, evaporados bajo una corriente de nitrógeno, reconstituidos con 0,15 ml de metanol y pasados a viales para su determinación por HPLC-MS/MS.

1.2.5 Aire

Para obtener las muestras personales de aire, se utilizaron 2 tipos de muestreadores. Por un lado, muestreadores pasivos de aire personal (PPAS) incorporados en forma de parche sobre los participantes para calcular la exposición y, por otra parte, muestreadores activos de bajo volumen (PLV-AAS), transportados por cada individuo y utilizados para la calibración de los PPAS.

Para eliminar cualquier posible contaminación previa de los broches de polidimetilsiloxano (PDMS) utilizados como PPAS, se pre-extrajeron dos veces en acetato de etilo mediante PLE con los siguientes parámetros instrumentales: 1500 psi de presión, 75 °C, 5 ciclos estáticos, *flush* 150 % y tiempo de purga 120 s. Después de su implementación, los broches se extrajeron en los viales en los que fueron recogidos mediante la adición de 30 ml de acetonitrilo. Los viales se agitaron durante 20 min utilizando un agitador de acción de muñeca y se dejaron en remojo durante toda la noche a temperatura ambiente para aumentar la eficiencia de extracción. Al día siguiente, los extractos se pasaron a los tubos de un sistema automatizado de evaporación (TurboVap) donde el volumen se

redujo hasta 1 ml. La cantidad restante se pasó a viales de GC-MS donde las muestras se evaporaron hasta los 0,1 ml mediante corriente de nitrógeno. Finalmente, se reconstituyeron hasta 0,5 ml con isooctano.

Los sorbentes compuestos por espuma de poliuretano y resina de copolímero estireno-divinilbenceno (PUF/XAD/PUF) utilizados en los PLV-AAS, no requirieron ningún tratamiento previo de limpieza. Tras su uso, PUF y XAD fueron extraídos con 20 ml de una mezcla de hexano, diclorometano y acetona (2:1:1, v/v), se sonicaron durante 20 minutos y se transfirieron los extractos a tubos de TurboVap. El proceso se repitió dos veces más añadiendo cada vez 10 ml de hexano, diclorometano y acetona (2:1:1, v/v). Una vez recogidos los extractos en los tubos, éstos se sometieron al proceso de evaporación hasta que alcanzaron un volumen de 0,5 ml y fueron transferidos a los viales de GC-MS.

1.3 Métodos de determinación

1.3.1 Cromatografía líquida acoplada a un espectrómetro de masas en tándem con analizador de triple cuadrupolo

La determinación de PFAS y PFR, salvo en muestras de aire, se realizó mediante HPLC-MS/MS con analizador de triple cuadrupolo (QqQ). La LC en fase reversa es la más utilizada en la separación de ePOP, utilizándose mayoritariamente como fase estacionaria apolar el gel de sílice (C18). La separación cromatográfica se realizó con una columna Kinetex C18 (50 × 2,1 mm i.d., 1,7 μm). Las fases móviles fueron metanol y agua, ambas con un 0,1 % de ácido fórmico en el análisis de PFR, o con 10 mM de formiato amónico en el análisis de PFAS, en gradiente con un flujo de 0,2 ml min^{-1} y un volumen de muestra inyectado de 5 μl. En los últimos trabajos (Capítulos 7-8) se observó una mayor definición de los picos cromatográficos utilizando metanol y agua, ambas con 2,5 mM de fluoruro amónico, como fase móvil para las PFAS.

El análisis se realizó con ionización por electrospray (ESI) en modo negativo para la detección de PFAS y positivo para los PFR. La adquisición de datos se llevó a cabo en el modo de monitorización de reacciones seleccionadas múltiples (MRM), para identificar y cuantificar, seleccionando dos o tres transiciones entre iones precursores e iones producto. La información relacionada con la determinación instrumental, incluyendo los tiempos de retención, las transiciones seleccionadas, las energías de colisión y del fragmentador, se puede consultar en las Tablas S6 (Capítulo 2), 2 (Capítulo 3), S2 (Capítulo 4), S5 (Capítulos 5 y 6), S6 (Capítulo 7), S1 (Capítulo 9) y S2 (Capítulo 10).

La validación del método se llevó a cabo en cada matriz y grupo de compuestos estudiado. Los límites de detección (LOD) y LOQ se muestran en las Tablas 1-4 (Capítulo 2), Tabla 3 (Capítulo 3), Tabla 1 (Capítulo 4), Tabla S7 (Capítulo 5) y Tabla 3 (Capítulo 9). El LOD se calculó como la masa de analito requerida para producir una relación señal/ruido (S/N) de 3 a 1, donde el ruido es calculado como tres veces la desviación estándar del ruido de fondo. El LOQ, la concentración más baja a la que el analito puede ser detectado de manera confiable, se estableció como aquel valor cuyo S/N fuera de 10 a 1.

La precisión, expresada como desviación estándar relativa (RSD) y calculada intra e interdía, fue en todos los casos inferior al 20 %. Las rectas de calibración presentaron coeficientes de determinación (R^2) superiores a 0,98.

Los efectos de matriz fueron comunes debido a la complejidad de las matrices ambientales utilizadas. Para compensar estos efectos indeseables, así como posibles pérdidas durante el procedimiento de extracción, la respuesta instrumental de cada analito se relacionó con la respuesta de los patrones internos incorporados al principio del proceso. La utilización de patrones internos en las muestras corrigió los efectos de matriz y logró una cuantificación adecuada sin la necesidad de usar patrones con ajuste matricial.

1.3.2 Cromatografía de gases acoplada a un espectrómetro de masas

La determinación de BFR y PAH en muestras de suelo y sedimento y de PFR y ftalatos en muestras de aire se llevó a cabo mediante GC-MS.

La separación de los BFR y PAH se realizó utilizando una columna de sílice fundida (60 m × 0,32 mm i.d.) recubierta con (5 % fenil)-metilpolisiloxano DB-5MS (espesor de película 0,50 μm) y helio como gas portador. El volumen de inyección fue de 2 μl y la temperatura de 60 °C, con un programa que alcanzaba 320 °C. La fuente de ionización fue el impacto de electrones (EI) con un potencial de ionización de 70 eV y una temperatura de la fuente de iones de 250 ºC. La adquisición de datos se realizó en modo de monitorización de iones seleccionados (SIM). Los compuestos se identificaron utilizando el software Xcalibur a través de los iones seleccionados y su intensidad relativa, teniendo en cuenta los tiempos de retención y/o por comparación con los datos de la literatura. Los LOD y LOQ fueron calculados al igual que en apartado anterior (**apartado 1.3.1**). Las rectas de calibración presentaron R^2>0,98.

La separación de los PFR y ftalatos se llevó a cabo utilizando un sistema con ionización química negativa (NCI) equipado con una columna DB-5MS de 15 m (0,25 mm i.d., 0,25 μm) para el análisis del TDCIPP e ionización EI equipada con una columna DB-5MS de 30 m (0,25 mm

i.d., 0,25 μm) para los ftalatos y el resto de PFR. Se añadieron patrones internos a los extractos de todas las muestras. Las recuperaciones de estos patrones oscilaron entre 68 y 129 % para todos los compuestos. Los resultados fueron corregidos mediante las recuperaciones para cada compuesto. Los LOD y LOQ para ftalatos (Saini *et al.*, 2015) y PFR (Sühring *et al.*, 2016) fueron calculados previamente. Cuando las concentraciones de los contaminantes analizados en los blancos fueron inferiores al 5 % de las encontradas en las muestras, los resultados no se corrigieron. Cuando los blancos presentaron niveles entre el 5 y el 35 %, los resultados en las muestras fueron corregidos, mientras que cuando los niveles fueron >35 %, los resultados no se incluyeron en el estudio.

2. Aplicación en ecosistemas acuáticos

2.1 Agua

2.1.1 Aguas superficiales

Se estudió la presencia de 21 PFAS en 48 muestras de aguas superficiales de las cuencas hidrográficas del Ebro y Guadalquivir. El ácido perfluorobutanoico (PFBA) es el compuesto que apareció en ambas cuencas a mayor concentración (742,9 ng l^{-1} en el Guadalquivir y 251,3 ng l^{-1} en el Ebro) y mayor frecuencia, aunque ésta fue mayor en el Guadalquivir (92 %) que en el Ebro (58 %). En ambos ríos, las PFAS de cadena larga (C>8) fueron menos detectadas en el agua que las de cadena corta, ya que éstas últimas son más producidas y consumidas. Además, la solubilidad de los compuestos de cadena larga es menor (Onghena *et al.*, 2012). Los niveles de contaminación por PFAS fueron muy altos en las muestras tomadas aguas abajo de estaciones depuradoras de aguas residuales (WWTP) (Guadalquivir: Córdoba, Écija y Morón; Ebro: Lleida y Zaragoza). Las altas concentraciones en el río Guadalquivir se localizaron sobre todo en la cuenca media y baja, donde se encuentran dos de las ciudades más importantes, Córdoba y Sevilla. Estos resultados coinciden con los de otros autores que también encontraron que las PFAS de cadena corta eran predominantes en y cerca de áreas urbanas e industriales (Myers *et al.*, 2012). La distribución espacial en el río Ebro fue más dispersa, con altas concentraciones en la parte baja de la cuenca, pero también en la superior. Las concentraciones detectadas en la cabecera pueden ser debidas a que el Ebro, al contrario de muchos ríos, fluye a través de un área muy industrializada y sus vertidos pueden ser una fuente puntual de PFAS. Sin embargo, también cabe remarcar la presencia de cierta contaminación por PFAS en el nacimiento del río Guadalquivir y en la zona media de la cuenca del Ebro. Una posible explicación de estos niveles residuales puede ser la existencia de fuentes difusas como la deposición atmosférica (Eschauzier *et al.*, 2013), o a otros procesos naturales como la escorrentía o la infiltración de agua. En el Guadalquivir, también se detectaron altas

concentraciones de PFBA y otras PFAS en puntos cercanos a zonas con industria textil, olivos y almazaras, y un campamento militar. Las concentraciones en este último fueron posiblemente debidas al uso de espumas formadoras de película acuosa (AFFF) utilizadas en la lucha contra incendios y que contienen grandes cantidades de ácido perfluorooctanoico (PFOA) y ácido perfluorohexanoico (PFHxA) (Eschauzier *et al.*, 2013). En el río Ebro, la concentración más alta (debida únicamente a la presencia de PFBA) se encontró en un lugar rodeado de estaciones de esquí. La alta concentración de PFBA pudo provenir de las ceras que se aplican a los equipos de montaña como esquís y tablas. Un estudio ya vinculó concentraciones de PFAS en sangre con la exposición a los aerosoles utilizados durante el enceramiento de los esquís (Freberg *et al.*, 2010). Las concentraciones acumuladas de PFAS en el río Ebro, con un máximo de 251,3 ng l^{-1}, fueron mucho más bajas que las encontradas en el Guadalquivir (máximo de 830,3 ng l^{-1}). La diferencia en el rango de concentraciones entre ambos ríos puede deberse a la diferencia de caudal, mientras que el Ebro es uno de los ríos más caudalosos de España (aproximadamente 600 m^3 s^{-1}), el Guadalquivir tiene un promedio mucho más bajo (unos 164 m^3 s^{-1}) y el efecto de dilución de la contaminación es menor. Las concentraciones de ácidos perfluorocarboxílicos (PFCA) encontradas en el agua del Guadalquivir fueron también más altas que las detectadas en el río Llobregat (Campo *et al.*, 2015), mientras que las de perfluorosulfonatos (PFSA), a pesar de que se encontraron los mismos compuestos, fueron más bajas. Respecto al río Ebro, las concentraciones de PFCA fueron similares y los PFSA fueron más bajos que los del Llobregat. El PFBA fue también el principal compuesto en agua en algunos estudios, encontrándose con una frecuencia de 93 % en el río Llobregat (Campo *et al.*, 2015), del 71 % en agua superficial de diferentes ciudades españolas (Llorca *et al.*, 2012), <60 % en el lago Tangxun (Zhou *et al.*, 2013) y del 52 % en la Bahía de Tokio (Ahrens *et al.*, 2010).

