Priority and emerging flame retardants in the aquatic environment: analytical development, occurrence and risk

Joyce Cristale

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Programa de Doctorado en Química Analítica del Medio Ambiente y la Polución

“Priority and emerging flame retardants in the aquatic environment: analytical development, occurrence and risk”

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Barcelona, septiembre 2013

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HACE CONSTAR:

que la presente memoria titulada “Priority and emerging flame retardants in the aquatic environment: analytical development, occurrence and risk”, presentada para optar al grado de Doctor por la Universidad de Barcelona, fue realizada por la Srta. Joyce Cristale en el Departamento de Química Ambiental del Instituto de Diagnóstico Ambiental y Estudios del Agua, adscrito al Consejo Superior de Investigaciones Científicas y bajo la tutoría de la Dra. Maria Teresa Galceran Huguet, Catedrática del Departamento de Química Analítica de la Universidad de Barcelona

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“O sucesso nasce do querer, da
determinação e persistência em
se chegar a um objetivo. Mesmo
não atingindo o alvo, quem busca
e vence obstáculos, no mínimo
fará coisas admiráveis.”

José de Alencar

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Preface

The field of study of this thesis regards the development of sampling and analytical techniques that permit to assess the impact of flame retardants on the aquatic ecosystem. Flame Retardants are used in polymers and textiles for diverse applications (electric and electronic equipments, construction, furniture, etc) and deserve attention due to their toxicity, widespread presence in environmental matrices and persistence. Thus, this study was performed in order to increase knowledge concerning the occurrence, sources, fate and environmental risk of priority and emerging flame retardants in rivers.

This thesis contains five main chapters. Chapter 1 presents an overall introduction describing flame retardants, their types and applications. Also, the introduction presents information on the presence of some flame retardants in the environment, their environmental fate and the reasons for selecting target compounds are justified. The objectives of this thesis are presented in the last section of this chapter. Chapter 2 presents the analytical methods developed during this thesis. Firstly a review on the main techniques described in the literature for the analysis of each studied flame retardant family is provided. Then, the methodology carried out for the development of the analytical methods is described. The results are presented in three published articles, which are fully annexed. Finally, an overall discussion of the results obtained, some difficulties found during the method development and additional data not published elsewhere are provided. Chapter 3 presents the results of the monitoring of flame retardants in rivers from UK and Spain, and the risk assessment performed in each case study. The introduction of this chapter presents a short review on published data regarding environmental levels of the studied compounds in rivers around the world, and considerations about risk assessment for aquatic organisms. Further, the monitoring methodology is described. The results obtained for the monitoring of the different rivers and the risk assessment study are published in two articles (annexed). The discussion of this chapter presents additional results regarding the presence and elimination of flame retardants in Wastewater Treatment Plants. Finally, the overall discussion is presented and compares the data obtained for the different monitoring studies and for all data base generated. Finally, Chapter 4 presents the main conclusions obtained in this thesis. Chapter 5 summarizes the methodologies and monitoring studies performed and highlight the main achievements (in Spanish).

Prefacio

El ámbito de estudio de esta tesis consiste en el desarrollo de técnicas de muestreo y de análisis que permitan evaluar el impacto de los retardantes de llama en el ecosistema acuático. Los retardantes de llama se utilizan en polímeros y tejidos para aplicaciones diversas (equipos eléctricos y electrónicos, construcción, muebles, etc.) y han generado cierta preocupación debido a su toxicidad, su presencia en las distintas matrices ambientales y por su persistencia. Por lo tanto, este estudio tiene como objetivo contribuir al conocimiento respecto la presencia, las fuentes, el destino y el riesgo ambiental de los retardantes de llama prioritarios y emergentes en el medio acuático.

Esta tesis contiene cinco capítulos principales. El Capítulo 1 presenta una introducción general que describe los retardantes de llama, los distintos tipos y sus aplicaciones. Además, se presenta información sobre la presencia de retardantes de llama en el medio ambiente, su destino ambiental y se justifica la selección de los compuestos objeto de estudio. Los objetivos de esta tesis se presentan en la última sección de este capítulo. El Capítulo 2 presenta los métodos de análisis desarrollados en esta tesis. En primer lugar se proporciona una revisión sobre las principales técnicas descritas en la literatura para el análisis de cada familia de retardantes de llama estudiados. A continuación, se describe la metodología llevada a cabo para el desarrollo de los métodos analíticos. Los resultados se presentan en tres artículos publicados, que se incorporan en el capítulo. Por último, se presenta un análisis global de los resultados obtenidos, de las dificultades encontradas durante el desarrollo analítico y datos adicionales no incluidos en las publicaciones. El Capítulo 3 presenta los resultados de la vigilancia ambiental de los retardantes de llama en ríos del Reino Unido y de España, y la evaluación del riesgo realizada en cada estudio. En la introducción de este capítulo se presenta una breve revisión respecto de los niveles ambientales de los compuestos estudiados en ríos de todo el mundo, y se discute la evaluación del riesgo para los organismos acuáticos. Además, se describe la metodología utilizada para la vigilancia ambiental en los diferentes ríos estudiados. Los resultados obtenidos para los diferentes ríos y el estudio de evaluación del riesgo se publican en dos artículos, los cuales se incluyen en la memoria. La discusión de este capítulo presenta resultados adicionales sobre la presencia y la eliminación de los retardantes de llama en las plantas de tratamiento de aguas residuales. Finalmente, se comparan y se discuten los datos obtenidos para los diferentes estudios de vigilancia ambiental y para toda la base de datos generada en esta tesis. Por último, el Capítulo 4 presenta las principales conclusiones obtenidas en esta tesis. En el Capítulo 5 se resume la metodología y los estudios de vigilancia ambiental realizados y se destaca los principales logros (en castellano).

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List of Acronyms

ABS	Acrylonitrile-butadiene-styrene
BDE-100	2,2',4,4',6-Pentabromodiphenyl ether
BDE-153	2,2',4,4',5,5'-Hexabromodiphenyl ether
BDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether
BDE-183	2,2',3,4,4',5',6-Heptabromodiphenyl ether
BDE-209	Decabromodiphenyl ether
BDE-28	2,4,4'-Tribromodiphenyl ether
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether
BDE-99	2,2',4,4',5-Pentabromodiphenyl ether
BEHTBP	Bis(2-ethyl-1-hexyl)tetrabromo phthalate
BTBPE	1,2 Bis (2,4,6-tribromophenoxy)ethane
DBDPE	Decabromodiphenyl ethane
Deca-BDE	Deca-BDE formulation
deca-BDE	Decabromodiphenyl ether (synonymous with BDE-209)
di-BDE	Isomers of dibromodiphenyl ether
DLLME	Dispersive liquid-liquid microextraction
DOC	Dissolved organic carbon
DPTE	2,3-Dibromopropyl 2,4,6-tribromophenyl ether
dw	Dry weight
DWTF	Drinking water treatment facility
EC_{50}	Half maximal effective concentration
ECD	Electron capture detection
ECHA	European Chemicals Agency
ECNI	Electron capture negative ionization
EHDP	2-Ethylhexyl diphenyl phosphate
EHTBB	2-Ethylhexyl-2,3,4,5-tetrabromobenzoate
EI	Electron ionization
EQS	Environmental quality standards
ESR	Existing Substances Regulation
EU	Europe Union
f	Assessment factor
f_{oc}	Organic-carbon fraction
FR	Flame retardant
GC	Gas chromatography
H	Henry’s law constant
HBB	Hexabromobenzene
HBCD	Hexachlorocyclododecane
HCDBCO	Hexachlorocyclopentadienyl-dibromooctane
hepta-BDE	Isomers of heptabromodiphenyl ether

hexa-BDE	Isomers of hexabromodiphenyl ether
HIPS	High impact polystyrene
HLB	Hydrophilic lipophilic balance
HPLC	High resolution liquid chromatography
HPV	High production volume
HRMS	High resolution mass spectrometry
iLOD	Instrumental limits of detection
ILS	Interlaboratory study
K_{DOC}	Dissolved organic carbon partition coefficients
K_{oc}	Organic-carbon/water partition coefficient
K_{ow}	Octanol-water partition coefficient
LC	Liquid chromatography
LC_{50}	Half maximal lethal concentration
LLE	Liquid-liquid extraction
log K_{oc}	Organic-carbon/water partition coefficient logarithm
log K_{ow}	Octanol-water partition coefficient logarithm
LPV	Low production volume
lw	Lipid weight
MAE	Microwave assisted extraction
MDL	Method detection limit
MEC	Measured environmental concentration
mono-BDE	Isomers of monobromodiphenyl ether
MS	Mass spectrometry
MS/MS	Mass spectrometry in tandem
m/z	Mass-to-charge ratio
n	Number of samples/replicates/observations
NBFRs	New brominated flame retardants
nd	Not detected
NOEC	No observed effect concentration
nona-BDE	Isomers of nonabromodiphenyl ether
NPD	Nitrogen–phosphorus detector
Octa-BDE	Octa-BDE formulation
octa-BDE	Isomers of octabromodiphenyl ether
OPFR	Organophosphorus flame retardant
PBB	Polybrominated biphenyl
PBDE	Polybrominated diphenyl ether
PBDF	Polybrominated dibenzofuran
PBEB	Pentabromoethyl benzene
PBT	Pentabromotoluene
PCI	Positive chemical ionization
Penta-BDE	Penta-BDE formulation
penta-BDE	Isomers of pentabromodiphenyl ether

PLE	Pressurized liquid extraction
PNEC	Predicted no effect concentration
POP	Persistent organic pollutant
PU	Polyurethane
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ	Risk quotient
RSD	Relative standard deviation
SBSE	Stir bar sorptive extraction
SD	Standard deviation
SIM	Single ion monitoring
SPE	Solid phase extraction
SPME	Solid phase microextraction
SRM	Selected reaction monitoring
SVHC	Substances of very high concern
TBBPA	Tetrabromobisphenol A
TBEP	Tris(2-butoxyethyl) phosphate
TBP	Tri-*n*-butyl phosphate
TCEP	Tris(2-chloroethyl) phosphate
TCP	Tricresyl phosphate
TCPP	Tris(2-chloro-1-methylethyl) phosphate
TDCP	Tris[2-chloro-1-(chloromethyl)ethyl] phosphate
TEHP	Tris(2-ethylhexyl) phosphate
tetra-BDE	Isomers of tetrabromodiphenyl ether
TGDRA	Technical Guidance Document on Risk Assessment
TiBP	Tri-*iso*-butyl phosphate
TOC	Total organic carbon
TPhP	Triphenyl phosphate
TPO	Thermoplastic polyolefin
tri-BDE	Isomers of tribromodiphenyl ether
UAE	Ultrasonic assisted extraction
UK	United Kingdom
UNEP	United Nations Environment Program
USA	United States of America
UV	Ultraviolet radiation
WFD	Water Framework Directive
ww	Wet weight
WWTP	Wastewater treatment plant

1 Introduction

1.1 Flame retardants: A general vision

Since the last century, technology advances changed human life. Nowadays, a huge variety of materials are manufactured and applied to different products to facilitate modern life or make it more comfortable. As a result, people are surrounded by a wide diversity of polymers in clothing, furniture, electronics, vehicles and computers. Most of these polymers are petroleum-based and flammable, and some applications can promote fire ignition. For example, miniature components in high-powered computers generate a high concentration of heat that can lead to fast overheating of internal components. Therefore, safety regulations have been adopted to prevent accidental fires and to protect human being [1]. Employment of products and materials in buildings and constructions, electrical and electronic equipments, furniture and transportations is regulated in European Countries, and has to follow fire safety standards and requirements [2-4]. Specific flammability tests are usually performed to evaluate ease of ignition, heat release, flame spread, smoke opacity and smoke toxicity [1]. Fire regulations are adopted in order to reduce the flame spread and the smoke emission rate, increasing the escape time. The level of performance required is usually proportional to the hazard situation, meaning that the higher has to be the escape time, the higher the requirements of the regulation. For example, materials used in aircrafts needs higher fire resistance levels than the ones used in cars [1]. In this context, flame retardants (FRs) are used in diverse types of materials to reach fire standards requirements.

1.1.1 Flame retardants types and mode of action

FRs are substances incorporated to materials to inhibit or slow down the spread of a fire. The term “flame retardant” does not refer to a specific class of chemicals but to a function, and different chemicals with different properties and structures, are included in this group. In fact, FRs have been used since ancient times. Egyptians (about 450 b.c.) used Alum to reduce the wood flammability, while Romans used a mixture of alum and vinegar (about 200 b.c.) [5]. Other examples of old flame retardant appliances include a mixture of clay and gypsum used in 1638 to reduce flammability of theatre curtains; a mixture of alum, ferrous sulphate and borax used on wood and textiles by Wyld in Britain (1735); Alum used to reduce flammability of balloons (1783); and a mixture of $(NH_4)_3PO_4$, NH_4Cl and borax were effective on linen and hemp (1821) [5]. Nowadays, these water soluble salts used in the past as FRs are of little utility since current materials and polymers are largely hydrophobic.

The large scale use of thermoplastics and thermosetting polymers over the past decades in buildings, transportations, and electronics lead to the development of different FRs. They consist mainly of inorganic and organic compounds based on bromine (e.g. high brominated aromatic and cycloaliphatic compounds), chlorine (e.g. chloroparaffins, declorane plus), phosphorus (e.g. phosphate-esters, phosphonates and phosphinates, ammonium phosphate), nitrogen (e.g. melamine and melamine derivatives), boron (e.g. sodium borate, borax and zinc borates), and metallic hydroxides (e.g. aluminium and magnesium hydroxides). FRs are either additive or reactive. Reactive FRs are added during the polymerisation process, modifying the polymer structure, and giving FR properties. Additive FRs are monomer molecules, not chemically bound to the polymer, incorporated mainly after polymerization [6]. A variety of FRs have been developed since different materials, with different physical nature and chemical composition, behave differently during combustion. Thus, the appropriate FR or combination of different ones have to be carefully selected for each material in order to give fire-resistancy and maintain their intended functionality and performance standards [7]. Table 1 presents different materials and desired final product performance that manufacturers take into account when choosing the suitable FR. Nowadays, the worldwide consumption of FRs is about 2 million tonnes/year, and an annual consumption growth of 4–5% is estimated. Approximately 85% of FRs are used in plastics, with textiles and rubber products accounting for the rest. Aluminium hydroxide is the most consumed FR (40.4%), followed by brominated FRs (19.7%), organophosphorus flame retardants (OPFRs) (14.6%), chlorinated flame retardants (11.3%), antimony oxide (8.7%), and others (4%) [8].

Depending on their chemical structure, FRs interact at different stages of the fire cycle. Figure 1 illustrates the fire cycle [9]. The fire starts with an ignition source (1) (e.g., heat, incandescent material, a small flame), that will preheat the material. When sufficiently heated, the material begins to degrade, the weakest bonds begin to break, and flammable gases are formed (2). If solid materials do not break down into gases, they remain in a condensed phase and often self-extinguish, especially if they “char” (3) (carbonated barrier formed between the flame and the underlying material). The concentration of flammable gases increases in the presence of the ignition source (4) until fuel, oxygen and free radicals (hydrogen, oxygen, hydroxide and peroxide radicals) combine in the combustion zone (5), creating exothermic chemical reactions that result in visible flames. Combustion products are released producing smoke (6). The fire then becomes self-sustaining since as it continues to burn the material, more flammable gases are released, feeding the combustion process [6-7].

Table 1 – Applications requiring fire-retardancy, typical required characteristics and most used FRs for different polymers [10].

Material	Applications requiring fire-retardancy	Typical required characteristics	Most used FR
Polyurethane (PU):			
Rigid PU foams	Insulation in building and construction (roofing, wall sheeting) and refrigeration.	Efficient flame retardancy, low viscosity for easier process ability, good green strength particularly in sandwich materials, high hardness	Brominated FR Phosphorus FR Nitrogen FR
Flexible PU foams	Furniture & upholstery, and in transportation	Low influence on foaming process, high viscoelasticity, uniform hardness distribution, low migration/ high FR retention with time, low fogging (automotive)	
Acrylonitrile-butadiene-styrene (ABS)	Electrical/electronics Typical applications are: enclosures*, chassis and casing	Easy processing, high gloss, high toughness combined with flexibility, good chemical resistance	Brominated FR Chlorinated FR
High impact polystyrene (HIPS)	Electrical/electronics (often used in enclosures*), applied when temperature does not exceed 80°C	Balance of properties and low cost, good flow, good impact and good dimensional stability	Brominated FR Phosphorus FR
Polyolefins:			
Polyethylene (and copolymers)	Wiring and cables (low and medium voltage), cable jacketing	The most used polymers in applications requiring flame retardancy. Selection of the best FR for polyolefins depends strongly on the requirements of the final applications such as FR efficiency, UV resistance, blooming, mechanical properties retention, process ability and recyclability	Brominated FR Metal Hydroxides
Polypropylene (and copolymers)	Cable conduit, power cables, connectors, public facilities seats (Stadiums...), fitting enclosures, fibers (carpets, seats...)		
Thermoplastic Polyolefin (TPO)	Roofing membranes, interior automotive applications, under the hood automotive applications, flexible cables, shrinkable films		
Thermoplastic elastomers	Wiring & cables, flooring, wall covering, hoses, conveyor belts, profiles, roofing membranes, pipes and tubes	Combine the performances of rubbers and the processability of thermoplastics	Metal Hydroxides
Rubber	Seals, gaskets, conveyor belts, cables, hydraulic tubes, hoses, profiles, foams or protective coverings	Low corrosivity of gases released during combustion, adjustable performances, good surface finish, reduced smoke density	Metal Hydroxides
Polyamides	Medium-voltage components, insulation components, switch components, switch casings, electromagnetic switches, terminal blocks, plug connectors	Ease of processing, favourable physical and electrical properties, high heat stability, good chemical resistance	Brominated FR Metal Hydroxides

[*] Electronic enclosures is a market segment which includes primarily the outer enclosures of equipments such as: televisions, desktop or notebook computers, monitors, printers, copiers, household appliances, etc.

Different FRs act in different stages of the fire cycle. They interfere during heating, decomposition, ignition or flame spread. FRs mechanisms were described in detail by Levchik (2006) [11]. FRs interrupt (chemically or physically) the combustion process, basically reducing the rate of heat transfer to the polymer and removing the fuel supply. FR mechanisms of action can be divided in two main groups, gas-phase-active and condensed-phase-active.

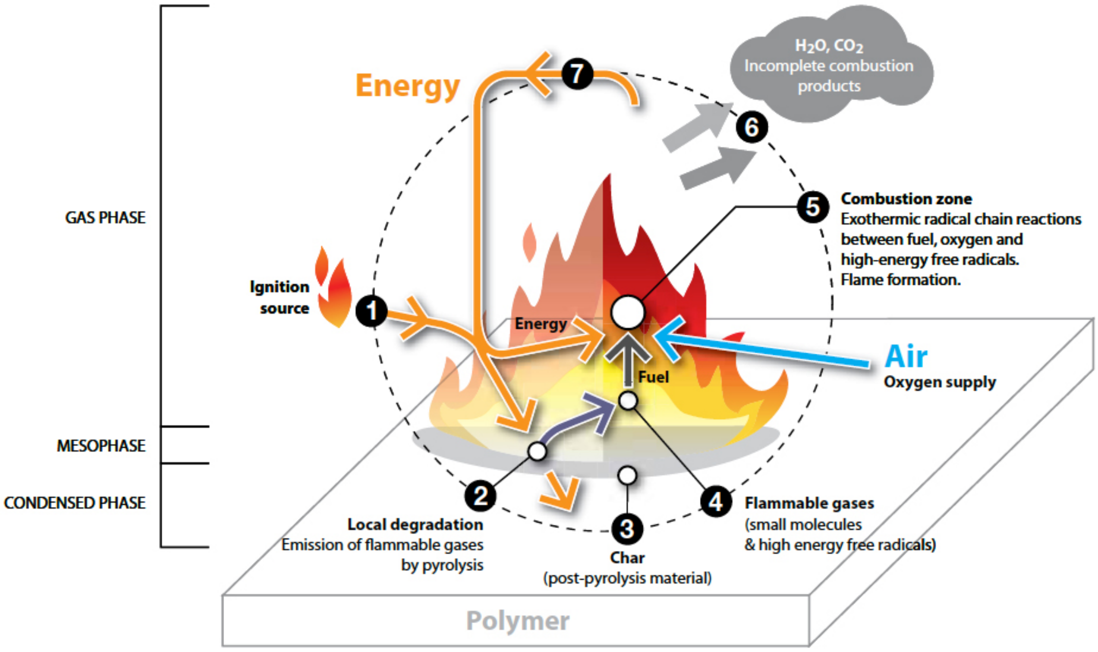

Figure 1 – Fire cycle [9].

Gas-phase-active mechanism: Some FRs act chemically in the gas-phase by quenching of high energy free radicals, interrupting radical chain reactions in the flame. Thus exothermic processes are stopped, the system cools down, and the supply of flammable gases is reduced and eventually completely suppressed. The best example of FRs acting by this mechanism are brominated and chlorinated FRs. Halogenated FRs release halogen radical and halogen halide at the same temperature range or below the decomposition temperature of the polymer. In the flame phase, the chain reactions of hydrogen radical with oxygen gas and the reaction of hydroxyl radical with carbon monoxide are the main exothermic reactions responsible for heat generation. Halogen halide will react with hydrogen and hydroxyl radicals, avoiding their high exothermic oxidation [12]. If the conditions are right, phosphorus FRs can also act in the gas-phase, by release of volatile phosphorus compounds and formation of phosphorus radicals (e.g. $HPO_2^{\bullet}$ and $PO^{\bullet}$) that will react with hydrogen and hydroxyl radicals [13]. Other FRs act physically in the gas-phase, generating large amounts of non combustible gases and diluting flammable gases. Sometimes they dissociate endothermically, absorbing heat, which eventually result in flame extinguishment. This mechanism is observed for metal hydroxides (aluminium hydroxide and magnesium hydroxide), that when heated release water by an endothermic decomposition [11].

Condensed-phase mechanisms: There are numerous modes of action in the condensed phase, but the most important one is charring. When a char is formed over the polymer surface, the carbonaceous layer acts as a physical barrier, absorbs part of the heat and therefore reduces the heat flux that would promote the emission of flammable gases. FRs can promote charring by chemical interaction with the polymer or by physical retention of the polymer in the condensed phase. Charring is the main mode of action of phosphorus based compounds. When heated, phosphorus FRs form phosphoric acid that acts in the condensed-phase forming char, mainly due to esterification and transesterification reactions between phosphoric acid and hydroxyl groups of the synthetic polymer or cellulose [14-15]. Nitrogen FRs (melamine and melamine derivates) and borate FRs also act in condensed phase by charring [11].

On the other hand, a complete understanding of how a certain FR acts is difficult, since they can undergo several mechanisms. A good example is melamine, which can act by cooling due to endothermic sublimation, by gas dilution due to the formation of N_2, by consumption of combustible gases (e.g. CH_4) in the flame zone due to reaction with NH_4 (degradation product of melamine), and by charring [16]. Other example are halogenated FRs, since charring and dilution of flammable gases (due to liberation of halogen halides) is believed to be a secondary mode of action [11]. Finally, different chemicals can be combined to give synergism effects, which result in more effective flame-retardancy. The most common examples of synergism are halogens with antimony and phosphorus with nitrogen [11].

1.1.2 Environmental concern over flame retardants

Despite the benefits associated to the use of FRs in terms of decreasing number of deaths and injuries related to fires [17], some of these compounds deserve environmental attention due to their toxicity and bioaccumulation potential. Halogenated FRs (especially brominated FRs), and more recently organophosphorus flame retardants (OPFRs), are considered as a potential threat to the environment since these FRs are high production and persistent chemicals and have been detected in all environmental compartments [18-20].

The fate of organic FRs in the environment is complex, and depends on their physicochemical properties (e.g. water solubility, log K_{ow}, vapour pressure, etc) and their degradability potential (e.g. biodegradation, photodegradation, etc). Since FRs, especially the additive ones, are continuously released from materials by volatilization, products containing

FRs are diffuse sources of these chemicals to the environment. Once released, FRs are partitioned between gaseous and particulate phases in atmosphere, which mainly depend on their vapour pressure and K_{ow} [21]. Associated to particles, high FR levels are found in dust particles from homes, offices, cars and planes [21-22]. Atmospheric transportation of halogenated FRs and OPFRs has been reported [23-24], and they can reach soil and waters by dry and wet depositions [25-26]. The highest FR concentrations in the air are found near urban centres [27], while the detection at trace levels in remote zones shows that FRs undergo long-range atmospheric transport [24, 28]. FRs have also been detected in rivers [29-30], coastal areas [31] and marine surface waters [32]. Wastewater treatment plants (WWTP) are punctual sources of FRs, since many of these compounds are not eliminated during the treatment. Soluble FRs reach the aquatic environment by WWTP discharges [33-34], while the lipophilic ones are accumulated in sewage sludge [35-36]. Sewage sludge is frequently applied to agricultural soils as fertilizers, and FRs can be mobilized from amended soils by desorption [37] and eventually reach groundwater [38]. Other punctual FR sources include discharges from industries that use FRs for manufacturing products, leaching from landfills, e-waste storage facilities as well as facilities for recycling of FR containing products [39-43]. Finally, lipophilic FRs can accumulate in biota and biomagnify along the food chain [44].

The first concern regarding brominated FRs implied the polybrominated biphenyls (PBBs), which are not marketed anymore, and a poisoning accident in Michigan (USA) in 1973[1] [45-46]. Polybrominated diphenyl ethers (PBDEs) are produced since 1965 [18] and took a prominent role in the market, in part because of PBBs bans, reaching a world production of 67 390 tonnes in 2001 [47]. Other two families of brominated FRs that have been deserved concern are hexabromocyclododecane (HBCD)[2] and tetrabromobisphenol A (TBBPA)[3] [48-49]. These brominated compounds, together with PBDEs, dominated the brominated FR market during the last decades. PBDEs, HBCD and TBBPA present octanol-water partition coefficient logarithm (log K_{ow}) ranging from about 6 to 10, and because of their lipophilicity, these compounds are accumulated in sediments, soil, sewage sludge and

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[1] An inadvertent mixing of a bag of Firemaster BP-6 and FF1® (commercial PBB mixture, containing mainly hexa- and heptabromobiphenyl congeners) into animal food exposed at least 2000 families to high levels of these compounds, due to consume of contaminated food from this farm. Due this accident and the toxic effects of PBBs, these formulations were removed from the market in USA after a total production of 6071 tonnes (1970 – 1976). The production of octabromobiphenyl and decabromobiphenyl formulations continued until 1979 in USA, while in Europe, PBBs were produced until 1985 in Germany and until 2000 in France.

[2] Commercial technical grade HBCD mixtures mainly consist of γ-HBCD (75-89%), α-HBCD and β-HBCD (10-13% and 1-12%, respectively) isomers. HBCD is an additive FR, mainly used in polystyrene insulation foams.

[3] TBBPA is mainly used as a reactive FR in the production of epoxy and polycarbonate resins, and a small percentage is transformed in TBBPA derivatives (e.g. dimethyl TBBPA and bis (2-hydroxyethyl ether) TBBPA) that are used in ABS resin, HIPS, paper and textile adhesives and coatings.

biota [50]. The first reported bioaccumulation study dates 1981 for fish from Swedish waters [51] and in 1987, PBDEs were first suggested as global contaminants because of their detection in biotic samples (seal blubber and bird muscle) from remote zones (Arctic Ocean) [52]. Since then, PBDEs, TBBPA and HBCD have been detected in organisms (e.g. mussels, fishes, mammals, birds, etc), while PBDEs and HBCD show high biomagnification potential [53-54]. However, the concern of the general public and the scientific community increased with the publication of data indicating high levels of PBDEs in breast milk, and the concentration of various congeners of PBDEs has doubled every year from 1972 to 1997 [55]. Human exposure routes are contaminated food and dust ingestion [56-57]. HBCD and TBBPA also have been detected in breast milk, while PBDEs tends to be the major FR, followed by HBCD and TBBPA [58]. PBDEs, TBBPA and HBCD are potential endocrine disruptors, and PBDEs also present development neurotoxicity [49, 59-60]. Environmental levels of PBDEs, HBCD and TBBPA in biotic and abiotic matrices and their toxic effects were intensively reviewed in literature [18, 46, 48, 50, 60-62].

Because of environmental concern regarding these “classical” brominated FRs, together with governmental regulations and bans related to PBDEs applications (see section 1.5), the use of other FRs has increased. In fact, large corporations such as IKEA, Apple, Sharp, Sony, Canon, Xerox, Dell and Samsung, among other, have phased out the use of PBDEs in some or all of their products, as a consequence of discontinuation of production of some PBDE formulations, and due adoption of green procurement guidelines which prohibit the use of PBDEs [47]. Therefore, manufacturers are actively identifying FR alternatives, which include the use of new brominated flame retardants (NBFR) and OPFRs. However, some of the formulations containing these alternatives FRs are potentially toxic. For example, Firemaster® 550 is produced by Chemtura Chemical Corporation (USA) since 2003 as a replacement for PBDEs in PU foams, and consists of a mixture of certain NBFRs and OPFRs [19]. Endocrine disruptor effects associated to Firemaster® 550 exposition was recently reported, and resulted in extreme weight gain, early onset of puberty and cardiovascular health effects in rats [63]. The concern of NBFRs and OPFRs in the environment has increased after studies reporting that some of them are present at high levels in dust [64-65], bioaccumulate and can biomagnify in biota [54], and can undergo long-range atmospheric transport [24, 66]. Very limited data is available concerning the presence, fate and risk of NBFRs and OPFRs in the environment. In this context, PBDEs, NBFRs and OPFRs need further studies to better understand their behavior in the environment and their potential risks.

1.2 Studied compounds, their applications and production volumes

PBDEs are priority contaminants in Europe since they are regulated (see section 1.5). NBFRs have been considered emerging[4] contaminants, since their presence in several environmental compartments have been reported in the last decade. Although OPFRs have been detected in the environment since 1970s, this FR family is considered as re-emerging contaminants because their use has increased after PBDE bans, and also because their increasing levels in the environment. The studied compounds were selected based on their use and production and potential negative effects. In the next sections, the studied compounds, the field of study, the state of art regarding their fate in the aquatic environment and legislation are described.

1.2.1 Polybrominated Diphenyl Ethers (PBDEs)

PBDEs are additive FRs used in a variety of plastics and foams. PBDEs are manufactured by the chemical reaction of bromine with diphenyl ether. Theoretically, 209 different congeners can be formed, which differ in number of bromines (1 to 10) and their position in the aromatic rings. The amount of bromine and the reaction time control the extent of bromination on the diphenyl ether molecule. On the other hand, commercial mixtures contain fewer PBDE congeners than the theoretical number possible, basically because the bromination of diphenyl ether is rather specific due to the directing properties of the oxygen in the molecule and due steric hindrance, resulting in limited number of congeners [46]. Three commercial mixtures of PBDEs were marketed, Penta-BDE, Octa-BDE and Deca-BDE, which were used for different purposes. The main environmental relevant congeners were selected in this thesis, and their chemical structure are presented at Figure 2. The world market demand of PBDEs in 2001 was 67 390 tonnes, consisting of 7500 tonnes of Penta-BDE, 3790 tonnes of Octa-BDE and 56 100 tonnes of Deca-BDE [47]. Nowadays, after Penta- and Octa-BDE bans in Europe, USA and China, manufacturers have phased out the production of these formulations, and so only Deca-BDE formulations are still produced [47].

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[4] Unregulated pollutants that have recently been analyzed and are believed to adversely affect human health or the environment.

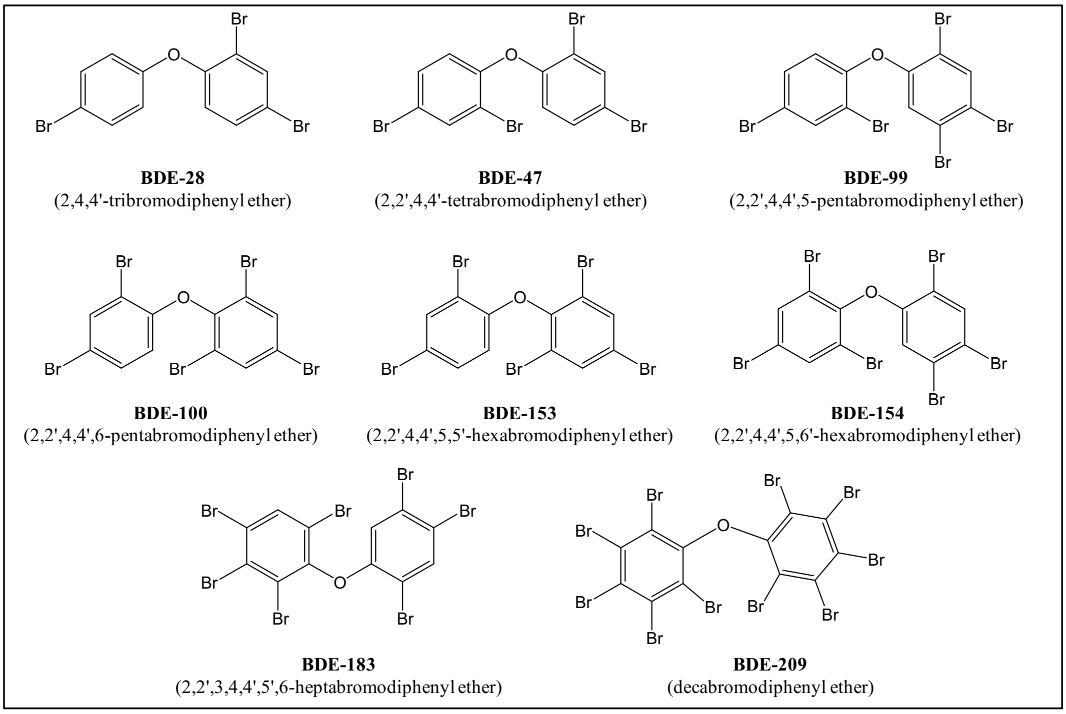

Figure 2 – Chemical structure of the studied PBDEs.

Penta-BDE (CAS 32534-81-9) has been mainly used in PU foam (95-98% of the total use). Other uses include unsaturated polyester, epoxy resins, laminates, adhesives and coatings. Typical products containing Penta-BDE are mattresses, seat cushions and other upholstered furniture, and rigid insulation [47]. Penta-BDE formulation consists of 41 – 42% tetra-BDE (mainly BDE-47), 44 – 45% penta-BDE (predominantly BDE-99 and to a lesser extend BDE-100), and 6 – 7% hexa-BDE (BDE-153 and BDE-154) [46]. Other minor components in Penta-BDE formulations include BDE-17, 28, 66, 85, 138, and 183 [67].

Octa-BDE (CAS Number 32536-52-0) has been mainly used in ABS (about 85% of total use). Other uses are nylon, thermoplastic elastomers, polyolefins, HIPS, polybutylene terephthalate, and polyamide polymers. Typical products containing Octa-BDE include electronic enclosures, automobile trim, and kitchen appliance casings [47]. Octa-BDE formulations are a complex mixture consisting typically of hepta-, octa- and nona-BDE isomers, while the congener profile differs among the commercial formulations. The formulation DE-79 (Great Lakes Chemical Corp., Indiana) presents as main components the BDE-175/183 (BDE-183 is the major component of the co-elution pair) (42%), BDE-197 (22.2%), BDE-207 (11.5%), BDE-196 (10.5%) and BDE-153 (8.66%), whereas Bromkal 79-8DE (Chemische Fabrik Kalk, Köln, Germany) present BDE-209 (49.6%), BDE-175/183 (12.6%), BDE-207 (11.2%), BDE-197 (10.5%), BDE-203 (8.14%) as main compounds [68].

Deca-BDE (CAS 1163-19-5) is used in textiles, electronic equipment, and building and construction materials. Deca-BDE is mainly used in HIPS, and other uses include high-impact polystyrene/polyphenylene oxide, polybutylene terephthalate, nylon, polypropylene, low-density polyethene, ethylene-propylene-diene rubber and ethylene-propylene terpolymer, unsaturated polyester, and epoxy [47]. Deca-BDE formulation consists mainly of BDE-209 (97–98%), with a small amount of nona-BDE (0.3–3%) [46].

1.2.2 New Brominated Flame Retardants (NBFRs)

NBFRs refer to brominated FRs other than PBDE, HCDBCO or TBBPA, that are new in the market or newly detected in the environment[5]. At least 75 brominated flame retardants have been produced [46], from which nine NBFRs were chosen for studying taking into account their reported occurrence in the environment. Figure 3 presents the chemical structure of the studied NBFRs, and details of their application and production are given in the following paragraphs.

Decabromodiphenyl ethane (DBDPE) (CAS 84852-53-9) is an additive FR used as Deca-BDE substitute and is available on the market since the mid-1980s [69]. Due to its good thermal stability and high bromine content, DBDPE is used in high temperature applications [70]. DBDPE is used in thermoplastic, elastomeric and thermoset polymer systems, such as HIPS, PBT, polypropylene, low-density polyethylene, ethylene propylene diene monomer rubber, unsaturated polyester, epoxy, and is also used in coatings and adhesive systems [71]. DBDPE is not produced in Europe, but an import volume between 1000 and 5000 tonnes was estimated in 2001, primarily to Germany [69]. The production volume of DBDPE in China in 2006 was 12 000 tonnes [69]. DBDPE is a classified as an LPV[6] chemical in the EU [72].

1,2 Bis(2,4,6-tribromophenoxy)ethane (BTBPE) (CAS 37853-59-1) is an additive FR especially efficient for applications in which thermal stability at high processing temperatures is necessary. BTBPE is used in HIPS, ABS, polycarbonate, thermoplastic, elastomers, unsaturated polyesters, adhesives, coatings, and textiles [73]. BTBPE has been produced since the mid-1970s and is now being used as a replacement for Octa-BDE [74]. BTBPE worldwide

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[5] Other terms for NBFRs that have been used in literature are “novel”, “emerging”, “current-use” and “non-PBDEs” flame retardants.

[6] LPV (low production volume) chemicals refer to chemicals produced below 1000 tonnes/year.

production/usage was estimated to be 16 710 tonnes in 2001 [47]. BTBPE and is an LPV chemical in the EU [72].

2,3-Dibromopropyl 2,4,6-tribromophenyl ether (DPTE) (CAS 35109-60-5) is used as a flame retardant in extrusion grade polypropylene [75]. No information was found as whether DPTE is used as an additive or reactive FR [76]. DPTE is the main component of the commercial product Bromkal 73-5 PE, which was produced by Chemische Fabrik Kalk (Cologne) from the mid-1970s. No information was found about production volumes. DPTE has not been reported by EU industry as an HPV[7] or LPV chemical [72].

Bis(2-ethyl-1-hexyl)tetrabromo phthalate (BEHTBP) (CAS 26040-51-7) is an additive FR with plasticizer properties for polyvinylchloride adhesives, coatings and elastomers including styrene-butadiene rubber, neoprene and ethylene propylene diene monomer rubber. BEHTBP is used in PVC applications such as wire and cable insulation, coated fabrics, film and sheeting [77]. BEHTBP is also one of the components in the commercial product Firemaster® 550 (Chemtura), produced since 2003 as a replacement for Penta-BDE in polyurethane foam applications [78]. USA production volumes of BEHTBP were 450 to 4500 tonnes/year from 1990 to 2006 [19]. BEHTBP is a classified as an LPV chemical in the EU [72].

2-Ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) (CAS 183658-27-7) is an additive FR and one of the components in the FR product Firemaster® 550 (Chemtura). Firemaster® 550 is a commercial mixture that gives phosphorus-bromine synergy effects, for application in polyurethane foam. The brominated compounds contribute to approximately 50% of the mixture, whereas the remaining 50% comprises an isopropylated triaryl phosphate and TPhP [78]. The approximate ratio EHTBB/BEHTBP is 4:1 (by mass) [78]. EHTBB has not been reported by EU industry as an HPV or LPV chemical [72].

Hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO) (CAS 51936-55-1) is an additive FR that contains chlorine and bromine atoms. HCDBCO is used in styrene polymers [79]. This substance has not been reported by EU industry as an HPV or LPV chemical [72].

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[7] HPV (high production volume) chemicals refer to chemicals produced above 1000 tonnes/year.

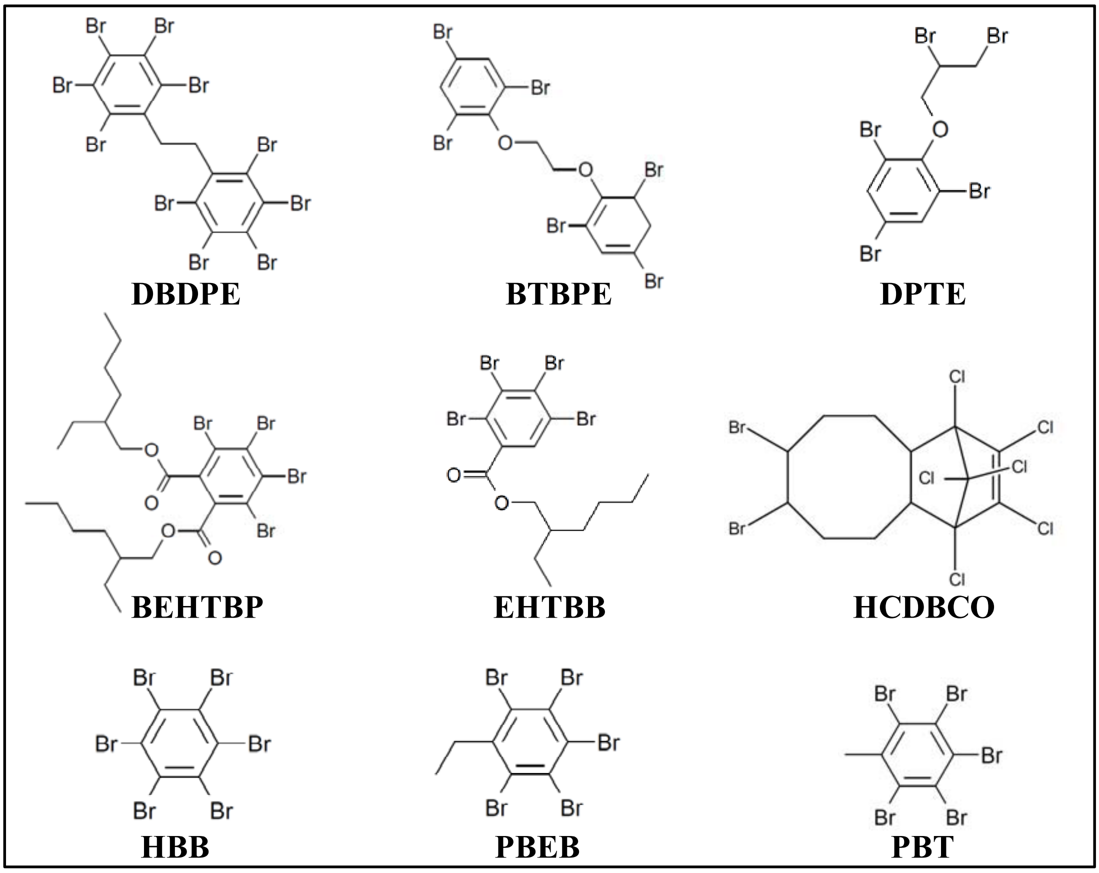

Figure 3 - Chemical structure of the studied NBFRs.

Hexabromobenzene (HBB) (CAS 87-82-1) is an additive FR used in polymers, plastics, textiles, wood and paper [75]. The USA production/import volume was listed as 10 000 - 500 000 pounds in 1998 [75]. HBB was widely used in Japan in the past (papers, wood, textiles, electronics, etc.) but at present it is used at lower volumes (350 tonnes in 2001) [19]. HBB has not been reported by the EU industry as an HPV or LPV chemical [72].

Pentabromotoluene (PBT) (CAS 87-83-2) is used in unsaturated polyesters, polyethylene, polypropylenes, polystyrene, textiles, rubbers and ABS [6]. No information was identified as to whether it is used as an additive or reactive flame retardant [76]. PBT is an LPV chemical in the EU [72].

Pentabromoethylbenzene (PBEB) (CAS 85-22-3) is an additive FR used in thermoset polyester resins (circuit boards, adhesives, wire and cable coatings, textiles, PU) [79]. PBEB was produced in US mainly in the 1970s and 1980s in the USA, while no USA production or import volumes was reported after 1986 [19]. PBEB is an LPV chemical in the EU [72].

1.2.3 Organophosphorus Flame Retardants (OPFRs)

OPFRs include phosphate esters, phosphonates and phosphinates. Only phosphate esters were studied, which are described below. Phosphate esters can be divided in three main groups: chloroalkyl phosphates and aryl phosphates that are mainly used as FRs; and alkyl phosphates that are used as plasticizers, anti-foaming, additives to lacquers, hydraulic fluids and floor polishing, though in some cases they are also used as FRs. A total of 10 OPFRs (3 chloroalkyl phosphates, 3 aryl phosphates, and 4 alkyl phosphates) were studied. Figure 4 presents the chemical structure of these OPFRs.

Chloroalkyl phosphates

Tris(2-chloroethyl) phosphate (TCEP) (CAS 115-96-8) is an additive FR that also acts as plasticizer and viscosity regulator in polyurethanes, polyester resins, polyacrylates and other polymers. The main application is in the building industry (e.g., roofing insulation, accounting for more than 80% uses in the EU), in furniture and in the textile industry (e.g., back-coatings for carpets and upholstery) [80]. TCEP is also used in the manufacture of cars, railways and aircrafts, in professional paints, varnishes and lacquers. TCEP production and use has declined since the 1980s, and TCEP has been progressively replaced by other flame retardants, such as TCPP and TDCP, mostly in the manufacturing of PU foam [80]. TCEP is classified as an HPV chemical in the EU [72].

Tris(2-chloro-1-methylethyl) phosphate (TCPP) (CAS 13674-84-5) is an additive FR mainly used in PU foam. Over 80% of TCPP application account for rigid PU foam used in constructions, while over 17% are applied in flexible foam used in upholstery and bedding for the UK and Irish markets [81]. Due its volatility and fogging potential, TCPP tends not to be used in flexible PU for automotive applications. TCPP is manufactured with a purity of 75 ± 10%, with bis(1-chloro-2-propyl)-2-chloropropyl phosphate (CAS 76025-08-6) (20-30%) and bis(2-chloropropyl)-1-chloro-2-propyl phosphate (CAS 76649-15-5) (3-5%) as major impurities [82]. The total production of TCPP in the EU was 36 000 tonnes in the year 2000, with production taking place in Germany and in the UK [81]. TCPP is classified as an HPV chemical in the EU [72].

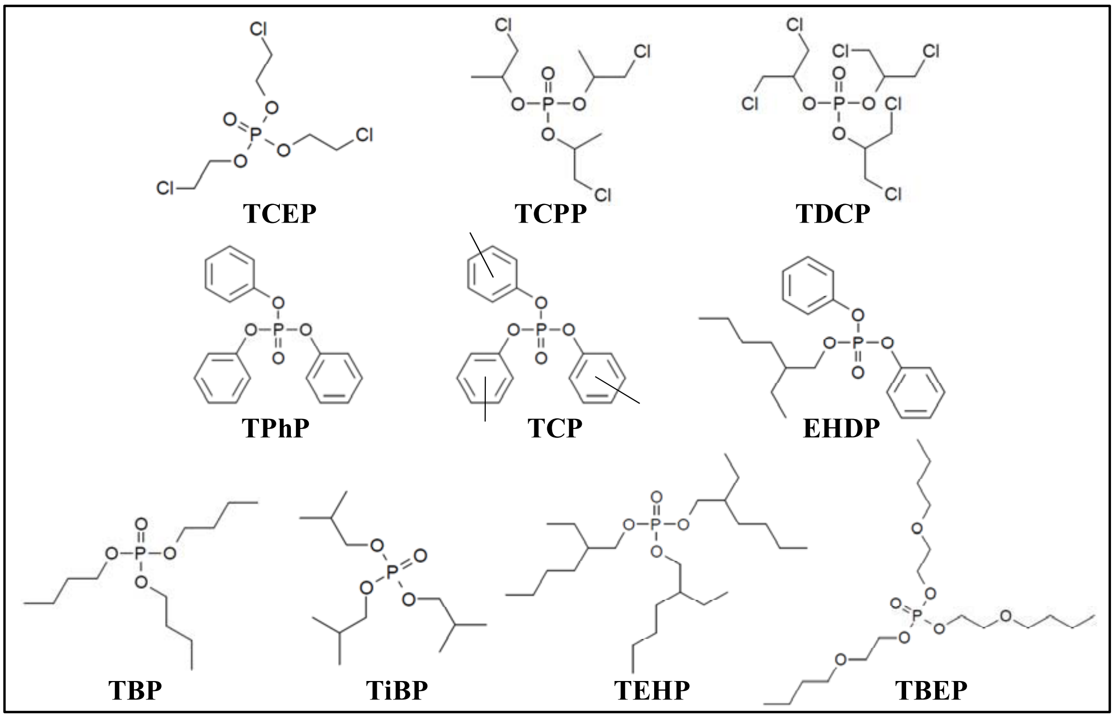

Figure 4 – Chemical structure of the studied OPFRs.

Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP) (CAS 13674-87-8) is an additive FR used in the PU foam mainly for applications by automotive industry, with some use in furniture. TDCP operates in the same marketplace as TCPP, but due to its higher price, TDCP is only used in those applications where a more efficient flame retardant is required to meet specific standards [83]. The total production of TDCP in the EU was 10 000 tonnes in the year 2000, with production taking place in Germany and in the UK [83]. TDCP is a classified as an HPV chemical in the EU [72].

Aryl phosphates

Triphenyl phosphate (TPhP) (CAS 115-86-6) is an additive FR with plasticizer properties mainly used in PVC (about 50% of total applications). TPhP is also applied in polymers (about 22%), printed circuit boards (about 11%) and in photographic films (about 7%). In a minor extend (less than 10%), TPhP is used in hydraulic liquids, adhesives, inks, and coatings [84]. The worldwide production (excluding East Europe) of TPhP was about 20 000 to 30 000 tonnes in the year 2000, and it was estimated that about 25% was produced in West Europe, about 40% in the USA, and about 35% in Asia (no information available about production/use in East Europe) [84]. TDCP is an HPV chemical in the EU [72].

Tricresyl phosphate (TCP) (CAS 1330-78-5) is an additive FR used as a flame retardant with plasticizing properties for PVC, phenolic resins, synthetic rubber and other polar polymers, and as a flame retardant component in hydraulic fluids [85]. Commercial TCP formulations are a complex mixture containing tri-*meta*-cresyl phosphate (TMCP), tri-*para*-cresyl phosphate (TPCP) as well as mixed tricresyl and dicresyl phosphate esters. Theoretically, the total number of tricresyl phosphate isomers is ten [86]. Concerning TCP worldwide production, 33 000 tonnes were produced in 1984 in Japan, 10 400 tonnes were produced in 1977 in the USA, while in China about 800 − 1000 tonnes per year were produced at the end of the 1980s [87]. TCP is a classified as an LPV chemical in the EU [72].

2-Ethylhexyl diphenyl phosphate (EHDP) (CAS 1241-94-7) is an additive FR with plasticizing and smoke suppressant properties. EHDP acts as a flame retardant in flexible PVC, thermoplastic polyurethane, nitrile butadiene rubber, cellulose acetate, and can be used in food contact applications [85]. EHDP is also used in hydraulic fluids [6]. EHDP is a classified as an HPV chemical in the EU [72].

Alkyl phosphates

Tri-n-butyl phosphate (TBP) (CAS 126-73-8) is an additive used as a flame-retarding plasticizer in cellulose based plastics and synthetic resins [85]. TBP is also used in the formulation of the fire-resistant aircraft hydraulic fluids, as a solvent for cellulose esters, lacquers and natural gums, as a plasticizer in the manufacture of plastics and vinyl resins, as an antifoaming agent in paper manufacturing plants, and as a herbicide and a defoaming agent for concrete and oil well drilling [86]. The production volume of TBP is estimated at 3000 – 5000 tonnes worldwide [88]. TBP is a classified as an HPV chemical in the EU [72].

Tri-iso-butyl phosphate (TiBP) (CAS 126-71-6) is used mainly as antifoam in various aqueous systems. TiBP is used as additive flame-retarding plasticizer in cellulose based plastics and synthetic resins. TiBP is also employed as a pasting agent for pigment pastes, it is used in hydraulic fluids for aircraft, and it is used by the textile industry in the field of adhesives [85]. TiBP is classified as an HPV chemical in the EU [72].

Tris(2-ethylhexyl) phosphate (TEHP) (CAS 78-42-2) is an additive flame-retarding plasticizer with low temperature flexibility properties used in PVC, polyurethane, nitrile butadiene rubber and other synthetic rubbers [85]. TEHP is also used as FR in cellulose acetate [89]. TEHP can be used as wetting agent for pigments and dyestuffs, as cooling lubricant and as a solvent for certain chemical reactions [85]. The world production of TEHP was estimated to be between 1000 to 5000 tonnes per year [89]. TEHP is a classified as an HPV chemical in the EU [72].

Tris(2-butoxyethyl) phosphate (TBEP) (CAS 78-51-3) is an additive flame retardant used mainly as a component in floor polishes, a solvent in some resins, a viscosity modifier in plastisols, an antifoam, and also as a plasticizer in synthetic rubber, plastics and lacquers. TBEP is widely used as a plasticizer in rubber stoppers for vacutainer tubes and plastic ware. The world global production has been estimated to be 5000 – 6000 tonnes, with less than 1000 tonnes in Europe [89]. TBEP is classified as an HPV chemical in the EU [72].

1.3 Field of study

The term "pollution" comes from the Latin verb “*polluere*”, which means “to soil or defile”. In a broader concept, pollution indicates the occurrence of adverse changes in the medium and can refer to water, air or soil. In the case of the aquatic environment, water pollution can be defined as the occurrence of phenomena that directly or indirectly alter the nature of a waterbody and thus impair their normal utilization, affecting humans and/or aquatic biodiversity. Waters can be polluted by the introduction of artificial or natural foreign substances, or by a change in the proportion or characteristics of their own constituents. In addition, pollution sources in waters can be punctual (concentrated in a specific point), as for example a pipe discharging sewage, or diffuse (distributed along the water body), as for example atmospheric deposition of contaminants [90].

Rivers are the source of water for drinking water facilities, agriculture and recreational activities around the world. Since ancient times, villages have been built on riverbanks. The wastes generated in these villages usually were thrown into the rivers to be carried away. Given that there was no other village downstream, rivers had the capacity to assimilate these pollutants and clean themselves. The growth of populations has led to an increased generation of wastewater, and so the autodepuration capacity of rivers became insufficient and they no longer could clean themselves. The expansion of industrial activities, also related to population growth, has promoted the transformation of raw materials into consumer goods in larger scale, and consequently large amounts of waste are generated. Nowadays, developed countries treat the waste generated in urban and industrial centers in order to improve the quality of their rivers. WWTPs remove approximately 85% of the solid and organic materials in the wastewater, kill bacteria and viruses, and then usually discharges it into the nearest waterway [91]. However, many pollutants are not eliminated during the treatment, and large amounts of undesired chemicals reach rivers [92]. Thus, WWTPs outputs are an important punctual source of contaminants. Innumerable contaminants reach river water by WWTP discharges, including synthetic organic compounds used for diverse applications, such as flame retardants, pharmaceuticals and personal care products, detergents, plasticizers, pesticides, etc [92-94]. Other important source of contamination in urban rivers is stormwater runoff. Rain events mobilize pollutants (e.g. automobile fluids, dust, etc) from roads and other impermeable surfaces and polluted stormwater enters storm gutters, pipes, ditches and ultimately reach rivers, many times without adequate treatment [95]. Thus, urban rivers suffer strong anthropogenic pressures by introduction of lots of contaminants that affect water quality and the ecological equilibrium.

Although analytical developments carried out in the last decades have allowed the detection of trace levels of contaminants in the aquatic environment, the environmental significance of these pollutants is poorly understood and there is a lack of information concerning their fate in the environment. When organic pollutants reach the aquatic environment (e.g. WWTP discharges to a river) they will be susceptible to several processes that will determine their fate. The tendency of a pollutant to undergo specific processes depends on their physicochemical properties (e.g. solubility, lipophilicity, vapour pressure, chemical stability) and also on the characteristics of the medium (e.g. pH, temperature, dissolved organic matter, organic carbon content) [96]. Figure 5 presents the main processes that can determine the fate of pollutants in river water.

Soluble pollutants tend to be transported in the dissolved phase, and considering rivers, these chemicals can be transported by the water flow far away from the emission source, and can reach other water bodies (e.g. the sea). Lipophilic pollutants tend to adsorb onto sediments or onto suspended particles, which can be transported along the river or can deposit on the bottom of the river [97]. Thus, river sediments act as a sink and a source of contaminants, since sediments can be resuspended or the compounds can desorb back to water. The physicochemical parameter that allows predicting the affinity of a compound to

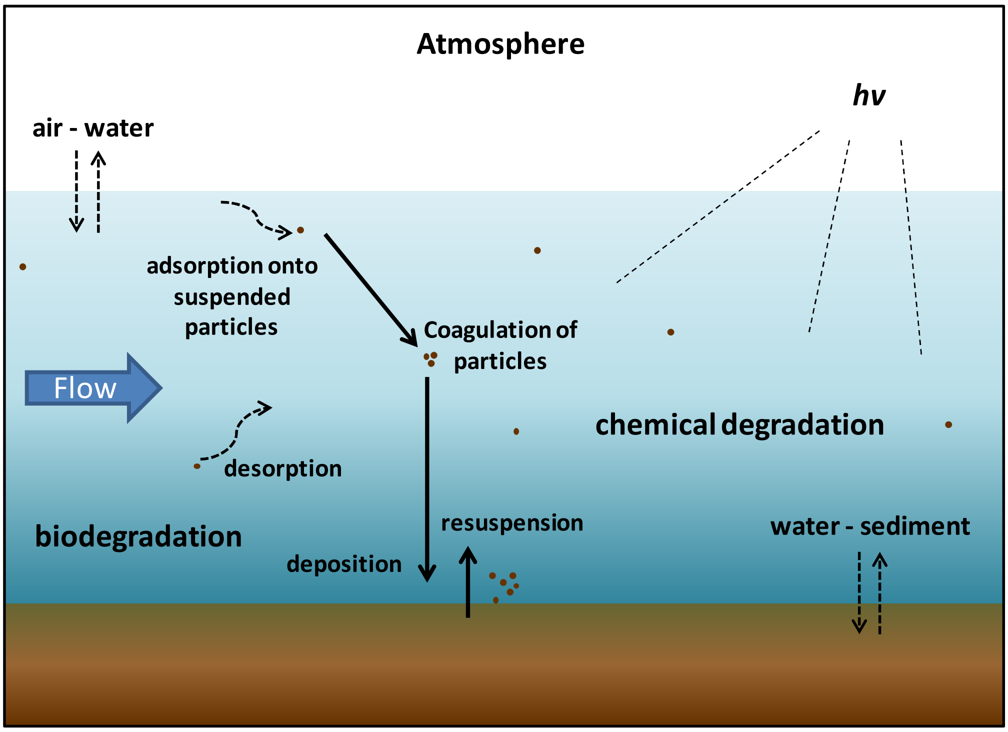

Figure 5 – Transport and transformation processes of organic compounds in river water.

organic matter is the organic-carbon/water partition coefficient (K_{oc}). The higher the K_{oc}, the higher the affinity to organic matter [98]. In addition, the higher the organic-carbon fraction (f_{oc}) of the sediment, the stronger the adsorption, and thus the harder the desorption of a pollutant back to water. Another important factor involved in the fate of lipophilic contaminants in river water is the dissolved organic carbon (DOC) content, since DOC acts stabilizing lipophilic molecules in the dissolved phase enhancing their solubility in water column [96].

Organic pollutants in water are also susceptible to air-water interactions, which depend on their vapour pressure and solubility, and also depend on the hydrodynamic characteristics of a particular environment. Air-water exchange is significant to the cycling of organic pollutants in the environment, especially considering long-range transport, where interactions with oceans are critical [99]. The less soluble compounds with higher vapour pressure will have a much greater tendency to be in the gas phase than soluble compounds with low vapour pressures [96], while turbulence in the water and air phases can enhance evaporative fluxes [100]. The Henry's law constant (H) allows the prediction of the distribution of a compound between aqueous solution and the vapour phase, and is defined as the partial pressure of a compound divided by its chemical concentration in the aqueous phase at equilibrium, which is highly dependent on the temperature [101]. The higher the H, the higher the tendency of evaporation loss from water.

The fate of an organic pollutant in the aquatic environment depends on its susceptibility to chemical and biological degradation. The most common chemical degradation processes in the aquatic environment are hydrolysis, photolysis and redox reactions. Hydrolysis is based on the attack of the compound by a water molecule or a hydroxide ion, and especially affect esters, carboxylic acids, amides, carbamates and organophosphates, while halogenated aromatics seem to be relatively immune [96]. By definition, "photodegradation is the decomposition of a compound by radiant energy" [102]. A pollutant in the environment can undergo direct photodegradation if the molecule absorbs energy from the emission spectrum of the sun. However, in river water there are natural organic compounds (e.g. humic substances) with photosensitizer activity, and organic pollutants that do not absorb solar radiation can be photodegradated by indirect reactions [96]. Redox reactions of organic pollutants have been observed in the environment, and polyhalogenated compounds have higher degradation rates under increased reducing conditions [101]. Finally, in order to assimilate organic carbon, microorganisms in the aquatic environment can modify the chemical structure of organic pollutants through metabolic or enzymatic action. Consequently, biodegradation processes may lead to environmental detoxification, the formation of new toxicants, or the biosynthesis of persistent products, while some compounds can be resistant to microbial attack [103]. The biodegradability potential of an organic pollutant in the environment is one of the most important parameters that affect their persistence.

Depending on the intensity and duration of exposure to a toxicant, direct effects to aquatic organisms (e.g. increased mortality, reduced fecundity, physiological stress, etc) can occur, which can initiate a trophic cascade effects that indirectly affect also resistant species [104]. Therefore, studies concerning the presence, persistence, fate, toxicity and associated risk of pollutants to the environment are necessary in order to identify hazardous pollutants and establish permissible levels of unregulated contaminants. In this context, pollutants in dissolved phase have received most of the attention, and regulations of several organic pollutants have been established [105]. More recently, the importance of incorporating sediment into water quality management has also been recognized [106], which allows to monitor the progressive reduction in the contamination of priority substances and to identify other hazardous contaminants.

1.4 Fate of PBDEs, NBFRs and OPFRs in the aquatic environment

As described in the previous section, depending on their physicochemical properties and their chemical structure, different organic compounds tend to undergo different transportation and transformation processes that will determine their fate in the aquatic environment. Three different classes of FRs were selected for study, and their partition in the environment is described in this section based on their physicochemical characteristics and reported studies concerning their occurrence and fate in the aquatic environment.

1.4.1 PBDEs

PBDEs have low vapour pressure and low water solubility, consequently it is estimated that once they are released to the environment they preferably will be associated to particles and to the organic matter of sediments and soils, while air and water are likely transport media. Table 2 presents the physicochemical properties of the studied PBDEs.

As discussed in the previous section, WWTPs can play an important role in the distribution of contaminants in the aquatic environment. Due their high lipophilicity, a high removing efficiency has been observed for PBDEs in WWTPs, since they are preferably accumulated in sludge [107-108]. However, PBDEs are detected in the effluents at ng – pg L^{-1} levels, and taking into account the high volumes discharged from these facilities, WWTPs are considered as an important source of PBDEs to the aquatic environment. For example, about 10.2 g day^{-1} (ΣPBDEs) reach the River Penobscot (USA) by discharges from a WWTP facility, which was estimated based on observed PBDE concentrations in the effluent (BDE-47, 71, 85, 99, 100, 138, 153, 154, 183) [107]. Once PBDEs reach river water, they can be transported along the river and reach oceans by runoff, which is an important transportation mode for these pollutants from terrestrial sources to oceans [109].

Atmospheric deposition has been indicated as a PBDE source to the aquatic environment, and it is considered the most important source in remote zones [110]. Vapour pressures of PBDEs will determine their partition in the atmosphere, where low brominated congeners will be rather transported in the gaseous phase and the high brominated congeners will tend to be associated to atmospheric particles. In fact, in air samples collected around the Great Lakes (North America), BDE-47 was predominant in atmospheric vapour phase, BDE-209 was predominant in atmospheric particles, and BDE-99 was present in the both phases at

Table 2 – Physicochemical properties of studied PBDEs.

	Water Solubility (mg L^{-1} at 25°C)	Vapour pressure (Pa at 25°C)	Henry's law const. ($Pa \cdot m^3/mol$ at 25°C)	log K_{ow}	log K_{oc}
BDE-28	0.334[a] 0.070[b]	1.78×10^{-4} [a] 0.00160 [a] 2.19×10^{-3} [a] 1.57×10^{-3} [a]	5.1[a] 1.924[a] 4.83±0.67[d]	5.98[a] 5.80[a]	5.74±0.00[c] 4.95±0.05[c] 5.21±0.06[c] 6.13±0.04[c]
BDE-47	0.0947[a] 0.015[b]	2.19×10^{-5} [a] 3.19×10^{-4} [a] 2.15×10^{-4} [a]	1.5[a] 1.107[a] 0.85±0.35[d]	6.02[a] 6.55[a] 6.39[a]	6.44±0.01[c] 5.77±0.01[c] 5.79±0.02[c] 5.85±0.03[c]
BDE-99	0.0389[a] 0.0094[b]	1.26×10^{-5} [a] 1.76×10^{-5} [a] 3.63×10^{-5} [a]	0.23[a] 0.530[a] 0.60±0.11[d]	6.81[a] 7.13[a] 6.76[a]	6.92±0.02[c] 6.19±0.01[c] 6.27±0.01[c] 6.25±0.02[c]
BDE-100	0.0541[a] 0.040[b]	2.86×10^{-5} [a] 3.68×10^{-5} [a]	0.069[a] 0.384[a] 0.24±0.06[d]	6.86[a] 6.53[a]	6.70±0.01[c] 5.77±0.12[c] 5.82±0.08[c] 6.33±0.00[c]
BDE-153	0.0167 [a] 8.70×10^{-4}[b]	8.43×10^{-6} [a] 8.87×10^{-6} [a]	0.067[a] 0.342[a] 0.26±0.08[d]	7.39[a] 7.62[a] 7.08[a]	7.30±0.01[c] 6.19±0.02[c] 6.22±0.03[c] 6.43±0.01[c]
BDE-154	8.70×10^{-4} [b]	3.80×10^{-6} [a]	0.24[a] 0.08±0.04[d]	7.39[a]	7.24±0.02[c] 6.10±0.12[c] 6.18±0.06[c] 6.46±0.00[c]
BDE-183	1.50×10^{-3} [b]	4.68×10^{-7} [a]	0.0074[a]	7.14[a]	7.52±0.01[c] 6.40±0.08[c] 6.96±0.05[c]
BDE-209	1.4×10^{-4} [e]	2.95×10^{-9} [a]	0.04±0.01[d]	9.97[a]	6.5[e]

[a] Revised values from literature, by Mackay et al (2006) [111].
[b] Experimental values reported by Titlemier et al. (2002) [112].
[c] Experimental values determined for four different sediments [113].
[d] Experimental values [114].
[e] SciFinder, calculated using Advanced Chemistry Development Software V11.02 (© 1994–2013 ACD/Labs).

similar concentration [115]. Wet and dry deposition of PBDEs in the aquatic environment have been reported [116-118], where wet deposition was pointed out as the most important removal mechanism of PBDEs from the atmosphere in periods of high atmospheric precipitation [116].

Regarding the aquatic environment, PBDEs in the water column may partition between three phases: particle phase, the truly dissolved phase, and the colloidal phase [119]. Increasing the bromination level of PBDE congeners, K_{ow} and K_{oc} increases while water solubility decreases. This means that low brominated PBDEs tend to be more easily transported in the dissolved phase than high brominated PBDEs, which tend to be associated to suspended particles and sediments. In addition, PBDEs have high dissolved organic carbon partition coefficients (K_{DOC}) and so they are rather associated to DOC. This indicate that, to some extent, DOC in the aquatic environment may contribute to the transport of even the less soluble PBDEs along rivers [113]. The correlation among brominated level of PBDEs and their partition in water column was reported by Zarnadze and Rodenburg (2008) [119] (New York/New Jersey Harbor - USA). The authors observed that BDE-17 was never detected in the particle phase but it was detected in 58% of dissolved phase samples, BDEs 47, 99, and 100 were detected in all particle- and dissolved-phase samples, BDE-153, 154, and 183 were detected in 12, 8, and 8%, respectively, of dissolved-phase samples and in 35, 79, and 38%, respectively, of particle-phase samples, and finally BDE 209 was detected in all particle-phase samples but only in 23% of dissolved-phase samples. A similar PBDE partition behaviour was observed in the River Predecele (France), except BDE-209 that was present at high concentrations in both the dissolved and the particulate phases [29]. A different partition for BDE-209 in river water and in harbour water is not surprising since it is expected that those waters present different content of DOC and dissolved salts, which affect the solubility of BDE-209 in the water column.

Sediments are the sink of PBDEs in the aquatic environment, especially the high brominated congeners. High concentrations of PBDEs in river sediments occur near industrial [39] and urban zones [120]. PBDEs in sediments were suggested as an anthropogenic marker of catchment’s urbanization process, since PBDEs follow all the criteria needed for markers of pollution: source specificity, widespread and massive usage, and environmental persistence [120]. In addition, PBDE monitoring in sediments permit to evaluate the temporal trends of these banned/regulated FRs. For example, in a temporal trend study performed from 1999 to 2009 in the River Detroit (USA), a decline in PBDE concentrations was observed over the period 2001 – 2003 followed by a subsequent general levelling off, which was attributed to the phasing out of the Penta- and Octa-BDE formulations, whereas the continued production and use of BDE-209 resulted in a general increase on the contribution of BDE-209 to the total PBDE load in sediments [121].

Among the chemical transformation processes that PBDEs can undergo in the aquatic environment, the most important one is photodegradation. PBDEs absorb light in the UV-A spectrum and undergo photodegradation, which often results in loss of bromine and thereby also a possibility for rearrangements [122]. Natural photodegradation rate of PBDEs is dependent on the bromination degree, where low brominated PBDEs tend to degrade slower than high brominated PBDEs [123]. The sum of these factors increases the concern about the presence of BDE-209 in the environment, since BDE-209 can undergo photodegadation and generate more toxic and persistent debrominated products. Söderström et al. (2004) [122] studied the photodegradation of BDE-209 in several matrices (toluene, silica gel, sand, sediment and soil) using artificial and natural sunlight, and observed that debromination of BDE-209 strongly depended on the matrix, and presented longer half-lives on natural matrices (sand, soil and sediments) than on the artificial ones (toluene and silica gel). This behaviour was attributed to the higher porosity of soil and sediments, which enabled BDE-209 to be absorbed into the particle where it was more shielded from UV-radiation, and to the high content of organic carbon, which can non-covalently bind planar organic compounds. The half-life time for BDE-209 in wetted sediment exposed to “discontinuous” natural sun light was 80 h. Nona- to tetra-BDEs were formed, including PBDE congeners present in commercial formulation (e.g. BDE-183, BDE-153 and BDE-154), as well as some brominated dibenzofurans (PBDFs). Hua et al. (2003) [124] found that humic acid acts decreasing degradation rates for UV irradiated BDE-209. Thus, a decrease of photodegradation rates of BDE-209 in rivers is expected due to the adsorption of BDE-209 onto sediments and due to the presence of DOC in water.

PBDEs are not easily biodegraded. Regarding aerobic biodegradation, tetra- to nona-BDE are predicted to be "recalcitrant" by the BIOWIN program [125-126]. Estimated half-lives using the EPIWIN program for Penta-BDE were 600 days in aerobic sediment, 150 days in soil, and 150 days in water [125]. PBDE persistence is supported by the fact that no degradation was seen in 29 days using a ready biodegradation test (OECD 301B) for Penta-BDE [125], and no degradation occurred in a 28-day in a closed bottle test (OECD 301D) for hexa- to nona-BDEs [126]. No significant aerobic biodegradation (period of 160 days) was found for BDE-209 in sewage amended soil [127]. Concerning anaerobic biodegradation, a very slowly PBDE biodegradation rate was observed [125-128]. No significant anaerobic biodegradation was observed for BDE-28 and BDE-209 during an incubation period of 160 days [127]. In another study, a half-life of 700 days was estimated for anaerobic biodegradation of BDE-209 in digested sewage sludge, and formation of nona- and octa-BDEs was observed [128].

Summarizing, a high persistence of PBDEs is expected in rivers due their chemical and biological stability, and due to their partitioning, which limit their transportation and decrease their degradation rate. In addition, the high brominated PBDEs can generate lower brominated PBDEs that are more persistent and toxic.

1.4.2 NBFRs

Few studies are available concerning the partition and fate of NBFRs in the aquatic environment. However, based on their solubility, K_{ow} and K_{oc} coefficients, it can be expected that NBFRs present a partition behaviour similar to PBDEs, tending to be associated to suspended particles and sediments, whereas different chemical and biological transformations can occur depending on their chemical structure. Table 3 presents the physicochemical properties of studied NBFRs.

Similarly to PBDEs, NBFRs have low water solubility and high K_{ow}, and so it is expected that they are accumulated in sludge in WWTPs. However, the impact of WWTPs to the aquatic environment as a source of NBFRs is not completely understood. DBDPE behaves similarly to BDE-209 in WWTPs since both are highly accumulated in sludge [129]. A removal higher than 99% for DBDPE was observed in a Swedish WWTP, and the average DBDPE concentration in the effluents ranged from 0.018 to 0.13 ng L^{-1} [129]. HBB was detected in sludge (<0.11 – 0.39 ng g^{-1} dw), influents (0.4 – 1.82 ng L^{-1}) and effluents (0.58 – 1.82 ng L^{-1}) in 3 WWTPs in Norway, while PBT and PBEB were not detected [130]. BTBPE, BEHTBP and EHTBB have been detected in sewage sludge [131] but no study is available concerning their evolution in WWTPs, and so the efficiency of these facilities and their impact on the waterway receiving their discharges is quite unknown. However, an American study indicated that WWTP discharges are an important source of NBFRs since BTBPE, BEHTBP, EHTBB were detected at high concentrations in river sediments collected near a WWTP outfall, whereas the concentrations decreased significantly in sediments collected downstream this point [132]. No data is available concerning the presence of DPTE and HCDBCO in WWTPs.

Several studies report the presence of NBFRs in air samples [19], which indicate that they are transported by the atmosphere and can reach the aquatic environment by atmospheric deposition. Möller et al. (2011) [133] analyzed air and seawater samples collected during a polar expedition cruise (from East China Sea to the Arctic Atmosphere) and observed that NBFRs undergo long range transport and that atmospheric deposition was a source of NBFRs to the ocean. In that study, PBT, HBB and DPTE were frequently detected in atmosphere (up to 5.9 pg m^{3}, mainly in gaseous phase) and in seawater (up to 1.6 pg L^{-1}), and EHTBB, BTBPE, and BEHTBP were seldom detected [133]. In other study, HBB, PBT, DPTE, EHTBB and BEHTBP were frequently detected (except EHTBB detected in 20% of samples) in the marine atmosphere from Southeast Asia toward Antarctica, and ranged from nd to 26

Table 3 − Physicochemical properties of studied NBFRs.

	Water Solubility (mg L^{-1} at 25°C)	Vapour pressure (Pa at 25°C)	Henry's law const. (Pa·m^3/mol at 25°C)	log K_{ow}	log K_{oc}
HBB	0.551[a] 3.0×10^{-3} [b] 1.10×10^{-4} [c]	1.14×10^{-4} [a] 7.5×10^{-4} [b] 3.17×10^{-4} [c]	0.14 [b] 10.8 [c]	6.111±0.674 [a] 6.07±0.8 [c]	4.7
PBT	0.48 [a] 0.78	6×10^{-4} [a] 1.22×10^{-3}	0.61 [d]	6.255±0.617 [a]	4.8
PBEB	0.21 [a]	1.56×10^{-4} [a]	0.37 [d]	6.764±0.617 [a]	5.1
DPTE	1.6×10^{-3} [a]	1.26×10^{-5} [a]	4.2 [d]	5.823±0.530 [a]	4.5
HCDBCO	8.7×10^{-3} [a]	8.27×10^{-7} [a]	5.1×10^{-2} [d]	7.619±0.420 [a]	5.5
EHTBB	0.16 [a]	3.71×10^{-7} [a]	103×10^{-3} [d]	7.279±0.619 [a]	5.3
BEHTBP	3.3×10^{-3} [a]	1.55×10^{-11}	3.3×10^{-06} [d]	9.344±0.697 [a]	6.5
BTBPE	1.6×10^{-2} [a]	3.88×10^{-10} [a]	1.7×10^{-05} [d]	8.312±0.716 [a]	5.9
DBDPE	9.5×10^{-5} [a]	2.93×10^{-15} [a]	3.0×10^{-08} [d]	11.679±0.853 [a]	7.0

[a] SciFinder, calculated using Advanced Chemistry Development Software V11.02 (© 1994-2013 ACD/Labs).
[b] Experimental values reported by Titlemier et al. (2002) [112].
[c] Experimental values reported by Kuramochi et al (2004) [137].
[d] Calculated: Henry's law const. = molecular weight (g mol^{-1}) × vapour pressure (Pa) / water solubility (mg L^{-1}).

pg m^{-3} [23]. BTBPE and DBDPE were detected in atmosphere of Great Lakes (North America) with average concentrations ranging from 0.5 to 1.2 pg m^{-3} and 1 to 22 pg m^{-3}, respectively, and were mainly associated to the particulate phase [134]. PBEB was also detected in air samples from the Great Lake region [135], and was mainly present in the gaseous phase [136].

Few studies report the partition of NBFRs in the aquatic environment. De Jourdan et al. (2013) [138] studied the partition and fate of EHTBB, BEHTBP, and BTBPE in an aquatic mesocosm (water + sediment) experiment performed along 70 days. In that study, EHTBB was detected only in suspended particles, while BTBPE and BEHTBP were detected in both, suspended particles and sediment, and resuspension from sediments was observed. Xie et al. (2011) [118] analyzed PBT, DPTE and HBB in the dissolved and particulate phases of seawater samples (Atlantic and the Southern Ocean), where PBT was not detected, DPTE was most abundant in the particulate phase (nd − 0.77 pg L^{-1}) and HBB was only detected in the dissolved phase (nd - 0.02 pg L^{-1}). At a natural pond in an e-waste recycling site (China), average amounts of DBDPE, BTBPE, HBB, PBEB and PBT in sediments were 1796±770 ng g^{-1} wet weight (ww), 4554±608 ng g^{-1} ww, 8672±1053 ng g^{-1} ww, 132±6.12 ng g^{-1} ww and 20.6±2.89 ng g^{-1} ww, respectively, and their respective concentration in the dissolved phase were <LOD, 0.02±0.01 ng L^{-1}, 0.52±0.04 ng L^{-1}, 0.06±0.00 ng L^{-1} and 0.03±0.01 ng L^{-1} [139].

NBFRs have been detected in sediments and their concentration is generally one to two orders of magnitude lower than the observed ones for PBDEs, especially for BDE-209 [140-142]. This fact can be associated to their lower production/use than PBDEs, their faster degradation in the environment, their harder transportation from sources to environmental water, or a combination of these factors. In surface sediment of the Great Lakes, DBDPE ranged from 0.11 to 2.8 ng g^{-1} dry weight (dw), HCDBCO ranged from 0.21 to 2.3 ng g^{-1} dw and BTBPE ranged from 0.13 to 8.3 ng g^{-1} dw, while BDE-209 ranged from 0.87 to 106 ng g^{-1} dw [142]. HBB, DBDPE, BEHTBP and EHTBB were not detected in sediments from San Francisco Bay (USA), whereas PBEB and BTBPE were detected at median concentrations of 0.01 ng g^{-1} dw and 0.02 ng g^{-1} dw, respectively, and ΣPBDE ranged from 2 to 8 ng g^{-1} dw [140]. In sediments from a Spanish River (n=19), PBEB ranged from 3.1 to 10 ng g^{-1} dw (n=13), HBB ranged from 0.4 to 2.4 ng g^{-1} dw (n=3), DBDPE ranged from 4.8 to 24 ng g^{-1} dw (n=19) and ΣPBDE ranged from 22 to 136 ng g^{-1} dw (n=19) [141]. The River Yadkin, a river historically contaminated by WWTP discharges from textile industries, BEHTBP ranged from 2000 to 19 200 ng g^{-1} TOC, EHTBB ranged from 80 to 3850 ng g^{-1} TOC, BTBPE ranged from 77 to 2000 ng g^{-1} TOC, while BDE-209 ranged from 61 500 to 298 000 ng g^{-1} TOC [132]. No data is available concerning DPTE concentration in sediments.

Regarding chemical transformations of NBFRs in the aquatic environment, it can be expected that these flame retardants undergo photodegradation since all of them (except HCDBCO) have aromatic groups [122]. EHTBB and BEHTBP have ester groups which are potentially susceptible to hydrolysis [96]. Wang et al. (2012) [143] studied the photodegradation of DBDPE under UV irradiation, and observed a slower degradation in humic acid/water than in the hexane or tetrahydrofuran solvents, which indicated that humic acid acts decreasing the degradation rate of DBDPE in the aquatic environment. Photodegradation of EHTBB and BEHTBP in three different solvents (methanol, tetrahydrofuran and toluene) was studied and the results indicated that the degradation rate of EHTBB was higher than of BEHTBP in all solvents (except in toluene), attributed to the greatest steric hindrance of BEHTBP, which affected its indirect photolysis [144]. In addition, the authors observed that BEHTBP and EHTBB photodegrade slower than nona-BDE, attributed to their lower aromatic content. De Jourdan et al. (2013) [138] observed that BEHTBP undergo hydrolysis in an aquatic mesocosm experiment, which resulted in formation of an anhydride. In this same study, generation of 2,4,6-tribromophenol was observed in the mesocosms treated with BTBPE, which indicates that ether cleavage would have occurred, but the mechanism of reaction was not proposed. No data concerning the photodegradation or other chemical transformation in water are available for the other studied NBFRs.

Few data is available concerning biodegradation of NBFRs. HBB presented faster biodegradation in soil under aerobic conditions than under anaerobic conditions, and presented half-life times of 22 and 120 days, respectively [127]. In another study, microbial activity and degradation of brominated flame retardants in soil was evaluated, and DPTE degraded (half-life time - 98 days) more rapidly than the PBDEs, which showed no declines over the 360 day period [145].

In conclusion, these recent studies indicated that the concentration of NBFRs tend to be lower than the ones observed for PBDEs, but the lack of information regarding their sources, partition and transformations in water do not allow a good understanding of their fate and impact in the aquatic system.

1.4.3 OPFRs

OPFRs present different chemical structures (substituent bound to oxygen) and physicochemical properties, which affect their fate in the aquatic environment. The chloroalkyl phosphates are more soluble than the aryl phosphates, while solubility and lipophilicity of alkyl phosphates vary. Table 4 presents the physicochemical properties of studied OPFRs.

WWTP discharges are considered the most important source of OPFRs to the aquatic environment [146]. The fate of OPFRs in WWTPs depends on their partition between water and sludge, and on their persistence. TCEP, TCPP and TDCP are not eliminated in WWTPs using conventional biological treatment, while alkyl and aryl phosphates are partially removed from water, but are still detected in the effluents [33, 147]. Of the total OPFRs amount reaching annually Swedish WWTPs, about 50% was estimated to be present in the effluents (27 tonnes) and only 1% (0.5 tonnes) end up in the sludge [33]. Lipophilic OPFRs tend to accumulate in sludge, but more soluble ones such as TCEP and TBEP (the most abundant compounds in wastewater) were also detected [33]. The presence of OPFRs in the raw water supplied to drinking water treatment plants have been reported, and because some of these compounds are not completely removed during the treatment in these facilities, they are detected in drinking water at ng L^{-1} levels [34, 148].

Table 4 – Physicochemical properties of studied OPFRs.

	Water Solubility[a] (mg L^{-1} at 25°C)	Vapour pressure[a] (Pa at 25°C)	Henry's law const.[a] (Pa·m^{3}/mol at 25°C)	log K_{ow}[a]	log K_{oc}[a]
TCEP	5000 at 20 °C	6.67	8.07×10^{-3}	0.54	2.04
	6000	8.22	1.5×10^{-5} at 20 °C	1.43	2.48
	7000 at 20 °C	7.9×10^{-4}	4.16×10^{-5}	1.48	
	7820 at 20 °C	0.0521	2.58×10^{-3}	1.7	
	8000 at 20 °C			1.78	
TCPP	1080	3.3	4.25×10^{-4}	2.59	2.44
	1100	1.4×10^{-3}	6.04×10^{-3}	2.68	2.76
	1200	7.52×10^{-3}		3.33	3.11
	1600				
TDCP	7.0	5.6×10^{-6}	1.33×10^{-4}	3.65	2.96
	18.1	3.97×10^{-5}	2.65×10^{-4}	3.69	4.09
	19.2	1.3 at 30 °C		3.74	3.96
	100 at 20 °C			3.76	
				3.8	
TBEP	1100	2.8×10^{-5}	1.22×10^{-6}	3.75	3.01
	1100-1300 at 20 °C	2.41×10^{-5}		3.65	5.67
		1.64×10^{-4}			
TBP	280	0.15	0.0152	2.5	3.13
	422	0.016	0.323	3.4	3.28
	400 at 20 °C	1.2		3.99	
	1000	0.904		4.00	
		9		4.01	
		0.465			
TiBP	265	0.95	0.323	3.72	2.99
		1.71			3.05
TEHP	<100 at 20 °C	1.1×10^{-5}	0.00796	4.1	5.79
	2	2.94×10^{-3}	9.69	4.23	6.36
	0.60	8.09×10^{-5}		5.04	
	<0.5 at 20 °C				
TPhP	1.9	4.1×10^{-3}	0.335	4.59	3.42
	2.1±0.1	2.4×10^{-3}	0.00403	4.61	4.00
	0.730	8.52×10^{-4}		4.63	3.93
	20	0.0707		4.76	
		6.29×10^{-5}		3.15	
				3.40	
				3.9	
TCP	0.36	6.6×10^{-5}	0.00542	5.11	3.67
	0.34±0.04	0.0133 at 20 °C	*m*-isomer: 8.38	5.12	1.618±993
	3.4 at 20 °C	8.76×10^{-6}		5.1-5.3	*o*- and *m*-isomers: 4.37
	0.260	*o*-isomer: 5.5×10^{-5} at 20 °C		5.9	
	p-isomer: 0.3 at 25 °C	*o*-isomer: 2.26×10^{-4}		5.93	*p*-isomer: 4.35
	p-isomer: 0.074	*o*-isomer: 1.95×10^{-3}		3.42	
		o-isomer: 6.33×10^{-3}		4.51	
		m-isomer: 9.9×10^{-5} at 20 °C			
		m-isomer: 1.84×10^{-6}			
		m-isomer: 1.21×10^{-5}			
		m-isomer: 3.74×10^{-6}			
		m-isomer: 1.45×10^{-5}			
		p-isomer: 4.4×10^{-5} at 20 °C			
		p-isomer: 2.94×10^{-6}			
		p-isomer: 4.65×10^{-6}			
EHDP	1.90[b]	8.67×10^{-5}[b]	0.025[b]	5.73[b]	4.21[b]

[a] Revised values (software estimated values for water solubility and K_{ow} not here included) by Verbruggen et al (2005) [87].

[b] Revised values by van der Veen and de Boer (2012) [20].

An evidence of long-range transport of OPFRs was recently reported [149], where they were monitored in the ocean atmosphere of the Northern Pacific and Indian Ocean toward the Polar Regions. OPFRs were mainly associated to atmospheric particles, TCPP and TCEP were the most abundant compounds (including the polar regions), and ΣOPFRs ranged from 120 to 2200 pg m^{-3} [149]. Precipitation after atmospheric transportation was suggested as a source of detected OPFRs in lake water (rural area) in Germany [150]. These same authors reported the presence of OPFRs in rainwater and pointed out the importance of precipitation as an entry-pathway, by wet deposition and runoff, for OPFRs in the aquatic environment [151].

Few studies regarding the presence of OPFRs in the aquatic environment are reported. Most of the available data concern the occurrence of OPFRs in the water phase, only some studies report their presence in sediments, whereas their partition in the aquatic environment (water/suspended particles/sediment) is still quite unknown. TCPP and TBEP are frequently the most abundant OPFRs in water [30, 34, 146], and their presence in sediments has been reported [30]. On the other hand, aryl phosphates were not detected or were at very low concentrations in water [30, 32, 34], and tend to accumulate in sediments [152]. Martinez-Carballo et al. (2007) [30] reported the occurrence of OPFRs in water and sediments from three Austrian rivers, and found that TCEP, TCPP, TBEP, TBP, TPhP and triethyl phosphate were detected in waters and sediments, TDCP was only detected in water, whereas TEHP and TCP were only detected in sediments.

Persistence among chloroalkyl, alkyl and aryl phosphates in the aquatic environment vary greatly since they behave differently under chemical and biological transformations. In theory, all organophosphate esters are potentially susceptible to undergo hydrolysis but it is expected to be slow in environmental relevant conditions, and the velocity of reaction varies among compounds [81]. Additionally, some compounds, such as aryl phosphates, can undergo direct photolysis, while others suffer indirect photodegradation. Concerning biodegradation, aryl phosphates appear to be more rapidly degraded than alkyl phosphates, while chloroalkyl phosphates are not biodegradable [153]. Thus, chloroalkyl phosphates are persistent in natural water since they are resistant to hydrolysis, indirect photodegradation and biodegradation [81, 83, 150]. Biodegradation and indirect photodegradation were indicated as the main transformation processes affecting the persistence of TBP, TiBP and TBEP in natural water (lake water) [150]. TPhP and TCP undergo hydrolysis, and reaction velocity increases with pH [87, 154]. TPhP and TCP are also easily biodegraded and can undergo direct photolysis [87]. Thus, considering OPFRs in the dissolved phase, it can be expected that their persistence follows as chloroalky phosphates > alkyl phosphates > aryl phosphates. However, their different partitions between dissolved phase and sediments need to be also considered and better understood for an appropriate assessment of their fate and persistence in the aquatic environment.

1.5 EU directives regarding flame retardant additives

The use of Penta-BDE and Octa-BDE formulations in Europe was prohibited in 2004, and more recently, in 2008, the use of Deca-BDE formulations in electric and electronic equipments was also banned. The directives and decisions adopted in the EU concerning the use of PBDEs are described below:

- Directive 2003/11/EC [105], banning the use/market of Penta-BDE and Octa-BDE formulations, or any other chemical formulation, or article, containing more than 0.1% by mass of these substances, from all uses after August 2004.
- Directive 2002/95/EC [155] (RoHS Directive) - restricting the use of substances considered hazardous to health in electrical and electronic equipment from July 2006. This directive prohibited the use of PBDEs in electrical and electronic equipments, among other substances.
- Directive 2002/96/EC [156] controlling the recovery and recycling of waste electrical and electronic equipment (WEEE directive), which included a regulation for wastes containing PBDEs.
- Commission Decision of 13 October 2005 [157] exempting Deca-BDE from Directive 2002/95/EC and as a consequence, also from the Directive 2002/96/EC. This exemption was annulled in 2008 [158], since then the use of Deca-BDE in electrical and electronic equipment was banned in Europe.
- Directive 2011/65/EU (RoHS 2) repeals the original RoHS Directive (Directive 2002/95/EC [155]) and introduces a methodology for substance restrictions and opens the scope covering all electrical and electronic equipment except for thoses that are specifically excluded. The maximum PBDE concentration tolerated in homogeneous materials is 0.1 % by weight.

1.5.1 Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

The REACH is an EU regulatory framework for **R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emicals, adopted on 18 December 2006, to improve the protection of human health and the environment while maintaining the competitiveness and enhancing the innovative capability of the EU chemicals industry. The REACH Regulation places responsibilities on industry to manage the risks from chemicals and to provide safety information on the substances. To comply with the regulation, companies have to register substances produced in or imported to the EU, provide information on the properties, uses and other necessary information on chemicals, make an assessment of the hazards and potential risks presented by the substance, and inform downstream users of the chemical composition of their products. The European Chemicals Agency (ECHA) is the responsible for the operation of REACH, which together with the Member States, will evaluate the information submitted by companies. If a given substance constitutes a risk for the human health or the environment, the Member State, or ECHA (on request of the Commission), may propose its identification as a Substance of Very High Concern (SVHC). Following the identification as SVHC, the substance is added to the Candidate List, which includes candidate substances for possible inclusion in the Authorisation List (Annex XIV of the REACH Regulation). A substance in the Authorisation List cannot be placed on the market or used after a given date, unless an authorisation is granted for their specific use, or the use is exempted from authorisation. If a chemical poses an unacceptable risk that needs to be addressed on an EU-wide basis, a Member State or ECHA (on request of the Commission) may propose a restriction on the manufacturing, placing on the market or the use of that chemical of concern [159].

Deca-BDE was successfully registered under REACH in September 2010. Deca-BDE was previously evaluated under the previous regime to REACH, the Existing Substances Regulation (ESR). An EU risk assessment previously conducted concluded that there was no need for restrictions as no significant risks were identified for the use of deca-BDE [160]. The results of the Deca-BDE Risk Assessment have been transferred into the REACH registration dossier submitted to the European Chemicals Agency (ECHA). Latter, on the 3rd of August 2012, the UK Competent Authorities submitted an Annex XV dossier to ECHA proposing deca-BDE as a substance for inclusion in the REACH Candidate List [161]. On 19 December 2012, deca-BDE was included in the Candidate List [162].

HBCD has already been subjected to an evaluation under the ESR Risk Assessment procedure, and was assigned as having persistent, bioaccumulative and toxic properties. In October 2008, HBCD was included by ECHA in the Candidate List [161]. In February 2011, the European Commission included HBCD in the amended Annex XIV of substances for authorisation under REACH [163].

TBBPA was registered under REACH in October 2010. On 18 June 2008, the European Commission officially published the Risk Assessment on TBBPA [164], which concluded that risks are not expected for workers, consumers and humans exposed via the environment, atmosphere, and micro-organisms in WWTPs, whereas a need for further information and testing for assessment of the risk to aquatic and terrestrial ecosystem was concluded. There are currently no ongoing processes under REACH for TBBPA [161].

On 22 December 2009, TCEP was included in the Candidate List for authorisation under REACH, due meeting the criteria for classification as toxic to reproduction [162].

1.5.2 Water Framework Directive (WFD)

The Water Framework Directive (WFD) [165], establishes a Community framework for water protection and management. Its main focus is the establishment of a strategy for dealing with chemical pollution of water. Among other purposes, this directive aims to contribute to the progressive reduction of emissions of hazardous substances to water, promotes sustainable water use, prevent further deterioration and protect aquatic ecosystems. In 2001, the Decision 2455/2001/CE [166] established a priority list of 33 substances in the field of Water Policy, including brominated diphenylethers (only Penta-BDE: 28, 47, 99, 100, 153, 154), identified as hazardous substances. Afterwards, the Directive 2008/105/EC [167] determined environmental quality standards (EQS) for the priority substances, and concerning brominated diphenylethers (only Penta-BDE), an EQS of 0.0005 $\mu g\ L^{-1}$ (annual average concentration in inland surface water) was established.

1.5.3 Stockholm Convention on Persistent Organic Pollutants (POPs)

The Stockholm Convention on Persistent Organic Pollutants (POPs), supported by the United Nations Environment Program (UNEP), is an international agreement that emerged as a result of international concerns regarding the threats posed by POPs to the environment. POPs are organic compounds that remain intact for long periods of time (years), are widely distributed, accumulate in the fatty tissue of living organisms (including humans), biomagnify, and are toxic to both humans and wildlife. The main objective of Stockholm Convention is to protect human health and the environment from the negative effects caused by POPs. The Stockholm Convention on POPs provides a framework, based on the precautionary principle, to ensure the safe disposal and decreased production and use of harmful substances. The Convention was initially adopted by 150 States, including EU Member States, and by the Council on behalf of the EU, at a conference held in Stockholm on 22 and 23 May 2001. Twelve POPs, the so called "dirty-twelve"[8], were established in that occasion, which included pesticides, industrial products and substances unintentionally produced. The Convention entered into force on May 17, 2004. Afterwards, in the fourth meeting held on May 2009 (Geneva), other nine compounds[9] were included in the POP list, which incorporated Penta- and Octa-BDEs. Nowadays, HBCD is proposed for listing under the Stockholm Convention [168].

The Regulation 850/2004/EC [169] was established in response to Stockholm Convention and also in response to the Decision 2004/259/EC[10] [170], and refers specifically to the production, marketing, use, discharge and disposal of POPs. In order to update the list of substances included in the Stockholm Convention meeting of 2009, this regulation has been amended by regulation 756/2010 [171] and regulation 757/2010 [172].