También se estudió la presencia de PFAS en 15 muestras de agua del río Júcar, así como de sus afluentes Magro y Cabriel. Todas las muestras presentaron concentraciones de alguna PFAS. De acuerdo con estudios realizados en otros ríos españoles (Campo *et al.*, 2015; Llorca *et al.*, 2012) y con los resultados del Capítulo 5, el PFOA (53,3 %) fue más frecuente que el PFOS (40 %). Al igual que en los ríos Ebro y Guadalquivir, las PFAS de cadena larga fueron menos frecuentes en agua que las de cadena corta, siendo la mayor concentración encontrada la de PFBA (644 ng l^{-1}). La familia de PFCA, al igual que en los ríos anteriores, fue la más detectada. Los niveles de contaminación en la cuenca del río Júcar son comparables a los determinados en los ríos Ebro y Guadalquivir (Capítulo 5) y fueron, en general, similares o incluso inferiores a los determinados en otros países y continentes, (Tabla S10, Capítulo 6). Las concentraciones acumuladas más altas se encontraron en la cabecera del río, tras las descargas urbanas e industriales provenientes de la

ciudad de Cuenca, así como en lugares cercanos a la desembocadura, con una mayor contribución de PFOS y ácido perfluorodecanoico (PFDA). Una fuente puntual importante de PFAS es la WWTP de la ciudad de Alzira, lo que explica las grandes concentraciones en la zona baja de la cuenca. Como ha sido demostrado en otras investigaciones, las WWTP son inefectivas eliminando las PFAS (Appleman *et al.*, 2014; Castiglioni *et al.*, 2015). Se calcularon ratios bajas de eliminación de PFOS (33 %) y PFDA (<20 %) en la WWTP de Alzira durante un muestreo realizado también en 2010. Otras posibles fuentes de PFAS fueron evaluadas mediante las ratios de ácido perfluoroheptanoico (PFHpA)/PFOA y PFOS/PFOA (Simcik y Dorweiler, 2005). La ratio PFHpA/PFOA >1 es indicativa de fuentes atmosféricas, mientras que cuando es <1 es indicativa de fuentes no atmosféricas asociadas con áreas urbanas. Esta ratio solo pudo calcularse para algunos puntos y de acuerdo a los valores obtenidos, la fuente principal de PFAS en esos puntos podría relacionarse con deposiciones atmosféricas. Igualmente, la ratio de PFOS/PFOA parece correlacionarse con los niveles de urbanización (Nguyen *et al.*, 2011). Esta ratio solo pudo obtenerse en dos puntos situados al final de la cuenca, indicando, en ambos casos, entradas importantes desde asentamientos humanos.

La carga de PFAS (g día^{-1}) fue calculada en los ríos Júcar y Cabriel, utilizando los datos de caudales de la fecha de muestreo. En el río Cabriel, la carga de PFAS se encontró entre los 22,1 × 10^{-3} y los 4,47 g día^{-1}, mientras que la carga en el río Júcar estuvo entre los 2,21 × 10^{-3} y los 204 g día^{-1}. Estos resultados demuestran la gran influencia del impacto humano en el aumento de las concentraciones de PFAS. También se observó una contribución de contaminación por PFAS del río Cabriel al Júcar y una concentración mayor después de los embalses de Contreras y Tous. Parece que estos sistemas reguladores del flujo de agua pueden jugar un papel importante en la redistribución de PFAS en el ecosistema acuático, aunque es necesaria una mayor investigación al respecto. Las normas de calidad ambiental (EQS) en aguas superficiales continentales (European Parlament, 2013) fijan la concentración máxima admisible y la concentración media anual para PFOS, y sus sales derivadas, en 36000 ng l^{-1} y 0,65 ng l^{-1}, respectivamente. En Italia se han fijado concentraciones medias anuales de EQS adicionales en aguas superficiales continentales para otros compuestos: 7000 ng l^{-1} para PFBA, 3000 ng l^{-1} para ácido perfluoropentanoico (PFPeA), 1000 ng l^{-1} para PFHxA, 3000 ng l^{-1} para sulfonato de perfluorobutano (PFBS) y 100 ng l^{-1} para PFOA. Aunque es necesario un mayor estudio para hacer comparaciones fiables, ya que los valores presentados en esta investigación provienen de una sola campaña, y los de la legislación son anuales, parece que las concentraciones máximas encontradas para PFOS y PFOA en las aguas del río Júcar son superiores a los EQS propuestos por la Unión Europea y el gobierno italiano, respectivamente.

Durante el invierno de 2016-2017, se tomaron 12 muestras de agua superficial en el área del parque natural de La Albufera así como en puntos clave de los ríos Turia, Júcar y Magro y se estudió la presencia de 9 PFR y 21 PFAS. Se encontraron valores medios de PFR entre 6,4 ng l^{-1} (TCEP) y 70,4 ng l^{-1} (TCIPP). La concentración más alta fue para TCIPP (330,2 ng l^{-1}). Los PFR más detectados en las muestras fueron TCIPP (67 %), seguido de fosfato de trifenilo (TPhP) (58 %), TCEP y fosfato de tripropilo (TPP) (ambos 50 %). La presencia de TPP solo en muestras de agua puede deberse a su alta solubilidad, 827 mg l^{-1} a 25 °C (van der Veen y de Boer, 2012). Debido a la falta de estudios sobre la concentración de PFR en humedales, los datos se compararon con estudios en otras aguas superficiales como ríos alrededor del Mar de Bohai en el norte de China (Wang *et al.*, 2015), y el río Elba y su tributarios en Alemania (Wolschke *et al.*, 2015), donde se encontraron niveles de TCIPP, TCEP y fosfato de tris(1,3-dicloro-2-propilo) (TDCIPP) más altos que los de este estudio. Los resultados son consistentes con las propiedades fisicoquímicas de los PFR (Tabla S1, Capítulo 7), ya que los detectados son altamente solubles en agua (por ejemplo, TPP) y los insolubles, como el fosfato de difenil cresilo (CDP) o el fosfato de tris(2-etilhexilo) (TEHP), no fueron encontrados.

Las PFAS detectadas con mayor frecuencia fueron PFOA (100 %), PFOS (92 %) y PFPeA (83 %). Las concentraciones más altas se encontraron para PFOS (47,8 ng l^{-1}) y ácido 2H-perfluoro-2-decenoico (FOUEA) (46,1 ng l^{-1}), en dos puntos del río Turia. Las concentraciones medias para los compuestos detectados oscilaron entre 1,0 y 31,6 ng l^{-1}. La Tabla 3 (Capítulo 7) muestra las concentraciones de PFAS en las aguas superficiales de humedales de otras partes del mundo. En el Delta del Ebro (Pignotti *et al.*, 2017), un humedal localizado en el este de España a unos 350 km al norte del de este estudio, el PFOA fue el compuesto más frecuente y el PFOS el más abundante. En la reserva natural de las marismas de Mai Po en Hong Kong (Loi *et al.*, 2011), los compuestos prevalentes fueron PFOA, PFBS y PFOS. En el humedal de Xixi, un área residencial agrícola y rural de China, las substancias perfluoroalquiladas detectadas en agua fueron PFOA, PFHpA y ácido perfluorononanoico (PFNA), siendo PFOA el que presentó una mayor frecuencia (100 %) y concentración (197,8 ng l^{-1}) (Xu *et al.*, 2016). Un estudio previo realizado en el parque natural de La Albufera (Picó *et al.*, 2012) encontró las mayores frecuencias de aparición para PFOA y PFOS (ambos 100 %). La mayoría de las PFAS mostraron concentraciones medias más bajas en este estudio que en el realizado en 2012, que detectó niveles medios de PFOA de 49,5 ng l^{-1}, mucho más altos que el valor medio actual (9,7 ng l^{-1}). Sin embargo, las concentraciones promedio de PFOS encontradas en aguas superficiales en este estudio (31,6 ng l^{-1}) son más altas que las de 2012 (14,2 ng l^{-1}), y ambas son relevantes teniendo en cuenta la EQS media anual de 0,65 ng l^{-1} establecida por la Unión Europea (2013). PFNA y PFDA fueron los únicos compuestos

de cadena larga detectados en aguas superficiales. Al igual que en los otros ríos estudiados, esto podría explicarse por su menor solubilidad (Onghena *et al.*, 2012) y reemplazo por PFAS de cadena corta.

2.1.2 Aguas residuales

En el muestreo realizado durante el invierno de 2016-2017 en el área del parque natural de La Albufera, también se tomaron muestras de influentes y efluentes de diez WWTP localizadas en el tramo final de los ríos Turia y Júcar y en localidades situadas alrededor del humedal.

Los PFR más frecuentes en influentes y efluentes fueron TPhP (100 % en ambos), TDCIPP (92 % y 85 %, respectivamente) y TCIPP (92 % y 77 %, respectivamente). El que presentó las concentraciones más altas (1543,5 ng l^{-1} en el influente de Pinedo I y 1908,5 ng l^{-1} en el efluente de Pinedo II) fue el TCIPP. Las depuradoras mostraron una menor eficiencia de eliminación para los PFR clorados (TCIPP, TDCIPP y TCEP) que para los no-clorados como TPhP y fosfato de tricresilo (TMPP). Como se muestra en la Figura 4 (Capítulo 7), las eficiencias de eliminación variaron de 14 a 66 %, excepto para TPP que solo se encontró en muestras de efluentes y TDCIPP que se encontró en concentraciones mucho más altas en muestras de efluentes (-74 %). Los PFR clorados también se detectaron en alta frecuencia y concentración en influentes y efluentes de otros estudios (Kim *et al.*, 2017; O'Brien *et al.*, 2015; Schreder y La Guardia, 2014). Este estudio confirma los resultados previos de Kim *et al.* (2017), quienes analizaron una WWTP en el Estado de Nueva York (EE. UU.) y mostraron una eficacia de eliminación negativa para TCEP, TCIPP y TDCIPP que relacionaron con la presencia de compuestos precursores y la baja biotransformación de los PFR.

De los 21 PFAS seleccionados, 13 se encontraron en influentes y 20 en efluentes de aguas residuales, siendo el sulfonato de perfluorodecano (PFDS) el único no detectado. El PFOA fue el más frecuente (100 %) en ambas matrices, seguido por el PFOS con un 54 % en influentes y un 92 % en efluentes. Las PFAS que aparecieron a mayores concentraciones fueron PFBS (101,3 ng l^{-1}) en el efluente Pinedo I y PFOS (63,1 ng l^{-1}) en el influente de Perellonet. Algunas PFAS como PFHxA, PFHpA, sulfonato de perfluorohexano (PFHxS), PFOA, sulfonato de perfluoroheptano (PFHpS), sulfonato de perfluoro-7-metiloctano (ipPFNS), PFOS y FOUEA presentaron mayores concentraciones en efluentes que en influentes, y otros como PFBS, ácido perfluoro-7-metiloctanoico (ipPFNA), PFNA, PFDA, ácido perfluoroundecanoico (PFUnDA), ácido perflurorotridecanoico (PFTrDA), ácido perfluorohexadecanoico (PFHxDA) y ácido perfluorooctadecanoico (PFODA) solo se detectaron en los efluentes. Las restantes mostraron eficiencias de eliminación entre 8 y 100 %. La cadena carbonada completamente fluorada de las

PFAS previene su descomposición aeróbica. Además, existe evidencia de que la biotransformación de precursores durante el tratamiento de fangos activos es una fuente adicional de PFAS en las WWTP (Lee *et al.*, 2010; Sinclair y Kannan, 2006). Las altas concentraciones de PFAS observadas en los efluentes sugieren que las WWTP son ineficaces para eliminar estos compuestos (Arvaniti *et al.*, 2012; Campo *et al.*, 2014). Otros estudios sugieren que las WWTP podrían ser las principales fuentes de contaminación de PFAS y PFR en las aguas superficiales y, por tanto, pueden contribuir a aumentar la concentración de estos compuestos en el entorno del parque natural de La Albufera.