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[8] The 12 initial POPs: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF).

[9] The new POPs: chlordecone, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, lindane, pentachlorobenzene, hexabromobiphenyl, tetrabromodiphenyl ether and pentabromodiphenyl ether (Penta-BDE), hexabromodiphenyl ether and heptabromodiphenyl ether (Octa-BDE), pentachlorobenzene, perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride, By-products: alpha hexachlorocyclohexane, beta hexachlorocyclohexane and pentachlorobenzene.

[10] This Decision approves the 1998 Protocol to the Convention on Long Range Transboundary Air Pollution on Persistent Organic Pollutants of 1979.

1.6 Objetives

Taking into account the concern about the presence of PBDEs in the environment, the increased use of alternative flame retardants, and the scarce available information concerning the fate and effects of those compounds in rivers, the overall aim of this thesis was to evaluate the occurrence and impact of priority and emerging flame retardants in the aquatic environment. To accomplish this main objective, the present thesis has two sections. The first includes the development and validation of multiresidue methods for the analysis of different families of flame retardants in water, sediment, dust and sewage sludge. The second part includes the study of the occurrence and distribution of PBDEs, NBFRs and OPFRs in Spanish and UK rivers with the purpose to identify hotspot zones along the rivers and to assess potential risks to aquatic organisms. Since WWTPs are indicated as an important source of flame retardants to the aquatic environment, the presence of flame retardants in influent waters, the elimination efficiency and the accumulation in sludge were studied, in order to evaluate the impact of WWTP discharges to surface waters.

The specific objectives were:

1. To develop a multiresidue method for the simultaneous determination of PBDEs, NBFRs, OPFRs, by-products and formulation intermediates, based on gas chromatography coupled to mass spectrometry. To compare the analytical performance under different ionization (EI and ECNI) and data acquisition modes. To calculate quality parameters such as limits of detection and repeatability. According to the results obtained, to propose a method for the determination of FR in water which provides good sensitivity and identification capabilities.

2. To develop and validate a multiresidue method for extraction and analysis of PBDEs, NBFRs and OPFRs in sediments, sewage sludge and dust, using GC-EI-MS/MS.

3. To calibrate and evaluate the performance of the Ceramic Dosimeter passive sampler for the monitoring OPFRs and NBFRs in surface water.

4. To study the occurrence and distribution of flame retardants in the River Aire (UK), and to evaluate the impact of anthropogenic sources and assess the risks to aquatic organisms.

5. To monitor the presence and partition of flame retardants in water and sediments along three Spanish rivers differing in anthropogenic pressures. To identify the most ubiquitous flame retardants in water and evaluate their joint effects using *D. magna* toxicity tests. To relate the flame retardant levels observed along the studied rivers to potential risk to aquatic organisms.

6. To evaluate the occurrence and elimination of OPFRs, NBFRs and PBDEs in five Spanish WWTPs.

2 Analytical Methods

2.1 Introduction

Organic contaminants in the environment are frequently present at trace concentrations[1]. Technologic advances in analytical chemistry that took place over the last 30 years, in terms of sample preparation and instrumental techniques, have permitted the determination of organic contaminants in environmental matrices at increasingly lower levels. The sample amount available, the extraction techniques and the instrumental detection limits have to be well scaled, and time and costs of analysis must be considered.

Multiresidue methods proved to be very advantageous for the analysis of environmental samples, since they allow the simultaneous determination of contaminants of several chemical families, decreasing analysis time and, often, cost. Therefore, the detection of a large number of compounds with the few number of extractions and clean-up steps is desirable. For this purpose, finding the best arrangement among methodology of extraction/purification and instrumental technique is essential in order to have an analytical method selective and efficient enough for the detection of all target compounds with minimum sample manipulation to avoid the presence of interferences and/or coelutions. One of the aims of this study was to develop multiresidue methods able to detect flame retardants of different families in water, river sediments and sewage sludge, with an efficient sample preparation. In addition, a preliminary study was performed for the development of a passive sampler for determination of flame retardants in river water.

In this introduction, a short review of the most used methods for analysis of PBDEs, NBFRs and OPFRs described in literature is presented, including considerations about the use of passive sampling techniques for organic contaminants in water.

2.1.1 Sample preparation

The analysis of trace contaminants in environmental samples require, in most of cases, a step of sample preparation, extraction and purification, in order to isolate the compounds of interest, allowing their detection and minimizing interferences. The selection of the appropriate techniques for the extraction, preconcentration and clean-up depends on the type of sample and on the chemical characteristics of the analytes.

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[1] In analytical chemistry, a trace substance is a substance in a sample that has an average concentration of less than 100 micrograms per gram.

FR extraction from water

Solid phase extraction (SPE) and liquid-liquid extraction (LLE) are the most traditional techniques used for the extraction of organic compounds from water samples, and have been used for PBDEs, NBFRs and OPFRs. However, extraction methods with no or minimum use of organic solvents such as solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and dispersive liquid-liquid microextraction (DLLME) have also been reported.

PBDEs: SPE cartridges [173] and SPE disk [174-175] with C_{18} adsorbent have been used for the extraction of PBDEs in water. Sánchez-Avila et al. (2009) [92] compared the performance of three SPE cartridges, ENV+ (hydroxylated polystyrene divinylbenzene co-polymer), LC-18 and Oasis HLB for the multiresidue analysis of EU priority contaminants (PBDEs included), and the best performance for PBDEs and remaining compounds was obtained using Oasis HLB cartridges [92]. Used solvents for PBDE elution include hexane, cyclohexane, dichloromethane, acetone, and ethyl acetate, which are used alone or as solvent mixtures. A LLE method using dichloromethane was reported for the extraction of PBDEs and other 61 compounds from river water [176]. Several authors propose the use of SPME [177-178], SBSE [179-180] or DLLME [181-182] methods for extraction of PBDEs in environmental water in replacement of classical SPE and LLE extractions for reducing handle time and solvent consumption, and allowing low limits of detection.

NBFRs: SPE is the most used technique for NBFRs extraction from water samples. A multiresidue method for analysis of several families of brominated flame retardants in wastewater was reported using Empore® Speed Disk and elution of analytes with an ethanol/toluene mixture, followed by a clean-up step using a multi-stage silica chromatographic column and an alumina column [183]. For the analysis of PBDEs and several NBFRs (HBB, PBEB, PBT, DPTE, BTBPE and BEHTBP) in sea water, about 1000 L of water was collected and directly pumped by a ship's intake system through a GFF filter (to collect the particulate phase) followed by a glass column packed with PAD-2 for the preconcentration of analytes [28]. Amberlite XAD-2 resin was also used for the collection of NBFRs (DBDPE, BTBPE and PBEP) from melted ice cores [184] and from larger volume water samples [54], and extracted using methanol and DCM.

OPFRs: Bacaloni et al. (2007) [185] compared the performance of three different cartridges, LC-18, Oasis HLB and Bakerbond (Hydrophilic-DVB), for the simultaneous extraction of 12 OPFRs from water, using methanol as elution solvent. Bakerbond and Oasis HLB cartridges allowed recoveries higher than 90% for 10 of 12 analyzed OPFRs, while the authors recommended the use of Bakerbond cartridges due its best performance for TCP and due its faster extraction. For the extraction of 7 OPFRs from wastewater, Meyer and Bester (2004) [147] used DVB-hydrophobic Speedisks and elution with methyl *tert*-butyl ether, followed by a clean-up step using silica gel column. SPE methods using Oasis HLB [186], Oasis Max [187] an styrene-divinylbenzene copolymer [150] cartridges for water extraction were reported. For surface water, recoveries from 89 to 107% for 7 OPFRs were obtained using LLE with toluene, but a low recovery (31%) was observed for TCEP [146]. LLE using dichloromethane for river water extraction was also reported [30]. García-Lopez et al. (2007) [188] reported a DLLME method for environmental water using acetone/tricloroethane (98:2).

FR extraction from sludge and sediment

Sludge and sediments are one of the most complex environmental samples. Solvents used for the extraction of flame retardants also co-extract large amounts of undesired non-polar and semi-polar compounds that can cause matrix interference, thus an efficient clean-up step is needed in order to allow their analysis by chromatographic techniques. Sample pre-treatment frequently include a freeze-drying step [189-190], which is a technique that allows water elimination by sublimation without losses of compounds. Another option is homogenise the wet sample with anhydrous Na_2SO_4 before extraction [191-192]. Sediments and sludge contain high content of sulphur, which can distort the chromatographic performance [193]. Sulphur can be eliminated using elemental cooper powder/granulates, to form a metal sulphide, which can be added before extraction [194], added to the extract [190], or placed in a column together with adsorbents used for clean-up [195]. Sulphur removal was also achieved using silver nitrate [196] and tetrabutylammonium sulphite [197]. Extraction and clean-up techniques used for the analysis of PBDEs, NBFRs and OPFRs in sediments and sludge are described in the following section.

PBDEs: Soxhlet extraction is the most classical and one of the most used techniques for PBDE extraction from sediments [31, 120, 198-199] and sludge [190, 200-201]. Soxhlet extraction is generally performed during 24 or 48 h, and used solvents include dichloromethane, toluene, or mixtures of solvents such as hexane/dichloromethane (1:1, v/v), hexane/acetone (1:1, v/v). The main disadvantage of this extraction technique is the large consumption of solvents, frequently ≥ 200 mL. Techniques that require a lower volume of solvent and a shorter extraction time, such as ultrasonic assisted extraction (UAE) [194], pressurized liquid extraction (PLE) [189, 192, 202] and microwave assisted extraction (MAE) [191, 203] have been successfully used. Many brominated FRs are stable under strong acid conditions, and sulphuric acid treatment is the most used clean-up technique due its efficiency for removal of the organic matter [195]. Sulphuric treatment can be performed by direct addition of the acid to the sample extract, followed by sequential LLE and centrifugation steps [191-192]. Another option is the use of a silica gel column impregnated with sulphuric acid [204]. This last approach avoids the formation of emulsions and reduces sample handling and solvent consumption [195]. Gel permeation chromatography [189, 203] and non acidified adsorbents, such as silica [194], alumina [205] and florisil [206] have also been used as clean-up.

NBFRs: The detection of NBFRs was mostly performed as additional information resulting from the analysis of major brominated FRs (PBDEs, HBCDs and TBBPA), and only few analytical procedures have been optimized specifically for NBFRs [19]. The extraction and clean-up protocol used for NBFRs in sediments and sludge were generally the same ones used for PBDEs. Extraction have been performed by Soxhlet or PLE, and used solvents are hexane/acetone, hexane/dichloromethane and toluene [207]. Both, destructive (sulphuric acid, acidified silica) and non-destructive (neutral silica, alumina, florisil and GPC) clean-up methods have been used [207].

OPFRs: Few analytical protocols were reported concerning the analysis of OPFRs in sediments and sludge. Martínez-Carballo et al. (2007) [30] reported a method for the extraction of nine OPFRs from sediments based on UAE with ethyl acetate/acetonitrile (30:70, v/v), and no clean-up step was used. Cao et al. (2012) [208] reported a method for the analysis of OPFRs in sediments which consisted in a primary sediment extraction by UAE using acetonitrile/water (25:75, v/v). After that, the extract was diluted to 500 mL using ultrapure water and subjected to SPE using OASIS HLB cartridges, followed by elution with ethyl acetate. In an interlaboratory study [209], UAE, ASE and Soxhlet were used for the extraction of OPFRs from a sediment sample. Most used solvents included mixtures of hexane, acetone, dichloromethane and ethyl acetate, and clean-up methods used by the participants included GPC and adsorbents such as florisil, HLB, NH_2-SPE and alumina. For sewage sludge, ASE with ethyl acetate was used, and the concentrated extract was subjected to clean-up using GPC and silica column [33].

2.1.2 Separation and detection

Gas chromatography (GC) and high resolution liquid chromatography (HPLC) are the principal separation techniques used for brominated FRs and OPFRs. Different instrumental conditions and detection techniques are used for the analysis of flame retardants, which depends on their volatility, thermal stability and chemical structure. GC is the main separation technique used for PBDEs and NBFRs due their high volatility. Non-polar or semi-polar columns with 15 to 30 m length have been used for the separation of PBDE congeners [210-211]. High brominated PBDEs (especially BDE-209) and DBDPE are thermally labile and undergo degradation in >15 m chromatographic columns. Limits of detection for BDE-209 and DBDPE increase significantly when using short columns (15 m or shorter) with 10 µm of film, due to minimization of residence time in the column, avoiding their thermal degradation [193]. For OPFRs, both GC and HPLC have been employed for the analysis of environmental extracts. For GC analysis, non-polar used columns include HP5-MS ((5%-phenyl)-methylpolysiloxane) [24, 212], DB5-MS (phenyl arylene polymer, equivalent to (5%-phenyl)-methylpolysiloxane) [33, 93, 147], HP1 (100% dimethylpolysiloxane) [213], and HT8 (8% phenyl (equiv.) polycarborane-siloxane) [214]. For HPLC separation, C18 [185-187, 215] and C8 [30] columns were used, and mobile phases generally consisted on water and methanol containing formic acid or ammonium acetate.

GC coupled to electron capture detection (ECD) or to mass spectrometry (MS) are typically employed for determination of brominated FRs in environmental samples. Halogenated compounds can be analyzed by GC-ECD, although it lacks of specificity and results have to be confirmed by GC-MS or either using a column of different polarity. Despite this technique provides low limits of detection and it is rather inexpensive, the selectivity is a problem for the analysis of complex samples, since coelutions with halogenated compounds can result in false positives [216]. Because of this disadvantage, this technique has been replaced by GC-MS techniques, operated in either electron ionization (EI) mode or, more frequently, in electron capture negative ionization (ECNI) mode. ECNI mode involves the ionization of a reagent gas (i.e. ammonia, methane, *iso*-butane) as a result of the collision with high energy electrons emitted from the source filament. This collision generates less energetic thermal electrons which ionize molecules with high electron affinity. Under ECNI, PBDEs are ionized through a dissociative electron capturing process, producing primarily bromide ions $[Br]^-$ [193]. Monitoring bromide ion gives very low instrumental limits of detections (iLOD) for PBDEs, reaching detection of less than 1 fg to about 400 fg (BDE-209) injected in the GC-MS system [193]. Bromide ion detection is selective for brominated compounds but frequently gives no further structural information, and so identification is based only in the retention time. Coelution with metabolic PBDE products and other natural or synthetic brominated compounds can be a problem for the analysis of complex environmental extracts [216]. Despite its higher iLOD, EI mode is also frequently applied for the analysis of brominated flame retardants in environmental extracts due its higher selectivity [92, 195, 205]. EI generally provide full spectral information for brominated FRs, and is more selective than ECNI when operating in single ion monitoring (SIM) mode. Mass spectrometry in tandem (MS/MS) operating in EI mode improves the selectivity and decrease background noise. Even if a co-eluting compound has the same precursor ion than the target analyte, misidentification is practically eliminated because the interfering compound often generates a different product ion under collision-induced dissociation. GC-EI-MS/MS have been employed for the analysis of PBDEs [217-219]. High resolution mass spectrometry (HRMS) offers both, good sensitivity and high selectivity. Thomsen et al. [220] obtained similar limits of detection for PBDEs in biological samples using both GC-EI-HRMS and GC-ECNI-MS. However, initial costs, operation and maintenance are higher for HRMS than by the more conventional MS techniques. Apart from the concept of sensitivity and selectivity, one of the major advantages of using EI for brominated FRs analysis is that it allows the application of ^{13}C-labeled standards for a more accurate determination of environmental concentrations [216].

The most used detectors for the analysis of OPFRs performed by GC are nitrogen–phosphorus detector (NPD) and MS [221]. GC-NPD presents a high sensitivity for phosphorus containing compounds, but selectivity can be unsatisfactory [222]. Another disadvantage of GC-NPD is that this technique does not permit the use of isotopically labelled standards for quantification. OPFRs have been determined by GC-MS operated in either EI or positive chemical ionization (PCI) mode. OPFRs, particularly many aliphatic triesters, present a poor fragmentation due to consecutive McLafferty rearrangements, resulting in an EI-MS spectrum with few fragments, and with a base peak at *m/z* 99 (protonated phosphoric acid). Monitoring this fragment can be disadvantageous since matrix can interfere with low mass ions, and so other less abundant fragments are frequently used for quantification, which result in higher iLOD [221]. GC-PCI-MS is suggested as a better alternative for the analysis of OPFRs in environmental samples given that lower energies are involved. Thus, OPFRs are less fragmented, resulting in limits of detection comparable to the ones obtained by GC-NPD, and lower than by GC-EI-MS [222]. Determination of OPFRs by LC-MS, generally operated in tandem MS and operated in positive mode, presents as advantage a higher selectivity and sensitivity than the one obtained by GC-EI-MS and GC-PCI-MS, since molecular ions are generated and because selected reaction monitoring (SRM) is more selective than SIM [221].

2.1.3 Passive sampling for pollutant monitoring in water

The development and application of passive sampling techniques for the monitoring of organic compounds in the aquatic environment have increased in the last decade. The advantages of this sampling technology for monitoring studies include: *in situ* pre-concentration of the analyte in the passive sampler device; reduction and/or elimination of matrix interference; cost effectiveness; little training required to handle the devices; no need of power sources for operation; and the time-weighted average concentration estimation, which is a valuable information in monitoring programs, given that pollutant concentration in water can vary continuously in the environment [223].

Passive sampling is a technique based on the free flow of an analyte from the sampled medium to a sorbent in a sampling device as a result of a difference between the chemical potential of the analyte in the two media [224]. Different passive sampler designs are available for the monitoring of contaminants in water, and most of them consist of a barrier phase between the sampled medium and the sorbent that will accumulate the analyte (also called receiving phase). The transport of analytes through the barrier occurs mainly by diffusion following Fick's laws, and the accumulated mass in the receiving phase is proportional to the average water concentration during the deployment time. Barrier phases for passive samplers for water monitoring are generally made of polymers such as polyethylene, polydimethylsiloxane, polysulfone, regenerated cellulose, silicone-polycarbonate, cellulose acetate, polytetrafluoroethylene (PTFE), nylon, polypropylene, polyvinyl chloride, etc, and by porous materials [223]. Based on the properties of the barrier, passive samplers fall into two categories: diffusion-based or permeation-based passive samplers.

When a passive sampler is deployed in water, the flow of a chemical substance into the passive sampling device continues until the equilibrium of the system is reached or until the sampling period is stopped. The uptake of an analyte can be represented as shown in Figure 6. In the initial time of deployment, the uptake of analytes is linear since their desorption rate from the receiving phase to water is negligible. When the exposure time is sufficiently long, the thermodynamic equilibrium between the water and the receiving phase is achieved. In the case of passive samplers for water deployment, the time for reaching the equilibrium depends on the amount and sorption capacity of the sorbent used [223]. Passive samplers can be designed to work in the kinetic zone, in the equilibrium zone, or between both. Most of the passive samplers for the aquatic medium work in the kinetic zone [224]. The main advantage of the kinetic (or integrative) passive samplers is that it sequesters contaminants from episodic events, and their use is especially interesting in environments where water concentration is variable.

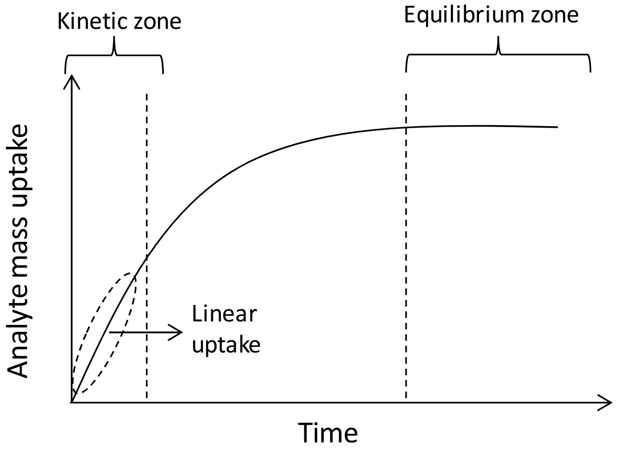

Figure 6 – Analyte uptake profile using passive sampling devices (based on [223]).

In kinetic passive samplers, it is assumed that the mass transfer rate of an analyte is linearly proportional to the difference of potential between the water and the receiving phase. The accumulated mass in the receiving phase (M_s) can be represented as:

$$M_s(t) = C_w R_s t$$

where R_s is the sampling rate, C_w is the water concentration (sampling medium) and t is the deployment time [224]. R_s can be understood as the volume of water extracted by the passive sampler per unit of time, for a certain analyte. For most passive sampler devices, R_s does not vary with C_w, but are often affected by water flow, temperature and biofouling. In practice, sampling rates of analytes for a certain passive sampler are determined by laboratorial calibration under various exposure conditions.

A boundary layer always exists at the medium/sampler interface. Depending on the passive sampling design and the water flow conditions, the boundary layer can play an important role on the uptake of analytes. Analyte transport through the boundary layer occurs by diffusion. Thus, two main processes are involved in the uptake of compounds: permeation/diffusion through the passive sampler barrier and diffusion through the boundary layer. The speed of these two processes determines to a great extent the net uptake rate. Since water turbulence affects the thickness of the boundary layer, water flow can affect the mass transfer of analytes, and the importance of this process depends on the passive sampling design. Another factor that can affect the uptake of compounds during passive sampler deployment in water is the formation of biofilm on the surface of passive samplers. Biofouling can affect the mass transfer of the analytes by increasing the thickness of the barrier or by blocking water-filled pores in diffusion limiting membranes. Temperature also influences the uptake of compounds since it affects the diffusion coefficient in the case of diffusive passive samplers, and the permeability in the case of permeation samplers. Thus, passive sampling performance needs to be evaluated regarding analytical recovery, sampling capacity, sampling rate, storage stability, and the effects of temperature, biofouling and water flow. Finally, passive and grab sampling results needs be compared in order to evaluate the effect of the environmental condition of deployment on the uptake of analytes [223].

2.2 Method development

In order to monitor the presence of different families of FRs in the aquatic environment, multiresidue methods for the simultaneous extraction and determination of these compounds in different matrices were developed. The first step of this study was to optimize instrumental conditions for the determination of all/most of proposed compounds. This first study included 4 OPFRs, 9 NBFRs, 8 PBDEs, 3 bromotoluenes (other than PBT), 4 bromoanilines, 2 bromoanisoles, 18 bromophenols[2] and 8 isotopically labelled standards. Taking into account the complexity of environmental samples and the low expected concentration of the target analytes, this study is aimed to obtain the best balance between detection limits and selectivity. GC was chosen as separation technique, and a DB5-MS column of 15 m (length) × 0.250 mm (I.D.) × 0.10 µm (film) was used given that this column decrease thermal degradation of BDE-209 and DBDPE in the column during analysis [193]. The oven temperature was optimized in order to have satisfactory separation and resolution of chromatographic peaks. Optimization of detection conditions were performed in a GC coupled to a triple quadrupole MS, operated in either ECNI or EI mode and comparison of these two ionization modes was undertaken. First of all, the fragmentation profile of each compound under each ionization mode was carried out using standard solutions. For EI mode, SIM and SRM acquisition modes were optimized. For ECNI mode, many brominated compounds presented only the ion $[Br]^-$ and so the use of SRM was impractical, thus SIM was used. GC-EI-MS/SIM, GC-EI-MS/MS and GC-ECNI-MS/SIM methods were compared in terms of iLOD, selectivity, repeatability and repetitivity. After selection of the best instrumental conditions for the determination of target compounds, GC-MS/MS was selected for the characterization of FR in water. To achieve this purpose, the hydrophilic lipophilic balance (HLB) SPE cartridges were selected taking into account previous reported studies demonstrating its feasibility for PBDEs [206] and OPFRs [186], and also based on the experience of our research group, which observed that the HLB sorbent presented the best performance for multiresidue extraction of organic compounds differing in polarity ranges [92]. Finally the proposed method was applied to the monitoring of FRs in a drinking water treatment facility (DWTF). The obtained results of this study were discussed in Paper 1.

The developed SPE method proposed in Paper 1 was tested for different types of water, including river water and wastewater (influent and effluent). Solubility and lipophilicity of studied compounds differ greatly among FR families and also among compounds belonging to the same family. Considering studied OPFRs, NBFRs and PBDEs, log K_{ow} range from about 1.5 to 12, and solubility vary from g L^{-1} to ng L^{-1}. As a broad concept, it is expected that compounds with log $K_{ow} > 5$ would be preferably associated to suspended particles, while compounds with a log $K_{ow} < 3$ would be present preferably in the dissolved phase, although it is important consider that different behaviours in partitioning for substances with similar K_{ow} frequently occur [225]. Thus, water samples were not filtered to

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[2] Bromophenols, such as 2,4 dibromophenol, 2,4,6-tribromophenol and pentabromophenol, are used as additive flame retardants in epoxy, phenolic and polyester resins, and polyolefins. Some bromoanilines, bromoanisols and bromotoluenes are degradation products and/or intermediates of flame retardant formulations.

avoid subestimation of water concentrations for the more lipophilic compounds, and thus both dissolved and particulate phases were considered. For water samples, a recovery study for evaluating matrix effects on the preconcentration performance and on instrumental response was carried out using spiked unfiltered samples. For this purpose, river water and wastewater (influent and effluent) were collected and spiked (in triplicate) at concentrations that ranged from 20 to 25 000 ng L^{-1} (see Table 5). Each original sample (non spiked) was analyzed in duplicate in order to subtract the initial sample contribution from spiked samples. The amount of sample extracted and the spiked level were chosen taking into account the expected levels of contaminants in the environment, the concentration of suspended particles (to avoid clogging of SPE cartridges), and the iLOD capabilities. 500 mL of river water, 250 mL of effluent and 100 mL of influent were extracted. In addition, internal standard quantification was performed using labelled surrogate standards in order to correct losses during extraction and matrix effects in the instrumental response.

A passive sampling method for monitoring of 4 OPFRs and 2 NBFRs in river water was developed. This study was performed during a visiting to the Lancaster University (UK), under supervision of the Professor Kevin C. Jones, who is an expert in the use of passive sampling techniques for the monitoring of organic pollutants in environmental matrices. Election of target compounds was based on their previous observed presence in the River Aire (UK). Included compounds in that study were TCEP, TCPP, TDCP, TPhP, HBB and PBEB. The ceramic dosimeter design was chosen for this purpose. It was the first study which evaluated the applicability of this passive sampler design for the monitoring of FR in river water, since to date, it had only been used for groundwater applications [226-227]. The main advantage of this device is that the uptake of compounds is independent of the water flow [228], simplifying calibration experiments, which is advantageous for monitoring studies of pollutants in river water, since water flow characteristics vary significantly among rivers, and can also vary in a same river during different periods (days, weeks). The HLB sorbent was used as receiving phase due its good performance for both, polar and apolar compounds. Laboratory calibration was carried out in order to determine analytical recovery, sampling capacity, storage stability and sampling rates. After the calibration step, the passive samplers were deployed in river water and grab sampling were collected during the deployment time. Figure 7 presents photos of the passive sampling deployment campaign in the River Aire (UK). A comparison of integrated concentration (obtained with the passive sampler) and snapshot concentration (grab sampling and SPE extraction) was performed to evaluate the efficiency of the passive sampler for the proposed FRs, and to evaluate the effects of deployment under environmental condition on the uptake of compounds. Details about theoretical bases of the ceramic dosimeter passive sampler, and the results obtained in the laboratorial calibration and field deployment were presented in Paper 2.

Figure 7 – Deployment of the ceramic dosimeter passive sampler in the River Aire (UK).

A method for the simultaneous determination of different FR families in river sediments, sewage sludge and dust was developed. Extraction was performed by UAE, which is a technique frequently applied for solid samples, using an appropriate solvent. The solid-liquid mixture (sample + solvent) is exposed to ultrasound waves, in an ultrasound bath, for a specific period of time. The extraction efficiency is due the formation of microscopic bubbles that explode generating strong shock waves, facilitating the penetration of the solvent in the sample. Clean-up of extracts was performed using commercially available florisil cartridges (10 g or 5 g), chosen due their efficiency in the elimination of fat and lipids, and due its applicability for OPFRs and PBDEs [206, 209]. Initially, two mixtures of solvents were proven: hexane/acetone (1:1, v/v) and ethyl acetate/cyclohexane (5:2, v/v). A sludge sample was spiked at 200 μg kg^{-1} of standards (except BDE-209 that was at 2000 μg kg^{-1}) and submitted to extraction with both mixtures of solvents by UAE. For all FR families, the better recoveries and the lowest relative standard deviation (RSD) were obtained using the mixture ethyl acetate/cyclohexane (5:2, v/v). The extraction with hexane/acetone (1:1, v/v) showed inconsistent results, with RSD ranging from 11 to 136%, and recoveries ranging from 14 to 240%. Thus ethyl acetate/cyclohexane (5:2, v/v) was chosen for the extraction of sediments, sludge and dust. The method development included different strategies in order to obtain adequate accuracy and precision, and included the use of spiked samples at two different levels, in triplicate. For OPFRs in sediments and dust, validation was performed by participating in an interlaboratory study (ILS) [209]. The accuracy of the method for PBDEs and NBFRs in dust was evaluated using a reference material for house dust (SRM 2585, NIST). The performance of the develop method and its performance for sediment, sewage sludge and dust samples were presented and discussed in Paper 3.

2.3 Results

2.3.1 Paper 1: "Gas chromatography/mass spectrometry comprehensive analysis of organophosphorus, brominated flame retardants, by-products and formulation intermediates in water"

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Gas chromatography/mass spectrometry comprehensive analysis of organophosphorus, brominated flame retardants, by-products and formulation intermediates in water

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ABSTRACT

A multiresidue method based on gas chromatography coupled to quadrupole mass spectrometry was developed to determine organophosphorus flame retardants, polybromodiphenyl ethers (BDEs 28, 47, 99, 100, 153, 154, 183 and 209), new brominated flame retardants, bromophenols, bromoanilines, bromotoluenes and bromoanisoles in water. Two ionization techniques (electron ionization – EI, and electron capture negative ionization – ECNI) and two acquisition modes (selected ion monitoring – SIM, and selected reaction monitoring – SRM) were compared as regards to mass spectral characterization, sensitivity and quantification capabilities. The highest sensitivity, at expenses of identification capacity, was obtained by GC–ECNI-MS/SIM for most of the compounds analyzed, mainly for PBDEs and decabromodiphenyl ethane while GC–EI-MS/MS in SRM was the most selective technique and permitted the identification of target compounds at the pg level, and identification capabilities increased when real samples were analyzed. This method was further used to evaluate the presence and behavior of flame retardants within a drinking water treatment facility. Organophosphorus flame retardants were the only compounds detected in influent waters at levels of 0.32–0.03 μg L^{-1}, and their elimination throughout the different treatment stages was evaluated.

1. Introduction

Halogenated and organophosphorus flame retardants (FR) are widespread in the environment due to their use in electronic goods and other industrial appliances such as thermoset polyester resins, circuit boards, textiles, adhesives, wire and cable coatings, polyurethanes, thermoplastic resins, extrusion polypropylene, styrene polymers, polyester, rubbers and plastics among others. The spill or release of products containing flame retardants are a main input to the environment. Most flame retardants are persistent, bioaccumulative and toxic or neurotoxic [1] and can be potentially dangerous for the public and environmental health as described in toxicological studies [2,3]. The Europe Union (EU) has taken actions as regards to the use and applications of flame retardants. The EU has banned the formulation and use of pentabromodiphenyl ether (pentaBDE), octabromodiphenyl ether (octaBDE) [4] and more recently, decabromodiphenyl ether (BDE 209) [5]. As a result of these bans, the use of others flame retardants is increasing. For example, decabromodiphenyl ethane (DBDPE) is used in replacement of BDE 209; 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) is used in replacement of octaBDE; bis(2-ethylhexyl)tetrabromophthalate (BEHTBP), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) and tris(1,3-dichloro-2-propyl)phosphate (TDCP) are used in replacement of pentaBDE. Due to their increasing use, traces of these compounds have been detected in environmental matrices [6,7]. Other new brominated flame retardants include pentabromoethyl benzene (PBEB) and hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO) detected in ambient air [8,9], as well as hexabromobenzene (HBB) and pentabromotoluene (PBT), detected in eggs of Great Lakes herring gulls [10].

On the other hand, organophosphorus flame retardants such as tri-chlororalkyl and tri-aryl phosphates are mostly employed in plastics, electronic equipment, furniture and construction. In most cases, these species are not chemically bound to the host materials and therefore, they can be easily emitted to the surrounding environment [11]. As a result, several organophosphorus flame retardants have been found in air, dust, water, wastewater and

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sediment [12–15]. Chlorinated-alkyl phosphates were included in the second (1995) and fourth (2000) Europe Union priority lists for risk assessment and were found to be rather persistent in the environment [16,17].

Another family of reactive flame retardants are bromophenols, mainly 2,4,6-tribromophenol. After use, bromophenols can be biotransformed into the corresponding bromoanisoles via *O*-methylation by bacterial microorganisms [18]. Bromotoluenes and aliphatic bromides may also be formed during the thermal decomposition of flame-retarded polystyrene or within the water treatment processes [19]. Several bromoanilines such as 4-bromoaniline (4 BA), 2,4-dibromoaniline (2,4 DBA), 2,6-dibromoaniline (2,6 DBA) and 2,4,6-tribromoaniline (2,4,6 TBA) are intermediates in the formulation of FRs incorporated to plastic materials [20]. Recent studies report the occurrence of di, tri and pentabromotoluenes, benzyl bromide, and tribromoanisoles in river water from Western Scheldt Estuary (The Netherlands) at levels from 0.0003 to 0.021 μg L^{-1} [21], while in another study, 2,4,6 tribromophenol (2,4,6 TBP) was detected in river water at levels ranging from 0.00051 to 0.0033 μg L^{-1}, and mono to tri bromophenols were detected in river sediment at 0.00055–0.00483 μg g^{-1} dry weight [22]. Bromophenols are also generated from the chlorination process in the water treatment systems [23].

The analysis of all these chemical families relies in the use of methods that permit their unequivocal identification in complex environmental matrices. Gas chromatography (GC) coupled to mass spectrometry operating in both electron ionization (EI) and electron capture negative ionization (ECNI) modes have been widely used for the analysis of brominated flame retardants [24,25]. For the analysis of organophosphorus flame retardants, LC–MS and GC coupled to EI-MS or nitrogen-phosphorous (NPD) detection are widely used [26]. Bromophenols have been determined in environmental samples using GC–EI-MS after derivatization with acetic anhydride [22] although Lópes et al. [21] reported a method without derivatization for bromophenols, bromotoluenes, bromoanilines and bromoanisoles.

The aim of this study was to develop a multiresidual chromatographic method for the determination of halogenated flame retardants, including PBDEs, new brominated flame retardants, tri-chlororalkyl and tri-aryl phosphates, bromophenols, bromoanilines and bromotoluenes using GC coupled to EI-MS, ECNI-MS and EI-MS/MS, with special emphasis to the characterization of flame retardants by MS/MS. Method performance and applicability are discussed for a set of 47 priority and emerging flame retardants and by-products and formulation intermediates. Finally, waters from a drinking waterwork facility were analyzed at the different treatment stages to determine their degradability and fate.

2. Experimental

2.1. Chemicals

The abbreviations of all compounds studied are shown in Table 1. Individual solution of BTBPE (50 μg mL^{-1}, 5% toluene in nonane), DBDPE at 25 μg mL^{-1} in toluene, BEHTBP, PBT, DPTE, HCDBCO at EHTBB at 50 μg mL^{-1} in toluene, and bromophenol mixture at 5 μg mL^{-1} in toluene containing 2 BP, 3 BP, 4 BP, 2,3 DBP, 2,4 DBP, 2,5 DBP, 2,6 DBP, 2,3,4 TBP, 2,3,6 TBP, 2,4,5 TBP, 2,4,6 TBP, 3,4,5 TBP, 2,3,4,5 TrBP, 2,3,4,6 TrBP; 2,3,5,6 TrBP, PBP were acquired from Wellington Laboratories (Guelph, Canada). A solution mixture of BDE 28, 47, 99, 100, 153, 154, 183 at 1 μg mL^{-1} and 209 at 10 μg mL^{-1} in n-nonane was acquired from Cambridge Isotope Laboratories (Andover, USA). 4 BA, 2,4 DBA, 2,6 DBA, 2,4,6 TBA, 3,5 DBT, 2,4,6 TBT, 2,4 DBAs, 2,4,6 TBAs, TPhP, HBB, PBEB purchased as solids and 2,5 DBT, TCEP, TCPP, TDCP purchased as liquid were acquired from Sigma–Aldrich (Germany). TCPP was acquired as a mixture of isomers (tris(2-chloro-1-methylethyl) phosphate, bis(1-chloro-2-propyl)-2-chloropropyl phosphate, and bis (2-chloropropyl)-1-chloro-2-propyl phosphate). Each standard solid or liquid was weighed and dissolved in hexane, except the TCEP, TCPP and TDCP that were dissolved in a mixture of ethyl acetate and methanol (1:1). Mass labeled surrogate standards (^{13}C) were MBDE 77 (50 μg mL^{-1}, 5% toluene in nonane), MBDE 209 (25 μg mL^{-1}, in toluene), MHBB (50 μg mL^{-1}, in toluene) and bromophenol mixture (4 MBP, 2,4 MDBP, 2,4,6 MTBP, 2,3,4,6 MTBP, MPBP) at 5 μg mL^{-1} in toluene, from Wellington. Gas chromatographic grade ethyl acetate, methanol and hexane were acquired from Merck (Darmstadt, Germany). Oasis HLB 60 mg solid phase extraction (SPE) cartridges were from Waters (Cerdanyola del Vallès, Spain).

2.2. Sampling procedure

Water samples were collected from a drinking water treatment plant (DWTP) located in NE Spain. Amber glass bottles were cleaned in an ultrasonic bath with Extran (Merck, Darmstadt, Germany), rinsed with distilled water and then with acetone and baked at 450 °C for 12 h to eliminate any external source of contamination. One liter of water was collected at each treatment step of the DWTP taken according its hydraulic retention time. The potabilization process consists on: dioxychlorination of river raw water, coagulation, flocculation, settling, sand filtration and groundwater addition to improve water quality. The filtered water is then splitted in two parallel potabilization lines one employing ozonization and GAC (granular activated carbon) filtration (∼70% of the total flow) and the other, ultrafiltration and reverse osmosis (∼30%). Both treated waters are then blended, chlorinated and distributed.

2.3. Extraction procedure

Samples were not filtered to avoid subestimation in the total concentration and thus both dissolved and particulate bound chemicals were analyzed jointly. Particulate matter was only relevant in raw river samples since sand filtration retained particulate matter, and thus its contribution in waters collected within the different treatment stages was very low. The surrogate standards were added to the raw water prior to preconcentration at 0.5 μg L^{-1}. For the preconcentration step, a Baker vacuum system (J.T. Baker, The Netherlands) was used. Oasis HLB 60 mg SPE cartridges were conditioned with 10 mL of hexane followed by 10 mL of dichloromethane, 10 mL of methanol and 15 mL of water HPLC, all at a flow rate of 5 mL min^{-1}. Then, 100 mL of the unfiltered sample were percolated through the cartridges at a flow rate of 5 mL min^{-1}. This relatively small preconcentrated volume avoided clogging. The cartridge was finally rinsed with 5 mL of HPLC water and gentle dried under vacuum during 30 min. Elution was performed with 10 mL of dichloromethane:hexane (1:1) followed by 10 mL of dichloromethane:acetone (1:1). The combined extract was evaporated at 25 °C under a nitrogen current using a TurboVap LV (Caliper Life Sciences, UK) to 250 μL. The water samples were analyzed by GC–EI-MS/MS. Procedural blanks ($n = 3$) were performed with 100 mL of HPLC water. TCPP and TPhP were detected at concentrations lower than method detection limits (MDL), while TCEP and TCPP were not detected. Brominated compounds were not detected in the blanks.

2.4. Instrumental conditions

GC–EI-MS, GC–EI-MS/MS and GC–ECNI-MS analysis were performed on a GC Agilent 7890A equipped with a 7000A Triple Quadrupole Mass Spectrometer. The column used was a DB-5MS

Table 1
Names, abbreviations and physical and chemical properties of studied compounds.

Compound name	Abbreviation	Formula	MW	Boiling point[a] (°C)	Solubility[a] (g L^{-1}) 25 °C, pH 7	Log K_{ow} [a]	pKa[a]
2,4,4′-triBDE	BDE 28	$C_{12}H_7Br_3O$	406.90	370.7 ± 32.0	7.7×10^{-4}	6.309 ± 0.563	
2,2′,4,4′-tetraBDE	BDE 47	$C_{12}H_6Br_4O$	485.79	395.5 ± 42.0	2.5×10^{-4}	6.680 ± 0.588	
2,2′,4,4′,5-pentaBDE	BDE 99	$C_{12}H_5Br_5O$	564.69	434.2 ± 45.0	6.2×10^{-5}	7.306 ± 0.686	
2,2′,4,4′,6-pentaBDE	BDE 100	$C_{12}H_5Br_5O$	564.69	415.9 ± 45.0	7.3×10^{-5}	7.103 ± 0.685	
2,2′,4,4′,5,5′-hexaBDE	BDE 153	$C_{12}H_4Br_6O$	643.58	471.1 ± 45.0	1.6×10^{-5}	7.933 ± 0.715	
2,2′,4,4′5,6′-hexaBDE	BDE 154	$C_{12}H_4Br_6O$	643.58	453.2 ± 45.0	1.9×10^{-5}	7.729 ± 0.715	
2,2′,3,4,4′,5′,6-heptaBDE	BDE 183	$C_{12}H_3Br_7O$	722.48	490.7 ± 45.0	5.6×10^{-6}	8.185 ± 0.788	
DecaBDE	BDE 209	$C_{12}Br_{10}O$	959.17	572.3 ± 50.0	1.4×10^{-7}	9.453 ± 0.871	
Pentabromoethylbenzene	PBEB	$C_8H_5Br_5$	500.65	413.3 ± 40.0	2.1×10^{-4}	6.764 ± 0.617	
2,3-dibromopropyl 2,4,6 tribromophenyl ether	DPTE	$C_9H_7Br_5O$	530.67	446.6 ± 45.0	1.6×10^{-3}	5.823 ± 0.530	
Hexabromobenzene	HBB	C_6Br_6	551.49	417.5 ± 40.0	5.51×10^{-4}	6.111 ± 0.674	
Hexachloro cyclopentadienyl dibromooctane	HCDBCO	$C_{13}H_{12}Br_2Cl_6$	540.76	480.7 ± 45.0	8.7×10^{-6}	7.619 ± 0.420	
2 ethylhexyl 2,3,4,5 tetrabromobenzoate	EHTBB	$C_{15}H_{18}Br_4O_2$	549.92	477.5 ± 40.0	1.6×10^{-4}	7.279 ± 0.619	
1,2 bis (2,4,6 tribromophenoxy)ethane	BTBPE	$C_{14}H_8Br_6O_2$	687.64	566.4 ± 50.0	1.6×10^{-5}	8.312 ± 0.716	
Bis(2-ethyl-1-hexyl)tetrabromo phtalate	BEHTBP	$C_{24}H_{34}Br_4O_4$	706.14	584.8 ± 45.0	3.3×10^{-6}	9.344 ± 0.697	
Decabromodiphenyl ethane	DBDPE	$C_{14}H_4Br_{10}$	971.22	676.2 ± 50.0	9.5×10^{-8}	11.679 ± 0.853	
Tris(2-choroethyl) phosphate	TCEP	$C_6H_{12}Cl_3O_4P$	285.49	347.4 ± 0.0	7.4	1.468 ± 0.356	
Tris(2-chloro-1-methylethyl) phosphate	TCPP	$C_9H_{18}Cl_3O_4P$	327.57	358.5 ± 22.0	1.0	2.529 ± 0.370	
Tris[2-chloro-1-(chloromethyl)ethyl] phosphate	TDCP	$C_9H_{15}Cl_6O_4P$	430.90	457.4 ± 40.0	0.12	3.275 ± 0.444	
Triphenylphosphate	TPhP	$C_{18}H_{15}O_4P$	326.28	412.4 ± 0.0	7.2×10^{-3}	4.590 ± 0.237	
3 bromophenol	3 BP	C_6H_5BrO	173.01	235.5 ± 0.0	3.6	2.232 ± 0.285	9.00 ± 0.10
4 bromophenol	4 BP	C_6H_5BrO	173.01	238.0 ± 0.0	2.8	2.578 ± 0.287	9.34 ± 0.13
2,3 dibromophenol	2,3 DBP	$C_6H_4Br_2O$	251.90	252.1 ± 20.0	1.4	2.899 ± 0.395	7.47 ± 0.10
2,4 dibromophenol	2,4 DBP	$C_6H_4Br_2O$	251.90	238.5 ± 0.0	0.73	3.466 ± 0.396	7.86 ± 0.18
2,5 dibromophenol	2,5 DBP	$C_6H_4Br_2O$	251.90	244.6 ± 20.0	1.2	3.100 ± 0.393	7.47 ± 0.10
2,6 dibromophenol	2,6 DBP	$C_6H_4Br_2O$	251.90	256.6 ± 0.0	1.7	3.265 ± 0.393	6.89 ± 0.10
3,4 dibromophenol	3,4 DBP	$C_6H_4Br_2O$	251.90	286.3 ± 20.0	0.91	3.100 ± 0.397	8.43 ± 0.18
3,5 dibromophenol	3,5 TBP	$C_6H_4Br_2O$	251.90	278.1 ± 20.0	1.1	2.975 ± 0.394	8.04 ± 0.10
2,3,4 tribromophenol	2,3,4 TBP	$C_6H_3Br_3O$	330.80	302.5 ± 37.0	0.50	3.818 ± 0.483	6.91 ± 0.23
2,3,5 tribromophenol	2,3,5 TBP	$C_6H_3Br_3O$	330.80	295.7 ± 35.0	1.0	3.673 ± 0.480	6.50 ± 0.15
2,3,6 tribromophenol	2,3,6 TBP	$C_6H_3Br_3O$	330.80	265.5 ± 35.0	2.7	3.817 ± 0.480	5.93 ± 0.15
2,4,5 tribromophenol	2,4,5 TBP	$C_6H_3Br_3O$	330.80	295.3 ± 35.0	0.40	4.018 ± 0.482	6.91 ± 0.23
2,4,6 tribromophenol	2,4,6 TBP	$C_6H_3Br_3O$	330.80	286.8 ± 0.0	0.76	4.404 ± 0.481	6.34 ± 0.23
3,4,5 tribromohenol	3,4,5 TBP	$C_6H_3Br_3O$	330.80	335.3 ± 37.0	0.32	3.673 ± 0.483	7.49 ± 0.23
2,3,4,5 tetrabromophenol	2,3,4,5 TrBP	$C_6H_2Br_4O$	409.70	344.9 ± 37.0	0.66	4.421 ± 0.556	5.96 ± 0.28
2,3,4,6 tetrabromophenol	2,3,4,6 TrBP	$C_6H_2Br_4O$	409.70	308.9 ± 37.0	1.7	4.787 ± 0.555	5.38 ± 0.28
2,3,5,6 tetrabromophenol	2,3,5,6 TrBP	$C_6H_2Br_4O$	409.70	309.6 ± 37.0	5.7	4.421 ± 0.553	4.97 ± 0.20
Pentabromophenol	PBP	C_6HBr_5O	488.59	352.3 ± 37.0	3.6	5.221 ± 0.621	4.43 ± 0.33
4-bromoaniline	4 BA	C_6H_6BrN	172.02	225.9 ± 13.0	1.5	2.279 ± 0.292	3.90 ± 0.10
2,4 dibromoaniline	2,4 DBA	$C_6H_5Br_2N$	250.92	264.8 ± 20.0	0.25	3.520 ± 0.409	1.83 ± 0.10
2,6 dibromoaniline	2,6 DBA	$C_6H_5Br_2N$	250.92	265.8 ± 20.0	0.251	3.566 ± 0.415	0.48 ± 0.10
2,4,6 tribromoaniline	2,4,6 TBA	$C_6H_4Br_3N$	329.81	300.0 ± 0.0	0.040	4.812 ± 0.500	−0.23 ± 0.10
2,5 dibromotoluene	2,5 DBT	$C_7H_6Br_2$	249.93	236.0 ± 0.0	0.014	4.292 ± 0.389	
3,5 dibromotoluene	3,5 DBT	$C_7H_6Br_2$	249.93	246.0 ± 0.0	0.013	4.302 ± 0.389	
2,4,6 tribromotoluene	2,4,6 TBT	$C_7H_5Br_3$	328.83	290.7 ± 35.0	2.8×10^{-3}	5.291 ± 0.475	
Pentabromotoluene	PBT	$C_7H_3Br_5$	486.62	394.4 ± 37.0	4.8×10^{-4}	6.255 ± 0.617	
2,4 dibromoanisole	2,4 DBAs	$C_7H_6Br_2O$	265.93	293.9 ± 0.0	0.25	3.770 ± 0.419	
2,4,6 tribromoanisole	2,4,6 TBAs	$C_7H_5Br_3O$	344.83	298.0 ± 0.0	1.8×10^{-4}	4.507 ± 0.502	
Isotopic standards							
4-bromo[$^{13}C_6$]phenol	4 MBP	C_6H_5BrO	178.96				
2,4-dibromo[$^{13}C_6$]phenol	2,4 MDBP	$C_6H_4Br_2O$	257.86				
2,4,6-tribromo[$^{13}C_6$]phenol	2,4,6 MTBP	$C_6H_3Br_3O$	336.75				
2,3,4,6-tetrabromo[$^{13}C_6$]phenol	2,3,4,6 MTrBP	$C_6H_2Br_4O$	415.65				
Pentabromo[$^{13}C_6$]phenol	MPBP	C_6HBr_5O	494.55				
$^{13}C_6$-Hexabromobenzene	MHBB	C_6Br_6	557.44				
3,3′,4,4′-tetrabromo[$^{13}C_{12}$]diphenyl ether	MBDE 77	$C_{12}H_6Br_4O$	497.70				
Decabromo[$^{13}C_{12}$]diphenyl ether	MBDE 209	$C_{12}Br_{10}O$	971.08				

[a] Data from SciFinder originating from calculated properties using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994–2011 ACD/Labs).

with 15 m (length) × 0.250 mm (I.D.) × 0.10 μm (film) (J&W Scientific, USA). The oven program was set at 40 °C (1 min), to 60 °C at 15 °C/min (8 min), to 220 °C at 10 °C/min and to 300 °C at 15 °C/min (8 min). Helium (purity 99,999%) was employed as carrier gas with a constant flow of 1.5 mL min^{-1}. The standards were injected (2 μL) into the CG system in splitless mode, with a splitless time of 1.5 min. The injector, quadrupole and transfer line were set at 300, 150, and 280 °C respectively. Electron ionization (EI) mode was set at 70 eV. The ion source temperature was tested at 225, 250, 275 and 300 °C and best response for most of compounds was obtained with 300 °C. Acquisition was performed using time scheduled Selected Ion Monitoring (SIM), monitoring the three most intense or selective ion fragments of each compound and using 10 retention time windows to obtain maximum sensitivity. For GC–EI-MS/MS acquisition, the base peak (precursor ion) of each compound was determined by scan acquisition mode, and then the optimum collision energy was increased from 5 and 60 V (maximum value), in intervals of 5 V, and the energy that produced the most abundant or selective precursor ion fragmentation and intense product ions was chosen. Two mass transitions were monitored for each compound. For GC–ECNI-MS, three ion source temperatures (220, 250 and 280 °C) and the ammonia gas flow (1, 1.75 and 2.25 mL min^{-1}) were optimized. The best response was obtained at 250 °C and 2.25 mL min^{-1}. The three most intense ions were monitored using time scheduled SIM.

2.5. Quality control analysis

For each method (GC–EI-MS/SIM, GC–EI-MS/MS and GC–ECNI-MS/SIM) a calibration curve was performed at five concentration levels, from 0.01 to 0.5 μg mL^{-1}, except for bromophenols that was from 0.1 to 1.4 μg mL^{-1} and for BDE 209 that was from 0.05 to 5 μg mL^{-1}. The repeatability was calculated by the injection of the standard solution at 0.3 μg mL^{-1} ($n = 5$). Limits of detection (LODs) were calculated using the lowest concentration standard of each compound that gave a signal to noise ratio of 3 (s/n = 3). Labeled standards were used for internal standard quantification. Among the surrogate standards, 4 MBP was used for monosubstituted bromophenols and bromoanilines; 2,4 MDBP for disubstituted bromophenols, bromoanilines, and also evaluated for bromotoluenes and bromoanisoles. 2,4,6 MTBP was chosen for trisubstituted bromophenols and also evaluated for bromoanilines, bromotoluenes and bromoanisoles. 2,3,4,6 MTBP was employed for tetrasubstituted bromophenols whereas MHBB was used for PBT, PBEB, DPTE, HBB, HCDBCO and EHTBB. The MBDE 77 was the surrogate standard for the BDEs 28, 47, 99, 100, 153, 154, 183 and also for BTBPE and BEHTBP. Finally, MBDE 209 was used as surrogate for both BDE 209 and DBDPE. Each surrogate standard was added in the calibration solution at 0.2 μg mL^{-1}. MPBP as surrogate standard for PBP was disregarded because it was not detected at 0.2 μg mL^{-1}. With GC–EI/MS and GC–ECNI/MS in SIM, 2 or 3 ions were monitored while with GC–EI-MS/MS, two transitions per compound were employed for the identification of each compound, as required by the 2002/657EC decision (2002) [27]. Recoveries ($n = 3$) were calculated using MilliQ water spiked at 0.4 μg L^{-1} for PBDEs (4 μg L^{-1} for BDE 209), 2.5 μg L^{-1} for bromophenols and 1 μg L^{-1} for the other compounds. MDL were calculated using spiked samples, as the concentration that give a signal to noise ratio of 3 (s/n = 3). To evaluate the extraction efficiency of waters (considering both dissolved and particulate fraction) from the drinking water plant, the recoveries of the surrogates were determined in each sample. Because of the high instrumental detection limit for PBP (not detected at 1000 pg injected), this compound was not studied in the water samples.

3. Results and discussion

3.1. Chromatographic performance

The optimization of the chromatographic separation is necessary for the multiresidue analysis of flame retardants belonging to different chemical families, which differ in physicochemical properties, polarity and K_{ow}. The 15 m DB-5MS was used to minimize the interaction of the bromophenols and the stationary phase, and also permitted the detection of BDE 209 and DBDPE without undergoing degradation in the GC column when reaching high temperatures [28]. The optimized oven temperature program permitted a good separation of the various flame retardants in 37 min, with BDE 209 eluting at 34.2 min and DBDPE at 35.9 min. In a first step, scan acquisition mode was used to characterize each compound. This information was used to optimize the SIM and SRM acquisition conditions. Coelution of two or more compounds was resolved by mass fragment selectivity or specific transitions, such as 2,5 DBT and 4 BA; 2,4,6 TBAs and 2,3,5 TBP; TPhP, DPTE and HBB; BDE 99, HCDBCO and EHTBB (Table 2 and Fig. 1). The coelution of some bromophenol isomers (3 BP and 4 BP at 11.8 min, 2,4 DBP and 2,5 DBP at 12.8 min, 2,4,5 TBP and 2,3,4 TBP at 17.7 min, 2,3,4,5 TrBP and 2,3,4,6 TrBP at 20.9 min) could not be resolved by mass fragment or SRM transition, so the result was expressed as a sum of isomers. On the other hand, 3,4,5 TBP could not be resolved by any MS method because of coelution with mass fragments of 2,3,5,6 TrBP, so this compound was not quantified. Fig. 1 shows the chromatogram of a standard mixture by GC–EI-MS/SIM, GC–EI-MS/MS and GC–ECNI-MS/SIM, respectively, where the isolation of the coeluted compounds by m/z fragments or MS/MS transitions is shown.

3.2. Mass spectral identification

The ions or SRM transitions monitored for GC–EI-MS/SIM, GC–EI-MS/MS, and GC–ECNI-MS/SIM are presented in Table 2. For brominated compounds, the [M+2] and [M+4] ion of each homologue group were usually selected.

3.2.1. Tri-chlororalkyl and tri-aryl phosphates

TCEP, TCPP, TDCP and TPhP showed good performance by GC–EI-MS mode both in SIM and SRM. In this study, the two main isomers of TCPP (tris(2-chloro-1-methylethyl) phosphate and bis(1-chloro-2-propyl)-2-chloropropyl phosphate) were quantified. The fragment at m/z 99 (corresponding to protonated phosphoric acid) [29] was a characteristic ion of all phosphates except for TPhP, and the base peak of TCPP and TDCP. For SIM acquisition, m/z 249 $[M-Cl]^+$, 205 $[M-OC_2H_4Cl]^+$ and 143 $[C_2H_5ClO_3P]^+$ were used for TCEP; m/z 99 $[H_4PO_4]^+$ and 125 $[C_2H_6O_4P]^+$ and 277 $[M-CH_2Cl]^+$ were used for TCPP; m/z 191 $[C_3H_6Cl_2O_3P]^+$, 381 $[M-CH_2Cl+2]^+$, and m/z 99 $[H_4PO_4]^+$ were used to determine TDCP; and 326 $[M]^{+\bullet}$, 325 $[M-H]^+$ and 77 $[C_6H_5]^+$ for TPhP.

On the other hand, only TPhP and TDCP were detected by GC–ECNI-MS with the optimized conditions. TPhP presented the fragments m/z 325 $[M-H]^-$ (base peak) and 249 $[M-C_6H_5]^-$. TDCP presented the isotopic fragment ions at m/z 317, 319, 321, corresponding to $[M-H_5C_3Cl_2]^-$. Since organophosphorus flame retardants were detected at low sensitivity by GC–ECNI-MS/SIM with the used conditions, the performance of TPhP and TDCP by this technique was not evaluated.

For the MS/MS determination of TCPP, the fragment m/z 99 was not chosen as precursor ion but the m/z 125 (abundance of 90%) to improve the selectivity. For TCEP, TDCP and TPhP, the m/z 249 $[M-Cl]^+$, m/z 99 and 326 $[M]^{+\bullet}$ were chosen as precursor ions, respectively. TCEP showed the product ions m/z 125 and 99; TCPP

Table 2

Elution number (indicated in the chromatograms at Fig. 1), retention time (RT), *m*/*z* and MS/MS transitions monitored by the analysis by GC–EI-MS/SIM, GC–EI-MS/MS and GC–ECNI-MS/SIM. In bold are indicated the *m*/*z* or MS/MS transitions chosen for quantification. The numbers given in parentheses are the relative abundance obtained by EI MS/SCAN and ECNI MS/SCAN.

Elution numbers	Compound	RT (min)	EI-MS/SIM			EI-MS/MS				ECNI-MS/SIM			
			Ion 1	Ion 2	Ion 3	T1	C. E.	T2	C. E.	Ion 1	Ion 2	Ion 3	Ion 4
1	3,5 DBT	11.2	**250 (100)**	248 (51)	171 (62)	**250 → 90**	30	250 → 169	20	**249 (51)**	79 (100)	81 (98)	
2	2,5 DBT	11.4	**250 (100)**	248 (51)	171[a]	**250 → 90**	30	250 → 169	20	**249 (36)**	79 (100)	81 (98)	
3	4 BA	11.5	**173 (98)**	171 (100)	92 (37)	**171 → 65**	40	171 → 92	20	**172 (42)**	79 (100)	81 (98)	
4′	4 MBP	11.8	**178 (100)**	180 (83)	99 (21)	**178 → 70**	20	178 → 99	20	[b]	[b]	[b]	[b]
4 and 5	3 BP + 4 BP	11.8	**174 (100)**	172 (99)	93 (24)	**172 → 93**	20	172 → 65	40	[b]	[b]	[b]	[b]
6′	2,4 MDBP	12.8	**258 (100)**	256 (52)	148 (15)	**258 → 68**	40	258 → 97.5	40	**256 (22)**	79 (100)	81 (98)	
6 and 7	2,4 DBP + 2,5 DBP	12.8	**252 (100)**	250 (52)	143 (16)	**252 → 63**	40	252 → 92	20	**252 (22)**	79 (100)	81 (98)	
8	2,3 DBP	12.9	**252 (100)**	250 (52)	143 (17)	**252 → 63**	40	252 → 92	20	**79 (100)**	81 (98)		
9	2,6 DBP	13.6	**252 (100)**	250 (51)	143 (16)	**252 → 63**	40	252 → 92	20	**79 (100)**	81 (98)		
10	2,6 DBA	14.4	**251 (100)**	249 (51)	170 (11)	**251 → 90**	35	251 → 63	35	**79 (100)**	81 (98)		
11	2,4 DBAs	15.5	**266 (100)**	251 (61)	223 (45)	**266 → 223**	20	266 → 251	20	**79 (100)**	81 (98)		
12	2,4 DBA	16.1	**251 (100)**	249 (51)	170 (17)	**251 → 90**	35	251 → 63	35	**79 (100)**	81 (98)		
13	2,4,6 TBT	16.5	**328 (100)**	330 (98)	249 (84)	**328 → 249**	20	328 → 89	35	**79 (100)**	81 (98)	327 (25)	
14	3,5 DBP	17.2	**252 (100)**	250 (51)	143 (20)	**252 → 63**	40	252 → 143	30	[b]	[b]	[b]	[b]
15	2,3,5 TBP	17.3	**330 (100)**	332 (97)	143 (18)	**330 → 141**	40	330 → 62	55	**330 (84)**	79 (100)	81 (98)	
16	2,4,6 TBAs	17.3	**343.5 (100)**	345.5 (97)	329 (87)	**343.5 → 329**	20	343.5 → 301	30	**343 (17)**	79 (100)	81 (98)	
17	3,4 DBP	17.4	**252 (100)**	250 (51)	143 (24)	**252 → 63**	40	252 → 143	30	[b]	[b]	[b]	[b]
18′	2,4,6 MTBP	17.6	**336 (100)**	338 (90)	148 (18)	**336 → 146**	50	336 → 67	50	**338 (17)**	79 (100)	81 (98)	
18	2,4,6 TBP	17.6	**330 (100)**	332 (98)	143 (21)	**330 → 141**	40	330 → 62	50	**330 (17)**	79 (100)	81 (98)	
19 and 20	2,4,5 TBP + 2,3,4 TBP	17.7	**330 (100)**	332 (98)	143 (20)	**330 → 141**	40	330 → 62	50	**79 (100)**	81 (98)	330 (97)	
21	2,3,6 TBP	17.9	**330 (100)**	332 (98)	143 (17)	**330 → 141**	40	330 → 62	55	**79 (100)**	81 (98)	330 (86)	
22	2,4,6 TBA	18.3	**329 (100)**	331 (98)	250 (22)	**329 → 168**	40	329 → 90	35	**79 (100)**	81 (98)	328 (36.5)	
23	TCEP	19.3	**249 (100)**	205 (70)	143 (75)	**249 → 125**	10	249 → 99	20	[b]	[b]	[b]	[b]
24	TCPP (isomers)	19.6 + 19.8	**99 (100)**	125 (90)	277 (13)	**125 → 99**	10	125 → 81	10	[b]	[b]	[b]	[b]
25	3,4,5 TBP	20.8	[c]	[c]	[c]	[c]		[c]		[c]	[c]	[c]	[c]
26	2,3,5,6 TrBP	20.8	**410 (100)**	408 (68)	221[a]	**410 → 221**	40	410 → 142	55	**410 (21)**	79 (100)	81 (98)	330[a]
27′	2,3,4,6 MTrBP	20.9	**415.5 (100)**	413.5 (56)	226 (24)	**415.5 → 225.5**	20	415.5 → 333.5	20	**336 (25)**	79 (100)	81 (98)	416 (20)
27 and 28	2,3,4,5 TrBP + 2,3,4,6 TrBP	20.9	**410 (100)**	408 (69)	221 (20)	**410 → 221**	40	410 → 142	60	**410 (18)**	79 (100)	81 (98)	330(60)[a]
29	PBT	23.5	**485.5 (100)**	487.5 (98)	406.5 (64)	**485.5 → 247**	45	485.5 → 325	35	**79 (100)**	81 (98)		
30	BDE 28	23.6	**246 (100)**	248 (96)	405.5 (47)	**407.5 → 247.5**	15	246 → 139	45	**79 (100)**	81 (98)		
31	PBEB	23.9	**484.5 (100)**	486.5 (97)	499.5 (94)	**484.5 → 325**	40	484.5 → 406	30	**79 (100)**	81 (98)		
–	PBP	24.0	**487.5 (100)**	489.5 (98)	139 (70)	**487.5 → 299**	40	487.5 → 219.5	55	**79 (100)**	81 (98)		
32	TDCP	24.3	**191 (80)**	381 (27)	99 (100)	**99 → 63**	40	99 → 81	30				
33	TPhP	24.9	**326 (100)**	325 (93)	77 (56)	**326 → 169**	25	326 → 215	25				
34	DPTE	24.9	**330 (100)**	332 (98)	201 (23)	**330 → 141**	45	330 → 62	55	**160 (98)**	79 (100)	81 (98)	291 (45)
35′	MHBB	24.9	**559.5 (68)**	557.5 (100)	238 (34)	**559.5 → 480.5**	30	557.5 → 398	30	**559.5 (15)**	79 (100)	81 (98)	557.5 (20)
35	HBB	24.9	**549.5 (79)**	551.5 (100)	232 (59)	**549.5 → 389.5**	50	551.5 → 312.5	45	**549.5 (17)**	79 (100)	81 (98)	471.5 (11)
36	BDE 47	25.6	**326 (100)**	324 (51)	485.5 (38)	**485.5 → 325.5**	15	485.5 → 219	45	**79 (100)**	81 (98)		
37	MBDE 77	26.4	**498 (100)**	496 (72)	228 (55)	**497.5 → 230**	60	497.5 → 149	60	**79 (100)**	81 (98)		
38	BDE 100	27.0	**405.5 (96)**	403.5 (100)	565.5 (51)	**405.5 → 137**	55	405.5 → 297	40	**79 (100)**	81 (98)	405 (15)	
39	BDE 99	27.4	**405.5 (94)**	403.5 (100)	565.5 (31)	**405.5 → 137**	55	405.5 → 297	40	**405 (10)**	79 (100)	81 (98)	
40	HCDBCO	27.5	**237 (90)**	268 (100)	107 (84)	**237 → 118.5**	35	237 → 167	35	**310 (46)**	79 (100)	81 (98)	237 (36)
41	EHTBB	27.5	**420.5 (60)**	418.5 (42)	112 (100)	**420.5 → 311.5**	40	420.5 → 233	40	**357 (19)**	79 (100)	81 (98)	469 (19)
42	BDE 154	27.9	**483.5 (100)**	481.5 (71)	643.5 (44)	**483.5 → 214.9**	60	483.5 → 377	55	**79 (100)**	81 (98)		
43	BDE 153	28.3	**483.5 (100)**	481.5 (63)	643.5 (23)	**483.5 → 324**	45	483.5 → 377	45	**79 (100)**	81 (98)		
44	BDE 183	29.0	**563.5 (98)**	561.5 (93)	723.5 (29)	**563.5 → 295**	55	563.5 → 404	50	**79 (100)**	81 (99)		
45	BTBPE	29.3	**356.5 (100)**	358.5 (98)	252 (85)	**356.5 → 118**	25	356.5 → 90	40	**79 (100)**	81 (98)	251(22)	329 (12)
46	BEHTBP	29.6	**464.5 (55)**	462.5 (46)	112 (100)	**464.5 → 380**	50	464.5 → 310.5	45	**79 (100)**	81 (98)	384 (25)	513 (28)
47′	MBDE 209	34.2	**811.5 (27)**	809.5 (62)		**811.5 → 651.5**	55	809.5 → 650	55	**494.5 (86)**	79 (100)	81 (98)	413.5 (12)
47	BDE 209	34.2	**797.5 (33)**	799.5 (37)		**797.5 → 638**	55	799.5 → 640	55	**486.5 (91)**	79 (100)	81 (98)	408.5 (17)
48	DBDPE	35.9	**484.5 (100)**	486.5 (98)	405 (25)	**484.5 → 324.5**	30	484.5 → 403.5	40	**79 (100)**	81 (98)		

C.E. – collision energy (V).

[a] Not determined due to co-elution interference.

[b] Not detected.

[c] Coelution separation no possible by specific ion.

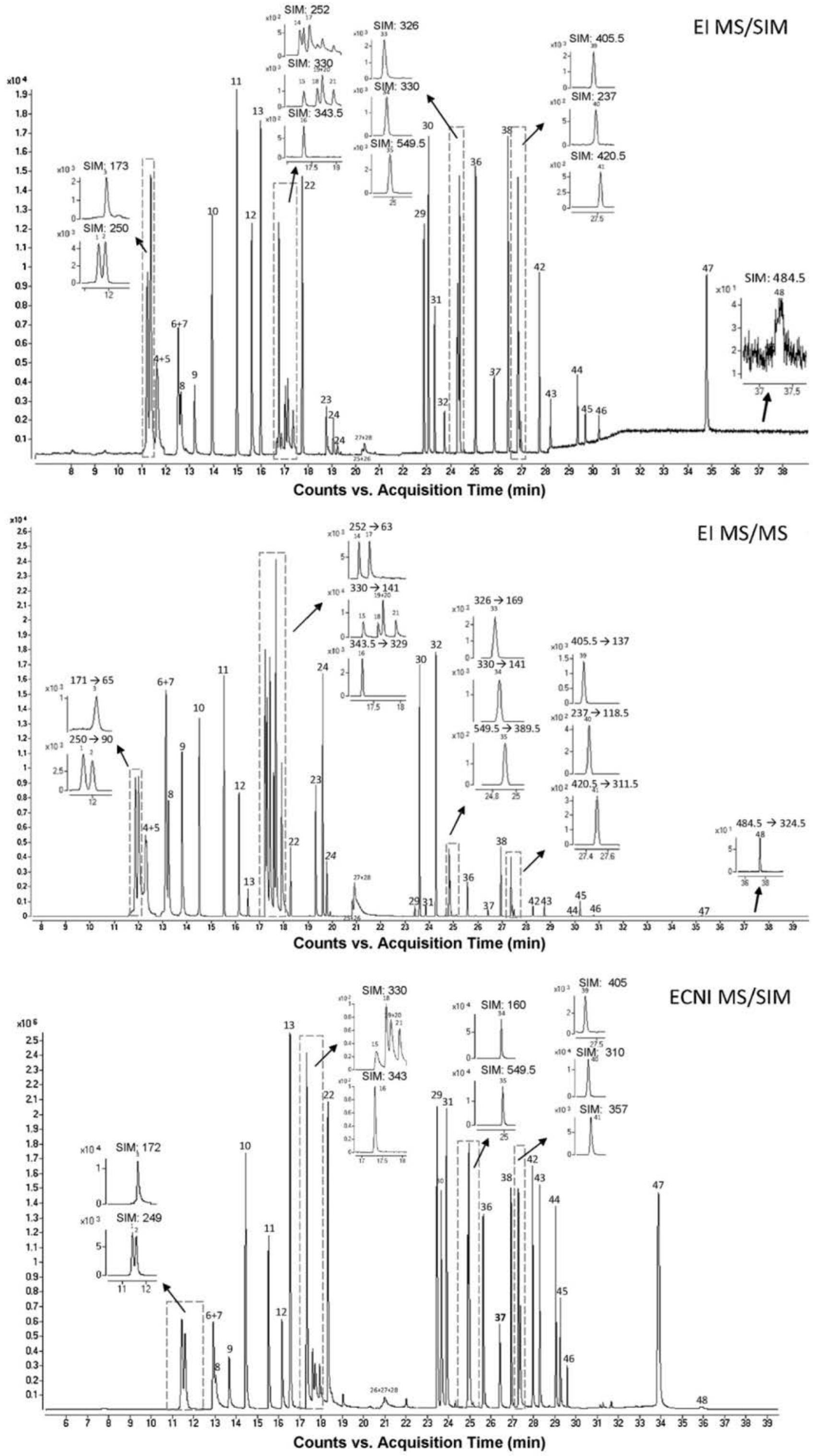

Fig. 1. GC–EI-MS/SIM, GC–EI-MS/MS and GC–ECNI-MS/SIM chromatograms obtained for flame retardants standards at concentration 5 ng μL^{-1} for BDE 209 and 0.5 ng μL^{-1} for the others compounds (except for bromophenols at GC–EI-MS/MS chromatogram, that were at 1.3 ng μL^{-1}). The correspondent numbered compound is shown in Table 2.

m/z 99 and 81 $[H_2PO_3]^+$; TDCP m/z 63 $[PO_2]^+$ and 81 and TPhP m/z 169 $[C_{12}H_9O]^+$ and 215 $[C_{12}H_8O_2P]^+$ [30], respectively.

3.2.2. Bromophenols

The major ions formed by GC–EI-MS in scan mode were the molecular fragments $[M]^{+\bullet}$, while the ions $[M-nBr]^+$ and $[M-CHO-nBr]^+$ could be observed at abundances lower than 30% (Table 2). As 2,3,5,6 TrBP coeluted with 3,4,5 TBP at 20.8 min, this compound was isolated by the fragment m/z 410 with GC–EI-MS/SIM and GC–ECNI-MS/SIM mode and by the transition 410 → 221 with GC–EI-MS/MS mode, permitting its identification.

$[Br]^-$ was the most abundant fragment ion obtained by GC–ECNI-MS. The use of labeled bromophenol surrogates was possible by GC–ECNI-MS/SIM using $[M]^{-\bullet}$ to isolate the labeled and the native bromophenol compounds, respectively. This method did not allow either to isolate dibromophenol coelutions at 17.2–17.4 min, so 3,5 DBP and 3,4 DBP could not be identified in the presence of 2,3,5 TBP and 2,4,6 TBP with the chromatographic conditions used in this study.

For the MS/MS determination of mono- to pentabromophenols, the $[M]^{+\bullet}$ (m/z 172 $[M]^{+\bullet}$, 252 $[M+2]^{+\bullet}$, 330 $[M+2]^{+\bullet}$, 410 $[M+4]^{+\bullet}$, and 487.5 $[M+4]^{+\bullet}$) were chosen as precursor ions, respectively. Product ions for monobromophenols were m/z 93 $[M-Br]^+$ and 65 $[M-CO-Br]^+$ whereas m/z 63 $[M-2Br-COH]^+$ and 92 $[M-2Br]^{+\bullet}$ were selected for dibromophenols. The product ions $[M-2Br-CHO]^+$ and $[M-3Br-CHO]^{+\bullet}$ were chosen for tribromophenols (m/z 141 and 62) whereas $[M-2Br-CHO+2]^+$ and $[M-3Br-CHO+2]^{+\bullet}$ were the product ions for tetrabromophenols (m/z 221 and 142) and for pentabromophenol (m/z 299 and 219.5), respectively.

3.2.3. Bromotoluenes, bromoanilines and bromoanisoles

The GC–EI-MS mass spectrum of bromoanilines and bromotoluenes were quite similar, yielding $[M]^{+\bullet}$ as base peak, being also observed the fragment $[M-Br]^+$. The bromoanisoles showed intense $[M]^{+\bullet}$ and $[M-CH_3]^+$ ions. By ECNI, the most intense peaks for bromoanilines, bromotoluenes, and bromoanisols were $[Br]^-$ isotopes, while the $[M-H]^-$ was observed for bromotoluenes, 4 BA, 2,4,6 TBA and 2,4,6 TBAs.

The $[M]^{+\bullet}$ was chosen as precursor ion for MS/MS determination of mono- to tri bromoanilines (m/z 171 $[M]^{+\bullet}$, 251 $[M+2]^{+\bullet}$ and 329 $[M+2]^{+\bullet}$), di-, tri- and pentabromotoluenes (m/z 250 $[M+2]^{+\bullet}$, 328 $[M+2]^{+\bullet}$, 485.5 $[M+4]^{+\bullet}$) and di- to tribromoanisoles (m/z 266 $[M+2]^{+\bullet}$ and 343.5 $[M+2]^{+\bullet}$). Bromoaniline's product ions were m/z 65 $[M-Br-CHN]^{+\bullet}$ and 92 $[M-Br]^+$ for 4 BA, m/z 90 $[M-2Br-H]^+$ and 63 $[M-2Br-CH_2N]^{+\bullet}$ for 2,4 DBA and 2,6 DBA, and m/z 168 $[M-2Br-H]^+$ and 90 $[M-3Br]^+$ for 2,4,6 TBA. Bromotoluene's product ions were m/z 90 $[M-2Br]^{+\bullet}$ and 169 $[M-Br]^+$ for 2,5 DBT and 3,5 DBT, m/z 249 $[M-Br+2]^+$ and 89 $[M-3Br]^+$ for 2,4,6 TBT, and m/z 247 $[M-3Br+2]^+$ and 325 $[M-2Br-H+2]^+$ for PBT. Finally, bromoanisol's product ions were $[M-C_2H_3O+2]^+$ and $[M-CH_3+2]^+$ for 2,4 DBAs (m/z 223 and 251) and 2,4,6 TBAs (m/z 301 and 329).

3.2.4. PBDEs

By GC–EI-MS/SIM the ions $[M]^{+\bullet}$ and $[M-2Br]^{+\bullet}$ were used in the identification and quantification of PBDEs. On the other hand, the most abundant ions formed by GC–ECNI-MS were m/z 79 and 81, corresponding to $[Br]^-$, as stated in earlier studies [31]. However, the lack of specificity using these ions leads to high erroneous identification and quantification. For quantification of BDE 99, the ion m/z 405 instead of m/z 79 or 81 was used to resolve coelution with HCDBCO and EHTBB. For BDE 209, besides the latter two ions, the pentabromophenoxide ion $[C_6Br_5O]^-$ was formed with enough relative abundance. Monitoring of this fragment ion gave a more reliable identification of BDE 209 [32] and also allowed the use of mass labeled BDE 209 (MBDE 209) as surrogate by ECNI.

In agreement with a previous study [33], the precursor ions chosen by GC–EI-MS/MS analysis were $[M]^{+\bullet}$ and the $[M-2Br]^{+\bullet}$, which originate product ions after the loss of nBr or nBr+CO groups. The ion $[M-2Br]^{+\bullet}$ was chosen as precursor ion in the analysis of BDE 183 (m/z 463.5 $[M-2Br+6]^{+\bullet}$) and 209 (m/z 797.5 $[M-2Br+6]^{+\bullet}$ and m/z 799.5 $[M-2Br+8]^{+\bullet}$), yielding product ions by the loss of 2Br groups.

3.2.5. New brominated flame retardants

For this group of compounds, the molecular ion was not always the most abundant by EI. In these cases, the most intense ion of each homologue group was selected as indicated below. By GC–EI-MS, the $[M]^{+\bullet}$ was formed for HBB, BTBPE and PBEB, and was the base peak for HBB. The PBEB showed the $[M+4]^{+\bullet}$ peak with 94% of abundance and a base peak at m/z 484.5 $[M-CH_3+4]^+$. DPTE presented a base peak at m/z 330 $[C_6H_3OBr_3+2]^{+\bullet}$, by elimination of the dibromopropyl group and rearrangement of the γ-hydrogen from the molecular ion [34]. The HCDBCO showed a very fragmented spectrum, with the intense peaks at m/z 268 $[M-C_5Cl_6+2]^{+\bullet}$, 237 $[C_5Cl_5+2]^+$ and 107 $[C_8H_{11}]^+$. Zhu et al. [9] have proposed the fragmentation routes of HCDBCO under EI-MS by retro Diels–Alder reaction. EHTBB showed a base peak at m/z 112 $[C_8H_{16}]^{+\bullet}$, and intense peak at 420.5 $[M-C_8H_{17}O+4]^+$. BTBPE presented a base peak at m/z 356.5, corresponding to the loss of the 2,4,6 tribromophenoxy group $[M-C_6H_2OBr_3+2]^+$. BEHTBP had m/z 112 $[C_8H_{16}]^{+\bullet}$ as base peak and an intense peak at m/z 464.5 $[M-C_{16}H_{33}O+4]^+$ which was used for quantification. The base peak of DBDPE was at m/z 484.5 $[M-C_7H_2Br_5+4]^+$, formed by the cleavage of the ethyl bond [35].

Most studies have used GC–ECNI-MS to quantify these compounds in environmental samples [8,35–38]. Guardia et al. [36] have proposed other ions besides of m/z 79 and 81 ($[Br]^-$ isotopes) to identify EHTBB, BTBPE, BEHTBP and DBDPE by GC–ECNI-MS. In this study, the mass spectrum obtained for these compounds by GC–ECNI-MS showed additional fragments for DPTE, HBB, HCDBCO, EHTBB, BTBPE and BEHTBP, with relative intensity from 12% to 98% and were chosen to improve the identification performance, although m/z 79 was used to quantify most of these compounds (Table 2). Both HBB and the MHBB showed the fragment $[M]^{-\bullet}$ by GC–ECNI-MS. When selecting the $[M]^{-\bullet}$ most intense isotope fragment, m/z 551.5 $[M+6]^{-\bullet}$ and m/z 557.5 $[M+6]^{-\bullet}$ for HBB and MHBB respectively, coelution between the isotopes were observed for both compounds, thus, detection of HBB was possible when injecting a MHBB solution at 0.2 $\mu g\,mL^{-1}$, and were resolved using the m/z 549.5 $[M+4]^{-\bullet}$ (17% abundance) and 559.5 $[M+8]^{-\bullet}$ (15% abundance), respectively.

The precursor ions chosen by GC–EI-MS/MS for HBB analysis were $[M]^{+\bullet}$ isotopes (m/z 549.5 $[M+4]^{+\bullet}$ and 551.5 $[M+6]^{+\bullet}$) which originate the product ions m/z 389.5 $[M-2Br+2]^{+\bullet}$ and 312.5 $[M-3Br+4]^+$, respectively. The m/z 484.5 $[C_7H_2Br_5+4]^+$ was selected as precursor ion for PBEB which give the product ions m/z 325 $[C_7H_2Br_3+2]^+$ and 406 $[C_7H_2Br_4+4]^{+\bullet}$. For DPTE analysis, the m/z 330 $[C_6H_3OBr_3+2]^{+\bullet}$ was used as precursor ion, yielding product ions m/z 141 $[C_5H_2Br]^+$ and 62 $[C_5H_2]^{+\bullet}$. The fragment m/z 237 $[C_5Cl_5+2]^+$ was chosen for HCDBCO, giving the product ions m/z 118.5 $[C_4Cl_2]^+$ and 167 $[C_5Cl_3+2]^+$. For EHTBB, the fragment m/z 420.5 $[C_7HBr_4O+4]^+$ was selected yielding the product ions m/z 311.5 $[C_6HBr_3+2]^{+\bullet}$ and 233 $[C_6HBr_2+2]^+$. The analysis of BTBPE was carried out by using m/z 356.5 $[C_8H_6OBr_3+2]^+$ as precursor ion, yielding the product ions m/z 118 $[C_8H_6O]^{+\bullet}$ and 90 $[C_6H_2O]^{+\bullet}$. For BEHTBP, the fragment m/z 464.5 $[C_8HBr_4O_3+4]^+$ was selected yielding the product ions m/z 380 $[C_5Br_4+4]^{+\bullet}$ and 310.5 $[C_6Br_3+2]^+$. Finally, the fragment m/z 484.5 $[C_7H_2Br_5+4]^+$ was chosen for DBDPE yielding the product ions m/z 324.5 $[C_7H_2Br_3+2]^+$ and 403.5 $[C_7H_2Br_4+2]^{+\bullet}$. These transitions are indicated in Table 2.

3.3. Method performance

Table 3 shows the instrumental LOD, the correlation coefficient (r^2) of the calibration curve and the repeatability obtained for each compound with each MS condition. The sensitivity of each GC–MS method was first compared using the instrumental limits of detection, calculated in pg injected that gave a s/n of 3.

GC–EI-MS/MS showed advantage for the analysis of TCEP, TCPP, TDCP and TPhP. This technique gave best linearity and lower detection limits (33, 3, 12 and 8 pg injected, respectively) than by GC–EI-MS/SIM (75, 34, 50 and 25 pg injected, respectively). GC–ECNI-MS was not sensitive enough to determine these compounds.

The LOD for bromophenols were similar using GC–EI-MS/SIM, GC–EI-MS/MS, and GC–ECNI-MS/SIM for most of compounds. Because of the low bromination level, 3 BP and 4 BP were not detected using GC–ECNI-MS/SIM. Good linearity was observed for all compounds using internal standard quantification, although 2,3,4,6 MTrBP as surrogate standard for tetrabromophenols produced poor linearity by all methods due to the bad chromatographic performance with the concentration and conditions used. Thus, this compound was disregarded as surrogate standard because poor resolution was observed at 0.2 μg mL^{-1}. We observed that high brominated bromophenols had worse chromatographic response than low brominated bromophenols. Tetra- to pentabromophenols elute late and show more tailed peaks, affecting the chromatographic resolution and the detection limits. This chromatographic limitation was observed in all GC–MS methods. Actually, the signal to noise ratio obtained for bromophenols monitoring the bromine ion by GC–ECNI-MS/SIM was comparable to those obtained by GC–EI-MS/SIM, or worse for some compounds. Furthermore, GC–ECNI-MS/SIM showed worse performance than both GC–EI-MS methods for the seven bromophenols eluting from 7.2 to 7.9 (3,5 DBP, 2,3,5 TBP, 3,4 BDP, 2,4,6 TBP, 2,4,5 TBP, 2,3,4 TBP, 2,3,6 TBP). Below 400 pg injected, the chromatographic resolution of these coeluted tribromophenols was very poor when monitoring both $[\mathrm{Br}]^-$ or $[\mathrm{M}]^{-\bullet}$, while 3,5 DBP and 3,4 DBP were not isolated. Thus the detection limits for these compounds where higher by GC–ECNI-MS than by the other methods. GC–EI-MS/SIM, GC–EI-MS/MS and GC–ECNI-MS/SIM optimization of PBP was carried out using a standard solution at 5 μg mL^{-1}. However, PBP showed the worst performance and was not detected by any method at the highest calibration level (1.4 μg mL^{-1}). Thus, the analysis of PBP was disregarded.

The analysis of bromotoluenes, bromoanilines and bromoanisols showed good performance and linearity. The LOD were similar comparing GC–EI-MS/SIM and GC–EI-MS/MS, while the lowest LOD were obtained by GC–ECNI-MS/SIM. Good linearity using internal standard quantification was obtained for mono- and di-substituted bromoanilines and bromotoluenes using 4 MBP and 2,4 MDBP, however for 2,4 DBAs, better linearity was observed by GC–EI-MS/MS and GC–ECNI-MS/SIM with external standard quantification. Poor linearity was obtained for 2,4,6 TBAs, 2,4,6 TBT and 2,4,6 TBA when 2,4,6 MTBP was used as surrogate standard and therefore these compounds were also quantified by external standard quantification.

Improvements in LOD for PBDEs could be observed by GC–EI-MS/MS when comparing to GC–EI-MS/SIM. However, GC–ECNI-MS/SIM was superior in sensitivity, exhibiting the lowest LOD (from 0.3 to 7 pg injected). Monitoring the bromide ions by GC–ECNI-MS is one of the most sensitive detection method for PBDEs [39] but it provides poor structural information for PBDEs, and thus, confirmation of results is dubious. Despite of the advantage of low LOD obtained by GC–ECNI/MS, special attention should be paid to the co-elution with others brominated species, like MeO-PBDEs or PBBs, that can interfere with PBDEs in a DB-5 column [24,40]. Ackerman et al. (2005) [41] reported an optimization of GC–ECNI-MS parameters (ions source temperature, electron energy, emission current, system pressure, reagent gas) for 39 mono- to heptaPBDE congeners, to obtain significant increases in the abundance of PBDE molecular and high mass fragments. By the optimized conditions, monitoring the ion $[\mathrm{M}{-}x\mathrm{H}{-}y\mathrm{Br}]^-$ allowed average LOD of 0.122 pg. Those limits were higher than the obtained by monitoring only the ion $[\mathrm{Br}]^-$, but were comparable to those reported in other studies also monitoring bromine ion by GC–ECNI-MS. On the other hand, GC–EI-MS/MS is suggested by various authors as a more selective technique to analysis PBDEs in environmental and human samples [33,42–44].

GC–ECNI-MS/SIM provided the lowest LOD for new brominated flame retardants, from 0.1 to 6 pg, while by GC–EI-MS/SIM and GC–ECNI-MS/MS the limits were from 2 to 1000 pg and from 0.4 to 200 pg, respectively.

Table 4 shows a simple comparison of the three GC–MS methods developed, addressing sensitivity and identification capabilities. All techniques provided acceptable performance and unique features for the analysis of flame retardants of differing chemical structure. GC–ECNI-MS/SIM showed low LOD for most of the compounds while GC–EI-MS/SIM and especially GC–EI-MS/MS were more selective and sensitive techniques. Given the low chromatographic noise of GC–EI-MS/MS in SRM, this technique was chosen for the analysis of priority and emerging flame retardants in water because of its superior selectivity compared to the other techniques. In addition, GC–EI-MS/MS allowed to eliminate background noise from the matrix which enhances greatly the limits of detection of the method. Considering that these compounds are reported in wastewater at a concentration of μg L^{-1} to ng L^{-1} [21,45–47], GC–EI-MS/MS proved suitable for the identification of priority and emerging FR with high precision and accuracy, without the risk of false positives, and therefore the performance of the method for the analysis of flame retardants in water was evaluated with this technique.

Fig. 2 shows the recovery ($n = 3$) for target compounds in spiked MilliQ water and extracted by SPE. When possible, the use of internal standards proved to be mandatory for the quantification of flame retardants in wastewaters. It permitted to control extraction efficiency and to quantify target compounds with high precision since possible losses during sample manipulation were corrected. Good method performance was obtained for organophosphorus by external quantification, with recoveries ranging from 88% to 121% and precision from 4% to 14%. MDL for organophosphorus were from 0.004 to 0.04 μg L^{-1}. These MDL values are in agreement with the reported levels of these contaminants in water samples [15,45,48].

Recoveries of bromophenols ranged from 50% to 111%, with relative standard deviations (RSD) from 11% to 22% (Fig. 2) except for 2,3 DBP, 2,6 DBP, 2,3,5 TBP, 2,3,6 TBP, 3,4,5 TBP and tetrabromophenols that were lower than 30%, so these compounds were not determined in the water samples, given basically to the poor chromatography of these underivatized compounds. MDL of bromophenols extracting 100 mL of water ranged from 0.04 to 0.74 μg L^{-1} using GC–EI-MS/MS. Some authors suggest the derivatization of phenols before the injection in the GC systems to improve the performance of the method, reaching low MDLs [49–51]. Sim et al. [22] reported MDL from 0.00008 to 0.0023 μg L^{-1} for 2 BP, 3 BP, 4 BP, 2,4 DBP, 2,6 DBP, 2,4,6 TBP and PBP after liquid/liquid extraction of 500 mL of water and a final step of concentration to 0.5 mL and analysis by GC–EI-MS/SIM after a derivatization step with acetic anhydride and indicate an important gain in detection limit for bromophenols. On the other hand, the derivatization step is laborious and time consuming, and cannot be adequate in multiresidue analysis because it could interfere in the chromatographic response of others compounds. López et al. [21] have analyzed bromophenols in water by SPE-GC–EI-MS without

Table 3
Instrumental detection limit (LOD), correlation coefficient (r^2) and repeatability obtained by GC–EI-MS/SIM, GC–EI-MS/MS and GC–ECNI-MS/SIM. Surrogate standards are indicated in italics. Method detection limit (MDL) for water samples is presented for the GC–EI-MS/MS method.

Compound	EI-MS/SIM			ECNI-MS/SIM			EI-MS/MS			
	LOD (pg)	r^2	Repeatability ($n=5$)	LOD (pg)	r^2	Repeatability ($n=5$)	LOD (pg)	r^2	Repeatability ($n=5$)	MDL (μg L^{-1}
3,5 DBT	5	0.999	0.72	1	0.979	3.2	7	0.999	4.8	0.01
2,5 DBT	6	0.999	1.6	1	0.939	1.6	7	0.999	7.3	0.01
4 BA	26	0.998	4.5	3	0.999	2.4	24	0.996	2.1	[c]
4 MBP	*32*		*3.0*	[a]	[a]	[a]	*62*		*7.9*	
3 BP + 4 BP	39	0.963	3.1	[a]	[a]	[a]	68	0.998	5.0	0.09
2,4 MDBP	*16*		*3.6*	*22*		*1.7*	*58*		*5.7*	
2,4 DBP + 2,5 DBP	16	0.998	1.7	22	0.992	1.4	36	0.996	7.9	0.04
2,3 DBP	24	0.997	1.1	30	0.974	2.1	53	0.999	5.8	[c]
2,6 DBP	20	0.994	3.2	51	0.982	1.1	23	0.998	8.2	[c]
2,6 DBA	2	0.991	2.4	0.1	0.972	0.6	5	0.979	0.7	0.01
2,4 DBAs	2	0.997	3.1	1	0.986	0.7	1	0.993	7.0	0.002
2,4 DBA	3	0.995	1.8	0.1	0.9905	3.1	12	0.997	6.9	0.02
2,4,6 TBT	2	0.989	2.6	0.1	0.978	1.3	5	0.968	7.4	0.009
3,5 DBP	400	0.999	8.2	[b]	[b]	[b]	400	0.999	12	0.62
2,3,5 TBP	94	0.999	8.8	400	0.994	8.0	67	0.995	7.7	[c]
2,4,6 TBAs	24	0.983	2.6	4	0.998	1.0	2	0.999	9.8	0.004
3,4 DBP	400	0.999	11	[b]	[b]	[b]	400	0.988	1.9	0.74
2,4,6 MDBP	*55*		*14*	*400*		*3.1*	*126*		*6.2*	
2,4,6 TBP	53	0.983	9.1	400	0.999	2.6	126	0.967	3.1	0.23
2,4,5 TBP + 2,3,4 TBP	51	0.989	14	400	0.993	3.1	184	0.988	10	0.35
2,3,6 TBP	70	0.982	5.7	400	0.957	1.5	126	0.931	1.5	[c]
2,4,6 TBA	4	0.986	0.7	0.2	0.923	0.8	4	0.964	0.8	0.007
TCEP	75	0876	6.9	[a]	[a]	[a]	33	0.9985	13	0.04
TCPP	34	0.931	2.3	[a]	[a]	[a]	3	0.993	2,1	0.004
3,4,5 TBP	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[c]
2,3,5,6 TrBP	307	0.998	25	400	0.827	5.9	360	0.997	3.4	[c]
2,3,4,6 MTrBP	*314*		*28*	*400*		*3.1*	*360*		*5.6*	
2,3,4,5 TrBP + 2,3,4,6 TrBP	314	0.943	12	400	0.977	1.9	360	0.997	1.5	[c]
PBT	4	0.992	3.6	0.1	0.981	1.6	24	0.995	2.9	0.03
BDE 28	3	0.982	2.4	0.6	0.989	2.5	0.4	0.998	15	0.0005
PBEB	4	0.996	1.9	0.2	0.980	1.8	0.4	0.995	2.7	0.0005
TDCP	50	0.986	4.2	[c]	[c]	[c]	12	0.996	2.6	0.02
TPhP	25	0.996	6.3	[c]	[c]	[c]	8	0.998	2.4	0.01
DPTE	9	0.970	6.8	0.4	0.938	3.1	6	0.999	2.3	0.01
MHBB	*2*		*1.7*	*1*		*1.5*	*2*		*11*	
HBB	2	0.989	1.6	0.6	0.989	0.7	2	0.998	0.9	0.003
BDE 47	3	0.991	3.8	0.6	0.989	3.0	6	0.999	12	0.008
MBDE 77	*3*		*5.7*	*0.2*		*5.4*			*7*	
BDE 100	7	0.999	4.1	0.4	0.990	5.4	2	0.998	0.9	0.003
BDE 99	7	0.999	7.2	7	0.990	3.2	1.8	0.998	14	0.003
HCDBCO	12	0.999	6.1	0.2	0.979	1.4	21	0.993	0.5	0.03
EHTBB	19	0.984	7.1	1.3	0.986	4.1	12.5	0.997	3.0	0.02
BDE 154	6	0.997	8.0	0.3	0.993	2.3	6	0.997	20	0.008
BDE 153	6	0.987	11	0.3	0.991	4.1	16	0.998	40	0.02
BDE 183	13	0.978	17	0.4	0.992	4.1	3	0.993	5.5	0.004
BTBPE	24	0.987	19	0.6	0.992	3.5	26	0.995	3.1	0.04
BEHTBP	15	0.979	5.1	2	0.991	4.5	20	0.996	3.8	0.03
MBDE 209	*173*		*21*	*3*		*3.5*	*100*		*6*	
BDE 209	173	0.997	23	3	0.981	3.5	100	0.983	12	0.13
DBDPE	1000	0.989	7	6	0.930	3.2	200	0.952	11	0.27

[a] Not detected at 1000 pg.
[b] Coelution separation not possible by mass fragment selectivity or specific transitions.
[c] Not determined.

a derivatization step and obtained MDL of 0.235 and 0.032 $\mu g\,L^{-1}$ for 2,4 DBP and 2,6 DBP, respectively, while MDL of 0.0001 $\mu g\,L^{-1}$ for both compounds were obtained by SPE-GC–ECNI-MS, using a SGE BPX5 column. These results show that it is possible to obtain low MDL for the analysis of bromophenols by GC–MS without a derivatization step if the extraction and the mass spectrometric and chromatography conditions are optimized. On the other hand, we have noted that the chromatographic resolution in the analysis of underivatizated bromophenols in a 15 m DB-5MS column is much affected by the increasing lifetime of the column, especially if highly polluted samples are analyzed, where the peaks tend to tail until they are undetected. A similar effect was also reported for bromo- and others phenols using a more polar phase column [52]. This lose of chromatographic resolution first affect the tri- to penta-bromophenols performance, but with the increasing lifetime of the column it also affect mono and di-bromophenols and 4 BA, eluting at the beginning of the chromatogram. We do not rule out that derivatization of bromophenols can be advantageous, especially for routine and/or high number of analysis, but the underivatizated method proposed in this study could be advantageous for the screening analysis of a wide range of flame retardants in environmental samples.