2.2 Suelo y sedimento

Se estudió la presencia de 21 PFAS en 46 muestras de sedimento de los ríos Ebro (22 muestras) y Guadalquivir (24 muestras). En el río Guadalquivir, 19 muestras tenían al menos una PFAS. El PFBA fue el compuesto predominante (67 %) seguido por PFPeA (38 %), PFOA y PFOS (ambos 33 %). Las concentraciones máximas fueron de PFBA (63,8 ng g^{-1}) y PFOA (27,1 ng g^{-1}). El resto de PFAS tuvieron concentraciones máximas por debajo de 1,1 ng g^{-1} (Tabla 1, Capítulo 5). De las 22 muestras del río Ebro, 20 resultaron positivas para PFAS. El PFBA fue predominante (86 %) seguido de PFOS (59 %) y PFPeA (55 %). Las concentraciones más altas fueron de PFOA (32,4 ng g^{-1}) y PFPeA (27,9 ng g^{-1}) (Tabla 1, Capítulo 5). Las altas concentraciones encontradas en sedimento del río Guadalquivir, al igual que ocurría con las de agua, corresponden a puntos de muestreo aguas abajo de las WWTP. La mayor frecuencia de PFOS y PFOA en sedimentos que en agua podría indicar los cambios recientes en la producción y el uso de PFAS ya que, en los últimos años, estos compuestos han sido reemplazados por otros de cadena más corta. Es difícil correlacionar la concentración de PFAS en agua con la que se encuentra en los sedimentos ya que el coeficiente de adsorción solo se puede calcular en equilibrio y los ríos son sistemas dinámicos. Las concentraciones más altas se observaron claramente en la sección final del río (excepto en un punto de la cabecera). La mayor concentración en los sedimentos del río Ebro se localizó en un punto rodeado de viñedos y pequeñas fábricas de vino en La Rioja. Los niveles de PFAS fueron bajos en la muestra de agua tomada en este punto. Sin embargo, la liberación de PFAS al agua puede ser intermitente dependiendo del trabajo de temporada en la bodega. En la sección final, existe una mejor correlación entre las concentraciones en agua y sedimentos. Las elevadas concentraciones pudieron estar relacionadas con la mayor densidad de población. Las concentraciones de PFAS en muestras de sedimentos del río Ebro fueron del mismo orden de magnitud que las encontradas en el Guadalquivir. Sin embargo, la frecuencia de aparición fue mayor en el Ebro. Por el momento, los datos sobre la presencia de PFAS en sedimentos de ecosistemas acuáticos mediterráneos son limitados. El PFBA fue también el compuesto

predominante, con una frecuencia de aparición del 100 %, en el río Llobregat (Campo *et al.*, 2015) y se encontró en concentraciones comparables a las de PFOA en el lago Michigan (Codling *et al.*, 2014).

También se estudió la presencia de 21 PFAS en 15 muestras de sedimento del río Júcar. Todas las muestras de sedimento mostraron contaminación por, al menos, una PFAS. Las frecuencias fueron bastante más altas que las observadas en muestras de agua, variando desde un 7 % hasta el 100 % (PFBA). Otros compuestos frecuentemente encontrados fueron PFOS (67 %) y PFPeA (60 %). La Tabla 2 (Capítulo 6) muestra frecuencias y concentraciones medias de PFAS superiores en sedimento en comparación con las muestras de agua, señalando la tendencia de estos compuestos a acumularse. Las PFAS de cadena corta presentaron las mayores concentraciones medias y se detectaron en muestras a lo largo de toda la cuenca (Fig. 3, Capítulo 6). Las concentraciones medias y máximas de PFOS y PFOA fueron similares a pesar de la mayor tendencia de PFOS a adsorberse en sedimento. Las concentraciones de PFAS detectadas están en el mismo rango, o incluso son ligeramente más altas, que las de otros estudios como se muestra en la Tabla S10 (Capítulo 6). Los niveles de contaminación fueron altos en ambos afluentes del Júcar, particularmente en el río Magro, alcanzando hasta 52,8 ng g^{-1}. En el Cabriel y el Júcar, las PFAS se acumularon aguas abajo. Al igual que en las muestras de agua, parece que los embalses que regulan el caudal en la cuenca tienen un efecto en la concentración de estos compuestos.

Durante el invierno de 2016-2017 se tomaron 19 muestras de sedimento en el área del parque natural de La Albufera, así como en puntos clave de los ríos Turia, Júcar y Magro, y se estudió la presencia de 9 PFR y 21 PFAS. Todas las muestras estaban contaminadas por al menos 4 PFR. El TCIPP fue omnipresente (100 %), seguido de TCEP y TPhP (95 %), TEHP (89 %), TDCIPP (79 %), CDP (68 %) y TMPP (47 %). La concentración media para los compuestos detectados varió de 2,5 a 53,8 ng g^{-1}, mientras que la máxima fue para TCIPP (246,5 ng g^{-1}). Los valores medios en este estudio fueron similares a los del estuario del río Perla en China (Hu *et al.*, 2017) y otros ríos europeos (Cristale *et al.*, 2013; Giulivo *et al.*, 2017) y chinos (Cao *et al.*, 2012). De las 19 muestras de sedimentos, 13 tenían al menos una PFAS (Tabla 2, Capítulo 7). El PFOS fue el compuesto predominante (58 %) mientras que las otras PFAS se detectaron en menos del 32 % de los puntos de muestreo. La concentración máxima fue para PFOS con 21,4 ng g^{-1}. PFOS también fue el compuesto más abundante en sedimentos de un humedal de Hong Kong (Loi *et al.*, 2011), el río Júcar (Capítulo 6) y en ríos y lagos coreanos (Lam *et al.*, 2014). Por el contrario, como se muestra en la Tabla 3 (Capítulo 7), el PFOA fue el compuesto encontrado a una mayor concentración en sedimentos del estudio previo en el parque natural de La Albufera (Picó *et al.*, 2012), en el río Ganges y el humedal Sundarban (India) (Corsolini *et al.*, 2012), y en el Delta del

Ebro (Pignotti *et al.*, 2017). Se detectaron PFAS de cadena larga como PFUnDA, ácido perfluorododecanoico (PFDoDA) y ácido perfluorotetradecanoico (PFTeDA) en el sedimento, pero no en el agua. Las PFAS de cadena larga tienen una alta afinidad por las partículas, y la superficie de los sedimentos podría actuar como un sumidero (Ahrens *et al.*, 2010).

Así mismo, se analizaron 12 muestras de sedimento del río Segura recolectadas en 2013, y 47 muestras de suelo de la cuenca del río Turia recolectadas en los años 2012 y 2013. La Figura 4 (Capítulo 2) presenta las concentraciones de PFAS detectadas. En los sedimentos del río Segura se encontraron PFCA en el 100 % de las muestras en un rango de concentración de 0,07 a 14,91 ng g^{-1}, siendo la concentración más alta la de PFBA. También se encontraron dos PFCA de cadena larga (PFTrDA y PFTeDA) en uno de los puntos de muestreo. Respecto a los PFSA, se encontraron niveles de PFOS de hasta 2,29 ng g^{-1}. PFBA y PFOS fueron los compuestos detectados más frecuentemente (100 % de las muestras), mientras que PFOA se encontró en el 50 % de las muestras. Como se señaló anteriormente, PFBA es la sustancia perfluoroalquilada dominante que reemplaza a PFOA debido a las restricciones de producción de esta última. La presencia de PFAS puede explicarse por su liberación a través de los efluentes de las WWTP, o el vertido de residuos industriales e incluso agrícolas (ya que las PFAS son utilizadas en algunas formulaciones de plaguicidas). Comparando las concentraciones en sedimentos del Segura con las de otros ríos, se encontraron valores comparables en sedimentos del río Llobregat (Campo *et al.*, 2015) donde las concentraciones medias eran similares, aunque ligeramente más altas para PFOA y PFOS, y también se encontró PFTrDA (0,19 ng g^{-1}) en un punto de muestreo. En ambos estudios, PFBA fue el compuesto encontrado a mayor concentración, aunque en el río Llobregat (3,67 ng g^{-1}), el promedio fue tres veces menor al detectado en el río Segura (10,47 ng g^{-1}). Otros estudios en sedimentos midieron las concentraciones de algunos PFAS como el del parque natural de La Albufera, con valores que variaron de 0,03 a 10,9 ng g^{-1} para PFOA, y de 0,10 a 4,80 ng g^{-1} para PFOS (Picó *et al.*, 2012). Los valores de PFOA fueron 10 veces más altos en La Albufera que en el río Segura, mientras que los niveles de PFOS fueron similares en ambos lugares. Sin embargo, otros autores han encontrado concentraciones más bajas, como Yang *et al.* (2011), quienes determinaron concentraciones de PFOA y PFOS en sedimentos del río Liao (China) 4 y 8 veces más bajas, respectivamente, que las del río Segura. En las muestras de suelo del río Turia, los valores de PFCA fueron mayores que los de PFSA. El PFBA presentó las concentraciones máximas en ambos muestreos, aunque en 2013 (64,04 ng g^{-1}) fueron mayores que en 2012 (17,96 ng g^{-1}). El PFBA fue también el compuesto encontrado en un mayor número de puntos de muestreo en 2012 y 2013 (77 y 42 %, respectivamente), seguido de PFOA (59 y 31 %) y PFOS (14 y 15 %), sin embargo, la frecuencia en 2013 fue menor que en 2012. Hay pocos artículos sobre las

concentraciones que las PFAS pueden alcanzar en suelo y los trabajos publicados se centran en PFOA y PFOS. En suelos agrícolas de Shanghai se encontró PFOA en un rango de 3,3 a 44 ng g^{-1}, y de 9,2 a 10,4 ng g^{-1} para PFOS (Li *et al.*, 2010). Un estudio piloto para analizar PFAS en suelos de seis países obtuvo rangos variables entre 0,95 a 12,4 ng g^{-1} para PFHxA, 0,76 a 31,7 ng g^{-1} para PFOA, y de 0,58 a 10,1 ng g^{-1} para PFOS (Strynar *et al.*, 2012). Sin embargo, no se han encontrado datos sobre la concentración en suelos de cuencas hidrográficas que puedan compararse con este estudio.

2.3 Peces

Debido a la complejidad de la pesca eléctrica, la escasez de peces en los ríos mediterráneos y a la voluntad de alterar lo menos posible el medio, el estudio en biota estuvo limitado por tamaños de muestra relativamente pequeños, lo que aumentó la posibilidad de potenciales sesgos de selección.

En el río Guadalquivir, se recolectaron especies como la boga del Guadiana (*Pseudochondrostoma willkommii*), el barbo andaluz (*Luciobarbus sclateri*) y la carpa común (*Cyprinus Carpio*). De los 17 compuestos analizados en peces, solo se detectó PFOS, aunque fue en el 100 % de las muestras. El valor medio de concentración de PFOS fue de 29,7 ng g^{-1} (Tabla 1, Capítulo 5). Las especies de peces recogidas en el río Ebro incluyeron el barbo de Graells (*Barbus graellsii*), la carpa común (*C. carpio*) y el siluro (*Silurus glanis*), en los que se detectaron 12 PFAS. PFOS y sulfonamida de perfluorooctano (PFOSA) fueron los compuestos más abundantes (ambos con una frecuencia de aparición del 81 %), seguidos de PFBS (69 %), PFHxA y PFOA (ambos 56 %). Mientras que PFBA, abundante en muestras de agua y sedimentos, estuvo presente en un 31 % de la biota analizada. La concentración más alta fue 1280,2 ng g^{-1} para PFHxA (Tabla 1, Capítulo 5). La observación más notable fue la alta frecuencia a la que se encontró PFOS en las muestras de peces de las dos cuencas fluviales. El K_{OW} de PFOS no justifica el alto potencial de bioacumulación encontrado. Sin embargo, algunos autores señalan cómo el uso de K_{OW} no es apropiado para predecir la bioacumulación de PFAS porque PFOS no se une a los lípidos, sino que se une a ciertas proteínas en animales (Jones *et al.*, 2003). Como resultado, el uso de la solubilidad en agua o K_{OW} puede mostrar resultados erróneos sobre su bioacumulación. El patrón de PFAS en biota no se correlaciona con el encontrado en agua o sedimento en cualquiera de los ríos. El PFOSA se detectó únicamente en muestras de biota. Este compuesto puede aparecer tras la metabolización del PFOS por aminación o hidrólisis o de precursores acumulados que son biodegradados en PFOSA y en otros compuestos (Dimitrov *et al.*, 2004). Los resultados presentados en este trabajo son similares a los de otros artículos en los que PFOS (100 %), PFOSA (75 %) y PFBS (67 %) son también las

sustancias perfluoroalquiladas encontradas a una mayor frecuencia en peces del Llobregat (Campo *et al.*, 2015), así como en el caso del PFBS en peces del Rin (Möller *et al.*, 2010). Sin embargo, son necesarios más estudios sobre la tendencia de acumulación en biota para poder explicar completamente estos resultados.