Bromotoluenes, bromoanilines and bromoanisols, were recovered from 52% to 125%, with RSD from 4% to 19%. Among these, the recovery for 4 BA, were below 30% due to worse chromatographic performance of this compound. MDL ranged from 0.009 to 0.03 $\mu g\,L^{-1}$, 0.007 to 0.02 $\mu g\,L^{-1}$ and from 0.002 to 0.004 $\mu g\,L^{-1}$ for bromotoluenes, bromoanilines and bromoanisols, respectively. The

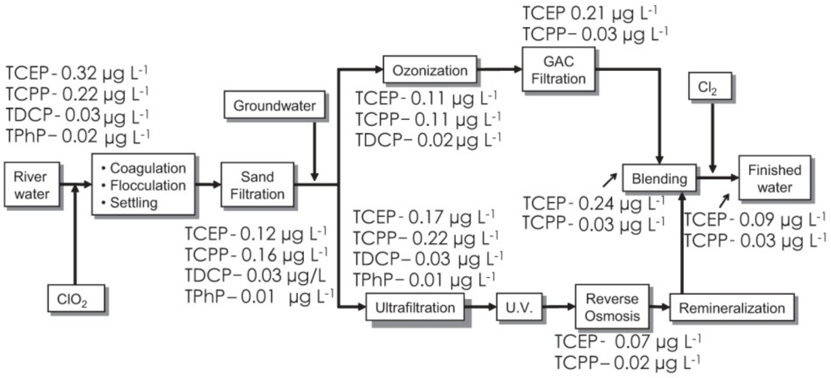

Fig. 3. Evolution of organophosphorus flame retardants within a drinking water treatment facility (Spain).

retardants, achieving at the end of the lines a removal near to 70%. In the case of TCEP, the lower removal rate suggests that this compound was more resistant to the treatment. Fig. 3 shows the preliminary results obtained and a scheme of the waterworks process. In accordance to a Andresen and Bester [48], ozonization was not effective to eliminate chlorinated organophosphates. On the other hand, a recent study [53] reports that activated carbon filtration was very effective to remove chlorinated and non chlorinated organophosphates, while in this study elimination rates after GAC filtration was lower than 75% for TCPP while the amount of TCEP before the treatment was lower than after filtration. This could be preliminary explained by differences between i.e. type, lifetime of carbon filters, contact times, etc. used in each drinking water treatment process. The above mentioned facility use a biologically active filter and contact time of 1 h, while at the facility of this study the average contact time in GAC filters is 15 min (flow rate 6 m^3/s). The filter is not biologically activated, although a biofilm is generated within the lifetime of filter, allowing some biological activity.

The concentrations of TCEP and TCPP in raw water were of 0.32 and 0.22 $\mu g\,L^{-1}$ and in finished water were 0.09 and 0.03 $\mu g\,L^{-1}$, respectively. These results are in agreement with a USA study reporting that organophosphorus flame retardants were resistant to the drinking water treatment [54]. In the American study, TCEP and TDCP were detected in raw, settled, filtered and finished water, in concentrations ranging from 0.08 to 0.24 $\mu g\,L^{-1}$ in raw water and from about 0.07 to 0.27 $\mu g\,L^{-1}$ in finished water.

4. Conclusions

47 flame retardants of different chemical families were characterized using several mass spectrometric techniques with the aim to resolve the uncertainties in the analysis of priority and emerging flame retardants in water. The performance of GC–EI-MS/MS, GC–EI-MS/SIM and GC–ECNI-MS/SIM did not vary considering linearity, reproducibility and sensitivity, however, GC–EI-MS/MS proved to be superior in the analysis of priority and emerging flame retardants in water samples due to its unique selectivity. However, some bromophenols such as 2,3 DBP, 2,6 DBP, 2,3,5 TBP, 2,3,6 TBP, 3,4,5 TBP and tetrabromophenols showed poor performance with the MS conditions used due to chromatographic problems and lack of sensitivity and were disregarded from the multiresidue method. This study reports the presence and evolution of several flame retardants within a waterworks facility, where some compounds were identified in finished water, although at trace level concentration. Finally, some of the compounds included in the study are legislated or banned, but most of them are included in the "new" generation of flame retardants. Thus, the method optimized will enable the monitoring of these contaminants in environmental matrices.

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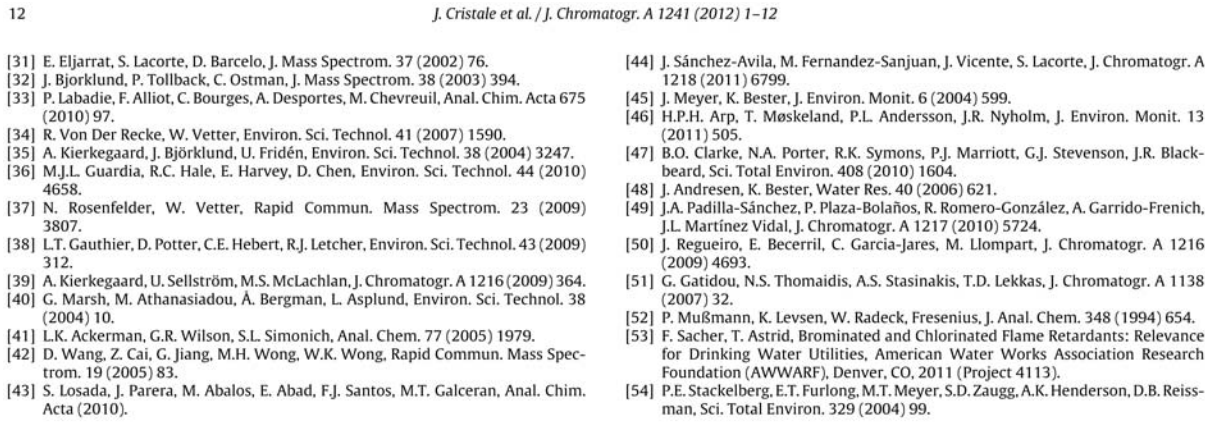

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2.3.2 Paper 2: “Assessment of flame retardants in river water using a ceramic dosimeter passive sampler”

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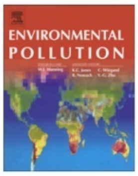

Assessment of flame retardants in river water using a ceramic dosimeter passive sampler

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ABSTRACT

Novel brominated (BFRs) and organophosphorus (OPFRs) flame retardants were monitored in river water using the ceramic dosimeter passive sampling device with HLB (hydrophilic–lipophilic balance) as sorbent. Laboratory calibrations were performed to determine sampling rates for each compound using the Archie's law exponent. The passive sampling device was used to determine the presence of 6 BFRs in the River Aire (United Kingdom), selected according to their ubiquitous presence in the River Aire. Passive sampling integrated river water concentrations ranged from 0.010 to 5.6 μg L^{-1} for all OPFRs, while BFRs were not detected with this specific passive sampler configuration. Decreased sampling rates were evidenced after 3 weeks of deployment, probably due to fouling. Good agreement between integrated and snapshot water concentrations was obtained, indicating the efficiency of the passive sampler for the monitoring of OPFRs in river water.

1. Introduction

The recent years, a big number of ubiquitous emerging organic pollutants have been detected in all environmental compartments as a result of the massive use of chemicals in industrial and other products (Dickhut et al., 2005; Cincinelli et al., 2005; Domine et al., 2007), rendering imperative the continuous environmental monitoring. In this direction, passive sampling techniques have emerged as an efficient way of sample collection, which leads to integrated, efficient and relatively inexpensive characterization of water pollutants. Many types of passive sampling techniques have been developed; the uptake of contaminants is usually achieved by sorption/binding onto a media with specific properties which facilitate this process. In aquatic environments, the properties that govern the uptake of chemicals by a passive sampler are usually the polarity, water solubility and lipophilicity of target pollutants (Alvarez et al., 2004; Huckins et al., 2006), and its performance depends on the type of water to be monitored (Zabiegała et al., 2010; Hildebrandt et al., 2006). Several passive sampler designs are commercially available for the monitoring of organic compounds in water, such as POCIS (polar organic chemical integrative sampler), SPMD (semipermeable membrane devices), and Chemcatcher (Vrana et al., 2005). Passive sampler devices have been used to monitor persistent organic pollutants (POPs) in various aquatic compartments (Katsoyiannis and Samara, 2007), pesticides (Mazzella et al., 2010), pharmaceuticals (Bartelt-Hunt et al., 2009; Chen et al., 2012) and endocrine disrupters (Arditsoglou and Voutsa, 2008; Fent et al., 2010), etc. Other devices such as the ceramic dosimeter have been used for monitoring of benzene, toluene, ethylbenzene, and xylenes (BTEXs) and Polycyclic aromatic hydrocarbons (PAHs) in groundwater, using the Dowex Optipore L-493 and the Amberlite IRA-743 as sorbent, respectively (Martin et al., 2003; Bopp et al., 2005).

Among all configurations, in this study we have chosen the ceramic dosimeter for the integrated monitoring of flame retardants. This device is characterized by an inert and rigid ceramic tube operating as barrier phase, having a diameter of 1 cm, 5 cm length, a wall thickness of 1.5 mm and a porosity of 5 nm. The advantages of this device are the easier calibration, since the water flow does not affect significantly the uptake of compounds, the long-term stability and the versatility of the system since (in theory) any class of compounds can be monitored by choosing the appropriate sorbent (Weiss et al., 2007). This unique design makes it very promising for monitoring organic contaminants in river water.

The aim of this study was to optimize and validate the ceramic dosimeter passive sampler for the monitoring of emerging and new flame retardants in river water. Firstly, grab samples from different

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points along the River Aire (England) were collected and analyzed for identifying the pervasive flame retardants. This study included polybrominated diphenylethers (PBDEs), new brominated flame retardants (BFRs) and organophosphorus flame retardants (OPFRs), compounds used in electronic devices and in many industrial formulations. Secondly, we optimized the sampler to determine 4 OPFRs and 2 BFRs, the most frequently detected in river water. For this purpose, the HLB (hydrophilic–lipophilic balance) sorbent, able to uptake simultaneously both polar and non-polar compounds, was chosen as sorptive phase. Under controlled laboratory and field conditions, and based on the detected compounds, we calibrated and tested the ceramic dosimeter passive sampler in terms of sampling rate, long stability and recovery. The system proved to be a valuable tool for the monitoring of flame retardants, compounds that have become ubiquitous in surface waters as a result of the continuous input from spills and waste disposal (Andresen et al., 2004).

2. Materials and methods

2.1. Chemicals and reagents

Individual solutions of 1,2 bis(2,4,6 tribromophenoxy)ethane (BTBPE) at 50 μg mL^{-1} in 5% toluene in nonane, decabromodiphenyl ethane (DBDPE) at 25 μg mL^{-1} in toluene, bis(2-ethyl-1-hexyl) tetrabromo phthalate (BEHTBP), pentabromotoluene (PBT), 2,3-dibromopropyl 2,4,6 tribromophenyl ether (DPTE), hexachloro cyclopentadienyl dibromooctane (HCDBCO) and 2 ethylhexyl 2,3,4,5 tetrabromobenzoate (EHTBB) at 50 μg mL^{-1} in toluene were acquired from Wellington Laboratories (Guelph, Canada). A solution mixture of BDE 28, 47, 99, 100, 153, 154, 183, and 209 in n-nonane at 1 μg mL^{-1} was acquired from Cambridge Isotope Laboratories. Solid standards of HBB (hexabromobenzene), PBEB (pentabromoethyl benzene), TCEP (tris(2-choroethyl) phosphate), TCPP (tris(2-chloro-1-methylethyl) phosphate), TDCP (tris[2-chloro-1-(chloromethyl)ethyl] phosphate) and TPhP (triphenyl phosphate), were acquired from Sigma–Aldrich (Germany). TCPP was acquired as a mixture of isomers (tris(2-chloro-1-methylethyl) phosphate, bis(1-chloro-2-propyl)-2-chloropropyl phosphate, and bis(2-chloropropyl)-1-chloro-2-propyl phosphate). Each solid standard was weighed and dissolved in hexane, except the TCEP, TCPP and TDCP that were dissolved in a mixture of ethyl acetate and methanol (1:1). The internal standards [$^{13}C_6$]hexabromobenzene (MHBB) at 50 μg mL^{-1} in toluene, 3,3′,4,4′-tetrabromo[$^{13}C_{12}$]diphenyl ether (MBDE-77) at 50 μg mL^{-1} in nonane and decabromo[$^{13}C_{12}$]diphenyl ether (MBDE-209) at 25 μg mL^{-1} in toluene, were acquired from Wellington Laboratories (Guelph, Canada), and PCB-65 (2, 3, 5, 6-tetrachlorobiphenyl) at 10 μg mL^{-1} from Dr. Ehrenstorfer (Augsburg, Germany). All standards were of >98% purity.

2.2. Grab sampling

The sample area was the River Aire (Yorkshire, England). This river is 114 km long, springs at Malham Tarn (North Yorkshire), and passes through the cities of Skipton, Keighley, Leeds and Castleford, among others. In this first step, an initial inventory of FR was performed by analyzing 3 sites along the river. 2.5 L of river water were collected at Point A (53° 50′ 37″ N and 01° 49′ 38″ W), Point B (53° 51′ 25″ N and 01° 43′ 43″ W) and Point C (53° 50′ 16″ N and 01° 42′ 21″ W), within the populated areas of Bradford and Leeds. 500 mL of unfiltered water sample was spiked with labeled surrogate (MHBB, MBDE 77 at 0.005 ng L^{-1}, and BDE 209 at 0.05 ng L^{-1}) and extracted using HLB 200 mg (WATERS, USA) solid phase extraction (SPE) cartridges, as described by Cristale et al. (2012). This sampling scheme permitted to identify the most ubiquitous compounds and their concentration ranges, for which passive sampling technique was thereafter developed.

2.3. Preparation, calibration, deployment and analysis of the passive samplers

SPE cartridges of 500 mg of HLB sorbent from Sigma–Aldrich (Germany) were conditioned with 12 mL of acetone and 12 mL of hexane, and dried under vacuum for 30 min. Then, around 400 mg of sorbent was removed from the SPE cartridge, rinsed with 1.5 mL of Milli-Q water and placed into the ceramic dosimeter, which was capped with PFTE caps and stored in Milli-Q water until deployment.

Laboratory calibration of the passive sampler was performed for TCEP, TCPP, TDCP, TPhP, HBB and PBEB, which accounted for the main flame retardants detected in river Aire. This was performed using 2-L beakers with 1.8 L of Milli-Q water spiked at 20 μg L^{-1} for OPFRs and at 1 μg L^{-1} for BFRs. Five containers, with two passive samplers each one, were used for the calibration. Each set of two samplers was removed from the beaker after 2, 4, 6, 8 and 10 days, and maintained in Milli-Q water until extraction. Then, 6 passive samplers were deployed in the River Aire (Point C) and were left for 1 (1 passive sampler), 3 (2 passive samplers) and 5 weeks (3 passive samplers). After deployment, the passive samplers were stored in Milli-Q water until extraction. Additionally, two blanks of the passive samplers were prepared together with the samplers for river water deployment and were kept in Milli-Q water for 5 weeks. The blanks were extracted and analyzed as described for the river samplers. For the extraction, the sorbent was removed from the dosimeter, rinsed with 5 mL Milli-Q water to achieve quantitative transfer and placed back to the empty SPE cartridge. The cartridges were dried under vacuum for 90 min and subsequently, the elution was performed with 12 mL of acetone and 12 mL of hexane/dichloromethane (1:1 v/v). The solvent was evaporated under a gentle N_2 stream until dryness and reconstituted in 100 μL of hexane containing MHBB and PCB-65 at 0.025 and 0.05 μg mL^{-1}, respectively, as internal standards.

For comparison between integrated and snapshot concentrations, two samples of 2.5 L of water were collected after the passive sampler deployment, and simultaneously with passive sampler collection after 1, 3, and 5 weeks, and were extracted as previously mentioned.

The analysis of the extracts was performed in a Thermo GC–MS (Thermo-Finnigan Trace), in EI mode, fitted with a ThermoQuest AS2000 autosampler. The GC–EI–MS/SIM conditions were adopted by Cristale et al. (2012). The oven program was set at 100–220 °C at 10 °C/min and to 300 °C at 8 °C/min (5 min).

2.4. Quality control

The extraction performance of HLB cartridges for TCPP, TCEP, TDCP, TPhP, HBB and PBEB was assessed using spiked HLB cartridges (500 mg, Sigma–Aldrich). The cartridges were rinsed with Milli-Q water, spiked with 0.05 μg of the standards (in acetone) and kept in contact overnight. The cartridges were dried under vacuum for 90 min and the extraction procedure was optimized using different solvents (acetone, hexane and dichloromethane). Acetone was found to be the most efficient solvent for OPFRs (recoveries of >90%), whereas for the non-polar compounds (HBB and PBEB) an additional elution with 12 mL of DCM/hexane 1:1 was performed, which helped achieve recoveries of 75% and 78% for HBB and PBEB, respectively.

To test the long-term stability of the passive sampler, six passive samplers were deployed in 2-L beakers, as described previously. Three passive samplers were immediately extracted and three more were kept in Milli-q water for 26 days, and then extracted.

2.5. Theoretical basis of the ceramic dosimeter passive sampler

The contaminant's accumulation in the ceramic dosimeter passive sampler occurs by diffusion from the contact water though the ceramic wall into the sorbent phase. Details about fundamentals of the ceramic dosimeter passive sampling are well described in previous studies (Martin et al., 2003; Bopp et al., 2005; Weiss et al., 2007). Basically, the only parameter that influences the sampling rate of contaminants is the effective diffusion coefficient (D_e) of the contaminant in the ceramic wall. The accumulated mass (M) in the sampler can be calculated from Fick's first law:

$$M = F \times A \times t = D_e \frac{\Delta C}{\Delta x} \times A \times t \qquad (1)$$

where, F is the mass flux through the ceramic membrane, A (8.4 cm^2) is the membrane surface area, t is the sampling time. The term ΔC is the difference in aqueous chemical concentration between the sampling environment (C_w) and the inert part of the ceramic tube, C_i. Because the water inside the ceramic tube is saturated by the receiving phase, $C_i \approx 0$, so ΔC can be simplified as C_w. Δx is diffusion path length that in this case is the thickness of the membrane (0.15 cm).

D_e is obtained as:

$$D_e = D_w \times \epsilon^m \qquad (2)$$

Where, D_w is the diffusion coefficient of the chemical in water, ϵ (0.305) is the porosity of the ceramic material, and m is the Archie's law exponent.

As temperature influences D_w, the accumulation of contaminants is temperature-dependent during the monitoring period. The D_w may be calculated according to Worch (1993) as:

$$D_w = 3.595 \times 10^{-7} \frac{T}{\eta \times MW^{0.53}} \qquad (3)$$

where, T (K) is the water temperature, η (mPa s) the water dynamic viscosity and MW the molecular weight of the chemicals. These parameters were used to calibrate and set up the optimum deployment conditions (Tables 1 and 2), for the selected compounds.

3. Results and discussion

3.1. Occurrence of flame retardants in the River Aire

From the 21 flame retardants analyzed, only OPFRs (TCEP, TCPP, TDCP, TPhP) and the brominated HBB, PBEB were detected in the

Table 1
Physical and chemical properties of the selected pollutants.

Compound name	Abbreviation	MW	Solubility[a] (g L^{-1}, at 25 °C, pH 7)	Log K_{ow}	Uses
Pentabromoethylbenzene	PBEB	500.6	2.1×10^{-4}	6.76 ± 0.617	Thermoset polyester resins (circuit boards, textiles, adhesives, wire and cable coatings, polyurethane foam)
Hexabromobenzene	HBB	551.5	5.5×10^{-4}	6.11 ± 0.674	Paper, woods, textiles, electronic and plastic goods
Tris(2-choroethyl) phosphate	TCEP	285.5	7.4	1.47 ± 0.356	Plastics, textiles, electronic equipment, furniture, construction
Tris(2-chloro-1-propyl) phosphate	TCPP	327.6	1.0	2.53 ± 0.370	
Tris[2-chloro-1-(chloromethyl) ethyl] phosphate	TDCP	430.9	0.12	3.28 ± 0.444	
Triphenylphosphate	TPhP	326.3	7.2×10^{-3}	4.59 ± 0.237	

[a] Data from SciFinder originating from calculated properties using Advanced Chemistry Development (ACD/Labs) Software V11.02 (©1994–2011 ACD/Labs).

water samples. The concentrations of OPFRs ranged from 0.016 to 26 μg L^{-1}, being TCPP and TPhP the most and least abundant compounds, respectively. PBEB was detected in point B and C at 0.0002 and 0.0003 μg L^{-1} and HBB was detected at point A at 0.0008 μg L^{-1}. Based on these results, the ceramic dosimeter passive sampler was calibrated and tested for monitoring of TCEP, TCPP, TDCP, TPhP, HBB and PBEB in river water.

3.2. Calibration and performance of the passive sampler

The passive sampler laboratory calibration results are given in Fig. 1. The uptake of all compounds was linear for, at least, 10 days. This deployment time did not allow determining the actual capacity of the passive sampler, but it indicated that the capacity is higher than ~500 ng for OPFRs and ~5 ng for BFRs. Also, the cumulative mass uptake was estimated for different values of Archie's law exponent (m) (Weiss et al., 2007), using Equations (1)–(3). For OPFRs, the best correspondence was obtained with m values of 1.6, 1.8, 1.7 and 1.9 for TCEP, TCPP, TDCP and TPhP, respectively. For BFRs, the best correspondence was obtained with m values of 3.2 and 2.8 for HBB and PBEB, respectively. Based on these m values and 12 °C (average river water temperature), the calculated sampling rates for OPFRs were 3.7, 2.7, 2.6 and 2.4 mL d^{-1}, for TCEP, TCPP, TDCP and TPhP, respectively and 0.66 and 0.39 mL d^{-1} for PBEB and HBB, respectively (Table 2). The sampling rates are smaller than the values reported by Li et al. (2011), who using HLB in POCIS samplers for ionic pharmaceuticals reported sampling rates in the range of hundreds of mL d^{-1}. However, to the best of our knowledge, there are no reported sampling rates for HLB contained passive samplers for BFRs and OPFRs, in order for a direct comparison to be feasible.

Fig. 2 (a, b) shows the correlation of the sampling rates with K_{ow} (Fig. 2a) and water solubility (Fig. 2b) of the studied compounds. The higher the water solubility, the higher is the uptake, because the latter takes place from the dissolved water phase. Contrarily, compounds with high K_{ow} have lower uptakes, due to the fact that a considerable fraction of these compounds will tend to partition to particulate matter, decreasing that way the uptake (Katsoyiannis et al., 2006).

The ability of the passive sampler to retain the pollutants after it has been deployed, collected and stored in the laboratory before analysis, was also assessed. It can be seen in Fig. 3 that the long-term stability of the passive sampler is quite satisfactory. TCEP exhibited a decrease after 26 days (~15%), HBB and TPhP an increase of ~40% and ~15%, respectively, while for the other compounds, no breakthrough was observed. It is important for a passive sampler to exhibit satisfactory long-term stability, as this is an indicator that there is neither back-diffusion to the sampling media, nor degradation of the pollutants of interest (Martin et al., 2003).

3.3. River deployment

Six passive samplers were deployed in the River Aire. All OPFRs were detected, while HBB and PBEB were below the limit of detection. Fig. 4a presents the OPFRs accumulated masses over 1, 3 and 5 weeks at point C of the River Aire, while the value plotted as 0 indicates the amount quantified in the blank passive samplers. Results after three weeks and after five weeks of deployment are averages of two and three passive samplers, respectively. After three weeks of deployment, the standard deviation varied between 3% (TCEP) and 21% (TDCP), whereas after five weeks it ranged between 10% (TPhP) and 19% (TDCP). Taking into account the deployment time and various other factors that can affect field sampling, the repeatability of this method is considered as very satisfactory. A first visual inspection of the results suggests that the linear uptake for all four OPFRs stops after ~3 weeks. The accumulated masses after five weeks have only slightly increased compared to the three-week deployment. In particular, the observed increase was 18%, −2%, 10% and 5%, for TCEP, TPhP, TDCPP and TCPP, respectively. Fig. 4a compares the correlation coefficient obtained for all the deployment period and Fig. 4b shows the same results excluding the five-week deployment. For all OPFRs, the highest correlation coefficient was obtained in the first 3 weeks of deployment, suggesting that OPFRs uptake was linear during this period and that sampling rates decreased from week 3 to 5. The uptake of chemicals can decrease mainly due to three factors: i) fouling, inhibiting the uptake by blocking the ceramic dosimeters

Table 2
Archie's law exponent, diffusion coefficient in water, effective diffusion coefficient in ceramic tube, sampling rate and detection limit calculated from data generated by laboratory calibration.

Abbreviation	Archie's law exponent (m)	Diffusion coefficient (D_w ($cm^2\ s^{-1}$) at 12 °C)	Effective diffusion coefficient (D_e ($cm^2\ s^{-1}$), at 12 °C)	Sampling rate (mL d^{-1}, at 12 °C)	Passive sampling detection limits[a] (μg L^{-1})
PBEB	2.8	3.8×10^{-6}	1.4×10^{-7}	0.66	0.007
HBB	3.2	3.6×10^{-6}	8.1×10^{-8}	0.39	0.007
TCEP	1.6	5.1×10^{-6}	7.6×10^{-7}	3.7	0.06
TCPP	1.8	4.7×10^{-6}	5.6×10^{-7}	2.7	0.02
TDCP	1.7	4.1×10^{-6}	5.4×10^{-7}	2.6	0.007
TPhP	1.9	4.8×10^{-6}	5.0×10^{-7}	2.4	0.002

[a] Minimum average water concentration needed to determine the integrated concentration in river water, based on its sampling rate and the instrumental detection limit, for a deployment time of 21 days.

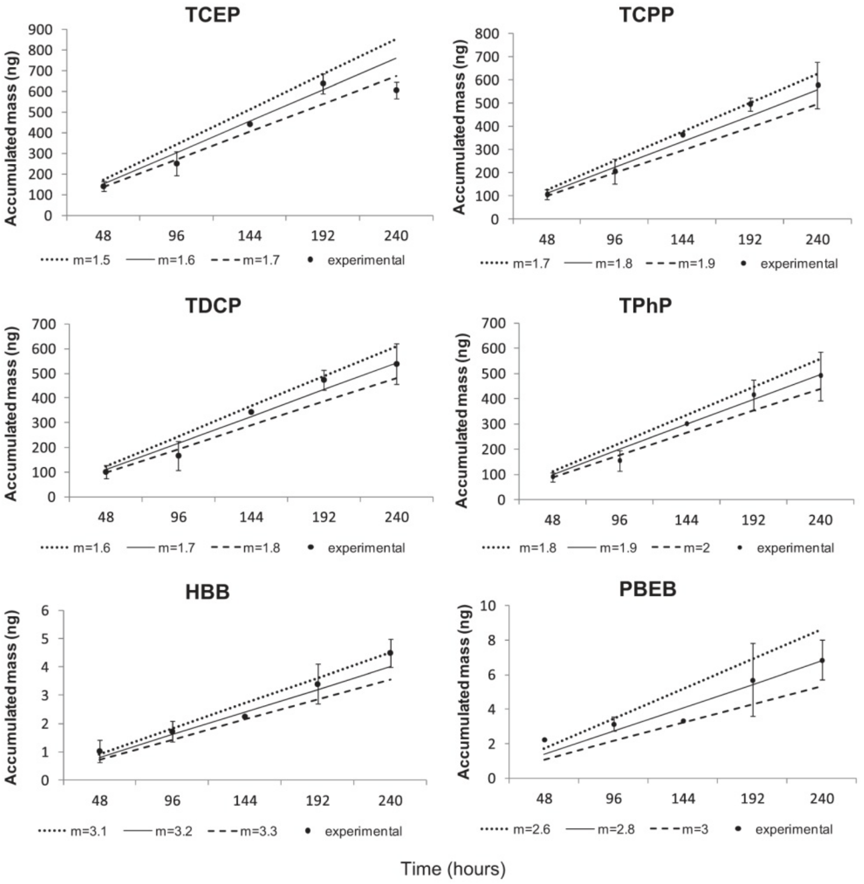


Fig. 1. Accumulated mass of OPFRs and BFRs in laboratory calibration experiments. The best fit for Archie's law exponent (m) is indicated by the continuous line.

pores, or acting antagonistically and uptaking the pollutants itself through the mechanism of sorption; ii) sampling equilibrium achievement; or iii) decreasing of chemicals' concentration in the water. Among these hypotheses, our laboratory calibration and grab sampling results indicate that fouling was the main factor that affected OPFRs uptake after 3 weeks of deployment. The laboratory calibration (Fig. 1) indicated that the uptake of these OPFRs was linear at least until around 500 ng of accumulated mass in the passive sampler. The average accumulated mass after five weeks was 14, 276, 3.2 and 1.3 ng for TCEP, TCPP, TDCP and TPhP, respectively, being much lower than 500 ng, so it can be concluded that the reaching of equilibrium was not the factor that caused the OPFRs sampling rate decrease for deployments longer than three weeks. Besides, as observed through grab water sampling, undertaken during the passive sampling deployment period (0, 1, 3 and 5 weeks), variations of water concentration was always up to a factor of three. OPFRs water concentrations ranged from 0.13 to 0.28 $\mu g\ L^{-1}$ for TCEP, from 2.9 to 6.7 $\mu g\ L^{-1}$ for TCPP, from 0.02 to 0.06 $\mu g\ L^{-1}$ for TDCP and from 0.01 to 0.02 $\mu g\ L^{-1}$ for TPhP. These values were also similar to the ones observed at the same sampling point for grab samples collected before the passive sampler deployment (TCEP – 0.28 $\mu g\ L^{-1}$, TCPP – 7.7 $\mu g\ L^{-1}$, TDCP –

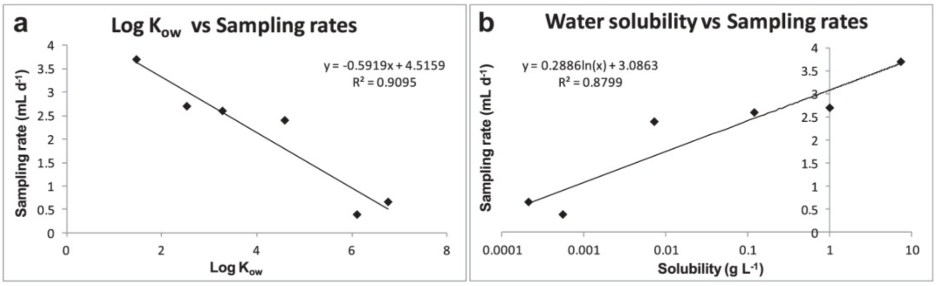


Fig. 2. Correlation of sampling rates with Log K_{ow} (a) and water solubility (b).

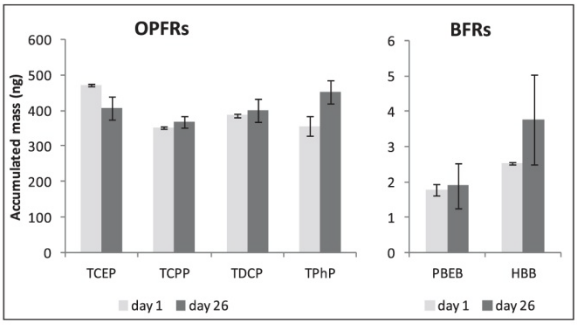


Fig. 3. Long-term stability of the passive sampler for TCEP, TCPP, TDCP, TPhP, HBB and PBEB.

0.09 µg L^{-1}, TPhP − 0.02 µg L^{-1}). Therefore, these results suggest that variations in water concentrations were not responsible for the decrease in the sampling rates after the third week of deployment. Fig. 5 shows pictures of the collected ceramic dosimeters after 1, 3 and 5 weeks of deployment. From the external appearance of the five-week sampler, it can be seen that almost all its surface is covered by fouling. In a lesser extent, this is observed also for the three-week sampling. Unlike what was observed for groundwater sampling, where deployment times of one year are reported for PAH analysis using the ceramic dosimeter (Bopp et al., 2005), our results indicate that for river water the deployment time is limited by the fouling effect, being likely the reason for the decreased uptake after the three weeks of deployment. Thus, our results indicate that for monitoring of trace pollutants in river waters, enhanced sampling rates may be necessary. Increase of the sampling rate can be achieved, for example, using larger samplers, thus increased surface area and consequently sampling rate. Table 2 shows the minimum average water concentration needed for each studied compound to be determined in river water by means of passive sampling.

In order to evaluate the efficiency of the passive sampler for flame retardants monitoring in river water, the estimated passive sampling water concentrations were compared to the concentrations deriving from grab sampling (Fig. 6). For this purpose, the OPFRs' amounts found in the blanks were subtracted from the passive sampling results. The estimated water concentrations were obtained using Equations (1)–(3), the average river water temperature of 12 °C, and the best correspondence value of m obtained from laboratory calibrations. As seen in Fig. 6, measured and estimated water concentrations are at the same order of magnitude, indicating that the passive sampler was efficient at least for the first 3 weeks of deployment. The actual river water concentrations of TCEP, TDCP and TPhP decreased notably between the 3rd week and the 5th week, whereas TCPP was higher in the 1st week and decreased during the 3rd week. This profile change may be due to the very heavy rain events observed in the area between the 3rd week and the 5th week. TCPP was the most abundant pollutant, exhibiting concentrations up to 7 µg L^{-1}, thus 2–3 orders of magnitude higher than the other three OPFRs. This difference suggests that TCPP is emitted mainly from important local sources. Its concentration may also be impacted by the heavy rain events registered during deployment. On the contrary, the other three OPFRs exhibited increased concentrations, suggesting that their occurrence is probably due to spills and atmospheric wash-out through precipitation. HBB was detected only in one grab sample at 0.0003 µg L^{-1}, while PBEB was detected in five of out of 10 grab samples analyzed, at concentrations from 0.0002 to 0.0019 µg L^{-1}. Concerning the calculated sampling rates for HBB and PBEB that are about 0.4 and 0.7 mL d^{-1}, and the observed levels of these contaminants in the river water, their non detection is probably due to the fact that for these compounds the deployment time (5 weeks) is short. Andresen et al. (2004) and Andresen and Bester (2004), reported concentrations of TCPP, TCEP and TDCP in German rivers. TCPP was in the levels of few tens to few hundreds of ng L^{-1}, thus many times lower than in River Aire, whereas the other two flame retardants were at the same order of magnitude.

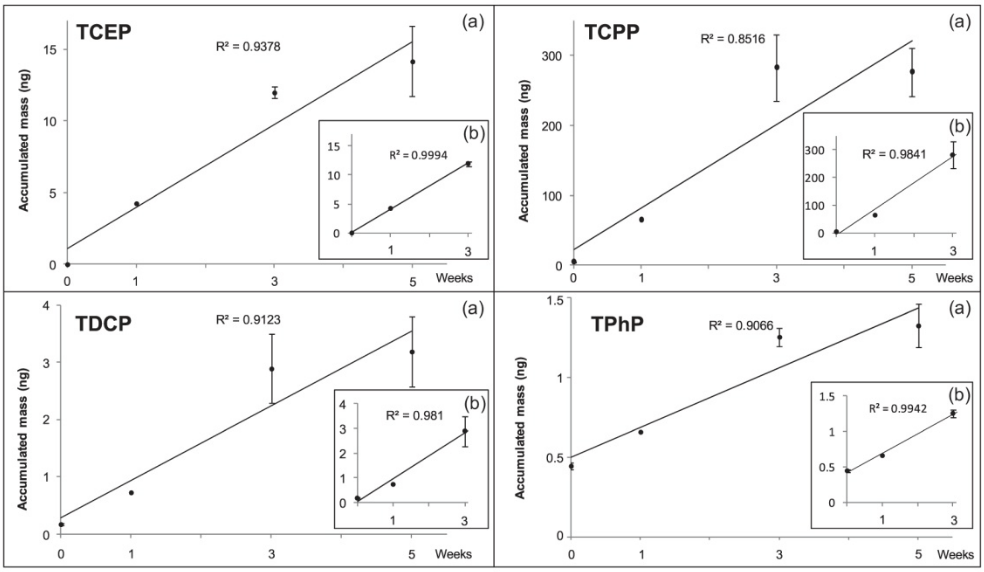
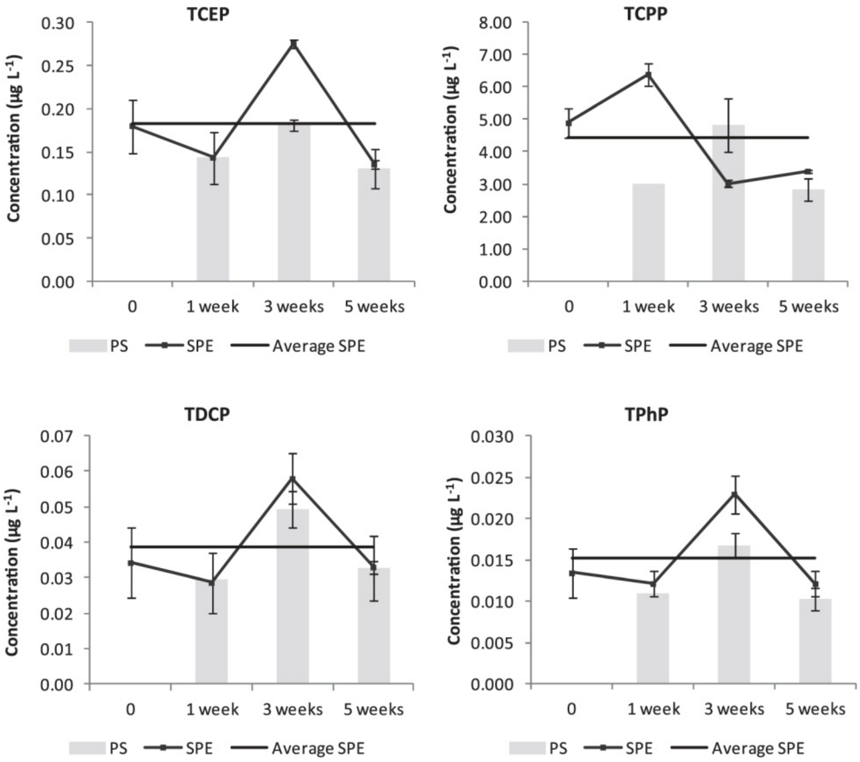


Fig. 4. OPFRs accumulated masses in the passive sampler after 1, 3 and 5 weeks deployment. (a) Correlation coefficient obtained for the entire deployment period. (b) Correlation coefficient obtained during the 3-week deployment period (excluding the five-week's result).

Fig. 5. Appearance of the passive samplers collected after 1, 3 and 5 weeks deployment.



PS – estimated passive sampling water concentration.
SPE – concentration derived from grab sampling, using solid phase extraction.
Average SPE – Average water concentration for all the sampling period, using grab sampling.

Fig. 6. Measured *vs* estimated (through passive sampling) water concentrations for OPFRs.

The good correlation between the integrated and snapshot concentrations is an indication of the robustness of this sampler design, resulting in satisfactory accuracy without any water flux calibration. For other passive sampler designs, such as POCIS and SPMD, the sampling rate is influenced by the water flow (Alvarez et al., 2004; Huckins et al., 2002). In the ceramic dosimeter, the uptake of chemicals is not affected significantly by water flow due to the fact that the 1.5 mm wall thickness of this device are much higher than any diffusion boundary barrier that could be formed in environmental waters. Therefore, the uptake of chemicals can be assumed as dependent only on the diffusion through the ceramic barrier (Weiss et al., 2007). This property of the ceramic dosimeter has been proved in this study for OPFRs in river water.

4. Conclusions

This pilot study presented preliminary results of the use of a novel passive sampler based on the ceramic tube and HLB sorbent as receiving phase. Our results suggest that it can be a promising alternative to existing passive sampling methods, efficient for both hydrophobic and hydrophilic substances. The in-laboratory calibration showed satisfactory long-term stability and the field deployment showed high repeatability characteristics and very good agreement with grab sampling. Based on the field deployment results, it can be estimated that the deployment times for this passive sampling method should not exceed three weeks. This study is also the first that assesses the occurrence of OPFRs in the River Aire, and the results highlight the existence of important sources especially for TCPP which was detected both by active and passive sampling in levels as high as 7 μg L^{-1}. As this was only a preliminary study, further experiments are considered necessary to accurately estimate deployment periods for surface waters with different organic content. Furthermore, the sampler should be evaluated for its efficiency on a wider range of chemicals.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2012.08.014.

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Assessment of flame retardants in river water using a ceramic dosimeter passive sampler

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SUPPORTING MATERIAL

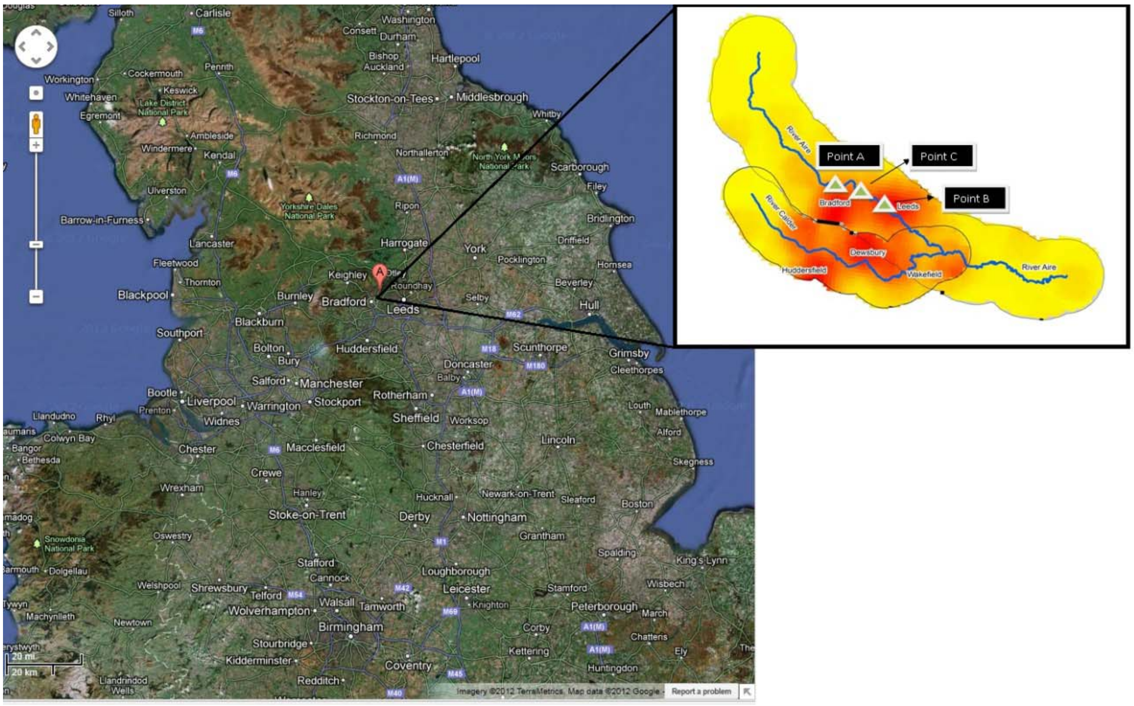

Figure S1: Map of the United Kingdom and of the River Aire. The A-flag of the big map is the Sampling point C of the present study.

2.3.3 Paper 3: “Development and validation of a multiresidue method for the analysis of polybrominated diphenyl ethers, new brominated and organophosphorus flame retardants in sediment, sludge and dust”

Joyce Cristale, Silvia Lacorte

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ABSTRACT

This study presents a multiresidue method for simultaneous extraction, clean-up and analysis of priority and emerging flame retardants in sediment, sewage sludge and dust. Studied compounds included eight polybrominated diphenyl ethers congeners, nine new brominated flame retardants and ten organophosphorus flame retardants. The analytical method was based on ultrasound-assisted extraction with ethyl acetate/cyclohexane (5:2, v/v), clean-up with Florisil cartridges and analysis by gas chromatography coupled to tandem mass spectrometry (GC-EI-MS/MS). Method development and validation protocol included spiked samples, certified reference material (for dust), and participation in an interlaboratory calibration. The method proved to be efficient and robust for extraction and determination of three families of flame retardants families in the studied solid matrices. The method was applied to river sediment, sewage sludge and dust samples, and allowed detection of 24 among the 27 studied flame retardants. Organophosphate esters, BDE-209 and decabromodiphenyl ethane were the most ubiquitous contaminants detected.

1. Introduction

Flame retardants are organic and inorganic substances added to materials to retard or eliminate the spread of fire. Some organic flame retardants were established as a threat to environment and human health, and lead governments to adopt regulatory laws. In this context, polybrominated diphenyl ethers (PBDEs) were banned in Europe [1,2]. The so called new (or novel) brominated flame retardants (NBFRs), many of them used as PBDEs substitutes, are considered emerging pollutants due to their accumulation in environmental matrices [3]. On the other hand, organophosphorus flame retardants (OPFRs) are considered as re-emerging pollutants because of their increased use after PBDE bans and their ubiquitous occurrence in all environmental compartments [4]. It is estimated that about two million tons of flame retardants are annually consumed (35% brominated and organophosphorus) [5], which are added to diverse materials, from where they are released [6,7]. Nowadays, after about fifteen years of intensive studies concerning the impact of flame retardants in the environment, their distribution mechanisms, fate and effects on biota and humans are far to be completely understood, especially when considering emerging and re-emerging flame retardants.

Because of their physico-chemical properties, flame retardants tend to accumulate on organic carbon rich matter, and have been detected in sewage sludge [8], sediments [9] or dust [10,11]. These matrices have environmental or human health relevance and there is a need for the full characterization of the content of flame retardants. Still, most studies refer to the analysis of a specific family, as pointed out in recent reviews [3,4,12–14]. Most traditionally, NBFRs and PBDEs are Soxhlet extracted from sediment, sludge and dust [3] although other techniques such as Pressurized-Liquid Extraction (PLE), Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) have become popular for the analysis of PBDEs, NBFRs and OPFRs [4,12]. For PBDEs and NBFRs, both non-destructive and destructive clean-up approaches have been used, such as gel permeation chromatography (GPC), and solid adsorbents like alumina and silica, which can be impregnated with sulphuric acid to improve lipid elimination [3]. For OPFRs, non-destructive clean-up by GPS, and adsorbents such as Florisil, alumina and primary-secondary amine have been used [4,14]. Gas chromatography (GC) coupled to mass spectrometry (MS) operating in electron capture negative ionization mode (GC-ECNI-MS), and monitoring of the ions m/z 79 and 81 $[Br]^-$ is the most used technique for determination of NBFRs and PBDEs [3] due to its low detection limits, whereas GC coupled to high resolution mass spectrometry (GC-HRMS) and GC coupled to tandem mass spectrometry in electronic ionization mode (GC-EI-MS/MS) have high specificity [15,16]. For OPFRs, GC coupled to MS operating in selected ion

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monitoring (GC-EI-MS(SIM)), GC coupled to nitrogen-phosphorous detection (GC-NPD), and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) are the most used techniques for the analysis of these compounds in environmental samples [14].