En el río Júcar, se tomaron 25 muestras de 9 especies diferentes incluyendo la trucha común (*Salmo trutta*), el gobio ibérico (*Gobio lozanoi*), la boga del Tajo (*Pseudochondrostoma polylepis*), la perca americana (*Micropterus salmoides*), el barbo mediterráneo (*Barbus guiraonis*), la perca sol (*Lepomis gibbosus*), el alburno (*Alburnus alburnus*), la anguila (*Anguilla anguilla*) y el lucio (*Esox lucius*). Se seleccionaron 17 PFAS y solo 5 fueron detectados con frecuencias entre 4 % y el 60 %, siendo PFOS el compuesto dominante. El patrón de distribución de PFAS fue diferente a otras matrices. El PFBA, por ejemplo, omnipresente en muestras de sedimento y agua, no se encontró en ninguna muestra de pez. Las concentraciones medias de PFCA fueron más altas que en sedimentos y agua indicando una posible bioacumulación (Tabla 3, Capítulo 6). Los valores variaron de 21,4 μg kg^{-1} de PFHpA a 274 μg kg^{-1} de PFPeA (valor máximo de 946 μg kg^{-1}). Por el contrario, las medias de PFSA fueron bajas (<2,16 μg kg^{-1}). Las concentraciones de PFAS fueron más altas que las encontradas en otras especies de peces y áreas (Tabla S10, Capítulo 6). Las concentraciones de PFOS detectadas en la biota del río Júcar no representan un riesgo para ella, ya que incluso el valor máximo obtenido (8,13 μg kg^{-1}) es menor que la EQS de 9,1 μg kg^{-1} establecida por la Unión Europea (2013). De acuerdo con las concentraciones acumuladas de PFAS en los diferentes puntos de muestreo, los valores más altos se observaron en peces capturados en la parte baja del río. Comparando los valores promedio obtenidos para cada especie, las especies invasoras como la perca americana o la perca sol presentaron las concentraciones más altas. La anguila, considerada una especie en peligro de extinción, presentó ΣPFAS de 87 μg kg^{-1}. Todas las especies parecieron acumular particularmente PFPeA y en una proporción menor PFHpA (Fig. 4b, Capítulo 6). También se determinó el factor de bioacumulación experimental (BAF, en l kg^{-1}). Las especies que muestran los BAF más altos (perca americana y perca sol) son depredadoras de la parte alta de la cadena trófica, lo que sugiere una posible biomagnificación. Sin embargo, las especies que muestran los BAF más bajos como las truchas, que se alimentan de invertebrados en su etapa juvenil, son también piscívoras en su etapa adulta. En base a esto, se exploraron las posibles correlaciones entre las concentraciones de PFAS, con valores medios y máximos, y los datos de los niveles tróficos sin obtener coeficientes significativos, probablemente porque los niveles tróficos son bastante similares, yendo desde 2,5 a 4,1 con la mayoría de ellos alrededor de 3,5. Estos resultados se obtuvieron para un muestreo particular y, en consecuencia, deben tomarse con

precaución y se necesitan más estudios, aumentando la frecuencia de muestreo y el número de muestras para confirmar las tendencias de PFAS descritas.

Durante el invierno de 2016-2017, se tomaron 10 muestras de peces del parque natural de La Albufera incluyendo anguila (*A. anguilla*), mújol (*Mugil cephalus*) y carpa común (*C. carpio*) para el análisis de PFR y PFAS. De los 9 PFR analizados, se encontraron 5, siendo TCEP (70 %) y TCIPP (50 %) los más detectados. Los clorados TCEP y TCIPP fueron detectados en todas las especies. La concentración más alta, 13,1 ng g^{-1} en peso húmedo (ww) de TCIPP, se encontró en una muestra de *A. anguilla*. La anguila ha sido considerada como una especie adecuada para el análisis de sustancias tóxicas porque tiende a bioacumular contaminantes en su tejido muscular como consecuencia de algunas características fisiológicas y ecológicas específicas (tamaño, larga vida útil, contenido de grasa, alimentación, hábitat, distribución, etc.) (Belpaire *et al.*, 2011; Belpaire y Goemans, 2007). No se han encontrado estudios sobre la presencia de PFR en peces que habiten en humedales. A diferencia de las PFAS, que son extremadamente estables a la degradación, la ausencia de PFR o las bajas concentraciones encontradas en este estudio pueden deberse a la degradación abiótica o a la metabolización por parte de los peces, como se ha notificado para TDCIPP (van der Veen y de Boer, 2012). La concentración real en los peces puede ser más alta que los niveles mostrados en este estudio porque es probable que los PFR se metabolicen, y estos metabolitos no han sido incluidos en el estudio (Greaves y Letcher, 2014). Respecto a las PFAS, el PFOS fue el más detectado (60 %). Al igual que en los PFR, la concentración más alta (194,5 ng g^{-1} ww para PFOS) se encontró en una muestra de anguila. El PFOS fue el compuesto más abundante en carpa y sus concentraciones medias (30,4 ng g^{-1} ww) fueron más altas que el valor de EQS de 9,1 ng g^{-1} establecido por la Unión Europea (European Parlament, 2013). El PFOS excedió los valores de EQS en el 50 % de las muestras (71,4 % en el caso de las anguilas). En el estuario del río Loira (Francia), con características similares a La Albufera, también se detectó presencia de PFOS excediendo los valores EQS en un 75 % de muestras de anguila (Couderc *et al.*, 2015). Del mismo modo, en el Delta del Ebro, el PFOS fue el compuesto más abundante detectado en la biota costera, aunque aguas arriba PFOA fue el compuesto principal (<LOQ-330 ng g^{-1} ww) (Pignotti *et al.*, 2017). El PFOS es conocido por su alto potencial de bioacumulación. Sin embargo, al tener características hidrofóbicas y lipofóbicas simultáneamente, no sigue el patrón típico de partición y acumulación en los tejidos grasos, sino que tiende a unirse a las proteínas y por lo tanto está más presente en tejidos altamente perfundidos (Lassen *et al.*, 2013). De acuerdo con Lassen *et al.* (2013), los PFCA no son muy bioacumulativos, pero algunos de sus precursores como los telómeros de alcoholes fluorados pueden bioacumularse y posteriormente, ser metabolizados a los ácidos correspondientes. La falta de detección de PFAS de cadena corta en la biota podría deberse

a su bajo potencial de bioacumulación. Esto contrasta con las concentraciones encontradas en aguas, donde también se detectaron PFAS de cadena corta. Ding y Peijnenburg (2013) establecieron que a igual número de carbonos fluorados, los PFSA tienden a bioacumularse más que los PFCA, lo que está en consonancia con los resultados obtenidos en este estudio para PFOS-PFOA e ipPFNS-ipPFNA.

3. Evaluación de la toxicidad para la biota acuática

Los modelos QSAR (relación cuantitativa estructura-actividad) se aplican para predecir la toxicidad acuática de los ePOP en ausencia de datos experimentales. Las estimaciones basadas en las relaciones matemáticas entre los valores K_{OW} y la correspondiente toxicidad medida pueden obtenerse mediante el software ECOSAR™ para cualquier compuesto químico, incluidos diferentes ePOP. Estos datos se usaron para calcular los cocientes de peligrosidad (HQ) para las PFAS. Los HQ se definen como la relación entre la concentración ambiental predicha o medida (MEC) y su toxicidad crónica, generalmente expresada como NOEC (concentración sin efecto observado) o PNEC (concentración sin efecto previsto), referidos a tres niveles tróficos diferentes (algas, dafnias y peces), tal como recomienda la Directiva Marco Europea del Agua (European Parlament, 2000). Cuando los valores NOEC no están disponibles, se utilizan los valores EC_{50} o LC_{50} de pruebas ecotoxicológicas estándar después de una estimación mediante un factor de corrección de 1000 (European Parlament, 2000). En general, HQ>0,1 indica riesgo medio y HQ>1 riesgo agudo.

Para determinar si los niveles de PFAS encontrados en las cuencas del Ebro, Guadalquivir, Júcar y afluentes podían ser tóxicos en ambientes acuáticos, se llevó a cabo una evaluación comparativa entre los niveles de PFAS en agua y su toxicidad, a través del HQ. Afortunadamente, los HQ fueron inferiores a 0,1 para la mayoría de las PFAS, considerando concentraciones máximas y medias, en los diferentes niveles tróficos considerados. En la cuenca del Ebro, solo el compuesto de cadena larga PFTeDA pudo suponer un riesgo agudo (HQ>1) para dafnias y peces tomando el valor máximo de concentración detectado (Tabla 2, Capítulo 5). En la cuenca del Júcar, ninguna de las PFAS presentó un riesgo agudo para los tres niveles tróficos estudiados, incluso cuando se consideraron los valores de concentración máximos (Tabla 4, Capítulo 6). Solo el valor máximo de PFDA presentó 0,1<HQ<1 y, por tanto, pudo suponer un riesgo potencial para dafnias y peces. Sin embargo, cabe mencionar que otras propiedades adversas de los ePOP como efectos de disrupción endocrina o su bioacumulación no están incluidas en este tipo de estimación del riesgo, así como el hecho de que la mayoría de los valores de PNEC se han estimado con el programa ECOSAR y no son valores experimentales.

4. Aplicación en ecosistemas forestales

En agosto de 2014 se produjo un incendio forestal en el municipio de Azuébar, junto a la sierra de Espadán, que quemó 10,59 ha de área boscosa. Un mes después del incendio, se tomaron muestras de suelo de dos laderas (BU: zona quemada y CO: zona control), en dos entornos (suelo bajo cubierta vegetal: UC y suelo desnudo: BS) y en dos profundidades (suelo superficial o TS: 0-2 cm y subsuelo o SS: 2-5 cm). Además, se tuvo en cuenta la posición de la ladera donde se tomaron las muestras, asociando la parte superior con la zona de erosión, la media con el transporte y la inferior con la deposición. Se consideraron cuatro episodios de lluvia erosiva ocurridos entre los tres y los doce meses posteriores al incendio, que produjeron 12,7, 143,6, 12,6 y 62,2 kg de sedimento. Estos fueron obtenidos mediante trampas recolectoras situadas en la parte inferior de la ladera quemada. En la ladera control no se produjeron sedimentos. En las muestras recogidas, se estudió la presencia de 21 PFAS, 11 PFR, 8 BFR (de las mezclas de tri- a hepta- bromodifenil éteres) y 16 PAH, estos últimos considerados los 16 prioritarios de la Agencia de Protección Ambiental de Estados Unidos (EPA) (Tabla S2, Capítulo 8).

Se observaron PBDE en ambas laderas, aunque los suelos en BU presentaron mayores concentraciones de ΣPBDE que en CO ($p<0,05$). El compuesto más frecuente fue BDE-47, que se encontró en todas las muestras quemadas y en la mayoría de las de la ladera control. La presencia de PBDE en CO podría estar relacionada con el transporte atmosférico y la deposición (Eljarrat *et al.*, 2008). BDE-85 solo se detectó en muestras de BU y fue, además, el PBDE encontrado a una mayor concentración en suelo (5,6 ng g^{-1}) y en sedimento (11,4 ng g^{-1}), lo que podría implicar su uso como extintor del fuego. Sin embargo, este compuesto solo representa alrededor del 2-3 % de las mezclas comerciales más utilizadas de penta-BDE. Los congéneres más abundantes en esta mezcla son BDE-47, -99, -100, -153 y -154 (La Guardia *et al.*, 2006) que no se encontraron, o se detectaron a bajas concentraciones, en las muestras de BU. Aunque se podría sugerir la hipótesis de que estos de congéneres se hubieran degradado por altas temperaturas, es cuestionable si se utilizó una mezcla de PBDE para facilitar la extinción del fuego. La posición en la ladera no tuvo una influencia significativa en la distribución de PBDE. La vegetación no influyó significativamente en la distribución de PBDE en el suelo. Sin embargo, los valores de BDE-85 tendieron a ser más altos en UC que en BS. En las áreas mediterráneas, la vegetación opera como un obstáculo para la erosión, actuando como sumidero, y causando la acumulación de suelo erosionado (Urgeghe y Bautista, 2015). El suelo erosionado puede transportar contaminantes, lo que explicaría las altas concentraciones encontradas, y podría ser la fuente de los PBDE detectados en UC. Es importante destacar la dificultad de determinar la contaminación real de PBDE en los

suelos en relación con los incendios forestales, ya que los estudios existentes han investigado su presencia principalmente cerca de sitios de reciclaje de desechos electrónicos y en campos agrícolas donde se aplican lodos de WWTP como fertilizante. En sedimento, las concentraciones de tri- a penta-BDE fueron bajas, aunque BDE-85 presentó el valor más alto (11,4 ng g^{-1}) y el BDE-47 se detectó en todas las muestras de sedimento. La suma de los valores de PBDE fue alta en el sedimento del primer evento erosivo (17,8 ng g^{-1}), siendo mayor que en los suelos (valor máximo 7,3 ng g^{-1}). Estas concentraciones disminuyeron hasta un orden de magnitud en los sedimentos recogidos en los siguientes episodios.