Whereas several methods are available for the extraction and analysis of each of these flame retardant groups separately, multiresidue analysis of brominated and organophosphate ester flame retardants in solid matrices needs advance. Advantages of multiresidue analysis include reduction of costs, time, solvents and required mass of sample. In this way, Van den Eede et al. [10] reported a method for the simultaneous extraction of PBDEs, NBFRs, OPFRs, and hexabromocyclododecanes (HBCD) in dust using UAE (hexane/acetone 1:1, v/v), clean-up with Florisil (fractionated elution) and analysis by GC-ECNI-MS (PBDEs and NBFRs), GC-EI-MS (OPFRs) and LC-MS/MS (HBCDs). To the best of our knowledge, no method has been reported before regarding simultaneous extraction, clean-up (without fractionated elution) and analysis (by a single technique) of PBDEs, NBFR, and OPFRs in solid matrices.

The objective of this study was to develop and validate a multiresidue method based on GC-EI-MS/MS for the simultaneous extraction, clean-up and analysis of 27 priority and (re-)emerging flame retardants (PBDEs, NBFRs and OPFRs) in sediment, sludge and dust. The accuracy of the method herein proposed was evaluated by using a certified reference material and by participating in an interlaboratory study, and method applicability was tested for surface river sediment, sewage sludge and dust samples.

2. Materials and methods

2.1. Chemicals and reagents

A solution mixture of BDEs 28, 47, 99, 100, 153, 154, 183 and 209 in n-nonane at 1 μg mL^{-1} was acquired from Cambridge Isotope Laboratories (Andover, USA). Individual solution of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) at 50 μg mL^{-1} in 5% toluene in nonane, decabromodiphenyl ethane (DBDPE) at 25 μg mL^{-1} in toluene, bis(2-ethyl-1-hexyl)tetrabromophthalate (BEHTBP), pentabromotoluene (PBT), 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE), hexachloro cyclopentadienyl dibromooctane (HCDBCO) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) (at 50 μg mL^{-1} in toluene) were acquired from Wellington Laboratories (Guelph, Canada). Pure standards of hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), tris(2-choroethyl) phosphate (TCEP), tris(2-chloro-1-methylethyl) phosphate (TCPP), tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP), triphenyl phosphate (TPhP), 2-ethylhexyl diphenyl phosphate (EHDP) and tributyl phosphate (TBP) were acquired from Sigma–Aldrich (Germany). TCPP was acquired as a mixture of isomers (tris(2-chloro-1-methylethyl) phosphate, bis(1-chloro-2-propyl)-2-chloropropyl phosphate, and bis(2-chloropropyl)-1-chloro-2-propyl phosphate). Tris(2-butoxyethyl) phosphate (TBEP), tris(2-ethylhexyl) phosphate (TEHP) and tricresyl phosphate (TCP) were acquired as pure standard from Dr. Ehrenstorfer (Augsburg, Germany). Tri-iso-butyl phosphate (TiBP) at 1000 μg mL^{-1} was acquired from Chiron (Trondheim, Norway). The surrogates [^{13}C$_6$]hexabromobenzene (MHBB) at 50 μg mL^{-1} in toluene, 3,3′,4,4′-tetrabromo[^{13}C$_{12}$]diphenyl ether (MBDE-77) at 50 μg mL^{-1} in nonane and decabromo[^{13}C$_{12}$]diphenyl ether (MBDE-209) at 25 μg mL^{-1} in toluene, were acquired from Wellington Laboratories (Guelph, Canada). Solid standard of triphenyl phosphate-D15 (TPhP-D15) was acquired from Sigma–Aldrich (Germany). Tributyl phosphate-D27 (TBP-D27) as solid standard was acquired from Cambridge Isotope Laboratories (Andover, USA). The internal standards 2,3,5,6-tetrachlorobiphenyl (PCB-65) and decachlorobiphenyl (PCB-209), both at 10 μg mL^{-1} in iso-octane, were acquired from Dr. Ehrenstorfer (Augsburg, Germany). Acetone, hexane, methanol, dichloromethane, ethyl acetate and toluene were acquired from Merck (Darmstadt, Germany). Cyclohexane was acquired from Scharlau (Sentmenat, Spain). Copper (particle size < 63 μm) was acquired from Merck (Darmstadt, Germany). Florisil cartridges were acquired from Phenomenex (10 g, 60 cc) and from Waters (5 g, 20 cc).

2.2. Extraction

Sediment, sludge and dust were analyzed following the same protocol, but different amounts of sample, surrogates, solvent and Florisil were used. The scaling of the amount of sample extracted, the amount of surrogate added and the amount of solvent used was necessary given the differing levels of flame retardants in sludge, sediment, dust and the differing amount of organic matter and organic carbon, which affect the extraction efficiency. Table 1 summarizes the amount of sample extracted, the surrogate added and the extraction conditions used for sediment, sludge and dust. The sample was placed in a glass centrifuge tube (30 mL), spiked with surrogate standards and kept in contact overnight. After that, the samples were extracted with ethyl acetate/cyclohexane (5:2, v/v) by vortex (1 min) followed by ultrasonic extraction (10 min). The extract was centrifuged (10 min at 3000 r.p.m.) and transferred to 40 mL amber vials. This procedure was repeated twice. Then, the total extract was concentrated to 1 mL under an N$_2$ flow in a Turbovap. The clean-up was performed using Florisil cartridges. The cartridges were conditioned and eluted with ethyl acetate/cyclohexane (5:2, v/v) (see amounts in Table 1). Finally, the extract was concentrated under N$_2$ flow to almost dryness and reconstituted in toluene containing PCB-65 and PCB-209 at 0.05 μg mL^{-1}.

For sediments, activated copper was used to minimize sulphur interference on GC–MS performance. Copper was activated using hydrochloric acid (25%) and ultrasonicated for 15 min. The acid was eliminated by several Milli-Q rinses until obtaining pH 7. The water was eliminated by two rinses with acetone and finally the activated copper was stored in hexane at −20 °C. About 200 mg of activated copper was added to the 1 mL concentrated extract (before clean-up step) and kept in contact overnight.

2.3. Analytical performance and validation

The accuracy of the method was determined using spiked samples (sediment, sludge and dust), a standard reference material (SRM 2585-NIST, for house dust) and by participating in an interlaboratory calibration.

Spiked samples at two concentration levels were extracted in triplicate. Sediment samples were spiked with 50 ng (level 1) or 10 ng (level 2) of NBFRs, OPFRs and PBDEs (except TBEP, BDE-209 and DBDPE that were 500 and 100 ng). Sludge samples were spiked with 100 ng (level 1) or 10 ng (level 2) of NBFRs, OPFRs and PBDEs (except BDE-209 and DBDPE that were 1000 and 100 ng). For dust samples, PBDEs and NBFRs were spiked with 100 ng (level 1) or 10 ng (level 2) (except BDE-209 and DBDPE that were 1000 and 100 ng), while OPFRs were spiked with 250 ng (level 1) or 50 ng (level 2) (except TBEP that were 500 and 2500 ng). The respective labeled surrogate amounts (Table 1) were also added. The spiked samples were kept in contact overnight before extraction. Since flame retardants were expected to be present in the original samples used for spiking, each sample was analyzed in duplicate and the sample contribution was subtracted from the spiked sample results.

The reference material SRM 2585 (NIST) for house dust was used to evaluate the accuracy of the method for PBDEs. In addition, the measured levels of BTBPE, EHTBB, BEHTBP and DPTE were

Table 1
Sample intake, surrogate, solvent and Florisil quantities used for sediment, sludge and dust extractions.

	Sample intake	Surrogate	Solvent extraction	Florisil	Conditioning and Elution	Final volume
Sediment	1.5 g	50 ng (MHBB, MBDE-77) 200 ng (TPhP-D15, TBP-D27, MBDE-209)	20 mL + 2 × 10 mL	10 g	60 mL	0.50 mL
Sludge	0.1 g	100 ng (MHBB, MBDE-77) 400 ng (TPhP-D15, TBP-D27, MBDE-209)	20 mL + 2 × 10 mL	10 g	60 mL	1.00 mL
Dust	0.1 g	25 ng (MHBB, MBDE-77) 100 ng (TPhP-D15, TBP-D27, MBDE-209)	3 × 10 mL	5 g	30 mL	0.50 mL

compared with indicative values from the literature for this reference material [10,18,19].

For OPFRs in sediments and dust, the method was validated by participating in an interlaboratory study (ILS) [20]. For this purpose, a standard solution, a sediment and a dust sample were sent to each participating laboratory (9 laboratories for the standard solution, 11 laboratories for the sediment sample and 10 laboratories for the dust sample). The test solution contained ten OPFRs (all of them were studied in this work) in the range of 40–140 μg kg^{-1} (in toluene). The sediment was a sterilized freeze dried and sieved (90 μm) sample collected from the Western Scheldt (The Netherlands). The house dust was the reference material SRM 2585 (NIST). The assigned values were compared to the results obtained in this study.

Procedural blanks were extracted and analyzed together with the samples. For such purpose, 6 procedural blanks were analyzed using the method for sediments, 6 using the method for sludge and 4 using the method of dust. To minimize blank contamination, glassware (centrifuge tubes, vials and pipettes) were backed overnight at 350 °C. Volumetric glass materials were solvent rinsed before using. Plastic materials were avoided. Also, all sample and glassware were covered with aluminum foil during the extraction steps, to avoid dust particles deposition. GC-EI-MS/MS system contamination was checked during the sample injection sequence by injection of toluene after each four sample set. Neither OPFRs nor any of other compounds were detected in the GC-EI-MS/MS blanks.

2.4. Calibration and analysis

Ten calibration levels were prepared ranging from 0.001 to 1 ng μL^{-1} (0.01–1 ng μL^{-1} for TBEP, DBDPE and BDE-209). Two calibration curves (generally from 0.001 to 0.025 ng μL^{-1}, and from 0.05 to 1 ng μL^{-1}), each one with at least 5 levels, were used. The syringe internal standards PCB-65 and PCB-209 were at 0.05 ng μL^{-1}. Internal standard quantification was performed with the labeled surrogates. TBP-D27 was used as surrogate for TiBP, TBP, TCEP and TCPP. TPhP-D15 was used as surrogate for TDCP, TPhP, TBEP, EHDP, TEHP and TCP. MHBB was used as surrogate for PBT, PBEB and HBB; MBDE-77 was used as surrogate for DPTE, HCDBCO, BEHTBP, EHTBB, BTBPE and for BDEs 28, 47, 99, 100, 153, 154 and 183. Finally, MBDE-209 was used as surrogate for BDE-209 and DBDPE.

GC-EI-MS/MS analysis was performed in a GC Agilent 7890A equipped with a 7000A GC–MS Triple Quadrupole. The column used was a DB-5MS with 15 m (length) × 0.250 mm (I.D.) × 0.10 μm (film) (J&W Scientific, USA). The oven program was set at 60–220 °C at 10 °C/min and to 315 °C at 15 °C/min (8 min). Helium (purity 99.999%) was employed as carrier gas with a constant flow of 1.5 mL min^{-1}. The injection volume was 2 μL by splitless, with a splitless time of 1.5 min. The injector, quadrupole, transfer line and ion source were set at 300, 150, 280 and 300 °C respectively. Electron ionization (EI) mode was set at 70 eV. The MS/MS transitions for PBDEs, NBFRs, TCEP, TCPP, TDCP and TPhP were the ones described by Cristale et al. [16]. In this study, the MS/MS transitions for TiBP, TBP, TBP-D27, TPhP-D15, TBEP, EHDP, TEHP and TCP were optimized. For this purpose, the base peak (precursor ion) of each compound was determined by scan acquisition mode, and then the optimum collision energy was increased from 5 to 60 V (maximum value), in intervals of 5 V, and the energy that produced the most abundant or selective precursor ion fragmentation and intense product ions was chosen.

2.5. Samples analyzed

To evaluate the feasibility of the method, sediment, sludge and dust samples were analyzed. Approximately 0.5 kg of surface sediments (n = 5) were collected with a Van Veen drag in the middle of the river bed at the river Besòs (Catalonia, Spain). Sludge samples were collected at five WWTPs close to Barcelona city, at different stages of the treatment (primary treatment: primary sludge; secondary treatment or anaerobic digestion: biological sludge). Sediment and sludge samples were freeze-dried, homogenized and sieved (120 μm). House dust samples were collected at five houses using a vacuum cleaner. Dust samples were sieved (500 μm) and homogenized. Samples were stored at −18 °C until analysis.

3. Results

3.1. GC-EI-MS/MS optimization for OPFRs

In this study, GC-EI-MS/MS conditions were optimized for TiBP, TBP, TBEP, EHDP, TEHP and TCP and also for the labeled surrogates TBP-D27 and TPhP-D15. Table 2 presents these optimized conditions (collision energies, precursor and product ions), and the previously reported ones for TCEP, TCPP (except for transition 2 (T2)), TDCP and TPhP [16]. Very low instrumental limits of detection (iLOD), calculated as 3 times the signal to noise ratio for a standard solution at low concentrations (from 0.01 to 0.0005 ng μL^{-1}), were obtained for the optimized OPFRs, ranging from 0.4 to 20 pg injected. Although the ion m/z 99 [H$_4$PO$_4$]$^+$ was the base peak for many OPFRs, several authors avoid use this ion for OPFRs analysis by GC-EI-MS with single ion monitoring (SIM) [21–23], since low mass fragment ions (m/z < 200) can be affected by complex environmental matrices, increasing the background noise. However, the optimized MS/MS conditions proved to be

Table 2
Optimized GC-EI-MS/MS transitions and instrumental limits of detection (iLOD) for OPFRs.

	T1			T2			iLOD (pg)
	Precursor ion (m/z)	Product ion (m/z)	C.E.	Precursor ion (m/z)	Product ion (m/z)	C.E.	
TBP-D27	103 $[D_4PO_4]^+$	63 $[PO_2]^+$	35	103 $[D_4PO_4]^+$	83 $[D_2PO_3]^+$	35	1.4
TPhP-D15	341 $[M]^+$	223 $[C_{12}D_8O_2P]^+$	25	341 $[M]^+$	180 $[C_{12}D_{10}O]^+$	25	1.0
TiBP	99 $[H_4PO_4]^+$	63 $[PO_2]^+$	35	99 $[H_4PO_4]^+$	81 $[H_2PO_3]^+$	35	1.0
TBP	99 $[H_4PO_4]^+$	63 $[PO_2]^+$	35	99 $[H_4PO_4]^+$	81 $[H_2PO_3]^+$	35	1.4
TCEP[a]	249 $[C_6H_{12}Cl_2O_4P]^+$	125 $[C_2H_6O_4P]^+$	10	249 $[C_6H_{12}Cl_2O_4P]^+$	99 $[H_4PO_4]^+$	20	5.0
TCPP[a]	125 $[C_2H_6O_4P]^+$	99 $[H_4PO_4]^+$	10	99 $[H_4PO_4]^+$	63 $[PO_2]^+$	35	1.2
TDCP[a]	99 $[H_4PO_4]^+$	63 $[PO_2]^+$	40	99 $[H_4PO_4]^+$	81 $[H_2PO_3]^+$	30	1.0
TPhP[a]	326 $[M]^+$	215 $[C_{12}H_8O_2P]^+$	25	326 $[M]^+$	169 $[C_{12}H_9O]^+$	25	1.0
EHDP	251 $[C_{12}H_{12}O_4P]^+$	51 $[C_4H_3]^+$	60	251 $[C_{12}H_{12}O_4P]^+$	77 $[C_6H_5]^+$	60	1.4
TBEP	125 $[C_2H_6O_4P]^+$	99 $[H_4PO_4]^+$	10	99 $[H_4PO_4]^+$	63 $[PO_2]^+$	35	20
TEHP	99 $[H_4PO_4]^+$	63 $[PO_2]^+$	35	99 $[H_4PO_4]^+$	81 $[H_2PO_3]^+$	35	0.4
TCP	368 $[M]^+$	165 $[C_{13}H_9]^+$	45	368 $[M]^+$	91 $[C_7H_7]^+$	45	16

C.E. – collision energy (V); T1 – transition 1 (quantification); T2 – transition 2 (confirmation).

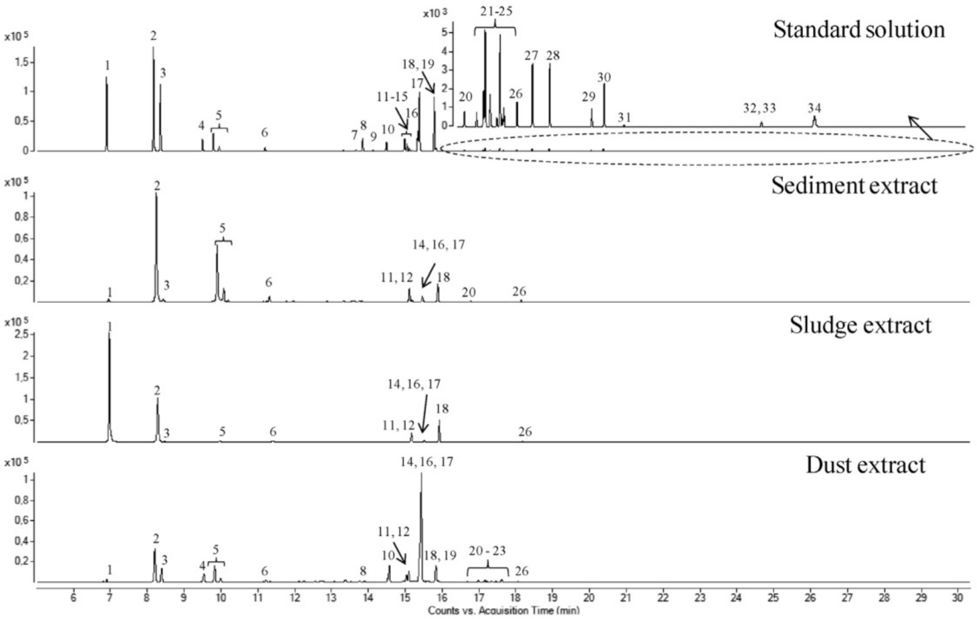
[a] Optimized transitions by Cristale et al. [16], except T2 for TCPP.

suitable for OPFRs analysis in sediment extracts using the ion m/z 99 as precursor ion, since selected reaction monitoring (SRM) are more specific than selected ion monitoring (SIM), minimizing matrix interference and background noise. These optimized transitions, together with the optimized ones reported in a previous study for OPFRs, NBFRs and PBDEs [16], were used for determination of flame retardants in sediment, sludge and dust samples.

3.2. Method performance

Fig. 1 presents a GC-EI-MS/MS chromatogram of a standard solution, a sediment, a sludge and a dust extracts. The optimized oven temperature program permitted a good separation of target flame retardants in 30 min, with DBDPE eluting at 26.2 min. Coelution of two or more compounds was resolved by specific transitions. In the case of sludge, background noise affected the detection of some compounds, and so other MS/MS transitions were optimized to solve this problem. Fig. 2 presents TCPP and BTBPE optimized transitions for sludge extracts. For TCPP, the confirmation transition 125 → 81 presented an interference in sludge extracts, and the transition 99 → 63 (C.E. 35 V) is proposed for TCPP confirmation in sludge. Similarly, the transitions 359 → 252 (C.E. 25 V) and 359 → 278 (C.E. 25 V) are proposed in this study for sludge analysis, since those ones optimized in a previous study [16] (356.5 → 90 and 356.5 → 118) were high affected by matrix interferences, due to low mass of product ions.

Several authors report blank contamination as an important issue concerning OPFRs analysis at trace levels [4,20,24,25]. OPFRs are ubiquitous contaminants in indoor environment, and are present in dust at μg kg^{-1} to mg kg^{-1} levels [4,23]. Also, TBEP can be adsorbed on glassware [26]. Since different blank profiles were observed among laboratories, with differing abundance of



1- TiBP, 2- TBP-D27, 3- TBP, 4- TCEP, 5- TCPP, 6- PCB-65, 7- PBT, 8- BDE-28, 9- PBEB, 10- TDCP, 11- TPhP-D15, 12- TPhP, 13- DPTE, 14- MHBB, 15- HBB, 16- EHDP, 17- TBEP, 18- TEHP, 19- BDE-47, 20- MBDE-77, 21- TCP, 22- BDE-100, 23- BDE-99, 24- HCDBCO, 25- EHTBB, 26- PCB-209, 27- BDE-154, 28- BDE-153, 29- BDE-183, 30- BTBPE, 31- BEHTBP, 32- BDE-209, 33- MBDE-209, 34- DBDPE.

Fig. 1. GC-EI-MS/MS chromatogram of a standard solution at 0.25 μg mL^{-1} (except TBEP, BDE-209 and DBDPE that was at 2.5 μg mL), a sediment, a sludge and a dust extract.

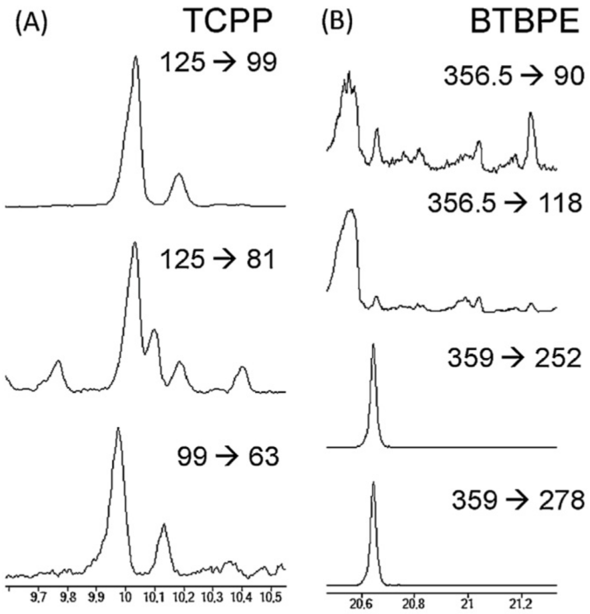


Fig. 2. Optimization of MS/MS transitions for TCPP (A) and BTBPE (B) in sludge.

OPFRs, it seems that the blank levels are influenced by different sources in each particular laboratory [20]. Despite the care adopted in this study to minimize background contamination, all OPFRs were detected in the blanks. Fig. 3 presents the amount of OPFRs detected in procedural blanks for each extraction method (sediment, sludge and dust). The most ubiquitous compounds in the blanks were TBEP, EHDP, while the other OPFRs were at similar levels. Among methods, the dust extraction performance was the one that presented the lowest OPFRs levels (no TCEP detection), which suggest that blank performance can be affect by the amount of extraction solvents and/or by Florisil cartridges acquired from different suppliers.

Since OPFRs are detected in procedural blanks, the blank levels were taken into account for the calculation of the limits of detection of the method (MDL). MDL for OPFRs was calculated as the average blank concentration plus three times the standard deviation. Table 3 presents the MDL obtained for each method in sediments, sludge and dust. MDLs for OPFRs ranged from 1.9 to 60 μg kg^{-1} in sediments, from 28 to 575 μg kg^{-1} in sludge and from 3.8 to 288 μg kg^{-1} in dust, and the highest MDLs were observed for TBEP and EHDP due to their high contribution in the blanks. Since OPFRs in procedural blanks are observed in most of laboratories that determine OPFRs in environmental samples [20], an MDL comparison between this and other studies is difficult given that MDL tend to be more influenced by laboratory blank levels than by instrumental capabilities, and also MDLs are not always calculated in the same way. Martínez-Carballo et al. [24] reported a method based on UAE and analysis by LC-MS/MS, and obtained iLOD of the same magnitude range than the ones obtained in this study (from 0.52 to 1.7 pg μL^{-1}) and method quantification limits (MQL) (determined as the maximum blank concentration) for sediments ranged from 0.61 to 1.5 μg kg^{-1}, which were obtained after blank performance improvements (only TCEP and TBP were detected). For sludge samples, Marklund et al. [22] reported a method based on ASE, GPC (clean-up) and analysis by GC-NPD, and obtained MDL (calculated as three times the noise level) ranging from 0.2 to 5.1 μg kg^{-1}, while the concentration in the procedural blanks ranged from 0.6 to 21 μg kg^{-1}. For dust samples, Van den Eede et al. [10] reported a multiresidue extraction method for several flame retardants families, fractionated clean-up with Florisil, and OPFRs analysis by GC-EI-MS(SIM). MQL for OPFRs (calculated as three times the standard deviation of blanks) ranged from 10 to 370 μg kg^{-1}.

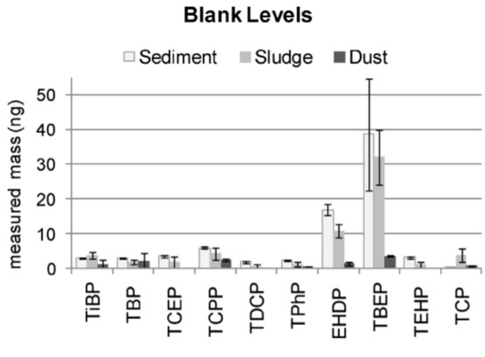


Fig. 3. Detected amount (ng) in procedural blanks performed for sediment (*n* = 6), sludge (*n* = 6) and dust (*n* = 4) extraction.

PBDEs and NBFRs were not detected in the blanks and the limit of detection was calculated as three times the signal/noise ratio obtained with spiked samples. MDL for PBDEs ranged from 0.29 to 1.5 μg kg^{-1} (40 μg kg^{-1} for BDE-209) in sediments, from 4.8 to 25 μg kg^{-1} (300 μg kg^{-1} for BDE-209) in sludge and from 2.0 to 5.3 μg kg^{-1} (275 μg kg^{-1} for BDE-209) in dust. For NBFRs, MDL ranged from 0.21 to 80 μg kg^{-1} in sediments, from 3.7 to 375 μg kg^{-1} in sludge, and from 5 to 237 μg kg^{-1} in sludge. GC-ECNI-MS is one of the preferred techniques for PBDEs and NBFRs measurements in environmental extracts due to its low iLOD, especially noticed for high brominated compounds such as BDE-209 and DBDPE. Reported MD(Q)L by GC-ECNI-MS for PBDEs ranged from 0.005 to 0.025 μg kg^{-1} (0.125 μg kg^{-1} for BDE-209) in sediments [9], from 1.6 to 4.1 μg kg^{-1} (6.1 μg kg^{-1} for BDE-209) in sludge [27], and from 0.04 to 1.6 μg kg^{-1} (17 μg kg^{-1} for BDE-209) in dust [10]. Considering NBFRs, MDL ranged from 0.002 to 0.04 μg kg^{-1} in sediments (PBT, BTBPE and DBDPE) [28], and from 0.1 to 7.1 μg kg^{-1} (BTBPE, HCDBCO, EHTBB, BEHTBB, DBDPE) in dust [10], all by GC-ECNI-MS. A disadvantage of brominated flame retardants analysis by GC-ECNI-MS is its low specificity. Although low limits of detection are obtained by monitoring the ions *m*/*z* 79 and 81 [Br]$^-$, poor structural information is given for PBDEs and many NBFRs [16] and so co-elution with non-target brominated compounds can generate false positives. In fact, co-elution problems among PBDEs, MeO-PBDEs and PBBs in a DB-5 column are reported in previous studies [29,30]. In addition, TCEP and TCPP were not detected when analyzed simultaneously with brominated flame retardants by GC-ECNI-MS [16], and so this technique cannot be applied for the simultaneous analysis of all the flame retardants proposed in this study.

The advantages of the developed multiresidue method are that: (i) 27 flame retardants are efficiently extracted from complex samples with only one clean-up step (Florisil); (ii) due to the high specificity of SRM transitions, the matrix effect was almost eliminated; (iii) precise identification capabilities are obtained using 2 SRM transitions plus retention time information; (iv) the method is sensitive enough to determine environmental relevant concentrations, although it shows MDL higher than GC-ECNI-MS.

3.2.1. Spiked samples

Analytical method efficiency for three solid matrices (sediment, sludge and dust) was evaluated using spiked samples. Table 3 presents the recoveries obtained for each case. The similar recovery for most of compounds in sediment, sludge and dust indicate that

Table 3
Spiked sample recoveries and method detection limits (MDLs) obtained for sediment, sewage sludge and dust. Spiked amounts (level 1 and level 2) are presented at Section 2.3.

Target compound	Sediment			Sludge			Dust		
	Level 1 % Rec (RSD)	Level 2 % Rec (RSD)	MDL $\mu g\,kg^{-1}$	Level 1 % Rec (RSD)	Level 2 % Rec (RSD)	MDL $\mu g\,kg^{-1}$	Level 1 % Rec (RSD)	Level 2 % Rec (RSD)	MDL $\mu g\,kg^{-1}$
TiBP	67 (1)	88 (11)	2.5	131 (27)	106 (7)	64	96 (3)	112 (35)	44
TBP	105 (6)	92 (5)	2.5	98 (4)	101(3)	38	98.6 (0.6)	113 (32)	77
TCEP	67 (6)	64 (11)	2.7	69 (5)	85 (19)	70	[b]	[b]	79
TCPP	107 (3)	138 (53)	4.5	78 (5)	[b]	102	78 (3)	[b]	31.4
TDCP	84 (6)	103 (7)	1.9	129 (5)	100 (5)	28	97 (4)	113 (35)	3.8
TPhP	84 (4)	60 (17)	2.0	118 (5)	119(2)	28	70 (13)	[b]	5.4
EHDP	77 (8)	97 (11)	15	119 (4)	109 (15)	165	102 (2)	141 (22)	27.7
TBEP	48 (8)	48 (6)	60	64 (19)	69 (40)	575	[b]	[b]	288
TEHP	93 (9)	135 (6)	2.8	120 (5)	107 (28)	40	98 (5)	117 (33)	5.4
TCP	105 (10)	93 (8)	6.7	121 (8)	116 (4)	73	108 (3)	[b]	9.1
BDE-28	72 (1)	82 (5)	0.48	111 (13)	112 (18)	4.8	85 (2)	82 (1)	4.3
BDE-47	69 (3)	81 (9)	0.35	98 (17)	95 (16)	6.2	84 (1)	104 (1)	2.1
BDE-100	76 (1)	77 (4)	1.0	100 (1)	114 (8)	15	87 (1)	89.8 (0.2)	5.3
BDE-99	84 (1)	62 (15)	0.87	105 (1)	90 (7)	13	85 (2)	121 (4)	5.0
BDE-154	75 (3)	77 (3)	1.5	100 (1)	121 (14)	7.1	91 (1)	89 (3)	2.0
BDE-153	82 (1)	91 (5)	0.8	101.7 (0.3)	115 (8)	17	92 (1)	105 (6)	2.3
BDE-183	99 (1)	74 (22)	0.29	105 (2)	90 (9)	25	98 (1)	129 (11)	4.4
BDE-209	103 (39)	93 (40)	40	98 (1)	100 (20)	300	75 (8)	[b]	275
PBT	90 (6)	84 (14)	0.21	108 (7)	108 (10)	14	73 (10)	68 (21)	5.0
PBEB	88 (6)	92 (7)	0.48	118 (6)	112 (4)	3.7	72 (7)	73 (6)	5.3
HBB	88 (5)	68 (18)	1.8	92 (10)	107 (9)	53	89 (3)	100 (8)	33
DPTE	104 (5)	125 (8)	0.91	120 (12)	140 (4)	9.1	103 (3)	103 (1)	7.4
HCDBCO	67 (18)	[a]	19	115 (4)	[a]	375	92 (5)	[a]	154
EHTBB	110 (6)	111 (14)	0.71	103 (12)	117 (5)	11	100 (8)	112 (14)	6.7
BEHTBP	90 (7)	123 (12)	16	99 (4)	[b]	43	155 (3)	137 (5)	8.3
BTBPE	140 (5)	99 (9)	5.0	103 (6)	99 (13)	6.8	153 (2)	126 (4)	18
DBDPE	57 (4)	[a]	80	126 (13)	126 (9)	353	68 (16)	123 (11)	237

[a] Not detected, spiked level lower than MDL.

[b] Not determined, amount in the matrix was similar or higher than spiking level.

the method was robust for the analysis of three flame retardants families in these samples.

For sediments, OPFRs recoveries ranged from 48 to 107% at spiking level 1 and from 48 to 138% at the level 2, with RSD lower than 15% in most of cases. The lowest OPFR recovery was obtained for TBEP (about 50%), and an RSD lower than 10% was obtained for both spiked levels. TCPP presented a recovery of 138% and RSD of 53% for the level 2 (10 ng). This variation can be explained by the presence of the TCPP in the sediment sample used for spiking ($25 \pm 15\ \mu g\,kg^{-1}$, $n = 3$), resulting in high recovery error. On the other hand, a recovery of 107%, with RSD of 3%, was obtained for TCPP at 50 ng spiking level. PBDEs recoveries ranged from 62 to 103%, with RSD < 20, except for BDE-209 that presented an RSD of 40%, probably due to its strong adsorption on sediments ($\log K_{ow}$ 9.5). Recoveries higher than 80% and RSD < 20 were obtained for all NBFR, except for DBDPE that presented a recovery of 57%. HCDBCO and DBDPE recoveries were not detected at the lowest spiked level, due their higher iLOD [16].

For sludge, OPFRs recoveries ranged from 64 to 131% at the level 1 and from 69 to 119% at the level 2, with average RSD of 9% and 14%, respectively. The highest RSD was observed for TBEP (40%), attributed to the interference from the spiked sample, since the amount present in the sample and the spiked amounts were at the same range of concentration. Recoveries for PBDEs ranged from 90 to 121%, and RSD ≤ 20. For NBFRs, recoveries ranged from 92 to 126% and from 99 to 140% for the level 1 and level 2, respectively, and RSD ≤ 13. TCPP and BEHTBP recoveries at the level 1 were not determined because the amounts present in the sludge sample were higher than the amount spiked, affecting the quantification.

For dust, OPFRs recoveries ranged from 78 to 108%, with RSD from 0.6 to 13% for the spiking level 1. It was not possible to calculate the recoveries of TCEP and TBEP in any spiked level due to their presence at high concentration in the dust sample used for spiking ($9023 \pm 448\ ng\,g^{-1}$ for TCEP, and $12{,}068 \pm 3160\ ng\,g^{-1}$ for TBEP, $n = 2$). For the spiking level 2, TiBP, TBP, TDCP, EHDP and TEHP were affected by the presence OPFRs in the sample (from $66 \pm 8\ ng\,g^{-1}$ to $2002 \pm 493\ ng\,g^{-1}$, $n = 2$), presenting recoveries from 112 to 141% and RSD from 22 to 35%, while recoveries of TCEP, TCPP, TPhP, TBEP and TCP could not be determined for the same reason. Good recoveries were obtained for PBDEs for the both spiked levels, ranging from 75 to 121%, with RSD from 0.2 to 11%. Finally, for NBFRs, recoveries ranged from 68 to 155% (RSD from 2 to 16%) at the level 1, and from 68 to 137 (RSD from 1 to 21%) for the level 2.

3.2.2. Reference material

The method performance for PBDEs was evaluated using the dust certified reference material SRM 2585 (NIST). Table 4 presents the measured and certified concentrations for PBDEs in the

Table 4
Measured, certified (PBDEs) and reported (BTBPE, EHTBB and BEHTBP) concentrations in SRM 2585 (NIST).

	Measured (SD) $\mu g\,kg^{-1}$	Certified or reported (uncertanty) $\mu g\,kg^{-1}$	Error[b] %
BDE-28	42 (1)	46.9 (4.4)	−10
BDE-47	486 (10)	497 (46)	−2.1
BDE-100	147 (5)	145 (11)	1.5
BDE-99	803 (45)	892 (53)	−10
BDE-154	77 (5)	83.5 (2.0)	−8.3
BDE-153	118 (8)	119 (1)	−1.2
BDE-183	44 (4)	43.0 (3.5)	3.1
BDE-209	2971 (333)	2510 (190)	18
BTBPE	76 (4)	39 (4.9) [18] 39 (14) [10]	
BEHTBP	857 (73)	1300 (94) [18] 574 (49) [10] 145 (16.7) [19]	
EHTBB	35 (6)	36 (2.4) [18] 26 (2) [10]	

[b] Calculated as ((average measured value – assigned value)/assigned value)*100.

Table 5
Assigned value, measured value and error obtained in the interlaboratory calibration (ILS) [20] of a standard solution, sediment and dust sample.

Target compound	GC solution			Sediment			Dust		
	Assigned value[a] μg kg^{-1}	Measured (SD) μg kg^{-1}, $n=2$	Error[b] (%)	Assigned value[a] μg kg^{-1}	Measured (SD) μg kg^{-1}, $n=3$	Error[b] (%)	Assigned value[a] μg kg^{-1}	Measured (SD) μg kg^{-1}, $n=3$	Error[b] (%)
TiBP	113	103.6 (0.4)	−8	NA			NA		
TBP	51	60.0 (0.4)	18	3.2	2.9 (0.1)	−9	269	264 (3)	−2
TCEP	141	156.5 (0.9)	11	4.2	2.1 (0.5)	−49	792	757 (26)	−4
TCPP	101	101 (2)	0	26	18 (4)	−30	944	939 (66)	−0.5
TDCP	119	108.1 (0.5)	−9	NA			1556	1658 (18)	7
TBEP	87	95 (1)	9	7.0	5.9 (0.5)	−16	73,464	14,663 (408)	−80
TPhP	50	50.1 (0.3)	0.2	NA			1104	1132 (46)	3
EHDP	48	46.0 (0.0)	−4	NA			963	974 (12)	1
TEHP	112	104.5 (0.2)	−7	18	18 (1)	0	265	295 (6)	11
TCP	NA			NA			NA		

NA – not assigned value by the ILS.

[a] Total error of 13%.

[b] Calculated as ((average measured value – assigned value)/assigned value)*100.

reference material. The method proved to be adequate for accurate analysis of PBDEs in dust, which can be verified by the calculated errors among measured and certified concentrations, which ranged from 1.2 to 18%. In addition, good precision was obtained for PBDEs in SRM 2585, with RSD from 2 to 11% among replicates ($n=4$). NBFRs are not certified in SRM 2585. On the other hand, several authors report BTBPE, EHTBB and BEHTBP concentrations in SRM 2585, and so our concentrations were compared to those studies (Table 4). EHTBB concentration was in good agreement with previous studies. For BTBPE, the calculated concentration was twice higher than reported concentrations, probably due to matrix enhancement, as observed before for the spiked dust samples (153% and 126% of BTBPE recoveries). BEHTBB reported concentration in SRM 2585 varied from 145 to 1300 μg kg^{-1}, and the concentration obtained in this study was 857 μg kg^{-1}. However, the accurate value is unknown, which reveals the necessity of certify concentrations for NBFRs using reference materials, which would allow validation of analytical methods for these flame retardants in environmental samples.

3.2.3. Interlaboratory study (ILS)

The OPFRs interlaboratory calibration for a standard solution, a sediment and a dust sample was used for the method validation. Table 5 presents the assigned value derived from the ILS, the measured value (blank contribution subtracted), and the error among the calculated and the assigned values. The obtained results for the standard solution were very satisfactory, with errors from 0.2 to 18%, being lower than 10% in most of cases. TCP had not an assigned value for the standard solution. For the sediment sample, only TBP, TCEP, TCPP, TBEP and TEHP had assigned values. Errors ≤ 30% were obtained for TBP, TCPP, TBEP and TEHP, while TCEP had an error of −49%. For dust samples, all the OPFRs except TiBP and TCP had ILS assigned values. Except for TBEP, the measured concentrations were very satisfactory for all the OPFRs, with error from 0.5 to 11%. Despite the recovery of TCEP could not be determined in spiked dust, very good agreement among our and ILS concentration was obtained for SRM 2585 (error of −4%), which indicate that the method was efficient for this compound. TBEP presented an error of −80%. Since TBEP recoveries with spiked dust could not be determined, and due their bad performance on ILS, TBEP validation was not completed and so it was not quantified in dust samples. The low recovery observed for TBEP might be due to the poor extraction efficiency with the clean-up cartridges and solvents used. Recoveries using spiked Florisil cartridges (no matrix) were 56% (RSD 8%) for the Phenomenex cartridges and 39% (RSD 20%) for the Waters cartridges. Particle diameter was possibly the main factor affecting extraction efficiency, indicating that probably a larger amount of solvent is needed to elute this compound. However, using a larger amount of solvent has serious drawbacks in the analysis of OPFRs due to higher blank contribution.

Thus, spiked samples, reference material SRM 2585 (dust), and ILS results showed that the method was efficient for simultaneous extraction, clean-up and analysis of PBDEs, NBFRs and OPFRs in sediments, sludge and dust (except TBEP), obtaining good precision and accuracy.

3.3. Analysis of flame retardants in sediment, sludge and dust

The developed method was applied to the analysis of flame retardants in sediment, sludge and dust samples. Surrogate recoveries were monitored in order to evaluate method efficiency and correct, by internal standard quantification, extraction loses and GC-EI-MS/MS variations. Fig. 4 presents the surrogate recoveries for each set of samples ($n=5$). Surrogate recoveries were satisfactory, with average recoveries from 79 to 125%, and RSD from 3 to 26% (average 13%). Comparing sample types performances, sludge samples (primary sludge and biological sludge) tend to have a higher surrogate variation (especially for MHBB and MBDE-77) due to the high organic matter content of sludge although RSD were acceptable (average 11%). MBDE-209 and TBP presented similar behavior for all samples.

A rigorous identification criteria was applied, where positives values were confirmed by comparing retention times and SRM transitions ratio (T1/T2), and the accepted variation range among standard and samples, for these parameters, was followed as recommended by the Commission Decision 2002/657/EC [17].

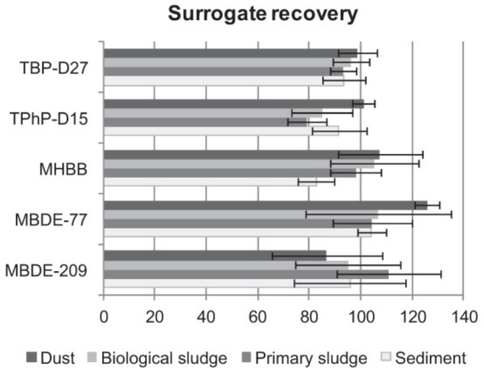


Fig. 4. Surrogate recovery (%) ($n=5$) in sediment, primary sludge, biological sludge, and dust samples.

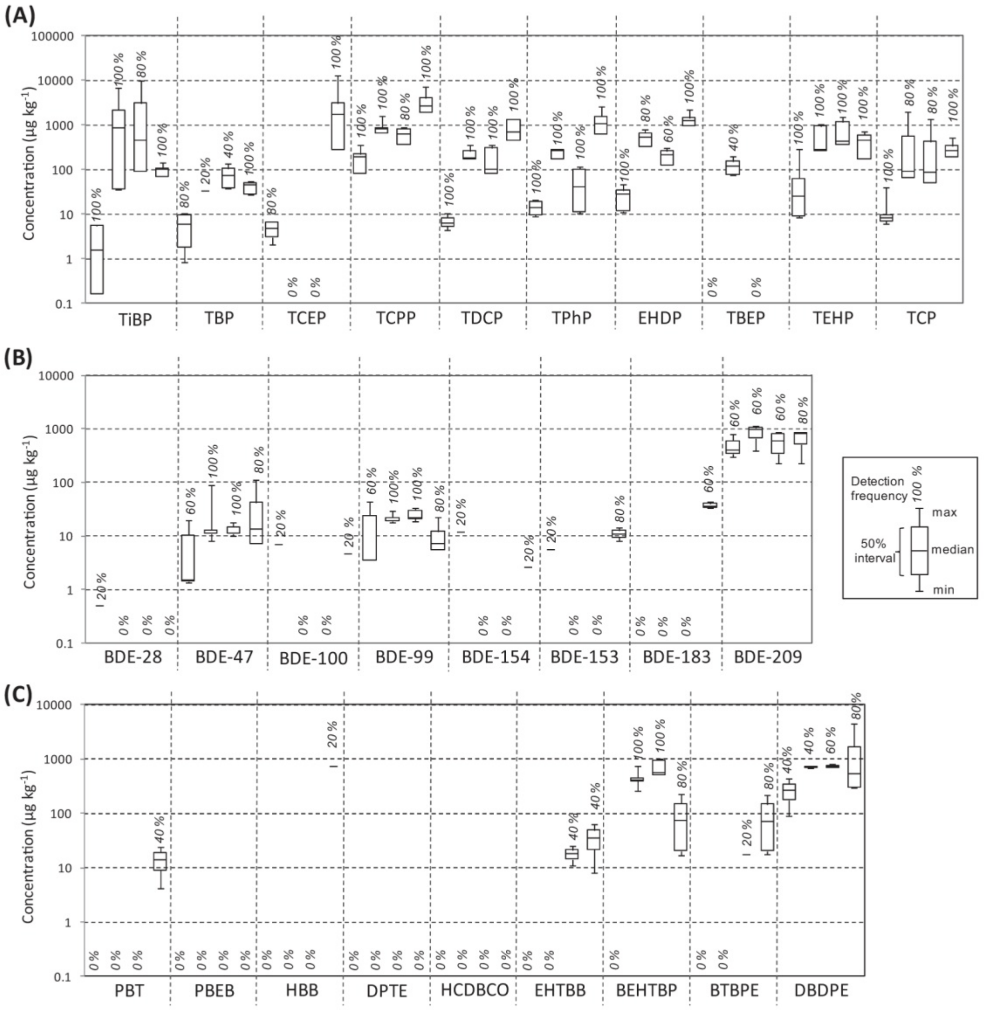


Fig. 5. Boxplot of measured concentrations of OPFRs (A), PBDEs (B) and NBFRs (C). For each compound, from left to right: sediment, primary sludge, biological sludge and dust. Blank contribution (average plus three times the standard desviation) was subtracted from OPFRs results.

Although this directive regulates the analysis of certain substances in food of animal origin, we consider that the identification criteria for determination by GC–MS/MS are appropriated for environmental samples. Fig. 5 shows the measured concentrations in sediment, primary sludge, biological sludge and dust samples as a boxplot graph. Among the 27 target flame retardants, 24 of them were detected. OPFRs presented the highest detection frequency and the highest levels, followed by PBDEs and DBDPE, while PBEB, HCDBCO and DPTE were not detected in any sample. The lowest OPFRs and PBDEs levels were observed in sediments collected in Catalonia (Spain), while DBDPE was the only NBFR detected in these samples. Sludge and dust samples presented similar levels in most of cases, while TPhP, EHDP, TDCP, TCPP, TCEP and DBDPE were more abundant in dust than in sludge samples. PBT and HBB were only detected in dust samples. Although only one sample (primary and biological sludge) were collected in each of the five Spanish WWTPs, a comparison among each set of samples suggest, in some cases, a concentration decrease tendency after or during biological treatment (TPhP, EHDP), while concentration decrease was not evident for some flame retardants (such as BEHTBP, BDE-209, DBDPE, TCP). The ubiquitous presence of flame retardants in these environmental compartments point out the necessity of more monitoring studies to have a better understanding of their distribution, fate and effects.

4. Conclusions

This study presents an efficient multiresidue method for the determination of 27 flame retardants (OPFRs, NBFRs and PBDEs) in sediments, sludge and dust. In this study, we have optimized a method based on ultrasonic extraction with ethyl acetate/cyclohexane (5:2, v/v) and GC-EI-MS/MS that provides excellent selectivity, identification criteria and good sensitivity for the analysis of flame retardants in solid samples. Issues such as scaling of the extraction conditions, external contamination and identification and quantification procedures are discussed to provide full details for the efficient analysis of these compounds. The method proposed has been validated by participating in an interlaboratory exercise and by analysing a reference material, and good performance was obtained. Finally, the feasibility of the method has been demonstrated by analyzing flame retardants in sediment, sludge and dust, where a wide range of contaminants have been detected at varying concentrations. The ubiquitous presence of PBDEs, NBFRs and OPFRs in these matrices call for future studies to determine their occurrence and the impact they pose, considering both concentration levels and toxicological data. This way, the real risk of these compounds in samples of environmental and health concern can be established.