El fuego en Azuébar agregó cantidades significativas de PAH al suelo (BU>CO, Tabla S6). BU tuvo mayores concentraciones de PAH ligeros (2-3 anillos aromáticos) y ΣPAH que CO ($p<0,05$). Por el contrario, los PAH pesados (4-6 anillos aromáticos) fueron dominantes en CO ($p<0,05$) y a mayor profundidad ($p<0,05$). El ΣPAH para BU osciló entre 133,5 y 1255 ng g^{-1} (Fig. 3, Capítulo 8). Las concentraciones de PAH en BU se encontraron entre las detectadas en muestras de suelo y de hojarasca de Corea por Choi (2014). La distribución de PAH en el suelo de Azuébar es similar a la obtenida en una región costera de Corea tras sufrir varios incendios forestales (150-1600 ng g^{-1}) (Kim *et al.*, 2003). Las concentraciones en el presente estudio son más altas que las encontradas por Vergnoux *et al.* (2011) en lugares del sur de Francia afectados por quemas repetidas (Σ14 PAH = 77-157 ng g^{-1}) y por Pizarro-Tobías *et al.* (2015) tras realizar quemas controladas en el parque natural de los Montes de Málaga (400 ng g^{-1} para Σ15 PAH). Los perfiles de los PAH en suelos quemados son constantes en todos los estudios y muy similares a los descritos aquí (Fig. 4a, Capítulo 8). Los niveles de PAH detectados en CO (33,8-120,2 ng g^{-1}), están en el mismo orden de magnitud que los valores obtenidos por Pizarro-Tobías *et al.* (2015): promedio de 58 ng g^{-1}, Kim *et al.* (2003): 49 ng g^{-1}, y Choi (2014): 26 ng g^{-1}. A pesar de que la vegetación parcialmente quemada es una fuente importante de PAH en el suelo (Choi, 2014), no hubo diferencias significativas basadas en la presencia o ausencia de vegetación. Las altas concentraciones de PAH en los sedimentos de la primera precipitación sugieren que la erosión del material orgánico quemado en superficie es un proceso importante para el transporte de PAH ya que el perfil de éstos en los sedimentos es similar al de BU (Fig. 4, Capítulo 8). Un signo de degradación y volatilización puede ser el incremento de PAH pesados en los sedimentos a lo largo del tiempo. En BU, los PAH ligeros y degradables disminuyeron probablemente en los meses posteriores al fuego (Choi, 2014). Esto también conduciría a una menor presencia de PAH ligeros en los sedimentos (Fig. 4b, Capítulo 8). Las concentraciones de PAH en los sedimentos del primer evento (ΣPAH = 3154 ng g^{-1}) fueron mayores que la concentración máxima encontrada en suelo

(ΣPAH = 1255 ng g^{-1}). Las concentraciones disminuyeron en los sedimentos de los siguientes episodios.

A pesar de que las concentraciones de PFR no se distribuyeron normalmente para ninguna variable (ladera, entorno, profundidad y posición de la pendiente), ΣPFR tendió a ser mayor en CO que en BU (Fig. 5, Capítulo 8). Esto hace suponer que estos compuestos no se utilizaron para facilitar la extinción del incendio (Mihajlović *et al.*, 2011; Wei *et al.*, 2015). Existen datos limitados sobre la presencia de PFR en sedimento y suelo y no existen datos en estas matrices relacionados con incendios forestales. Tal y como se esperaba, los PFR no detectados en suelo, tampoco estuvieron presentes en sedimento. TDCIPP y TEHP solo se detectaron en muestras de sedimentos del segundo evento, que fue el más erosivo. TnBP mostró la concentración (26,5 ng g^{-1}) y la frecuencia de aparición más alta. Al contrario que los contaminantes mencionados anteriormente, el ΣPFR en el primer evento de lluvia, no fue superior al valor máximo encontrado en muestras de suelo.

Respecto a las PFAS, UC mostró concentraciones más altas de ΣPFAS que BS ($p<0,05$, Fig. 6, Capítulo 8). A pesar de que las PFAS se han encontrado generalmente en instalaciones de lucha contra incendios por estar presentes en las AFFF, nunca se han estudiado en un área afectada por un incendio forestal. Estudios acerca de las concentraciones de PFAS en instalaciones de entrenamiento contra incendios mostraron altos niveles de PFOS, entre 21 y 8520 ng g^{-1} (Filipovic *et al.*, 2015; Hale *et al.*, 2017; Houtz *et al.*, 2013; Kupryianchyk *et al.*, 2016). Estas concentraciones están muy lejos de las de este estudio (entre 0,1 y 1,7 ng g^{-1}), lo que sugiere que las PFAS tampoco estaban presentes en los materiales utilizados para extinguir el incendio forestal. El transporte de precursores de PFAS volátiles en la atmósfera es una de las principales vías de distribución de estos compuestos en el medio ambiente (Bossi *et al.*, 2016; Lai *et al.*, 2016; Strynar *et al.*, 2012). Aunque, como ya se ha comentado en otros apartados, las descargas de las WWTP también se han citado comúnmente como fuentes de PFAS (Campo *et al.*, 2014; Hu *et al.*, 2016). Las PFAS no encontradas en suelo, tampoco estuvieron presentes en sedimento, mientras que PFBA, ipPFNA, PFNA, PFDoDA, PFODA, PFDS y PFHpS estuvieron presentes en suelo, pero no en sedimento. La concentración de PFAS acumulada fue un orden de magnitud mayor en el suelo (máximo ΣPFAS = 16,9 ng g^{-1}) que en sedimento (ΣPFAS = 4,2 ng g^{-1}).

Respecto a la peligrosidad de las concentraciones encontradas, en España no existe una legislación que regule niveles admisibles de ePOP en el suelo. Kalf *et al.* (1995) estimaron una concentración máxima permisible (MPC) para PAH en suelo, que debía proteger al 95 % de especies en un ecosistema. En Azuébar, solo el valor de MPC para naftaleno (Nap) propuesto por

estos autores (140 ng g^{-1}) fue excedido. Sin embargo, los niveles a los que las especies se ven afectadas de forma aguda son probablemente mucho más altos que este MPC. Por otra parte, se puede afirmar que, en algunas partes de la ladera, los niveles de PAH podrían ser posiblemente dañinos para humanos y animales si se expusieran durante largos periodos de tiempo. Algunos procesos pueden aumentar el peligro de contaminación después del fuego. En primer lugar, los resultados muestran que los procesos de erosión pueden concentrar PBDE y PAH localmente donde se depositan los sedimentos. En segundo lugar, los PAH podrían acumularse en el suelo tras incendios recurrentes. Los PAH ligeros pueden ser producidos por el fuego en cantidades tales que sus niveles permanezcan elevados durante años a pesar de su degradabilidad y volatilidad (Vergnoux *et al.*, 2011). Los PAH pesados, aunque se producen en pequeñas cantidades, también podrían permanecer en el suelo durante largos períodos (Duan *et al.*, 2015). La persistencia de los PAH pesados podría explicar sus niveles relativamente altos en los suelos de CO, pudiendo ser un remanente de un incendio ocurrido en el pasado. Respecto a la relación entre las concentraciones de ePOP y el carbono orgánico del suelo (SOC), en CO, solo los PAH presentaron una correlación significativa mientras que, en BU, tanto los PAH como los PBDE se correlacionaron significativamente con el contenido de SOC. Estas correlaciones entre los niveles de SOC y PAH respaldan la influencia de la vegetación en la distribución de estos compuestos. En ambas laderas, más SOC se asocia con más PAH. Esto está en consonancia con la idea de que se agrega material orgánico con alto contenido de PAH al suelo durante los incendios.

5. Aplicación a la exposición en humanos

5.1 Leche materna y otros alimentos infantiles

Se estudió, por primera vez en la Comunidad Valenciana, la exposición infantil a PFAS a través de la dieta incluyendo: leche materna, fórmulas de inicio, continuación y crecimiento y otros alimentos infantiles (potitos a base de carne, pescado o frutas y papillas de cereales deshidratados). De los 20 PFAS incluidos en este estudio, se detectaron 17 en las muestras analizadas. PFBA (100 %) y PFOA (92-100 %) fueron los compuestos más detectados en todas las matrices, mientras que PFOS fue más irregular (17-92 %). Teniendo en cuenta las concentraciones promedio, en cada matriz predominaron diferentes compuestos. En general, las concentraciones fueron más bajas en leche materna y más altas en fórmulas y papillas de cereales deshidratados.

En leche materna, todas las muestras contuvieron PFBA y PFOA. Los compuestos que presentaron un mayor porcentaje respecto a la concentración total de PFAS fueron PFOA (29 %), PFHpA (16 %) y PFUnDA (14 %), mientras que PFOS y PFBA representaron un 8 % cada uno. A diferencia de otros ePOP que se acumulan en los tejidos grasos, las PFAS circulan en la sangre

principalmente unidas a proteínas, y tienden a acumularse en tejidos con alto contenido en ellas, como hígado y riñones. Una de las proteínas más abundantes en la leche humana, la albúmina sérica, no puede ser sintetizada por las glándulas mamarias. Se especula que podría ser transferida desde el plasma a la leche y, por tanto, podría ser el vehículo que transporta las PFAS a la leche humana (Lönnerdal *et al.*, 2017). Según Kang *et al.* (2016), los altos niveles de PFCA de cadena corta observados en leche materna pudieron reflejar el patrón reciente de uso de estas PFAS como reemplazo de las de cadena larga. Estos autores también relacionaron la presencia de PFHpA y PFOA con los utensilios de cocina antiadherentes, y la presencia de PFHpA y PFOS con el uso de productos de cuidado personal. Por otra parte, en nuestro estudio, los altos niveles de PFBA podrían explicarse porque es un producto utilizado como antimanchas y antigrasa en embalajes de alimentos, sofás y alfombras. Estos resultados están en consonancia con otros trabajos realizados durante el desarrollo de la tesis que describieron la contaminación ambiental generalizada con estos PFAS de cadena corta en el entorno del área estudiada (Capítulos 5-8). El patrón de PFAS en leche humana depende del observado en suero, que a su vez depende de la exposición, acumulación y vida media de los compuestos. Como sugirió Kang *et al.* (2016), las PFAS de cadena corta reflejan exposiciones recientes debido a que sus semividas en plasma humano son de pocos días (por ejemplo, 3 días para PFBA). Sin embargo, estas semividas se prolongan con el aumento de la longitud de la cadena (por ejemplo, entre 2 y 8 años para PFOA en suero/plasma humano).

En las fórmulas infantiles, al igual que en la leche materna, se detectaron PFBA y PFOA en el 100 % de las muestras analizadas. Las concentraciones de PFBA variaron de 14 a 496 ng kg^{-1} y las de PFOA de 27 a 2490 ng kg^{-1}. Sin embargo, la concentración promedio más alta (1228 ng kg^{-1}) correspondió a PFDS porque, a pesar de ser poco frecuente (5 muestras), se encontró en concentraciones muy elevadas. Las fuentes de PFAS en fórmulas podrían ser las mismas que en la leche materna sumando, además, la posible contaminación a través de la cadena de procesado de alimentos y el material de empaquetado, que podrían justificar las altas concentraciones de PFDS.