Acknowledgements

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2.4 Discussion

In Paper 1, a simultaneous method for the analysis of bromophenols, bromoanilines, bromotoluenes, bromoanisoles, PBDEs, NBFRs and four OPFRs (TCEP, TCPP, TDCP and TPhP) was developed. Since a standard mixture of all bromophenol congeners was commercially available, all of them were included in that study to evaluate their occurrence in water. The analysis of bromophenols by GC without derivatization was possible, but detection limits were higher than the ones obtained for derivatizated bromophenols [229]. No bromophenol were detected, neither in the samples collected in each step of the treatment in a DWTF (Sant Joan Despí), nor in the raw water from the River Llobregat (Catalonia, Spain). Those results indicated that the detection limits were probably too high for the detection of bromophenols in river water, and so these compounds were not included in the next studies performed during this thesis. Bromoanilines, bromotoluenes and bromoanisoles presented lower detection limits than bromophenols, but were neither detected in the analyzed samples. The extract evaporation step under N_2 flow was critical for bromoanilines, bromoanisoles and bromotoluenes (except PBT), and so this step needed to be performed very carefully to avoid significant loses, and samples could not be evaporated to total dryness. Since solvent evaporation was not critical for the other flame retardants, bromotoluenes (except PBT), bromoanilines and bromoanisoles should be analyzed separately from the other FRs, and so were not further analyzed. In Paper 3, other six OPFRs were included due their increased concern in literature, since these compounds have been frequently detected in the aquatic environment. The included OPFRs were TiBP, TBP, TBEP, EHDP, TEHP and TCP.

2.4.1 Instrumental performance

In Paper 1, three instrumental methods (GC-EI-MS/SIM, GC-EI-MS/MS and GC-ECNI-MS/SIM) were developed and compared in terms of detection limits and repeatability, using standard solutions. The lowest limits of detection for brominated compounds were obtained by GC-ECNI-MS/SIM, monitoring the ions *m/z* 79 and 81 $[Br]^-$. However, many brominated flame retardants presented no other characteristic ion at abundance higher than 20% for its use as confirmation ion. Figure 8 and Figure 9 compare the mass spectrum obtained for BDE-47 and PBEB by EI and ECNI, in SCAN mode. For both compounds, ECNI spectrum presented only the ions *m/z* 79 and 81 as base peaks, while several characteristic fragments could be observed in the EI spectrum for both compounds. The monitoring of the single ion $[Br]^-$ for PBDEs and NBFRs can result in false positives, since any co-eluting brominated compound may interfere with the detection of the target compounds. The selectivity using EI is enhanced when working in SRM operation mode, where the most abundant ions suffer a further fragmentation, which generate characteristic product ions. Thus, GC-EI-MS/MS was the chosen method for the analysis of several families of FRs in environmental samples (water, sediments, sludge and dust), since this technique permitted a simultaneous determination of all target compounds with high selectivity. In addition, and after a study of matrix interference, all developed GC-MS methods can be potentially used for the determination of flame retardants in different environmental samples. However, significant differences in detection response can occur when comparing standard solutions to sample extracts. Despite that the use of MS/MS transitions or characteristic ions (SIM) increase significantly the selectivity of the method, co-extracted interferents eluting together with the analyte sometimes can enhance the response. For this reason, the use of spiked samples is needed for evaluating the matrix effect. The GC-EI-MS/MS method presented a good performance for the analysis of waters, sediments, sludge and dust, except for TCPP and BTBPE in sludge samples, where other transition had to be optimized to eliminate matrix interferences (discussed in Paper 3).

The applicability of the GC-EI-MS/SIM conditions for the analysis of river water samples was evaluated using spiked river water samples. This study was performed during a visiting at Lancaster University (UK), using a Trace GC-MS (Thermo Finnigan). The method was adequate for analysis of all proposed FRs in river water extracts, but matrix interferences for TCPP and TDCP using the ions *m/z* 99 were observed, and so these compounds were quantified using the ions *m/z* 277 and *m/z* 381, respectively. This method was used for the analysis of river water samples (River Aire, UK) using both SPE extraction and the ceramic dosimeter passive sampler (Paper 2 and Paper 4).

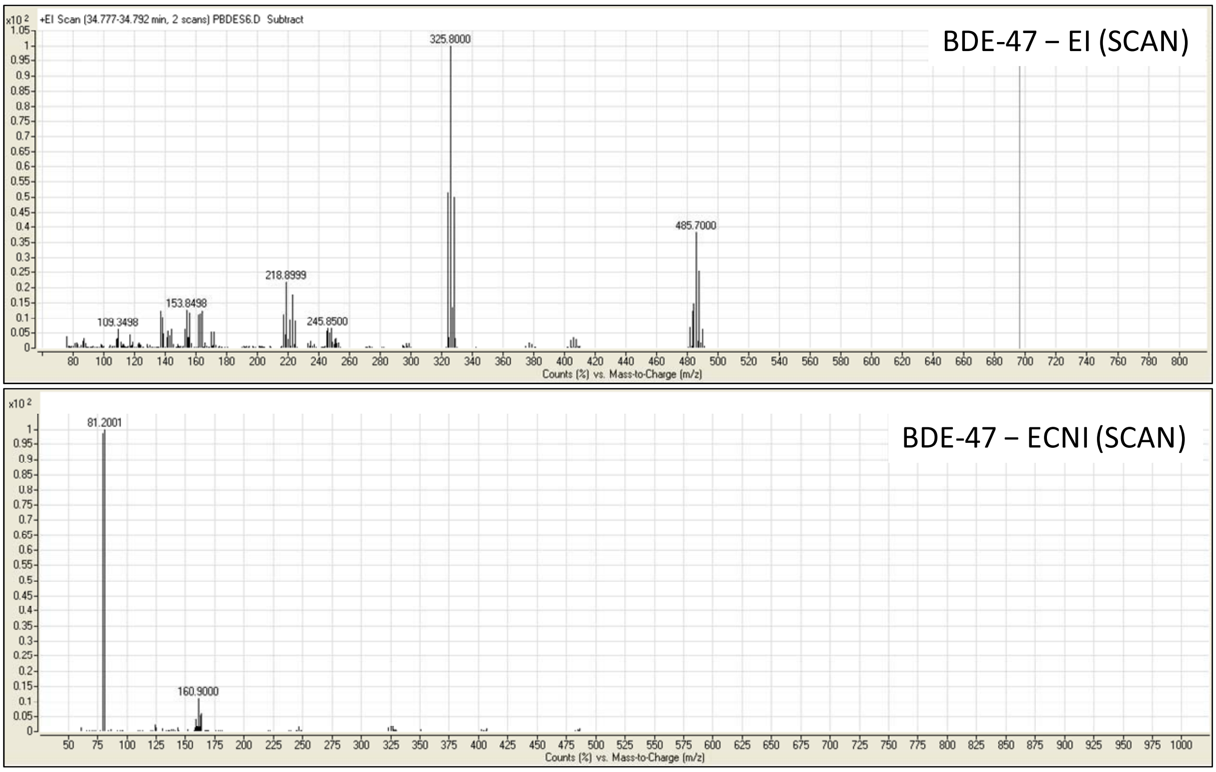

Figure 8 – EI and ECNI mass spectrum of BDE-47.

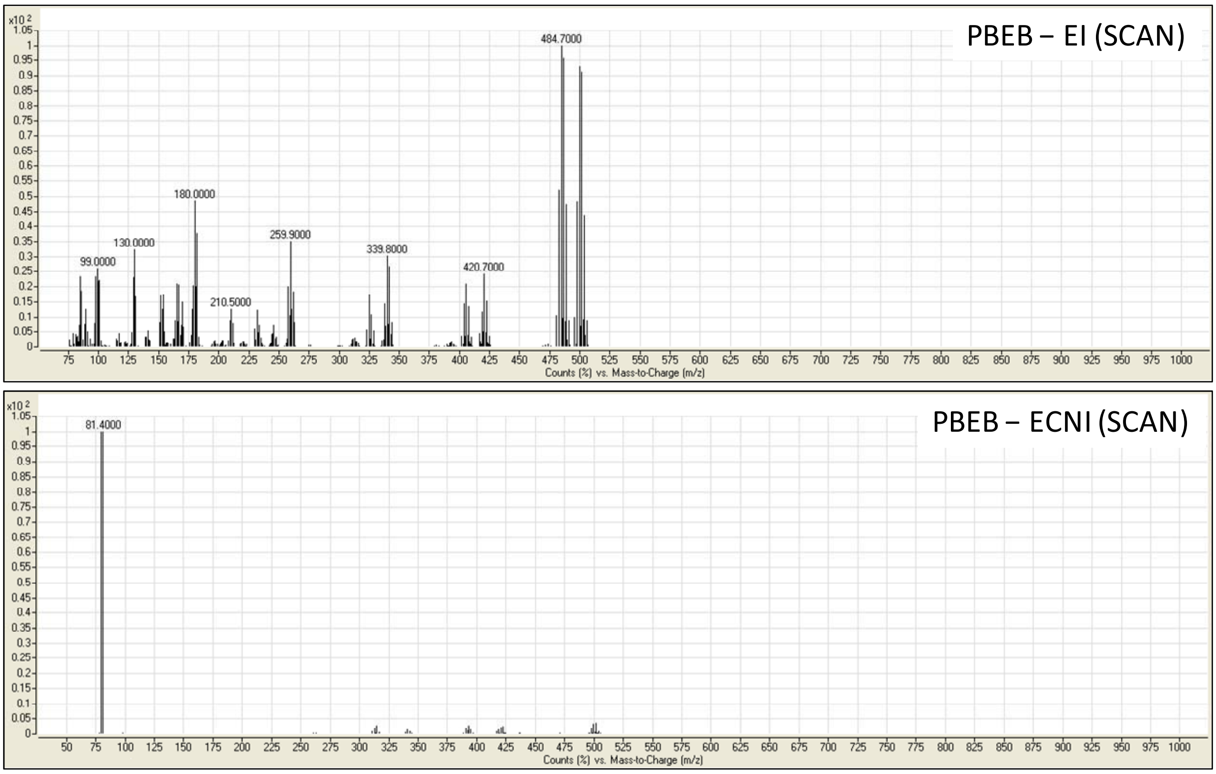

Figure 9 – EI and ECNI mass spectrum of PBEB.

2.4.2 Procedural blanks

The presence of OPFRs in laboratory blanks has been reported in the literature [209], and was also observed during method development and analysis of real samples. As discussed in Paper 3, OPFRs were systematically detected in the procedural blanks performed for sediments, sludge and dust. In addition, some influence of the solvent amount and/or florisil cartridge brands on blank performance was evidenced in that study (Paper 3). Similarly, OPFRs were detected in procedural blanks performed for water extraction. Figure 10 presents the MS/MS transition (T1, used for quantification) of each OPFR detected in the blanks performed for waters and for sediments. Figure 11 compares the relative contribution of OPFRs in the laboratory blanks (waters[3] and sediments). Despite the differences in terms of type of cartridge and solvents used for waters (SPE with HLB cartridges, hexane/acetone 1:1 v/v, dichloromethane/acetone 1:1 v/v) and for sediments (ultrasonic extraction, ethyl acetate/cyclohexane 5:2 v/v), the profile of OPFRs was very similar, where EHDP and TBEP were the most abundant compounds in both cases. This behaviour indicates that the main sources of OPFRs in sediments and water blanks are the same. However, the average ΣOPFRs amount in water and sediment blanks were 13 ng and 87 ng, respectively. Since the extraction of sediments require much more handling than the extraction of waters, the higher contribution of OPFRs in sediments blanks indicate that minimizing the sample handling can potentially decrease the contamination of procedural blanks. Some compounds, such as EHDP and TBEP, presented high variation in terms of observed levels in laboratory blanks performed in different days. This variation for EHDP and TBEP can be observed by the error bars presented in Figure 11. The results of laboratory blanks for water and sediment were performed in three different days (two blanks per day) together with a set of samples. Thus, these results indicate the importance to perform laboratory blanks together with each set of samples in order to take into account possible daily variations, which can affect the results.

In Paper 1, some OPFRs contamination was observed in the procedural blanks performed together with the extraction of the water samples from a DWTP. In that occasion, TCPP and TPhP were detected at levels near the method detection limits (MDL), while TCEP and TDCP were not detected. However, an important improvement of iLOD for OPFRs by GC-EI-MS/MS was obtained in the following studies, which resulted in the detection of all OPFRs in the water blanks. This improvement in instrumental detection limits was attributed

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[3] These procedural blanks were performed during the extraction of river water and sediments samples collected in three Spanish rivers, and the results of that study were described at Paper 5 (Chapter 3).

to the use of a new chromatographic column, which resulted in better chromatographic resolution (peak shape) and in lower iLOD. Therefore, the sensitivity and state of the GC-MS can influence the levels of OPFR in procedural blanks. GC-EI-MS/MS system contamination was checked during sample injection sequences by injection of solvent (toluene) after each four sample set, and no OPFRs were detected.

Blank contamination of OPFRs was also observed using the ceramic dosimeter passive sampler used for the analysis of OPFRs (TCEP, TCPP, TDCP and TPhP) and NBFRs (PBEB and HBB) in river water, as described in Paper 2. Two passive sampler blanks (not deployed) were extracted, and the absolute amount of TCPP, TDCP and TPhP present in the blanks were of 4.5±0.4 ng, 0.17±0.02 ng and 0.47±0.02 ng, respectively. These absolute values were of the same order of magnitude than the ones obtained for SPE waters blanks (Paper 5). The samples of these two studies were extracted in different laboratories (Lancaster University and IDAEA-CSIC). The observation of blank contamination in both of these studies agree with the study reported by Brandsma et al. (2013) [209], which indicated that blank contamination was observed in most of the laboratories participating in an OPFRs interlaboratory study.

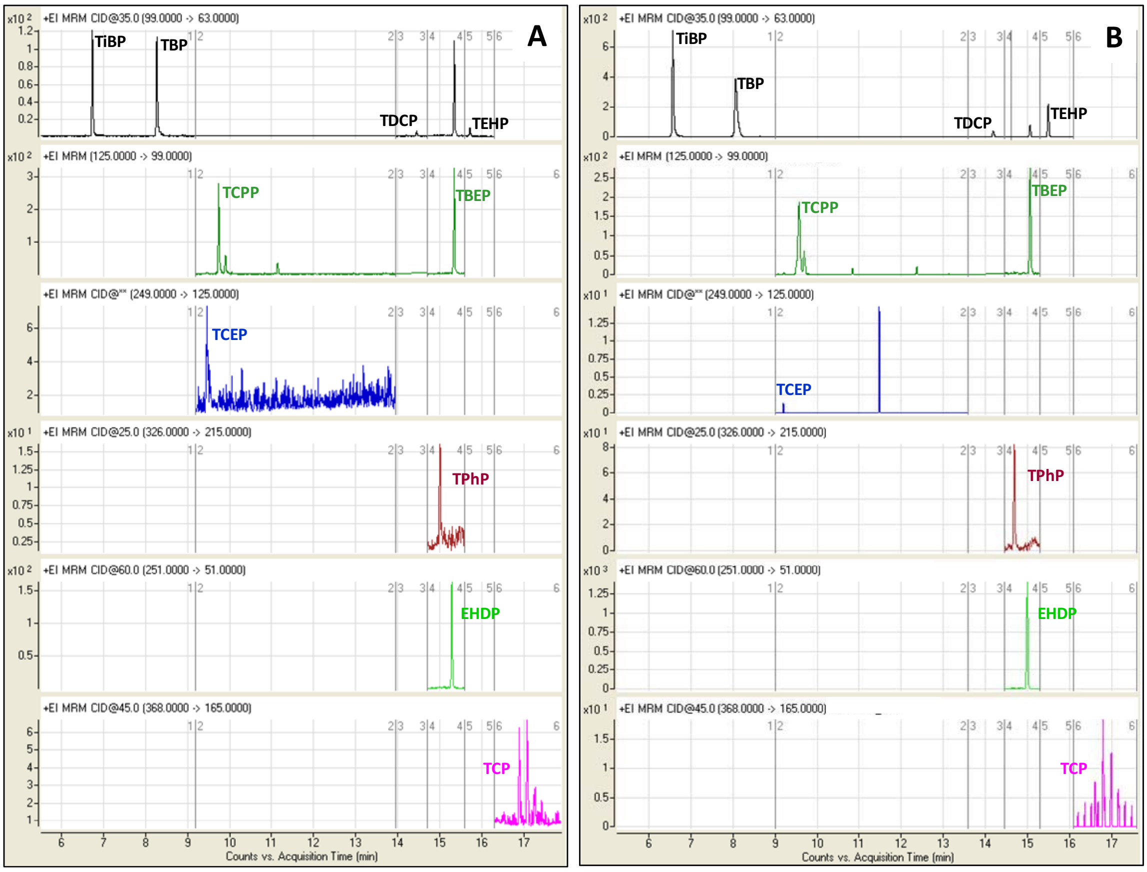


Figure 10 – Chromatogram of each OPFR detected in procedural blanks performed for waters (A) and for sediments (B). No other compound was detected in the blanks.

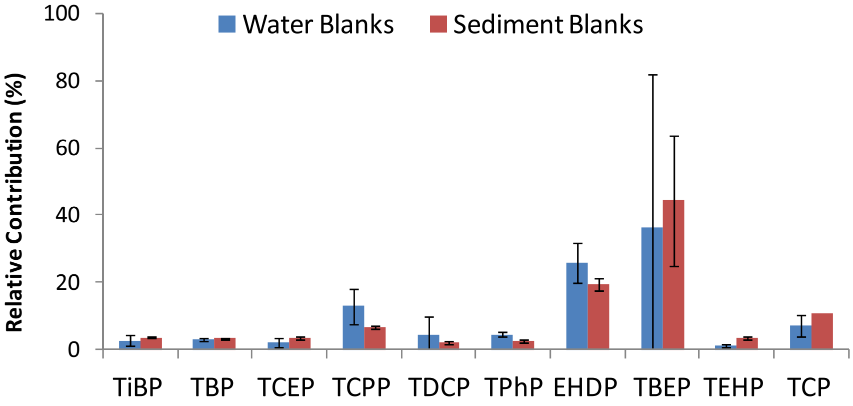


Figure 11 – Relative abundance of OPFRs in the blanks (n=6) for waters and for sediments.

2.4.3 Interlaboratory study (ILS)

During this study, our laboratory was invited to participate in an interlaboratory study for the determination of OPFRs in different environmental matrices [209], which I performed. The experiments included the preparation of standard solutions, extraction of samples, analysis and data processing. The participants received a standard solution, a sediment sample, a spiked fish oil sample (not analyzed by our laboratory) and a dust sample. Each participant analyzed the samples using the method developed in each laboratory. Different standard solutions, prepared in different solvents, were supplied to laboratories to be used for quantification either using GC or LC. All the participants were asked to give results as individual triplicates, together with triplicate of laboratory blanks for each method.

Data assessment (generated by ILS participants) and determination of assigned values were performed statistically using the Cofino model [230], which uses a normal distribution assumption and include all data. For data comparison, the blank contribution obtained by each laboratory was subtracted from the measured values. Laboratory performances for each OPFRs were given in terms of calculated z-score, which is an error estimation taking into account the assigned and measured value and the total measuring error. A certificate, reporting our lab code and obtained z-scores was provided by the interlaboratory study organizers, and a copy is presented in Appendix I. Figure 12 presents the z-scores, for the different matrices, obtained by each participating laboratory on the ILS, where our laboratory code is 2GC. A big variation among laboratories was observed, and so not all compounds could have assigned values. Reported factors that can affect the quantification of OPFRs by chromatographic techniques are matrix effects and blanks levels [186, 209]. However, for most of cases, satisfactory results (|Z| < 2) were obtained in this study using the method developed within the frame of this thesis. All OPFRs quantified in the standard solution presented satisfactory results, with |Z| ranging from 0 to 1.4. TCPP and TPhP had |Z| = 0 for the standard solution, which means that the measured and assigned value were coincident. For sediments, TCPP had questionable results (2 < |Z| < 3) and TCEP had unsatisfactory result (|Z| > 3). For dust, all OPFRs had satisfactory results, except for TBEP (Z = -6.4). Since the results for the standard solution was satisfactory, the unsatisfactory results were not due calibration errors, but rather due specific matrix effects. Moreover, different florisil cartridges were used for dust (5 g, Waters) and sediments (10 g, Phenomenex), which could also affect the analytical performance of these compounds.

The laboratories that presented all results in the satisfactory zone (-2 < Z < 2) were the 13GC, followed by the 6GC (almost all results in the satisfactory zone). In a non published document sent to each participant with details of methods adopted by each participant, 13GC and 6GC informed that used five labelled OPFRs internal standards, three additional to the two recommended ones, TPhP-D15 and TBP-D27. This indicate that accuracy of the method can be improved using the highest possible number of surrogates.

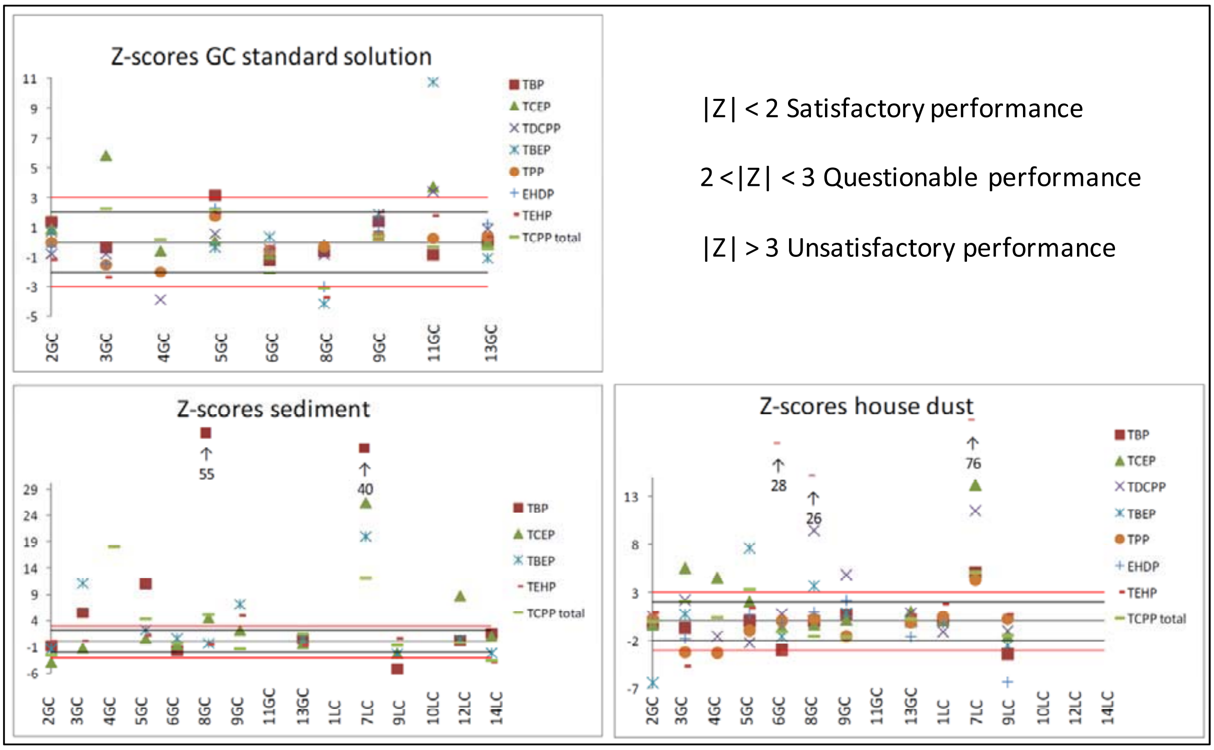


Figure 12 – Obtained z-scores for standard solution, sediment and dust by each participating laboratory on the ILS. Our laboratory code is 2GC. Data from a non published report, which was sent to each ILS participant.

2.4.4 Method performance

As described in Paper 1, the method developed for water samples used SPE extraction with Oasis HLB cartridges and elution with mixtures of acetone, hexane and dichloromethane. This method was applied to the analysis of water samples from a drinking water treatment facility. This method was also tested for the analysis of OPFRs, NBFRs and PBDEs in river water and in wastewater, using OASIS HLB 200 mg cartridges. The efficiency of the method and the matrix effect in different types of water were evaluated using spiked river water (500 mL) and wastewater (influent – 250 mL, effluent – 100 mL) samples. Taking into account the complexity of the different types of water studied, differing in suspended particles and DOC content, the use of surrogates was mandatory in order to correct variations on SPE efficiency and also to correct matrix effects on the chromatographic response. BEHTBP, however, presented better precision and accuracy when quantified by external standard quantification than when using the surrogates MHBB or MBDE-77 for internal standard quantification. This was due to the poor response of these surrogate standards for a compound with very different chemical structure. This means that to quantify this compound by internal standard, a more suitable compound should be used, an option could be a labelled phthalate. Table 5 presents the recoveries for spiked water samples, which ranged from 80 to 120%, with RSD lower than 20% in most of cases, indicating that the method was efficient for the analysis of different families of flame retardants in different types of water. HCDBCO was not recovered in spiked wastewater (influents and effluents). TEHP presented a poor recovery in spiked influents (29 ± 2%), while its recovery in river water and effluents were acceptable. This method was used for the determination of OPFRs, NBFRs and PBDEs in river water (Spain and UK) and wastewater (Spanish WWTPs), whose results were described at Chapter 3. Additional recovery results using spiked Milli-Q and river water samples, which were analyzed by GC-EI-MS/SIM, were presented in Paper 4 (Chapter 3).

For the analysis of environmental samples, method efficiency was evaluated taking into account the recovery of the surrogates. The surrogate recoveries obtained for river water and wastewater samples are presented in Figure 13. Average recoveries for TBP-D27 and TPhP-D15 were higher than 80% in most of cases. A lower recovery was obtained for MHBB, MBDE-77 and MBDE-209 in wastewater samples (about 60%), probably due matrix interferences, as these waters are rich in terms of DOC and suspended particles content (samples were not filtered), affecting the performance of the more lipophilic compounds. On the other hand, recoveries higher than 70% were obtained for brominated surrogates (MHBB, MBDE-77, MBDE-209) in river water samples, while MBDE-209 presented the highest RSD (26%), probably due differences among analyzed samples (DOC and suspended particle content). Thus, the use of surrogates for water samples was essential for evaluating the performance of the method and for correcting the results taking into account losses during extraction.

Table 5 – Recoveries (% Rec.) obtained in spiked river water and wastewater (n=3).

Compound	River Water[a]		WWTP Effluent[b]		WWTP Influent[c]	
	% Rec. (SD)	MDL (ng L^{-1})	% Rec. (SD)	MDL (ng L^{-1})	% Rec. (SD)	MDL (ng L^{-1})
TiBP	62 (3)	1.9	85 (4)	60	79 (3)	150
TBP	105 (2)	1.2	92 (1)	9.5	86 (1)	24
TCEP	90 (16)	1.5	105 (1)	19	95 (4)	47
TCPP	91 (18)	7.2	96 (13)	46	85 (12)	115
TDCP	104 (7)	5.3	102 (4)	1.6	94 (1)	4.0
TPhP	102 (2)	1.6	94 (2)	1.8	88 (2)	4.6
EHDP	94 (18)	11	65 (3)	27	70 (1)	67
TBEP	107 (23)	44	111 (9)	23	85 (5)	57
TEHP	75 (13)	0.8	48 (7)	7.6	29 (2)	19
TCP	97 (12)	4.2	70 (1)	3.7	77 (2)	9.2
BDE-28	90 (1)	0.4	127 (3)	4.3	145 (8)	4.7
BDE-47	112 (6)	0.3	98 (8)	1.4	113 (6)	6.4
BDE-100	92 (6)	0.4	93 (9)	3.9	88 (3)	18
BDE-99	87 (8)	0.4	95 (8)	3.7	87 (4)	20
BDE-154	89 (1)	1.2	107 (12)	1.5	97 (13)	14
BDE-153	95 (5)	0.6	109 (10)	2.2	95 (2)	5.9
BDE-183	90 (14)	3.0	92 (14)	6.0	84 (2)	41
BDE-209	114 (17)	60	113 (30)	187	124 (21)	550
PBT	108 (13)	0.8	85 (3)	3.4	100 (17)	12
PBEB	101 (12)	0.5	80 (5)	2.0	76 (10)	18
HBB	98 (24)	1.5	89 (6)	13	96 (29)	48
DPTE	126 (3)	0.4	129 (21)	2.0	136 (24)	7.5
HCDBCO	86 (2)	3.0	[d]		[d]	
EHTBB	92 (6)	1.5	99 (3)	3.0	78 (6)	21
BEHTBP	86 (7)	7.5	114 (20)	17	79 (2)	38
BTBPE	89 (6)	1.5	139 (10)	5.7	123 (12)	23
DBDPE	88 (9)	50	124 (4)	171	128 (15)	230

[a] Spiked at 200 ng L^{-1} for OPFRs and 20 ng L^{-1} for NBFRs and PBDEs (except BDE-209 and DBDPE that were at 200 ng L^{-1}). Volume of final extract: 250 µL.

[b] Spiked at 1000 ng L^{-1} for OPFRs and 40 ng L^{-1} for NBFRs and PBDEs (except BDE-209 and DBDPE that were at 400 ng L^{-1}). Volume of final extract: 250 µL.

[c] Spiked at 2500 ng L^{-1} for OPFRs and 100 ng L^{-1} for NBFRs and PBDEs (except BDE-209 and DBDPE, that were at 1000 ng L^{-1}). Volume of final extract: 500 µL.

[d] Not detected.

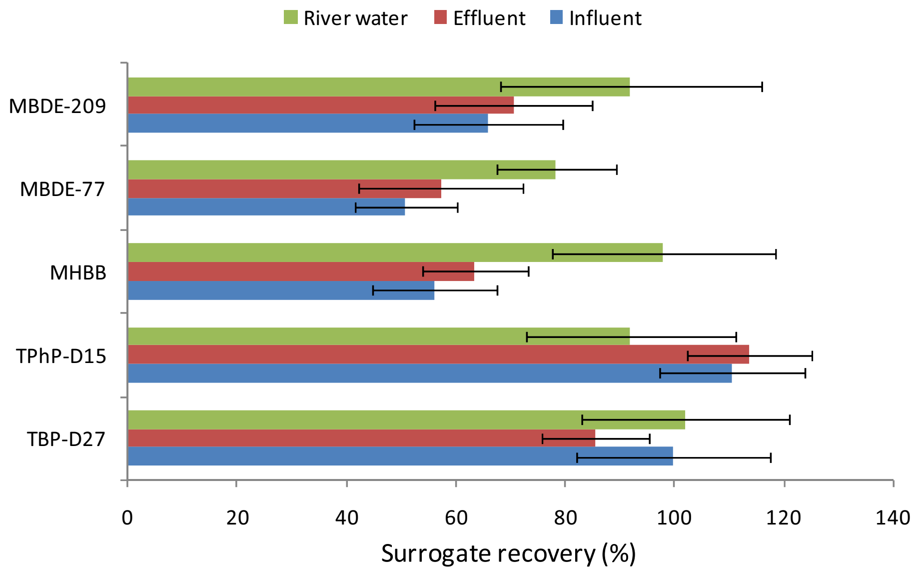


Figure 13 – Surrogate recoveries obtained for river water (Spanish Rivers, n=32) and wastewater samples, including effluent (n=10) and influent (n=10).

The ceramic dosimeter passive sampler allowed the detection of all studied OPFRs (TCEP, TCPP, TDCP and TPhP) in river water, and integrated water concentrations were in agreement with the measured concentrations using SPE extraction and grab sampling. Those results prove the efficiency of the ceramic dosimeter design for FR surface water monitoring. However, the fouling effect needs be better understood to identify and control the surface phenomena that can potentially affect the uptake of compounds: biofilm formation, biodegradation in the biofilm and scaling (precipitation of minerals which plug the ceramic pores).

The efficiency of the developed method for detection of OPFRs, NBFRs and PBDEs in river sediments, sewage sludge and domestic dust was shown in Paper 3. This method was applied for the monitoring of flame retardants in 25 river sediment samples (results presented in the Chapter 3). Those samples were collected in different points of three Spanish Rivers. The recovery of surrogates obtained for those samples are presented in Figure 14. Surrogate recoveries ranged from 80 to 120% for most of the samples indicating that the method was robust for sediments samples differing in organic carbon content. Among used surrogates, MBDE-209 presented the lowest recovery and the highest RSD, but considering the complexity of this kind of environmental sample, MBDE-209 recoveries were considered acceptable.

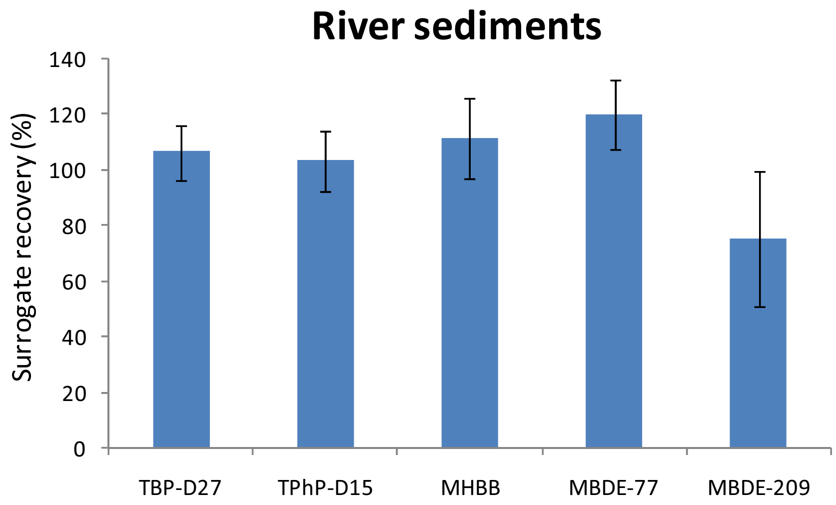


Figure 14 – Surrogate recovery obtained for river sediment samples (n=25).

In summary, the developed method allowed the simultaneous extraction and determination of several families of flame retardants in water, sediment and sludge, which was advantageous in terms of costs and time. These methods were applied for the monitoring of these flame retardants in rivers and in WWTPs, and results are described in the following chapter.

3 Monitoring Studies

3.1 Introduction

As presented in Chapter 1, urban rivers are affected by anthropogenic pressures, and sources of contaminants include discharges from WWTPs, industrial releases, runoff, atmospheric deposition, etc. Contamination of rivers by FRs has been described in the literature. However, only knowledge of environmental levels is not very useful if it is not related to their potential risk for aquatic organisms. This chapter presents an overview of environmental levels of FRs in rivers in different countries and some consideration about the most important sources of flame retardants. Additionally, theoretical bases of the risk assessment methodology used during this thesis are described.

3.1.1 Occurrence of priority and emerging flame retardants in rivers

The occurrence of FRs in rivers depends on the degree of anthropogenic impact that a river basin suffers. FR concentrations tend to be higher in the proximity of urban centres and industrial areas, and lower (or below limits of detection) in areas far from these sources. In addition, the stricter the regulation of a country concerning flame-retardancy of materials and furniture, the higher the use of flame retardants in the commercialized products and consequently, the higher their emissions to the environment. On the other hand, it is important to take into account that the environmental levels of FRs in the aquatic environment do not depends only on the anthropogenic impact, but also on the characteristics of the medium (e.g. DOC, f_{oc} of sediments and suspended particles, water volume, light incidence, temperature, climatic conditions, etc).

Concerning PBDEs, most studies report their occurrence in sediments because of their high lipophilicity, while a lower number of studies report their concentration in the water column. On the other hand, few data are available regarding the occurrence of NBFRs and OPFRs in rivers. Table 6 presents concentrations of PBDEs, NBFRs and OPFRs in rivers around the world. In general, BDE-209 is the most abundant PBDE congener in the environment [29, 231-232], which is in agreement with the higher use of Deca-BDE than Penta- and Octa-BDE formulations. Reported PBDE concentrations in rivers (Table 6) indicate that concentrations tend to be higher in North America and Asia than in Europe (except UK). This tendency is in agreement with the higher consumption of PBDE formulations in those regions than in Europe and rest of world. The global consumption of

PBDEs (in 2001) in different parts of the world is presented in Table 7. Also in accordance with these levels, an international survey of BDE-209 in sewage sludge indicated that the concentrations in samples from North America, UK and Asia were higher than in samples from European countries, South Africa and Australia [36]. The authors attributed the similarities of BDE-209 levels in samples from USA and UK to the UK's history of stringent fire regulations.

High PBDE concentrations were reported in rivers located near industrial areas and in areas affected by electronic waste. Sediments in the Pearl River Delta, and some of its tributary rivers, present one of the higher brominated FR levels reported in the literature, since an electronic manufacturing centre is located in this area. The impact of this industrial centre on PBDE and NBFR concentrations in sediments was evidenced by Chen et al. (2013) [231], who reported much higher concentrations (up to about 50 times higher) in the rivers flowing through the electronics manufacturing centre than in the rivers flowing through rural or less-industrialized areas. High PBDE and NBFR concentrations were also observed in rivers located near electronic waste areas [231, 233], and in the proximity of a waterfall of a WWTP that received effluents from a textile industry [132]. Concerning OPFRs, most of studies indicate that WWTPs are the most important source of these contaminants in rivers, since these compounds are not completely removed during the treatment [147]. TCPP, TBEP, TBP and TCEP tend to be the most abundant compounds in river water [30, 34, 234-235].

A recent study indicated an increase tendency for NBFR concentrations in river sediment associated to a decrease tendency for PBDE concentrations, as a result of regulatory laws concerning the use of PBDEs in China. Chen et al. (2012) [231] reported that concentrations of PBDEs in sediments from the Pearl River Delta collected in 2009 − 2012 decreased significantly compared with those for sediments collected in 2002 in that region. The authors also observed that the levels of DBDPE have exceeded those of BDE-209 in the majority of sediments (2009 − 2012 samples), which suggested a different contaminant pattern of brominated FRs in current sediments due to the replacement of the Deca-BDE mixture by DBDPE in that region. These findings highlight the importance of the simultaneous monitoring of priority and emerging flame retardants in order to compare their levels and evaluate their trends in the environment. In addition, a risk assessment must be performed in order to evaluate whether regulatory laws and the use of alternative flame retardants have been effective for minimizing/eliminating hazardous effects to aquatic organisms.

Table 6 – Levels of PBDEs, NBFRs and OPFRs in rivers around the world.

	Compounds	**Environmental levels**	**Ref.**
Europe			
River Prédecelle (France)	ΣPBDE (28, 47, 99, 100, 153, 154, 183, 209)	2.3 – 4.3 ng L^{-1} (dissolved + particulate phases) 3.1 – 15.1 ng g^{-1} (sediment)	[29]
Clyde Estuary (UK)	ΣPBDE (28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 209)	1 – 2645 ng g^{-1} (sediment)	[236]
Llobregat River basin (Spain)	ΣPBDE (38 congeners, di- to deca-BDE)	22 – 136 ng g^{-1} (sediment)	[141]
	PBEB	3.1 –9.6 ng g^{-1} (sediment)	
	HBB	0.4 – 2.4 ng g^{-1} (sediment)	
	DBDPE	4.8 – 23 ng g^{-1} (sediment)	
Rivers Danube, Liesig and Schwechat (Austria)	ΣOPFRs[a] (9 compounds)	141 – 922 ng L^{-1} (water) 2.4 – 1938 ng g^{-1} (sediment)	[30]
Rivers Rhine, Elbe, Main, Oder, Nidda, Schwarzbach (Germany)	TBP	100 – 1510 ng L^{-1} (water)	[234]
	TCEP	nd – 220 ng L^{-1} (water)	
	TBEP	103 – 663 ng L^{-1} (water)	
North America			
River Niagara (Canadian and American sides)	ΣPBDE (47, 49, 99, 100, 153, 154, 209)	0.25 – 188 ng g^{-1} (sediment)	[232]
River Detroit (Great Lakes system, North America)	ΣPBDE (46 congeners, mono- to deca-BDE)	6.802 – 388.4 ng g^{-1} (suspended sediment)	[121]
Saginaw River Watershed (USA)	ΣPBDE (28, 47, 66, 85, 99, 100, 138, 153, 154, 209)	0.05 – 49.4 ng g^{-1} (surface sediment)	[237]
River Yadkin (USA)	ΣPBDE (20 congeners, tri- to deca-BDE)	64 291 – 329 000 ng g^{-1} TOC (sediment)	[132]
	BEHTBB	2000 – 19 200 ng g^{-1} TOC (sediment)	
	EHTBP	80 – 3850 ng g^{-1} TOC (sediment)	
	BTBPE	77 – 2000 ng g^{-1} TOC (sediment)	
	TOC (sediment)	0.41 – 1.60%	
Raw water of a DWTF (USA)	TCEP, TDCP, TBP, TBEP	75 (TBP) – 290 (TBEP) ng L^{-1} (water)	[238]
Asia			
Nanyang River (China) polluted by electronic waste	ΣPBDE (14 congeners, mono- to deca-BDE)	4434 to 16 088 ng g^{-1} (surface sediment) 55 to 445 ng g^{-1} (bottom sediment)	[233]
Pearl River Delta (China)			[231]
River Dongjiang (electronics manufacturing centre)	ΣPBDE (16 congeners, tri- to deca-BDE)	3.83 – 2517 ng g^{-1} (sediment)	
	DBDPE	nd – 1728 ng g^{-1} (sediment)	
	BTBPE	0.03 – 20.1 ng g^{-1} (sediment)	
	PBT + HBB + PBEB	0.09 – 2.96 ng g^{-1} (sediment)	
River Zhujiang (densely populated city)	ΣPBDE (16 congeners, tri- to deca-BDE)	10.7 – 910 ng g^{-1} (sediment)	
	DBDPE	11.8 – 1180 ng g^{-1} (sediment)	
	BTBPE	0.13 – 41.8 ng g^{-1} (sediment)	
	PBT + HBB + PBEB	0.11 – 2.30 ng g^{-1} (sediment)	
River Dayanhe (adjacent to a e-waste site)	ΣPBDE (16 congeners, tri- to deca-BDE)	13.5 – 161 (sediment)	
	DBDPE	22.4 – 362 ng g^{-1} (sediment)	
	BTBPE	nd – 73.4 ng g^{-1} (sediment)	
	PBT + HBB + PBEB	0.13 – 2.30 ng g^{-1} (sediment)	
River Beijiang (rural or less-industrialized areas)	ΣPBDE (16 congeners, tri- to deca-BDE)	9.99 – 183 (sediment)	
	DBDPE	nd – 263 ng g^{-1} (sediment)	
	BTBPE	0.47 – 1.68 ng g^{-1} (sediment)	
	PBT + HBB + PBEB	0.13 – 1.00 ng g^{-1} (sediment)	
River Xijiang (rural or less-industrialized areas)	ΣPBDE (16 congeners, tri- to deca-BDE)	4.12 – 65.0 ng g^{-1} (sediment)	
	DBDPE	2.86 – 80.4 ng g^{-1} (sediment)	
	BTBPE	nd – 1.22 ng g^{-1} (sediment)	
	PBT + HBB + PBEB	nd – 0.38 ng g^{-1} (sediment)	
Pearl River Estuary	ΣPBDE (16 congeners, tri- to deca-BDE)	3.67 – 45.6 ng g^{-1} (sediment)	
	DBDPE	nd – 30.5 ng g^{-1} (sediment)	
	BTBPE	0.04 – 0.53 ng g^{-1} (sediment)	
	PBT + HBB + PBEB	0.09 – 1.98 ng g^{-1} (sediment)	
Songhua River (China)	ΣOPFRs (12 compounds)	265.4 – 4777.2 ng L^{-1} (water)	[235]
Rest of world			
Diep River (South Africa)	ΣPBDE (28, 47, 99, 100, 153, 154, 183, 209)	2.60 – 4.83 ng L^{-1} (water)	[239]
Pangani River Basin (Tanzania)	ΣPBDE (47, 99, 100, 153, 154, 183)	0.038 – 2.175 ng g^{-1} (sediment)	[240]

[a] Calculated sum, based on reported levels for each individual compound.

Table 7 – The usage of PBDEs in different areas of the world in 2001 (in tonnes) [241].

Formulation	America	Europe	Asia	Rest of the world	total
Penta-BDE	7100	150	150	100	7500
Octa-BDE	1500	610	1500	180	3790
Deca-BDE	24 500	7600	23 000	1050	56 100

3.1.2 Risk assessment

Undesirable ecological effects can occur depending on the concentration and life time of a contaminant in the aquatic environment. For example, high amounts of a contaminant can be discharged to surface water only once but result in water concentrations high enough to reach acute toxic levels and causing death of organisms. On the other hand, contaminants can reach the aquatic environment by diffuse sources and be present in water at low concentrations for long periods of time. Sometimes those "constant" low concentrations do not reach acute toxic levels, but can be high enough to cause long term effects, such as endocrine disruption effects, reproductive effects, etc. Thus, an evaluation of exposure and effects of contaminants to different organisms, in different ecosystems, taking into account sources and distribution pathway, is not a simple issue. Therefore, the European Community elaborated the Technical Guidance Document on Risk Assessment (TGDRA) - Part 2 [242] for assisting the competent authorities to carry out the environmental risk assessment of contaminants in the environment.

In order to assess the risk that a contaminant poses to aquatic organisms, predicted no effect concentration (PNEC) needs to be estimated. PNEC refer to a concentration below which an unacceptable effect will most likely not occur, and is calculated by dividing the toxic concentration by an appropriate assessment factor (f). PNEC estimation takes into account that ecosystem sensitivity depends on the most sensitive species, and that protecting ecosystem structure protects community function. A potential risk to aquatic organisms is suspected if the measured environmental concentration (MEC) of a toxicant exceeds their respective PNEC [242].

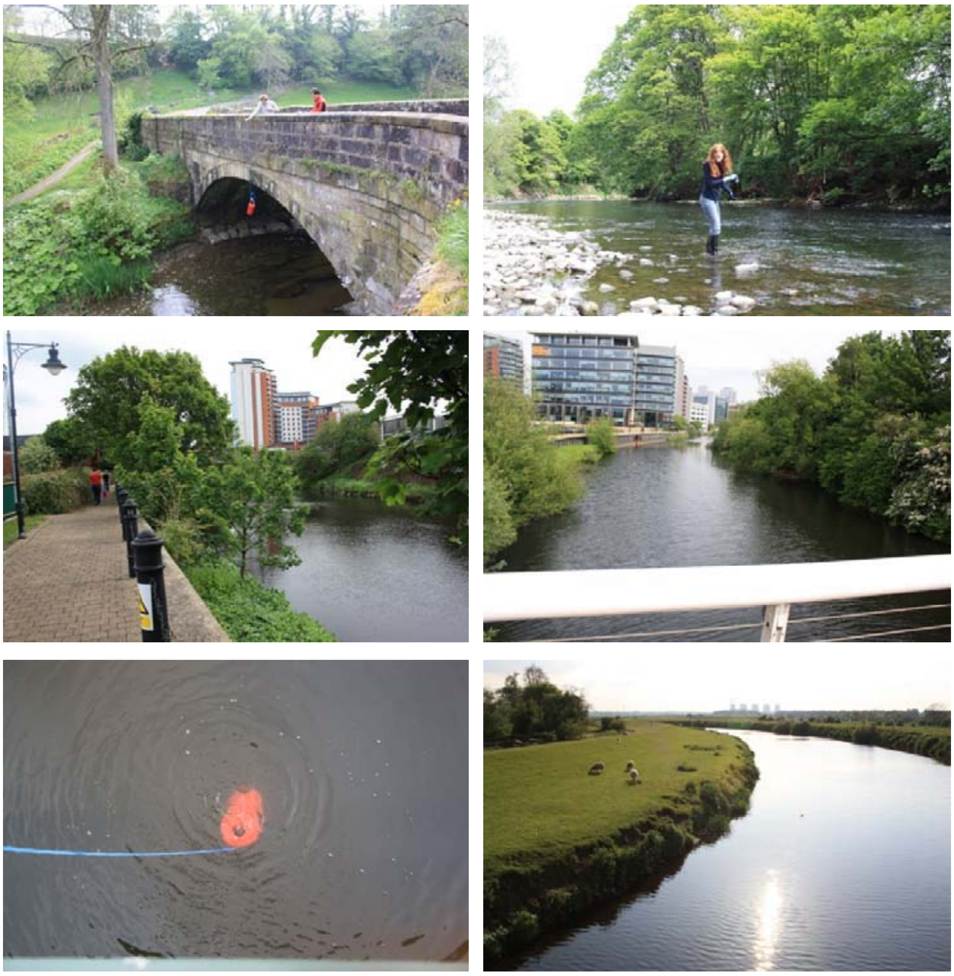
The toxic concentration is generally determined using biological assays where the organism is exposed to a certain concentration (dose) of the contaminant and a dose-response relationship is established. The response to a toxicant can be related to diverse effects on the organism, such as mortality, growth, behaviour, reproduction, etc. Dose-response endpoints of toxicants are usually expressed as the concentration that causes mortality in 50% of the population of tested organisms (LC_{50}), or as the concentration that has an effect on 50% of the population (EC_{50}). No observed effect concentration (NOEC) is another terminology, used to describe concentrations that have a nonexistent effect [243]. LC_{50} and EC_{50} are frequently used for the assessment of short-term risks, while NOEC is recommended for assessment of long-term risks [242]. However, those parameters are established for model species exposed to a toxicant under laboratory conditions, and so a direct use of these values to other aquatic species under environmental conditions is not reliable. TGDRA recommends the use of an assessment factor for PNEC calculations, where this factor aims to address the uncertainties associated to the extrapolation from single-species laboratory data to a multi-species ecosystem. Such uncertainties include intra- and inter-laboratory variation of toxicity data; intra- and inter-species variations (biological variance); short-term to long-term toxicity extrapolation; laboratory data to field impact extrapolation, considering that additive, synergistic and antagonistic effects may occur [242]. The higher the uncertainty of the toxic concentration, the higher the magnitude of the assessment factor. TGDRA propose different f values to be used for PNEC estimation according to the available toxicity data.