Para las papillas de cereales deshidratados, el PFBA se detectó en el 100 % de las muestras en concentraciones de 1,4 a 968 ng kg^{-1}, mientras que PFOS y PFOA fueron detectados en el 92 %. Los valores medios muestran que las PFAS más relevantes fueron PFBA, PFHpA, PFOA, PFOS y PFDS. La dificultad para conocer el origen de los cereales ya ha sido señalada en otros estudios (D'Hollander *et al.*, 2015). Finalmente, en potitos, se detectaron PFBA y PFOA en el 100 % de las muestras analizadas con concentraciones de hasta 5013 ng kg^{-1}, seguidas de PFDA (83 %) con hasta 387 ng kg^{-1}. Sin embargo, el PFOS se detectó solo en el 17 % de las muestras. En esta matriz, tres compuestos representaron casi todo el perfil de contaminación: PFBA (49 %), PFOA (21 %) y PFDA (20 %).

Las concentraciones en la leche materna, fórmulas y otros alimentos infantiles fueron, en términos generales, del mismo orden de magnitud que las recogidas en otros estudios de PFAS (Tabla 6, Capítulo 9). PFOS y PFOA se detectaron por debajo de 200 ng l^{-1}, también en línea con los niveles de estudios previos (Antignac *et al.*, 2013; Barbarossa *et al.*, 2013; Croes *et al.*, 2012; Völkel *et al.*, 2008). Un estudio de 2009 que analizó 6 PFAS en leche materna, fórmulas y papillas de cereales deshidratados de Barcelona (Llorca *et al.*, 2010) mostró rangos de PFOA y PFOS similares, o algo superiores, a los de este estudio.

El perfil de PFAS en las diferentes muestras (Fig. 1, Capítulo 9) señala que los PFCA son más relevantes que los PFSA en todas las matrices estudiadas, como ocurre en otros estudios (Llorca *et al.*, 2012). Las leches de fórmula son las que presentan la contribución más importante de PFSA (principalmente debido a PFOS y PFDS). Los patrones de distribución de PFAS en la leche materna observados en este estudio son consistentes con otros estudios previos (Kadar *et al.*, 2011; Liu *et al.*, 2010; Llorca *et al.*, 2010). Los PFCA de cadena larga son los más prevalentes en la leche materna y los cereales, mientras que los de cadena corta lo son en leche de fórmula y potitos.

De acuerdo con la directriz de la Autoridad Europea de Seguridad Alimentaria (EFSA, 2008), la ingesta diaria tolerable (TDI) establecida es de 150 ng kg^{-1} $día^{-1}$ para PFOS y 1500 ng kg^{-1} $día^{-1}$ para PFOA. Estos datos podrían utilizarse como una referencia sobre la ingesta diaria total de PFAS. Con el fin de evaluar los posibles riesgos para la salud infantil asociados a la ingesta de PFAS, se calculó la ingesta diaria estimada (EDI) para los primeros dos años, teniendo en cuenta la concentración de PFAS en el alimento, el consumo promedio diario del alimento y el peso corporal del bebé. La tabla S6 (Capítulo 9) fija el consumo diario estimado de alimentos infantiles según la ingesta dietética de referencia (DRI) desarrollada por la Comisión de Nutrición y Alimentación del *Institute of Medicine of the National Academies* (2005). Las EDI estuvieron muy lejos de las TDI establecidas por la EFSA para PFOS y PFOA. Sin embargo, hay otros PFAS que están presentes en las muestras y pueden presentar un rango de toxicidad diferente. Además, las TDI establecidas por la EFSA son provisionales hasta que existan más datos disponibles para realizar una evaluación de riesgos precisa.

En la Figura 2 (Capítulo 9) se ilustra la ingesta diaria de PFAS para recién nacidos y bebés (hasta 2 años) que se alimentan con leche materna (a) o fórmulas (b). Los resultados son similares y muestran que la lactancia artificial no presenta ninguna ventaja respecto a la ingesta de estos contaminantes a través de la dieta. Las ingestas fueron, en general, mayores para los bebés que se alimentaron con leche de fórmula, excepto durante los primeros seis meses de vida, en los que la ingesta fue ligeramente superior para aquellos que se alimentaban con leche materna (debido al

alto contenido de PFOA). Esto ya se destacó en otros estudios (Antignac *et al.*, 2013; Barbarossa *et al.*, 2013; Llorca *et al.*, 2010). Otro aspecto a tener en cuenta es que las muestras de leche de este estudio fueron cedidas por madres primerizas. Otros autores como Barbarossa *et al.* (2013) tuvieron en cuenta en su estudio si las mujeres donantes de leche eran primíparas o multíparas, señalando que la concentración y la frecuencia media fue más alta para los analitos estudiados en leche de madres primíparas. Esto puede explicar los altos niveles de PFOA encontrados en este estudio, así como confirmar la mayor exposición a PFAS para los primogénitos.

5.2 Aire en ambientes interiores

La inhalación puede ser otra importante vía de exposición a ePOP semivolátiles. La exposición por inhalación en microambientes (como las oficinas), se evalúa midiendo las concentraciones del aire en una localización estática. Sin embargo, estas mediciones pueden no reflejar la exposición personal ya que excluyen la influencia de las actividades individuales. La exposición personal por inhalación se puede establecer de manera representativa mediante el uso de muestreadores activos personales. Sin embargo, son caros, molestos y poco prácticos por la necesidad de energía, mantenimiento, calibración frecuente y ruido. Los PPAS son una alternativa útil a los muestreadores activos porque no presentan estas desventajas logísticas al funcionar mediante difusión y deposición. Sin embargo, los PPAS presentan otros inconvenientes como los elevados tiempos de exposición necesarios para acumular niveles detectables analíticamente y la necesidad de calibrarlos con muestreadores de aire activos para obtener sus ratios de muestreo. Este estudio, llevado a cabo en la *University of Toronto* (Canadá) bajo la supervisión de la Profesora Miriam Diamond, forma parte del trabajo realizado durante la estancia requerida para obtener la mención internacional. El objetivo fue caracterizar broches de PDMS como PPAS para mejorar la estimación de la exposición por inhalación a ePOP semivolátiles en un ambiente interior no industrial. Para ello, en primer lugar, se calibraron los broches de PDMS como PPAS y después, se utilizaron para medir las concentraciones personales de la exposición por inhalación a ePOP semivolátiles en participantes que trabajaban en una oficina. Los compuestos seleccionados fueron PFR, ftalatos y BFR (nuevos y PBDE), por ser ePOP semivolátiles utilizados como plastificantes y retardantes de llama en muchos materiales utilizados en interiores. Sin embargo, los BFR no fueron detectados a concentración suficiente en ninguna muestra.

5.2.1 Calibración de los broches

El propósito del estudio de calibración fue medir las tasas de muestreo para convertir la masa de los compuestos recolectados por los broches de PDMS en concentraciones volumétricas de aire y, por tanto, en la exposición por inhalación. Se utilizaron PLV-AAS como dispositivos de

referencia para calibrar los broches de PDMS. Los PLV-AAS consistieron en una bomba conectada a un tubo de sorbente PUF/XAD/PUF. Cinco participantes llevaron los PLV-AAS y los broches de PDMS durante 8 horas de trabajo en oficina en 4 días consecutivos para la calibración.

Las concentraciones promedio ± desviaciones estándar medidas usando PLV-AAS oscilaron entre 2450 ± 400 y 2870 ± 500 ng m^{-3} para Σftalatos, y 490 ± 50 a 1180 ± 620 ng m^{-3} para TCIPP. Las concentraciones diarias personales de aire de los cinco participantes variaron durante los cuatro días entre 6 y 27 % para todos los compuestos, excepto DnBP para un participante, y DEHP y TCIPP, para tres participantes, cuyas concentraciones oscilaron entre 32 y 64 %. Esta mayor variabilidad pudo deberse a cambios en las concentraciones de partículas suspendidas totales, especialmente para DEHP y TCIPP que tienen propensión a acumularse en ellas o a las diferentes actividades diarias realizadas por los participantes.

Para obtener la tasa de muestreo (Rs, m^3 $día^{-1}$ o m^3 h^{-1}) de cada compuesto, la masa recogida por el broche de PDMS se dividió entre la concentración de aire derivada de PLV-AAS, correspondiente, para obtener el volumen de aire equivalente (Veq). El Veq (m^3) se trazó frente al tiempo de implementación del broche para generar una curva de captación. La pendiente de la curva representa el Rs del compuesto. Las Figuras 2 y 3 (Capítulo 10) muestran las curvas de captación y los valores de Rs (m^3 h^{-1}), respectivamente. La captación de todos los compuestos fue lineal, lo que es consistente con la gran capacidad de captación de PDMS para los ePOP semivolátiles (Okeme *et al.*, 2016a). Los valores de Rs oscilaron entre 0,20 ± 0,16 (TCIPP) y 0,66 ± 0,17 m^3 $día^{-1}$ (DiBP). La Rs varió en un rango de 17 a ≤ 26 % para DEP, DiBP y DnBP y en ~80 % para DEHP y TCIPP entre los participantes, y entre 42 y 74 % dentro de los participantes para todos los compuestos medidos. La alta variabilidad de Rs observada dentro y entre los participantes refleja, en parte, la variabilidad en las concentraciones de aire de DEHP y TCIPP. La variabilidad de Rs pudo ser debida a que el PPAS recolectó con diferentes eficacias los compuestos de la fase gaseosa y particulada (Bohlin *et al.*, 2014; Harner *et al.*, 2014), lo que puede explicar las menores Rs medidas para DEHP y TCIPP en relación con el resto de los compuestos. Se sabe que DEHP y TCIPP son abundantes en las partículas en suspensión (Okeme *et al.*, Enviado) mientras que DEP, DiBP y DnBP son compuestos predominantes en la fase gaseosa (Saini *et al.*, 2015). Estas caracterizaciones gas-partícula se basan en la detección del filtro frente a la del sorbente durante el muestreo activo, aunque la eficiencia del muestreo de partículas no se investigó aquí. El promedio de Rs medido fue 0,43 ± 0,14 m^3 $día^{-1}$ y el valor de Rs normalizado al área de superficie, Rs', tuvo un valor de 0,86 ± 0,29 m^3 $día^{-1}$ dm^{-2}. El Rs' es comparable al valor de 0,84 ± 0,40 m^3

día^{-1} dm^{-2} (Okeme *et al.*, 2016b) y aproximadamente la mitad del valor de 1,5 ± 1,1 m^3 día^{-1} dm^{-2} (Okeme *et al.*, Enviado) obtenidos para PDMS estacionarios probados en dos ubicaciones diferentes. Se esperaría una Rs' más alta para un PPAS, en comparación con el PAS estacionario, porque las actividades personales suelen aumentar la circulación de aire alrededor del individuo. Los valores de Rs' medidos para el broche de PDMS, comparables o más bajos a los valores de PDMS estacionarios de la literatura, fueron causados probablemente por la naturaleza del trabajo de oficina, en el cual los participantes se sentaron en su escritorio durante la mayor parte del período de muestreo. Además, parece que el flujo de aire se redujo a la única superficie expuesta del broche, ya que se usó en el pecho, lo que disminuiría la tasa de absorción difusiva. Por el contrario, los muestreadores estacionarios utilizados en Okeme *et al.* (2016b) y Okeme *et al.* (Enviado) no tuvieron ninguna barrera para la circulación de aire.

5.2.2 Concentración de aire personal derivada del broche de PDMS y estimación de la exposición por inhalación

Se aplicó la tasa de muestreo genérica de 0,43 m^3 día^{-1} medida en el estudio de calibración, a la masa de los compuestos muestreados usando broches de PDMS, para derivar las concentraciones personales de aire y las estimaciones de la exposición por inhalación de los participantes involucrados en el estudio de campo (Tabla 1, Capítulo 10). Los ftalatos fueron aproximadamente tres veces más abundantes que los PFR. Las concentraciones variaron desde 1210 a 2650 ng m^{-3} en los ftalatos y 264 a 663 ng m^{-3} en los PFR. No se encontraron datos en la literatura sobre muestreos personales de ftalatos para su comparación. La concentración promedio de ΣTCIPP + TDCIPP + TCEP de 334 ng m^{-3} fue comparable al valor de 426 ng m^{-3} medido para los mismos tres PFR usando PLV-AAS en un estudio de EE. UU. (Schreder *et al.*, 2016). El TCIPP representó >90 % de los PFR, lo que fue consistente con otros estudios (van der Veen y de Boer, 2012), y pudo respaldar la hipótesis del la mayor utilización de TCIPP como retardante de llama tras la disminución del uso de TCEP y TDCIPP, que han sido categorizados como carcinógenos (Schreder *et al.*, 2016). La exposición a la inhalación se estimó para los tres participantes en el estudio de campo con una tasa de inhalación estándar de 16 m^3 día^{-1} (EPA, 2011). Las estimaciones fueron 19400 a 42400 ng día^{-1} para los ftalatos y 4066 a 10613 ng día^{-1} para los PFR. Las medianas de la exposición estimada de 4140, 518 y 292 ng día^{-1} para TCIPP, TCEP y TDCIPP, respectivamente, fueron comparables a los valores medianos de 4540, 186 y 102 ng día^{-1}, o aproximadamente dos veces más altas que estos valores estimados para los mismos PFR en EE. UU. por Schreder *et al.* (2016) utilizando los resultados de PLV-AAS. El estudio de estos autores mostró que la exposición por inhalación fue significativamente más alta que la exposición por ingestión de polvo.

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CONCLUSIONES GENERALES

CONCLUSIONES

Según los objetivos establecidos en la presente tesis doctoral, la investigación llevada a cabo y los resultados obtenidos, se han alcanzado las siguientes conclusiones:

Primera. Las sustancias perfluoroalquiladas —iónicas— y los retardantes de llama fosforados —de polaridad intermedia— se determinan por cromatografía líquida de alta eficacia acoplada a la espectrometría de masas en tándem con triple cuadrupolo. Los procedimientos optimizados proporcionaron límites de cuantificación instrumentales en un rango de 0,3 a 3,3 ng ml^{-1} para sustancias perfluoroalquiladas y de 0,3 a 2,5 ng ml^{-1} para retardantes de llama fosforados. Para estos últimos, se utilizó una columna "trampa", colocada antes del inyector del cromatógrafo, para distinguir los niveles de TCIPP de las muestras, de aquellos provenientes del equipo.

Segunda. La extracción sólido-líquido asistida por ultrasonidos o agitación mecánica demostró ser una técnica muy robusta para la extracción de las sustancias perfluoroalquiladas y los retardantes de llama fosforados en las distintas matrices ambientales sólidas. La extracción por fase sólida mostró recuperaciones adecuadas cuando se utilizó en las muestras acuosas o para la purificación de las muestras sólidas. Los mejores resultados se obtuvieron cuando se utilizó metanol, con o sin acidificar, como disolvente de extracción.

Tercera. Los compuestos semivolátiles como hidrocarburos aromáticos policíclicos, ftalatos, retardantes de llama bromados y fosforados, se separan, identifican y cuantifican adecuadamente por cromatografía de gases acoplada a espectrometría de masas. Además, se extraen cuantitativamente por extracción sólido-liquido asistida por agitación mecánica o por extracción líquida presurizada, dependiendo de la complejidad de la matriz utilizada.

Cuarta. En los ríos Ebro, Turia, Júcar, Segura y Guadalquivir y en el parque natural de La Albufera, las sustancias perfluoroalquiladas más detectadas y a una mayor concentración fueron PFBA, PFOA y PFOS. Generalmente, solo los sedimentos y la biota presentaron compuestos de cadena larga, sugiriendo su acumulación. En ningún caso se superaron los valores de concentración máxima admisible establecidos por la Directiva 2013/39 de la Unión Europea. Teniendo en cuenta el valor establecido por dicha Directiva para la media anual de PFOS (0,65 ng l^{-1}) y los valores medios obtenidos en los ecosistemas acuáticos

estudiados, sería conveniente establecer muestreos periódicos que permitan establecer la media anual de PFOS.

Quinta. Respecto a los retardantes de llama fosforados, el TCIPP fue el compuesto encontrado a mayores concentraciones y en más muestras en todas las matrices analizadas. Compuestos como el TPP, más hidrosolubles, solo fueron encontrados en muestras de agua. Por otra parte, compuestos como el CDP y el TEHP, con valores de coeficiente de reparto octanol-agua mayores, solo se encontraron en muestras de sedimento. La ausencia o baja concentración encontrada en la biota pudo deberse a su metabolización.

Sexta. Las elevadas concentraciones de sustancias perfluoroalquiladas y retardantes de llama fosforados en los efluentes de las estaciones depuradoras de aguas residuales sugieren la presencia de precursores en el agua residual y su ineficaz eliminación a través de los tratamientos de dichas instalaciones.

Séptima. El riesgo que las sustancias perfluoroalquiladas pueden suponer en los ecosistemas acuáticos se evaluó a través del cociente de peligrosidad en tres niveles tróficos. Afortunadamente, teniendo en cuenta los valores medios de concentración detectados, ninguna sustancia supuso un riesgo para la biota. Solo cuando se consideraron los valores máximos, PFTeDA pudo suponer un riesgo agudo en el Ebro y PFDA pudo presentar un cierto riesgo en el Júcar, para dafnias y peces. Sin embargo, en este tipo de estimación de riesgo, los efectos de disrupción endocrina o su bioacumulación no son considerados.

Octava. La norma de calidad ambiental para biota establecida por la Directiva 2013/39 de la Unión Europea sobre la concentración de PFOS, fue excedida en un 50 % de los peces muestreados en La Albufera y, concretamente, en un 71 % de las anguilas.

Novena. El incendio forestal de Azuébar incrementó las concentraciones de hidrocarburos aromáticos policíclicos en suelo quemado. Además, el contenido de carbono orgánico se correlacionó con su presencia y distribución. Las concentraciones de polibromodifenil éteres en muestras del suelo quemado fueron mayores que las obtenidas en la ladera control. Sin embargo, no se pudo confirmar que se aplicara una mezcla de estos compuestos como agente extintor en el incendio. Las concentraciones de sustancias perfluoroalquiladas y retardantes de llama fosforados, fueron similares a las encontradas en

otros estudios no relacionados con incendios, sugiriendo que no se usaron durante la extinción del mismo.

Décima. Los resultados del primer estudio en la Comunidad Valenciana sobre la presencia de sustancias perfluoroalquiladas en leche materna, leche de fórmula y otros alimentos infantiles, muestran una amplia distribución de estos compuestos, especialmente PFBA, PFOA y PFOS. Respecto a la exposición a través de la dieta, los valores de ingesta diaria estimada no superaron en ningún caso los límites máximos de ingesta tolerable establecidos para PFOA y PFOS por la Autoridad Europea de Seguridad Alimentaria. Sin embargo, los valores de ingesta tolerables pueden verse modificados si se demuestran nuevos efectos nocivos de estas sustancias a una menor concentración. Además, la presencia de otras sustancias perfluoroalquiladas en las muestras puede plantear una toxicidad adicional.

Undécima. Las tasas de muestreo de los broches de polidimetilsiloxano fueron suficientes para evaluar la exposición personal por inhalación a ftalatos y retardantes de llama fosforados en ambientes interiores de oficina, durante un periodo de 24 horas. La exposición individual estuvo en un rango de concentraciones de ng m^{-3} a μg m^{-3}, resultando en una exposición por inhalación estimada de 20 a 40 μg $día^{-1}$ para ftalatos y de 4 a 11 μg $día^{-1}$ para retardantes de llama fosforados.

Como **conclusión general**, los resultados de esta tesis doctoral permiten establecer la presencia y distribución de los contaminantes orgánicos persistentes emergentes analizados en distintos ecosistemas acuáticos y forestales. La presencia de estas sustancias supone un cierto riesgo para la fauna de las áreas estudiadas. Afortunadamente, en seres humanos, y considerando lo que en estos momentos se conoce sobre su toxicidad y efectos, los valores no representan un riesgo para su salud. Sin embargo, se trata de un problema ambiental emergente, ya que su persistencia, posible bioacumulación y toxicidad, facilita que puedan encontrarse durante décadas en el medio ambiente, llegando a alcanzar niveles que supongan un riesgo significativo para el medio acuático y los seres humanos que se abastecen de él.

CONCLUSIONS

According to the objectives established in this thesis, the research carried out and the results obtained, the conclusions are:

First. Perfluoroalkyl substances —ionic— and phosphorous flame retardants —of intermediate polarity— are determined by high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. The optimized procedures provided instrumental limits of quantification in a range from 0.3 to 3.3 ng ml^{-1} for perfluoroalkyl substances and from 0.3 to 2.5 ng ml^{-1} for phosphorus flame retardants. For the latter, a "trap" column, placed before the chromatograph injector, was used to distinguish whether TCIPP came from the samples or the equipment.

Second. Solid-liquid extraction assisted by ultrasound or mechanical agitation proved to be a robust technique for the extraction of perfluoroalkyl substances and phosphorus flame retardants in different solid environmental matrices. Solid-phase extraction showed adequate recoveries when used either in aqueous samples or for solid samples clean-up. The best results were obtained using methanol as extraction solvent, with or without acidification.

Third. Semi-volatile compounds such as polycyclic aromatic hydrocarbons, phthalates, brominated and phosphorus flame retardants, are separated, identified and quantified properly by gas chromatography coupled to mass spectrometry. Furthermore, they are extracted quantitatively by solid-liquid extraction assisted by mechanical agitation or by pressurized liquid extraction, depending on the complexity of the matrix.

Fourth. In the Ebro, Turia, Jucar, Segura and Guadalquivir rivers, and in the Albufera Natural Park, the perfluoroalkyl substances most detected and at a higher concentrations were PFBA, PFOA and PFOS. Generally, only sediments and biota presented long-chain compounds, suggesting their accumulation. The maximum allowable concentration values established by Directive 2013/39 of the European Union were not exceeded. Considering the annual average level of PFOS established by this Directive (0.65 ng l^{-1}) and the mean values obtained in the aquatic ecosystems, it would be convenient to establish periodic samplings to determine the annual average of PFOS.

Fifth. Regarding phosphorus flame retardants, TCIPP was the most frequent in all the matrices and at the highest concentrations. Compounds such as TPP, more water soluble, were only found in water samples. Furthermore, compounds such as CDP and TEHP, with higher octanol-water partition coefficient values, were only found in sediment samples. The absence or low concentrations of phosphorus flame retardants in biota could be due to their metabolization.

Sixth. The high concentrations of perfluoroalkyl substances and phosphorus flame retardants in effluents of the wastewater treatment plants suggest the presence of precursors in wastewater and their ineffective elimination through the treatments of these plants.

Seventh. The risk that perfluoroalkyl substances can pose to aquatic ecosystems was assessed through the hazard quotient at three trophic levels. Fortunately, taking into account the mean concentration values detected, any substance posed acute risk. Only when the maximum values were considered, PFTeDA could pose an acute risk in the Ebro River and PFDA present certain risk in Jucar River, for daphnia and fish. However, in this type of risk estimation, the effects of endocrine disruption or its bioaccumulation are not considered.

Eighth. The environmental quality standard for biota, established by Directive 2013/39 of the European Union for PFOS concentration, was exceeded by 50 % of fish samples in the Albufera and, specifically, in 71 % of eels.

Ninth. The forest fire of Azuébar increased the concentrations of polycyclic aromatic hydrocarbons in burned soil. In addition, the soil organic carbon correlated with their presence and distribution. The concentrations of polybromodiphenyl ethers in samples of burned soil were higher than those obtained in the control soil. However, it could not be established that a mixture of these compounds was applied as a fire extinguisher. The concentrations of perfluoroalkyl substances and phosphorus flame retardants were similar to those found in other studies unrelated to fires, suggesting that they were not used during the extinction of the fire.

Tenth. The results of the first study in the Valencian Community about the presence of perfluoroalkyl substances in breast milk, infant formula, and baby food show a wide distribution of these compounds, especially PFBA, PFOA and PFOS. Regarding exposure through diet, the estimated daily intake did not exceed the tolerable daily intake established by the European Food Safety Authority for PFOA and PFOS. However, tolerable intake values could be modified if new harmful effects of these substances are demonstrated at a lower concentration. In addition, the presence of other perfluoroalkyl substances in the samples may pose additional toxicity.

Eleventh. The polydimethylsiloxane brooch sampling rates were appropriate to assess the personal exposure by inhalation to phthalates and phosphorus flame retardants in indoor office environments over a 24-hour period. The individual exposure was in a range of concentrations from ng m^{-3} to μg m^{-3}, resulting in an estimated exposure by inhalation of 20 to 40 μg day^{-1} for phthalates and 4 to 11 μg day^{-1} for phosphorus flame retardants.

As a **general conclusion**, the results of this thesis have established the presence and fate of analysed emerging persistent organic pollutants in different aquatic and forest ecosystems. The presence of these substances supposes a certain risk for the biota of the studied areas. Fortunately, considering the current knowledge about their toxicity and effects, the values do not represent a health risk to humans. However, the presence of these pollutants is an emerging environmental concern because their persistence and potential bioaccumulation facilitate they can be in the environment for decades. Thus, they can easily reach levels that pose a significant risk to aquatic environments and humans that use these water supplies.

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ÍNDICE DE FIGURAS

Sección 1. Introducción

Capítulo 1. *Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems.*

Figura 1 Water sample: PFBA was identified using Formula Finder and combining TOF-MS and TOF-MS/MS information. Reproduced with permission from Ref. [60]. Copyright (2015) Springer.

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Sección 2. Desarrollo de metodología analítica

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(b) Turia River sampling (2013). Made with gvSIG 2.0.

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Figura S6 Cumulated PAH concentrations in the sediments.
Figura S7 Cumulated PFR concentrations in the sediments.
Figura S8 Cumulated PFAS concentrations in the sediments.

Sección 4. Aplicación a la exposición en humanos

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Figura 1 Contribution of long and short-chain PFCAs and PFSAs (%) to the total concentrations in baby food. (*) Where PFCAs are perfluoroalkyl carboxylates (PFBA, PFPA, PFHxA, PFHpA, PFOA, ipPFNA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFHxDA)and PFSAs are perfluoroalkyl sulfonates (PFBS, PFHxS, PFHpS, PFOS and PFDS) and short-chain include compounds with less than 8 carbons and long-chain with equal or more than 8 carbons in the structure.
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ÍNDICE DE ABREVIATURAS

A continuación se describen las abreviaturas más representativas de esta tesis doctoral, así mismo, en cada capítulo se detallan todas la abreviaturas utilizadas.

General acronyms – acrónimos generales

AFFF	Aqueous film-forming foam – espumas formadoras de película acuosa
BAF	Bioaccumulation factor – factor de bioacumulación
BFR	Brominated flame retardant – retardante de llama bromado
BS	Bare soil – suelo desnudo
BU	Burned hillslope – ladera quemada
CO	Control hillslope – ladera control
DB-5MS	(5 %-phenyl)-methylpolysiloxane – (5 % fenil)-metilpolisiloxano
DRI	Dietary reference intake – ingesta dietética de referencia
EDI	Estimated daily intake – ingesta diaria estimada
EFSA	Autoridad Europea de Seguridad Alimentaria – European Food Safety Authority
EI	Electron impact ionzation – ionización por impacto de electrones
EPA	Environmental Protection Agency – Agencia de Protección del Medio Ambiente
ePOP	Emerging persistent organic pollutant – contaminante orgánico persistente emergente
EQS	Environmental quality standard – norma de calidad ambiental
ESI	Electrospray ionization – ionización por electrospray
GC	Gas chromatography – cromatografía de gases
HPLC	High performance liquid chromatography – cromatografía líquida de alta eficacia
HQ	Hazard quotient – cociente de peligrosidad
IS	Internal standard – patrón interno
LC	Liquid chromatography – cromatografía liquida

LOD	Limit of detection – límite de detección
LOQ	Limit of quantification – límite de cuantificación
MEC	Measured environmental concentration – concentración ambiental medida
MPC	Maximum permissible concentration – concentración máxima permisible
MRM	Multiiple reaction monitoring – monitorización de reacciones seleccionadas múltiples
MS	Mass spectrometry – espectrometría de masas
MS/MS	Tandem mass spectrometry – espectrometría de masas en tándem
NCI	Negative chemical ionization – ionización química negativa
NOEC	No observed effect concentration – concentración sin efecto observado
PAH	Polycyclic aromatic hydrocarbon – Hidrocarburo aromático policíclico
PAS	Passive air sampler – muestreador pasivo de aire
PBDE	Polybromodiphenyl ether – polibromodifenil éter
PDMS	Polydimethylsiloxane – polidimetilsiloxano
PFAS	Perfluoroalkyl substance – sustancias perfluoroalquiladas
PFCA	Perfluoroalkyl carboxylic acid – ácido perfluorocarboxílico
PFR	Phosphorus flame retardant – retardante de llama fosforado
PFSA	Perfluorosulfonate –perfluorosulfonato
PLE	Pressurized liquid extraction – extracción líquida presurizada
PLV-AAS	Personal low volume active air sampler – muestreador activo de aire personal de bajo volumen
PNEC	Predicted no effect concentration – concentración sin efecto previsto
PPAS	Personal passive air samplers – muestreador pasivo de aire personal
PUF	Polyurethane foam – espuma de poliuretano
QqQ	Triple quadrupole – triple cuadrupolo
QSAR	quantitative structure–activity relationship – relación cuantitativa estructura-actividad

R^2	Coefficient of determination – coeficiente de determinación
Rs	Sampling rate – tasa de muestreo
RSD	Relative standard deviation – desviación estándar relativa
S/N	Signal-to-noise ratio – relación señal/ruido
SIM	Monitorización de iones seleccionados – selected ion monitoring
SLE	Solid-liquid extraction – extracción sólido-liquido
SOC	Soil organic carbon – carbono orgánico del suelo
SPE	Solid-phase extraction – extracción en fase sólida
SS	Subsoil - subsuelo
TBAS	Tetrabutylammonium hydrogensulfate – tetrabutilamonio hidrogenosulfato
TDI	Tolerable daily intake – ingesta diaria tolerable
TFC	Turbulent flow chromatography – cromatografía de flujo turbulento
TS	Topsoil – suelo superficial
UC	Under canopy soil – suelo bajo cubierta vegetal
USE	Ultrasound assisted extraction – extracción asistida por ultrasonidos
Veq	Equivalent air volume – volumen de aire equivalente
WWTP	Wastewater treatment plant – estación depuradora de aguas residuales
XAD	Hydrophobic copolymer of styrene-divinylbenzene resin – resina de copolímero estireno-divinilbenceno

Target compounds – compuestos objetivo

Ace	Acenaphthene – acenafteno
Acy	Acenaphthylene – acenaftileno
Ant	Anthracene – antraceno
BaA	Benzo[a]anthracene – benzo[a]antraceno
BaP	Benzo[*a*]pyrene – benzo[*a*]pireno

BbF	Benzo[*b*]fluoranthene – benzo[*b*]fluoranteno
BDE-100	2,2',4,4',6-penta-bromodiphenyl ether – 2,2',4,4',6-penta-bromodifenil éter
BDE-153	2,2',4,4',5,5'-hexa-bromodiphenyl ether – 2,2',4,4',5,5'-hexa-bromodifenil éter
BDE-154	2,2',4,4',5,6'-hexa-bromodiphenyl ether – 2,2',4,4',5,6'-hexa-bromodifenil éter
BDE-183	2,2',3,4,4',5',6-hepta-bromodiphenyl ether – 2,2',3,4,4',5',6-hepta-bromodifenil éter
BDE-28	2,4,4'-tri-bromodiphenyl ether – 2,4,4'-tribromodifenil éter
BDE-47	2,2',4,4'-tetra-bromodiphenyl ether – 2,2',4,4'-tetra-bromodifenil éter
BDE-85	2,2',3,4,4'-penta-bromodiphenyl ether – 2,2',3,4,4'-penta-bromodifenil éter
BDE-99	2,2',4,4',5-penta-bromodiphenyl ether – 2,2',4,4',5-penta-bromodifenil éter
BghiP	Benzo[ghi]perylene – benzo[ghi]perileno
BkF	Benzo[*k*]fluoranthene – benzo[*k*]fluoranteno
BzBP	Benzylbutyl phthalate – ftalato de bencilo y butilo
CDP	Cresyl diphenyl phosphate – fosfato de difenil cresilo
Chr	Chrysene – criseno
DahA	Dibenz[*a,h*]anthracene – dibenzo[*a,h*]antraceno
DEHP	Bis(2-ethylhexyl) phthalate – ftalato de bis(2-etilhexilo)
DEP	Diethyl phthalate – ftalato de dietilo
DiBP	Diisobutyl phthalate – ftalato de diisobutilo
DiNP	Diisononyl phthalate – ftalato de diisononilo
DnBP	Di-n-butyl phthalate – ftalato de di-n-butilo
Flt	Fluoranthene – fluoranteno
Flu	Fluorene – fluoreno
FOUEA	2H-perfluoro-2-decenoic acid – ácido 2H-perfluoro-2-decenoico

Ind	Indeno[*1,2,3-cd*]pyrene – indeno[*1,2,3-cd*]pireno
ipPFNA	Perfluoro-7-methyloctanoic acid – ácido perfluoro-7-metiloctanoico
ipPFNS	Perfluoro-7-methyloctanesulfonate – sulfonato de perfluoro-7-metiloctano
Nap	Naphthalene – naftaleno
PFBA	Perfluorobutanoic acid – ácido perfluorobutanoico
PFBS	Perfluorobutanesulfonate – sulfonato de perfluorobutano
PFDA	Perfluorodecanoic acid – ácido perfluorodecanoico
PFDoDA	Perfluorododecanoic acid – ácido perfluorododecanoico
PFDS	Perfluorodecanesulfonate – sulfonato de perfluorodecano
PFHpA	Perfluoroheptanoic acid – ácido perfluoroheptanoico
PFHpS	Perfluoroheptanesulfonate – sulfonato de perfluoroheptano
PFHxA	Perfluorohexanoic acid – ácido perfluorohexanoico
PFHxDA	Perfluorohexadecanoic acid – ácido perfluorohexadecanoico
PFHxS	Perfluorohexanesulfonate – sulfonato de perfluorohexano
PFNA	Perfluorononanoic acid – ácido perfluorononanoico
PFOA	Perfluorooctanoic acid – ácido perfluorooctanoico
PFODA	Perfluorooctadecanoic acid – ácido perfluorooctadecanoico
PFOS	Perfluorooctane sulfonate – sulfonato de perfluorooctano
PFOSA/ FOSA-M	Perfluorooctanesulfonamide – sulfonamida de perfluorooctano
PFPeA	Perfluoropentanoic acid – ácido perfluoropentanoico
PFTeDA	Perfluorotetradecanoic acid – ácido perfluorotetradecanoico
PFTrDA	Perfluorotridecanoic acid – ácido perfluorotridecanoico
PFUnDA	Perfluoroundecanoic acid – ácido perfluoroundecanoico
Phe	Phenanthrene – fenantreno
Pyr	Pyrene – pireno

TBEP	Tris(2-butoxyethyl)phosphate – fosfato de tris(2-butoxietilo)
TCEP	Tris(2-chloroethyl) phosphate – fosfato de tris(2-cloroetilo)
TCIPP	Tris(2-chloroisopropyl) phosphate – fosfato de tris(2-cloroisopropilo)
TDBPP	Tris(2,3-dibromopropyl) phosphate – fosfato de tris(2,3-dibromopropilo)
TDCIPP	Tris(1,3-dichloro-2-propyl) phosphate – fosfato de tris(1,3-dicloro-2-propilo)
TEHP	Tris(2-ethylhexyl) phosphate – fosfato de tris(2-etilhexilo)
TMPP	Tricresyl phosphate – fosfato de tricresilo
TnBP	Tributyl phosphate – fosfato de tributilo
TPhP	Triphenyl phosphate – fosfato de trifenilo
TPP	Tripropyl phosphate – fosfato de tripropilo

Tesis doctoral

Determinación de contaminantes orgánicos persistentes emergentes en el medio ambiente y evaluación de la exposición en humanos.

Determination of emerging persistent organic pollutants in the environment and human exposure assessment.

Autora

María Lorenzo Martínez

Directores de tesis

Yolanda Picó García y Julián Campo Velásquez

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