3.2 Environmental monitoring methodology

The monitoring of OPFRs, NBFRs and PBDEs in the aquatic environment included rivers located in UK and in Spain. Rivers highly affected by anthropogenic pressures were chosen in each study. A comparison among polluted rivers located in those countries is interesting since UK has more restrictive fire safety regulations for furniture than EU, and so environmental FR levels and profiles may possibly differ. A risk assessment was performed for all studied rivers, correlating measured concentrations to toxic concentrations for aquatic organisms. Since WWTPs are considered one of the most important sources of FRs to surface water, the occurrence and removal of FRs in these facilities was also studied.

3.2.1 Sampling campaign

The occurrence of flame retardants in rivers in UK and Spain was evaluated, totalling 4 monitored rivers, one river in UK (Aire) and three rivers in Spain (Nalón, Arga and Besòs). In order to evaluate the impact of flame retardants on river water quality, and the potential of rivers for transporting contaminants, all rivers were monitored from the source to the mouth, and sampled points were chosen taking into account WWTP discharge points and the proximity of urban and industrial centres. Concerning all the four studied rivers, a total of 45 sites were monitored: River Aire – 13 sampling points, River Nalón – 11 sampling points, River Arga – 8 sampling points, and River Besòs – 13 sampling points. Since NBFRs and PBDEs tend to accumulate in sediments due their lipophilicity, the presence of FRs in sediments was also monitored in the Spanish rivers. Characteristics of each studied river, and a map indicating the sampled points, WWTPs, urban and industrial centres along each monitored river were presented in Paper 4 (Aire) and Paper 5 (Nalón, Arga and Besòs). Table 8 – 9 presents the geographical coordinates of sampled points along each river. Figure 15 and 16 presents photos taken during the sample campaigns at the River Aire and the Spanish rivers, respectively.

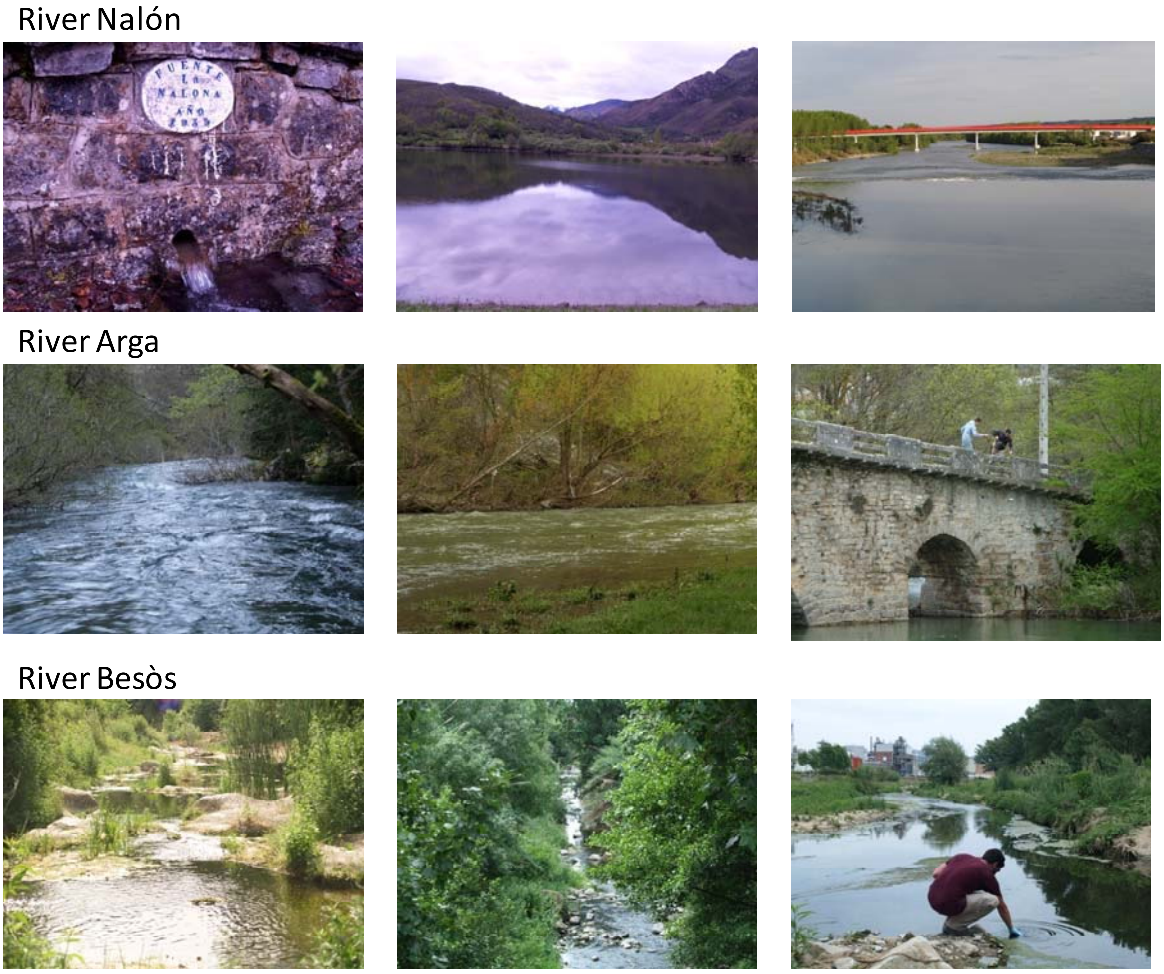


River Aire

Figure 15 – Sampling campaign at the River Aire (UK).

Table 8 – Geographical coordinates of sampled points along the River Aire (UK)

River Aire			
P1	54° 1' 44" N, 2° 8' 54" W	P8	53° 47' 48" N, 1° 33' 19" W
P2	53° 58' 59" N, 2° 6' 11" W	P9	53° 46' 52" N, 1° 31' 23" W
P3	53° 54' 30" N, 1° 59' 04" W	P10	53° 45' 0" N, 1° 25' 29" W
P4	53° 51' 48" N, 1° 51' 20" W	P11	53° 44' 20" N, 1° 18' 45" W
P5	53° 50' 37" N, 1° 49' 38" W	P12	53° 43' 26" N, 1° 11' 38" W
P6	53° 51' 25" N, 1° 43' 43" W	P13	53° 43' 43" N, 1° 7' 28" W
P7	53° 50' 16" N, 1° 42' 21" W		



River Nalón

River Arga

River Besòs

Figure 16 – Sampling campaign at the rivers Nalón, Arga and Besòs (Spain).

Table 9 – Geographical coordinates of sampled points along the Spanish rivers Nalón, Arga and Besòs.

River Nalón			
N1	43° 6' 1" N, 5° 13' 19" W	N7	43° 18' 42" N, 5° 53' 29" W
N2	43° 13' 45" N, 5° 28' 30" W	N8	43° 21' 30" N, 5° 57' 41" W
N3	43° 14' 15" N, 5° 33' 20" W	N9	43° 23' 23" N, 6° 0' 18" W
N4	43° 17' 21" N, 5° 38' 16" W	N10	43° 29' 28" N, 6° 6' 14" W
N5	43° 19' 1" N, 5° 42' 48" W	N11	43° 31' 11" N, 6° 4' 34" W
N6	43° 19' 25" N, 5° 44' 1" W		
River Arga			
A1	43° 0' 53" N, 1° 28' 37" W	A5	42° 48' 23" N, 1° 40' 59" W
A2	42° 58' 44" N, 1° 30' 44" W	A6	42° 48' 51" N, 1° 44' 53" W
A3	42° 51' 35" N, 1° 34' 27" W	A7	42° 18' 54" N, 1° 48' 6" W
A4	42° 49' 10" N, 1° 37' 39" W	A8	42° 14' 15" N, 1° 45' 25" W
River Besòs			
B1	41° 38' 54" N, 2° 26' 25" E	B8	41° 33' 1" N, 2° 15' 6" E
B2	41° 47' 31" N, 2° 14' 14" E	B9	41° 29' 30" N, 2° 9' 23" E
B3	41° 38' 4" N, 2° 9' 37" E	B10	2° 11' 14" E, 41° 29' 17" N
B4	41° 41' 59" N, 2° 16' 58" E	B11	41° 28' 50" N, 2° 11' 26" E
B5	41° 41' 32" N, 2° 11' 45" E	B12	41° 27' 29" N, 2° 11' 26" E
B6	41° 33' 43" N, 2° 14' 2" E	B13	41° 25' 20" N, 2° 13' 39" E
B7	41° 33' 1" N, 2° 16' 4" E		

Five Spanish WWTPs were sampled in order to evaluate the occurrence of FRs in wastewater, and to evaluate the removal efficiency. Characteristics of WWTPs including inhabitant equivalents, water influent flow, treatment type and water destination are indicated in Table 10. WWTP locations are indicated in Figure 17. Influent and effluent samples were collected in two consecutive days (except $WWTP_4$, n=1) during September-October 2012. Twenty four h composite 1 L samples were taken by personnel of the WWTP using an automated position sample collector. After collection, water samples were stored at 5ºC and extracted as soon as possible, within a period no longer than five days.

Table 10 – Inhabitant equivalents, water influent flow, treatment type and water destination of studied WWTPs (Spain).

WWTP	Inhabitants	Inhabitant equivalents	Water influent (m^3 day^{-1})	WWTP treatment	Wastewater Destination
$WWTP_1$	146 024	186 666	87 500	Secondary treatment	River Segre
$WWTP_2$	226 932	296 333	33 000	Secondary treatment	River Sec
$WWTP_3$	181 147	451 250	57 000	Secondary treatment	Mediterranean Sea
$WWTP_4$	1 161 906	2 275 000	420 000	Secondary treatment	Mediterranean Sea
$WWTP_5$	1 233 385	2 843 750	525 000	Secondary treatment	Mediterranean Sea

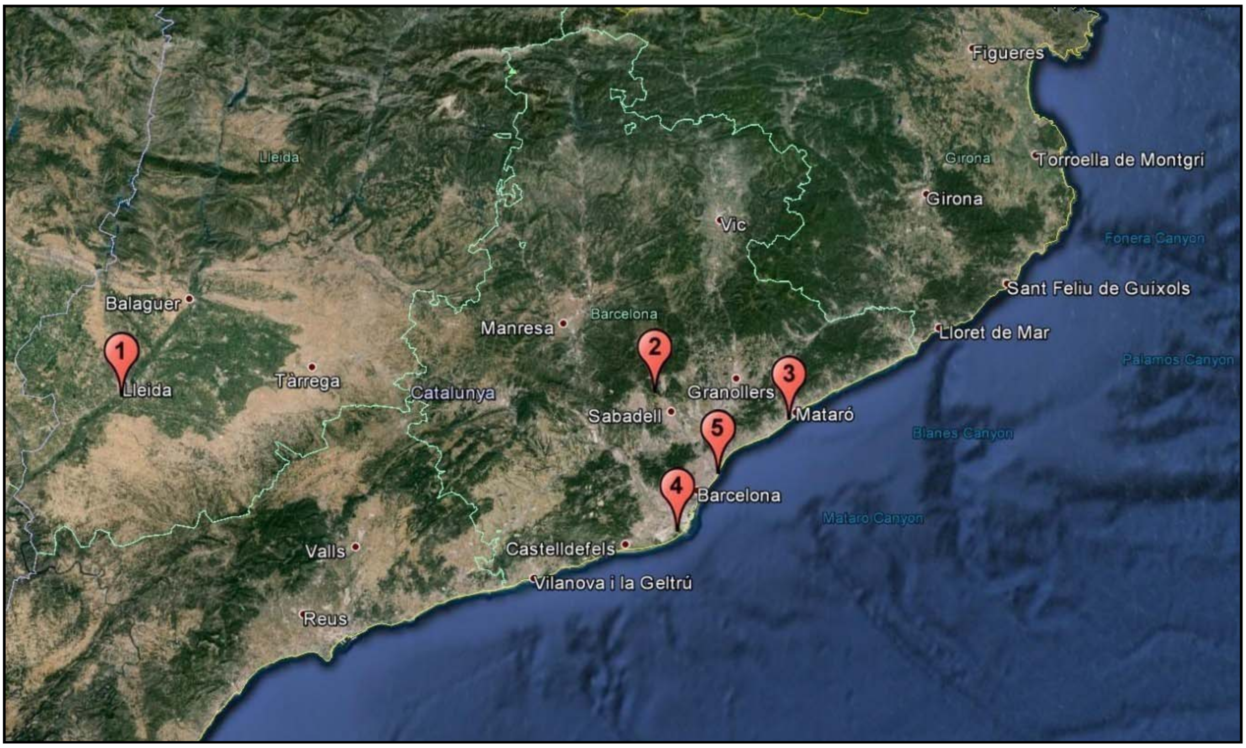


Figure 17 – Location of the studied WWTPs (1–5).

3.2.2 Analysis

Water and sediment samples were extracted following the protocols described in the Chapter 2. Sampling, extraction and analysis performed for the River Aire monitoring (Paper 4) were carried out during a visiting in the Lancaster University (Lancaster, UK). In that study, analyses were performed by GC-EI-MS/SIM, using a simple quadrupole MS detector (Trace GC-MS, Thermo-Finnigan). Samples collected for the monitoring of Spanish rivers (Paper 5) and WWTPs (influent and effluent) were processed in the IDAEA-CSIC (Barcelona, Spain), and analyzed by GC-EI-MS/MS, using a triple quadrupole MS detector (GC Agilent 7890A equipped with a 7000A GC-MS triple quadrupole).

3.2.3 Risk estimation

The risk associated with the presence of FR along the studied rivers was assessed. For PNEC estimation, acute toxicity (LC_{50} or EC_{50}) was used with an assessment factor of 1000, as proposed by the TGDRA [242]. The risk assessment performed for the River Aire (Paper 4) took into account reported LC_{50} or EC_{50} values for aquatic organisms from three different trophic levels: algae, daphnid and fish. Further, a risk map was drawn in order to indicate the river zones where adverse effects are more probably to occur.

In the monitoring of Spanish rivers (Paper 5), co-existence of ten OPFRs in water column was observed in some locations. It is known that mixtures of toxicants may interact to produce additive, synergistic or antagonistic effects, and that these effects depend on the characteristics of the chemicals and the physiological condition of the organism [243]. Thus, for a more accurate risk assessment, experiments were carried out in order to evaluate the effects of OPFR multicomponent exposure to aquatic organisms. The hypothesis tested in this study was that OPFRs act by a similar mode of action and that exposure to mixtures of these contaminants causes concentration-addition effects. For this purpose, the acute toxicity test was performed with *Daphnia magna,* which was chosen as a model organism. *D. magna* is commonly used as a representative pelagic invertebrate test organism in freshwater toxicity test, supporting environmental monitoring and ecological risk assessments [244]. Tests using *D. magna* organisms were performed following the OECD Guideline 202. Details of the acute assays were described in Paper 5. The results of *D. magna* toxicity tests were applied for a risk assessment in three Spanish rivers. The concentration of OPFRs in water and the obtained

EC_{50} were used for calculation of Risk Quotients (RQ) along each studied river. A risk assessment was also performed considering the detected flame retardants in sediments collected in Spanish rivers, by estimating pore water concentrations and relating it to the obtained EC_{50} to *D. magna* for OPFRs, while reported EC_{50} was used for PBDEs.

Results obtained in the monitoring of the River Aire and in the Spanish rivers are presented in Paper 4 and Paper 5, respectively. Concentrations of FRs in influents and effluents of the studied WWTPs were presented in the discussion section of this chapter (section 3.4.1). Additionally, section 3.4 provides an overall comparison and discussion concerning all monitoring data generated during this thesis, including the results of method application described in Papers 1 and in Paper 3 (Chapter 2).

3.3 Results

3.3.1 Paper 4: “Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK)”

Joyce Cristale, Athanasios Katsoyiannis, Andrew J. Sweetman, Kevin C. Jones,
Silvia Lacorte

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Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK)

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ABSTRACT

This study presents the occurrence and risk of PBDEs, new brominated and organophosphorus flame retardants along a river affected by urban and industrial pressures (River Aire, UK). Tris(2-choroethyl) phosphate (TCEP), tris(2-chloro-1-methylethyl) phosphate (TCPP), tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP) and triphenyl phosphate (TPhP) were detected in all samples, with TCPP present at the highest concentrations, ranging from 113 to 26 050 ng L^{-1}. BDE-209 was detected in most of the sampled sites, ranging from 17 to 295 ng L^{-1}, while hexabromobenzene (HBB) and pentabromoethyl benzene (PBEB) were seldom detected. A risk quotients based on predicted no effect concentrations (PNEC) and flame retardants water concentration proved significant risk for adverse effects for algae, *Daphnia* and fish in sites close to industrial and urban sewage discharges. This study provides a protocol for the risk estimation of priority and new generation flame retardants based on river concentrations and toxicological values.

1. Introduction

The term new brominated flame retardants (NBFRs) refers to compounds which are new in the market, have only recently been detected in the environment, or are brominated flame retardants other than polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) or tetrabromobisphenol A (TBBPA). NBFRs include hexabromobenzene (HBB), pentabromoethyl benzene (PBEB), pentabromotoluene (PBT), 2,3-dibromopropyl 2,4,6 tribromophenyl ether (DPTE), 2-ethylhexyl 2,3,4,5 tetrabromobenzoate (EHTBB), bis(2-ethyl-1-hexyl)tetrabromophthalate (BEHTBP), 1,2 bis (2,4,6 tribromophenoxy)ethane (BTBPE), and decabromodiphenyl ethane (DBDPE), among others, and have been used in different types of polymers, plastics, resins and textiles (Covaci et al., 2011). Organophosphorus flame retardants (OPFRs), instead, is a family of chemicals that became a re-emerging environmental issue, after PBDEs were banned in Europe (Directive 2003/11/EC; European Court of Justice, 2008). Tris(2-choroethyl) phosphate (TCEP), tris(2-chloro-1-methylethyl) phosphate (TCPP), tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP) and triphenyl phosphate (TPhP) are used in flexible and rigid polyurethane foams, plastics, and textiles (van der Veen and de Boer, 2012).

The annual global consumption of flame retardants is estimated to be around two million tons a year, among which 35% are brominated and organophosphorus flame retardants (Flame retardants-online, 2012). These compounds are released from materials (Kemmlein et al., 2003) and reach surface water from industrial and wastewater treatment plants (WWTP) discharges (Anderson and MacRae, 2006; Meyer and Bester, 2004), and from atmospheric depositions (Möller et al., 2011b), and are found in water at levels of ng to μg L^{-1} (Arp et al., 2011; Labadie et al., 2010; Wang et al., 2011).

The occurrence and fate of flame retardants in aquatic environments is of concern and a prioritized issue according to EU Water Framework Directive (Directive 2008/105/EC). PBDEs are endocrine disrupters, neurotoxic, bioaccumulative and exhibit potential for biomagnification (Vonderheide et al., 2008). BTBPE, EHTBB, PBT, PBEB and HBB have high accumulation and biomagnifications factors (Wu et al., 2011). Among OPFRs, TCPP accumulates in the liver and kidneys and is considered to be potentially carcinogenic; TCEP is carcinogenic, neurotoxic and may cause chronic adverse effects to aquatic organisms; TDCP is indicated as carcinogenic, is neurotoxic and exhibits higher acute toxicity for aquatic organisms than TCEP and TCPP; TPhP is very toxic to aquatic ecosystems and bioaccumulative (van der Veen and de Boer, 2012). Thus, taking into account that many flame retardants are high-production volume chemicals, the limited

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available data on the occurrence of PBDEs, NBFRs, and OPFRs in water and their toxicity and bioaccumulation properties, more studies are considered necessary in order for the scientific community to gain insight about their presence, behavior and fate in fresh waters. The aim of this study was to determine the occurrence of PBDEs, NBFRs and OPFRs in the River Aire (UK), a river historically impacted by chemical pollution and that can represent a case study where management actions can be put into place to improve water quality. Specifically, the study provides (i) a monitoring protocol that covers the whole river basin and takes into account the main drivers of environmental pollution (urban, industrial and WWTP discharges); (ii) a database to be used to define most ubiquitous compounds, their geographical distribution, transport along the river and identifies hot spot areas and (iii) risk data to be used to determine the impact of chemicals in the specific study area. This integrated protocol that combines a comprehensive sampling, chemical analysis and risk assessment can be used as a toolbox which may contribute to prioritize emerging contaminants and will serve in water management issues.

2. Methods

2.1. Sampling

In this study, the River Aire was chosen as a model of rivers that are high affected by anthropogenic pressures. Around twenty years ago, this river was considered dead since no fish could survive in the areas where local raw sewage outflows were discharged (Information Britain, 2012). The River Aire is a major river in Yorkshire, UK, running 114 Km through one of the most populated areas in UK, West Yorkshire (2 079 211 inhabitants). Aire flows through cities such as Leeds (443 247 inhabitants), Keighley (49 453 inhabitants), Shipley (28 162 inhabitants), Bingley (19 884 inhabitants) and Skipton (14 313 inhabitants). In Castleford (37 525 inhabitants) there is the confluence of the rivers Aire and Calder. The mouth of the Aire is the River Ouse in Airmyn (East Riding of Yorkshire), with an average discharge flow of 35.7 $m^3\ s^{-1}$.

All bottles used for sampling were previously washed and rinsed with Milli-Q water, acetone and hexane. Two and a half liters of river water were collected from each of the 13 points along the River Aire in spring 2011. The sampling points included a site near the river source, and after WWTPs discharges, covering a stretch of >100 km, from Malhan to Eggborough. Fig. 1 presents the sampling points along the River Aire; the outputs from wastewater treatment plants are indicated.

2.2. Chemicals

A solution mixture of BDEs 28, 47, 99, 100, 153, 154, 183 and 209 in n-nonane at 1 $\mu g\ mL^{-1}$ was acquired from Cambridge Isotope Laboratories (Andover, USA). Individual solution of BTBPE (at 50 $\mu g\ mL^{-1}$ in 5% toluene, 95% nonane), DBDPE (at 25 $\mu g\ mL^{-1}$ in toluene), BEHTBP, PBT, DPTE and EHTBB (at 50 $\mu g\ mL^{-1}$ in toluene) were acquired from Wellington Laboratories (Guelph, Canada). Pure standards of HBB, PBEB, TCEP, TCPP, TDCP and TPhP were acquired from Sigma Aldrich (Germany). TCPP was acquired as a mixture of isomers (tris(2-chloro-1-methylethyl) phosphate, bis(1-chloro-2-propyl)-2-chloropropyl phosphate, and bis (2-chloropropyl)-1-chloro-2-propyl phosphate). HBB, PBEB and TPhP were weighed and dissolved in hexane, while TCEP, TCPP and TDCP were dissolved in a mixture of ethyl acetate and methanol (1:1 v/v). The surrogates [$^{13}C_6$]hexabromobenzene (MHBB) at 50 $\mu g\ mL^{-1}$ in toluene, 3,3′,4,4′-tetrabromo[$^{13}C_{12}$]diphenyl ether (MBDE-77) at 50 $\mu g\ mL^{-1}$ in nonane and decabromo[$^{13}C_{12}$]diphenyl ether (MBDE-209) at 25 $\mu g\ mL^{-1}$ in toluene were acquired from Wellington Laboratories (Guelph, Canada). The internal standards 2, 3, 5, 6-tetrachlorobiphenyl (PCB-65) and decachlorobiphenyl (PCB-209), both at 10 $\mu g\ mL^{-1}$ in iso-octane, were acquired from Dr. Ehrenstorfer (Augsburg, Germany).

Calibration curves were performed at nine levels, ranging from 0.025 to 2.5 $ng\ \mu L^{-1}$ for OPFRs, DBDPE and BDE-209, and ranging from 0.0025 to 0.25 $ng\ \mu L^{-1}$ for PBDEs and NBFRs, in hexane. When all the nine calibration levels do not allowed an acceptable linearity ($r^2 > 0.99$), more than one calibration curve, with at least 5 levels, were used. Each calibration level contained the surrogates MHBB and MBDE-77 at 0.025 $ng\ \mu L^{-1}$, MBDE-209 at 0.250 $ng\ \mu L^{-1}$, and the internal standards PCB-65 and PCB-209 at 0.05 $ng\ \mu L^{-1}$. When necessary, sample extracts were diluted to allow their quantification.

2.3. Extraction and analysis

Five hundred milliliters of unfiltered water sample was spiked with labeled surrogate standards (MHBB, MBDE 77 at 5 $ng\ L^{-1}$, and MBDE 209 at 50 $ng\ L^{-1}$) and extracted using OASIS HLB 200 mg cartridges (WATERS, USA). The cartridges were conditioned with 15 mL of hexane followed by 15 mL of dichloromethane, 15 mL of methanol and 15 mL of Milli-Q water. After the preconcentration step, the cartridges were dried using a vacuum manifold, eluted with 15 mL of dichloromethane/hexane (1:1 v/v) and dichloromethane/acetone (1:1 v/v), concentrated under N_2 flow to almost dryness and reconstituted in 100 μL of hexane with the internal standard PCB-65 and PCB-209 at 0.05 $ng\ \mu L^{-1}$. To minimize background contamination, vials and pipettes were baked at 380 °C, overnight.

Five hundred milliliters of spiked Milli-Q and river water samples were used for method validation. For this purpose, two litters of water were collected at the River Aire (P1). The spiking was performed at two levels, in triplicate. The NBFRs and PBDEs were spiked at 1 and 10 $ng\ L^{-1}$ (except for BDE 209 and DBDPE that were spiked at 10 and 100 $ng\ L^{-1}$). The OPFRs were spiked at 100 and 1000 $ng\ L^{-1}$. Five

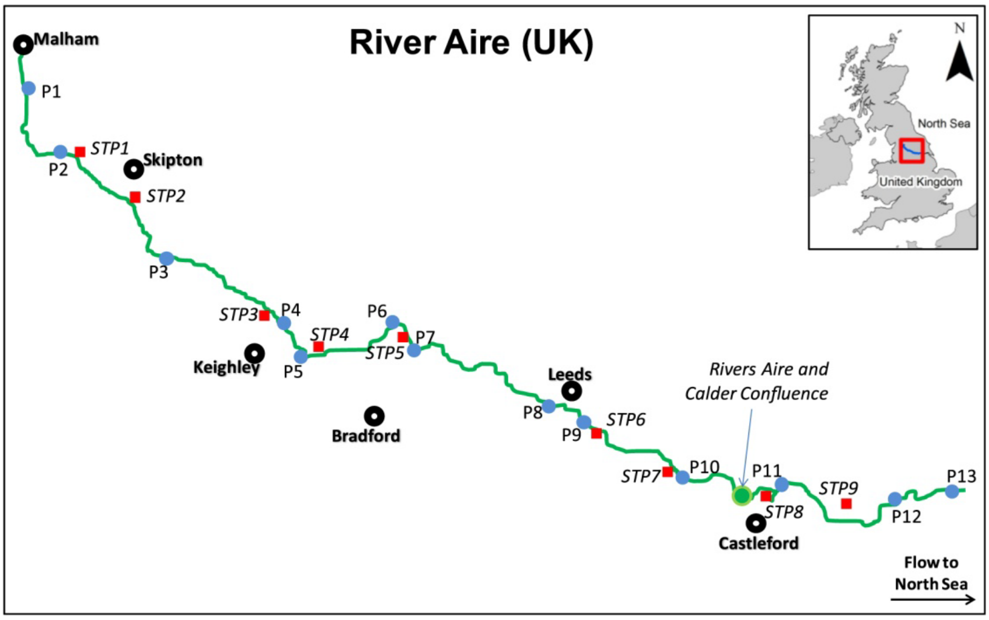

Fig. 1. Sampled points (P1 to P13) and sewage treatment plants outputs (STP) along the River Aire (UK).

procedural blanks, prepared with 500 mL of Milli-Q water, were extracted and analyzed together with the samples. The limits of detection of the method were calculated as 3 times the signal/noise ratio obtained by the lowest spiked level (river water). For the compounds detected in the procedural blanks (TCPP and TPhP), the limits of detection were calculated from mean blank values plus three times the standard deviation.

Analysis was performed in a gas chromatograph coupled to a quadrupole mass spectrometer, in electron ionization (EI) mode, using a Trace GC–MS (Thermo-Finnigan) with a ThermoQuest AS2000 autosampler. The column used was a DB-5MS with 15 m (length) × 0.250 mm (I.D.) × 0.10 μm (film) (J&W Scientific, USA). Helium (purity 99.999%) was employed as carrier gas with a constant flow of 1.5 mL min^{-1}. The standards were injected (2 μL) into the GC system in splitless mode, with a splitless time of 1.5 min. The injector, quadrupole, transfer line and ion source were set at 270, 150, 300 and 275 °C, respectively. Electron ionization (EI) mode was set at 70 eV. Emission current was set at 300 μA. The oven program was set at 60–220 °C at 10 °C/min and to 315 °C at 8 °C/min (5 min). Acquisition was performed by Selected Ion Monitoring (SIM) and the monitored ions were the ones described by Cristale et al. (2012).

2.4. *Risk assessment*

The risk evaluation for water organisms was performed based on the concentrations of the detected flame retardants in water. In this study, the risk quotient (RQ) was assessed on non target organisms, as described in previous studies (Santos et al., 2007; Sánchez-Avila et al., 2012). The RQs were calculated as a quotient of the measured environmental concentration (MEC) and the predicted no effect concentration (PNEC). PNEC was estimated as a quotient of toxicological relevant concentration and a security factor (*f*). For this purpose, the LC_{50} or EC_{50} for fish, *Daphnia* and algae associated to TCEP, TCPP, TDCP, TPhP and BDE-209 was used for RQ calculation. RQ was calculated according to:

$$RQ = \frac{MEC}{PNEC} = \frac{MEC}{\left(\frac{L(E)C_{50}}{f}\right)}$$

For data interpretation, the maximum probable risk for ecological effects from contaminated water was followed as recommended by Wentsel et al. (1996):

$RQ < 1.0$ (i.e., the exposure point concentration is less than the risk screening benchmark) indicates no significant risk;

$1.0 \leq RQ < 10$ (i.e., the exposure point concentration is between one and ten times the risk screening benchmark) indicates a small potential for adverse effects;

$10 \leq RQ < 100$ (i.e., the exposure point concentration is between ten to one hundred times the risk screening benchmark) indicates significant potential for adverse effects;

$RQ \geq 100$ (i.e., the exposure point concentration is equal to or greater than one hundred times the risk screening benchmark) indicates that potential adverse effects should be expected.

3. Results

3.1. *Method performance*

Recovery study was performed using spiked Milli-Q and river water samples. OASIS HLB SPE cartridges were efficient for the pre-concentration of polar (OPFRs) and apolar (NBFRs and PBDEs) flame retardants for a water volume of 500 mL. The matrix effect was evaluated using spiked river water and the recoveries were comparable to the ones obtained using Milli-Q spiked water. The recoveries for spiked Milli-Q and river water samples were satisfactory, ranging from 70 to 120% with standard deviation lower than 20, in most of cases. TCPP and TPhP were detected in procedural blanks at 15.5 ± 1.3 ng L^{-1} and 3.5 ± 0.3 ng L^{-1}, respectively, and these concentrations were subtracted from the actual samples concentration. The limits of detection (LOD) ranged from 0.18 to 19 ng L^{-1} for OPFRs, from 0.08 to 104 ng L^{-1} for NBFRs and from 0.14 to 2.5 ng L^{-1} for PBDEs. Table 1 presents the recoveries and limits of detection for each compound.

To evaluate the extraction efficiency of waters from River Aire, the recoveries of the surrogates were determined in each water sample. Surrogate recoveries were satisfactory, with average recoveries of 92 ± 23% for MHBB, 105 ± 21% MBDE 77 and 60 ± 20% for MBDE 209. Since the water samples were not filtered, probably the variability of surrogate recoveries, especially observed for MBDE-209, was a result of differences in particulate matter concentrations among the water samples. Internal standard quantification using labeled surrogates was performed for the brominated compounds, and so their concentrations were corrected by the surrogate recoveries.

3.2. *Organophosphorus flame retardants in River Aire*

OPFRs were detected in all sites with water concentrations decreasing as follows: TCPP > TCEP > TDCP > TPhP. Fig. 2 presents the evolution of OPFRs along the River Aire. TCPP was the only contaminant detected near the river source (126 ng L^{-1} (average value, $n = 2$) at P1 and 167 ng L^{-1} at P2). The highest TCPP

Table 1
Recoveries and standard deviation (SD) of organophosphorus and brominated flame retardants in spiked Milli-Q and river water samples.

Compound	Spiked Milli-Q water				Spiked river water				LOD (ng L^{-1})
	Level 1		Level 2		Level 1		Level 2		
	Recovery (%)	SD	Recovery (%)	SD	Recovery (%)	SD	Recovery (%)	SD	
TCEP	99	11	120	5	113	3	115	15	2.4
TCPP	90	12	96	6	114	3	113	10	19
PBT	81	4	87	3	86	3	85	4	0.08
BDE-28	124	21	98	23	65	7	89	5	0.88
PBEB	67	10	78	6	74	4	83	10	0.15
TDCP	92	9	87	9	97	5	73	5	0.18
DPTE	119	6	[a]	[a]	119	6	[a]	[a]	1.2
HBB	84	0.04	124	1	92	2	116	7	0.11
TPhP	86	13	83	7	89	3	65	5	4.5
BDE-47	103	6	119	10	65	1	112	3	0.14
BDE-100	76	2	70	20	49	1	91	7	0.5
BDE-99	80	2	106	7	44	1	102	13	0.92
EHTBB	48	11	57	7	67	5	79	21	1.3
BDE-154	56	3	67	5	45	1	89	11	0.48
BDE-153	59	3	109	14	50	1	80	13	0.9
BDE-183	43	9	39	11	37	1	70	8	0.18
BTBPE	45	6	69	9	83	12	92	6	1
BEHTBP	43	8	[a]	[a]	95	14	[a]	[a]	7.9
BDE-209	128	3	123	12	70	14	106	16	2.5
DBDPE	91	5	87	3	100	20	[a]	[a]	104

Level 1: 10 ng L^{-1} for all compounds, except for BDE-209, DBDPE that were at 100 ng L^{-1}, and TCEP, TCPP, TDCP, and TPhP that were at 1000 ng L^{-1}.
Level 2: 1 ng L^{-1} for all compounds, except for BDE-209, DBDPE that were at 10 ng L^{-1}, TCEP, TCPP, TDCP, and TPhP that were at 100 ng L^{-1}.
[a] Not detected.

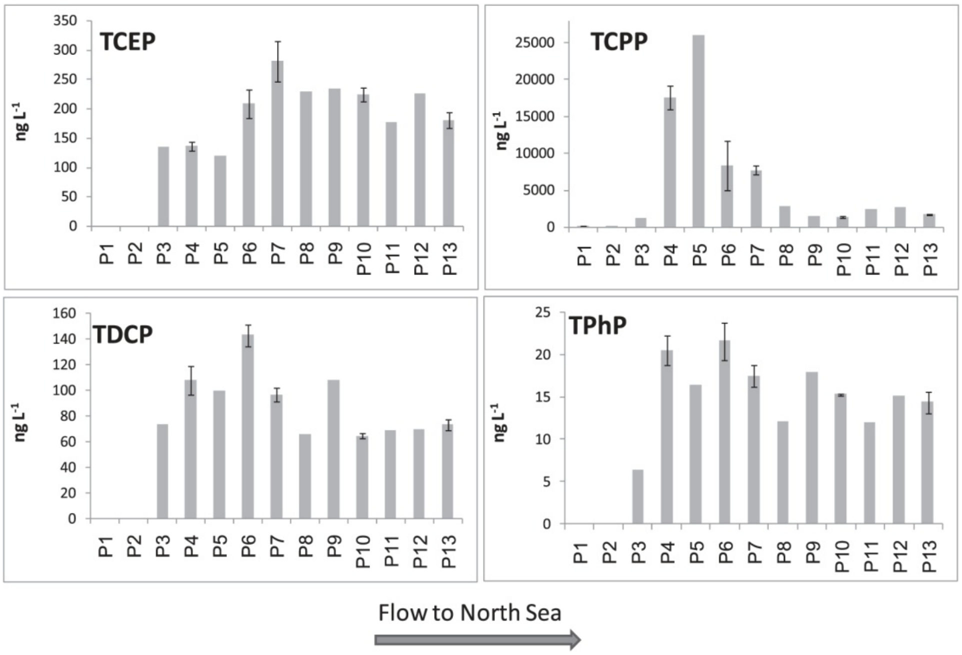

Fig. 2. Occurrence of OPFRs along the river Aire (UK). The samples P1, P4, P6, P7, P10 and P13 were collected in duplicate, and the bar indicates the maximum and minimum measured value.

concentration was measured at sites 4 and 5, and then showed decreasing concentrations along the river. TCPP concentrations ranged from 113 to 26 050 ng L^{-1} (average 6040 ng L^{-1}). A different behavior was observed for the other flame retardants. No big variations in terms of concentration along all sampling points was observed for TCEP, TDCP and TPhP, ranging from 119 to 316 ng L^{-1}, 62 to 149 ng L^{-1} and 6.3 to 22 ng L^{-1}, respectively.

The levels of TCEP, TDCP and TPhP in River Aire water reflect the widespread distribution of this family of contaminants in rivers impacted by anthropogenic pressures, as previously reported for other sites. In Spain, TCEP and TDCP average concentrations in river water were 85 ± 10 ng L^{-1} and 70 ± 10 ng L^{-1}, respectively (García-López et al., 2010). In the Austrian rivers Danube, Schwechat and Liesig, water concentrations ranged from 13 to 130 ng L^{-1}, from <MQL (lower than method quantification limit) to 19 ng L^{-1} and from <MQL to 10 ng L^{-1} for TCEP, TDCP and TPhP, respectively (Martínez-Carballo et al., 2007), attributed to WWTPs discharges. In River Ruhr (Germany), TCEP concentrations ranged from 13 to 130 ng L^{-1}, 50 ng L^{-1} for TDCP, and up to 40 ng L^{-1} for TPhP (Andresen et al., 2004). To the best of our knowledge, the River Aire TCPP levels are the highest ever reported in surface waters and wastewaters. In studies from various countries (Spain, Germany, China), the observed TCPP river water concentration never exceeded 430 ng L^{-1} (García-López et al., 2010; Andresen et al., 2004; Wang et al., 2011). In Spanish wastewater samples (both raw and treated), TCPP concentrations ranged from 290 to 680 ng L^{-1} (García-López et al., 2010), while in effluents from 16 Austrian municipal WWTPs, TCPP concentrations ranged from 270 to 1400 ng L^{-1} (Martínez-Carballo et al., 2007). The mean TCPP concentration in Swedish and Germany WWTP's effluents were 4400 and 3000 ng L^{-1}, respectively (Marklund et al., 2005; Meyer and Bester, 2004). In our study, the TCPP concentrations from the most contaminated river points (P4 and P5) were 17 640 and 26 050 ng L^{-1}, respectively, being four to six times higher than WWTPs reported values, and forty to sixty times higher than river water concentrations reported in literature. The higher concentration of TCPP in comparison with TCEP can be explained by the industrial replacement of TCEP by TCPP in Europe (Quednow and Püttmann, 2009). TCPP consumption in the year 2000 by UK industries accounted for 4800 tons, being 71% of this used in flexible foam (European Commission, 2007).

The high concentration of OPFRs in River Aire point to treated or untreated sewage discharges. Several studies indicate WWTPs as an important source of OPFRs in water (Andresen et al., 2004; Martínez-Carballo et al., 2007). TCEP, TCPP and TDCP are not sufficiently eliminated in WWTPs, while TPhP has an elimination rate of around 60% (Marklund et al., 2005). In this study, WWTPs located in more populated areas, from Keighley to Leeds, are likely the most important sources of OPFRs, exhibiting their highest concentrations between P4 and P9. On the other hand, TCPP presented high levels at P4 and P5, indicating a local source. TCPP is not easily biodegradable and has a low octanol–water coefficient ($\log K_{ow} = 2.59$) (Reemtsma et al., 2008), thus probably the decrease of concentration observed between P4 and P8 is due to the dilution that occurs during the advection of the contaminant along the river. Between P9 and P13, the profile of TCPP is similar to the others OPFRs, indicating WWTPs as the dominant source of this compound. The detection of TCPP in P1 and P2 (low density population) suggests that atmospheric deposition may also be a secondary source of OPFRs in river waters. The latter has been pointed in a number of studies as the only mechanism responsible for the detection of OPFRs in air from very remote areas, like in the North Sea (Möller et al., 2011a), in lake waters from German remote areas (Regnery and Püttmann, 2010) and soil samples (Mihajlović et al., 2011).

3.3. Brominated flame retardants in River Aire

BDE-209 was the only PBDE detected along the River Aire (Fig. 3). BDE-209 concentrations in water (dissolved + particulate

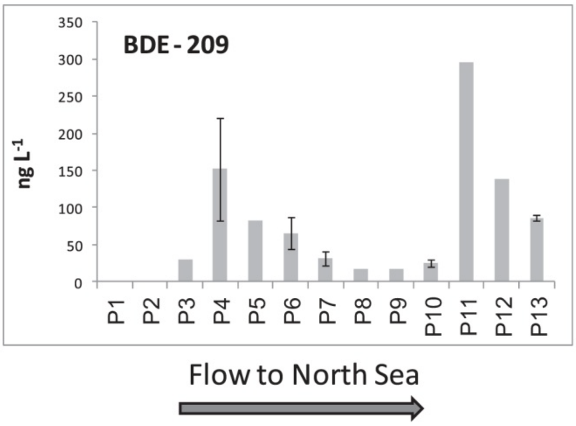

Fig. 3. Occurrence of BDE-209 along the river Aire (UK). The samples P1, P4, P6, P7, P10 and P13 were collected in duplicate, and the bar indicates the maximum and minimum measured value.

phase) ranged from 17 to 295 ng L^{-1}. These results are ten to one hundred times higher than BDE-209 levels in River Seine (Paris, France), where BDE-209 concentrations in water (dissolved + particulate phase) ranged from 2.1 to 2.5 ng L^{-1} (Labadie et al., 2010). The highest levels of BDE-209 were observed at high population density sites (P4 to P6) and at the sites after the Calder and Aire confluence (P11 to P13). Since the Calder River flows through high industrial and populated areas such as Dewsbury (54 341 inhabitants), Wakefield (76 886 inhabitants) and also has the influence of Huddersfield (146 234 inhabitants) by its tributary River Colne, the increase of BDE-209 concentrations after Aire and Calder confluence suggests that the River Calder was the responsible for the high levels observed at the points 11 to 13.

High BDE-209 concentrations near urban centers indicate human activities as main PBDE sources in air, water, sediment and soil (Vonderheide et al., 2008). An international survey of DBDPE and BDE-209 in sewage sludge samples found the highest levels of BDE-209 in UK and USA samples (19 000 ng g^{-1} and 12 000 ng g^{-1}, respectively) (Ricklund et al., 2008). Even though PBDE concentrations in effluents from WWTPs are low, due to accumulation in sludge, the high water volumes released from these facilities can be an important PBDE source to receiving waters (Hale et al., 2006). BDE-209 was the dominant PBDE congener in UK sediment core, with reported concentrations from 2.3 to 98 125 ng g^{-1} dry weight (Webster et al., 2008). BDE-209 is also reported as the dominant PBDE congener in UK dust samples, with concentrations ranging from 120 to 520 000 ng g^{-1} (Harrad et al., 2008). The dominance of BDE-209 in UK dust, sediment and in the water samples indicates a widespread use of Deca-BDE formulations. On the other hand, non detection of other PBDEs in River Aire is in accordance with Birgul et al. (2012) that observed a declining in PBDEs (BDE-209 not studied) air concentrations over the last years in UK.

PBEB and HBB were seldom detected, and concentrations ranged from 0.16 to 0.40 ng L^{-1} ($n = 5$; P1, P6, P7, P8 and P9) and 0.76 ng L^{-1} ($n = 1$; P5), respectively. These values are comparable to the ones reported by Arp et al. (2011) for water samples from wastewater facilities in Norway. HBB wastewater concentrations ranged from 0.40 to 1.82 ng L^{-1}, while PBEB was not detected in wastewater samples but was detected in seepage water from a metal recycling factory at 0.94 ng L^{-1}.

3.4. *Risk assessment*

The risk that flame retardants can pose to aquatic environment is still unknown. Continuous inputs and intrinsic toxicity are two main parameters that influence their effects towards the ecosystem. As discussed earlier, flame retardants have been, or are being widely used and released from various activities carried out in UK. The risk assessment to aquatic organisms was estimated, taking into account the water concentration of the most detected flame retardants (TCEP, TCPP, TDCP, TPhP and BDE-209) along the River Aire. The risk assessment was performed considering $L(E)C_{50}$ of fish, *Daphnia* and algae, following the recommendation of the Technical Guidance Document on Risk Assessment (European Commission, 2003), that requires at least three trophic levels from the assessed environment. For OPFRs, the toxicity data were the ones selected from the Environmental Risk Limits report for organophosphorus flame retardants (Verbruggen et al., 2005). For BDE-209, the toxicity data were those reported by the Ministry of the Environment of Japan for an initial assessment of ecological risk (MOE, 2012). For RQ calculation, the lowest $L(E)C_{50}$ and a factor of 1000 was used (European Commission, 2003). Since in this study, the water samples were not filtered, for the RQ calculation it was assumed that the detected compounds in water (dissolved + particulate phases) are 100% bioavailable to aquatic organisms. Table 2 presents the toxicological relevant concentrations (LC_{50} or EC_{50}) used for RQ calculations. Tables S1–3 (Supplementary material) present the RQs results for each compound and sampling point. No significant risk ($RQ < 1$) was observed along the River Aire associated to TCEP, TCPP, TDCP or TPhP for any of the three organisms. RQs for TCEP, TCPP, TDCP and TPhP varied from 0.0013 to 0.87 for fish, from 0.00036 to 0.29 for *Daphnia*, and from 0.0013 to 0.58 for algae, respectively. On the other hand, low ($1.0 \leq RQ < 10$) to significant ($10 \leq RQ < 100$) potential for adverse effects associated to BDE-209 were observed along the river, with RQs from 3.8 to 65 for fish, from 3.6 to 62 for *Daphnia*, and from 3.3 to 57 for algae, respectively.

To estimate the joint effect for these flame retardants along the River Aire, a sum of RQs of each detected compound was performed

Table 2
Acute toxicity (LC_{50} or EC_{50}) used for the risk assessment for Fish, *daphnia* and Algae.

Compound	Fish			*Daphnia*			Algae		
	$L(E)C_{50}$ (mg L^{-1})	Species	Ref.	$L(E)C_{50}$ (mg L^{-1})	Species	Ref.	$L(E)C_{50}$ (mg L^{-1})	Species	Ref.
TCEP	90	*Carassius auratus*	(Verbruggen et al., 2005)	330	*Daphnia magna*	(Verbruggen et al., 2005)	51	*Scenedesmus subspicatus*	(Verbruggen et al., 2005)
TCPP	30	*Poecilia reticulata*	(Verbruggen et al., 2005)	91	*Daphnia magna*	(Verbruggen et al., 2005)	45	*Scenedesmus subspicatus*	(Verbruggen et al., 2005)
TDCP	1.2	*Oncorhynchus mykiss*	(Verbruggen et al., 2005)	4.2	*Daphnia magna*	(Verbruggen et al., 2005)	39	*Pseudokirchneriella subcapitata*	(Verbruggen et al., 2005)
TPhP	0.42	*Oncorhynchus mykiss*	(Verbruggen et al., 2005)	1.1	*Daphnia magna*	(Verbruggen et al., 2005)	0.5	*Scenedesmus quadricauda*	(Verbruggen et al., 2005)
BDE-209	0.00455	*Oryzias latipes*	(MOE, 2012)	0.00479	*Daphnia magna*	(MOE, 2012)	0.00520	*Pseudokirchneriella subcapitata*	(MOE, 2012)

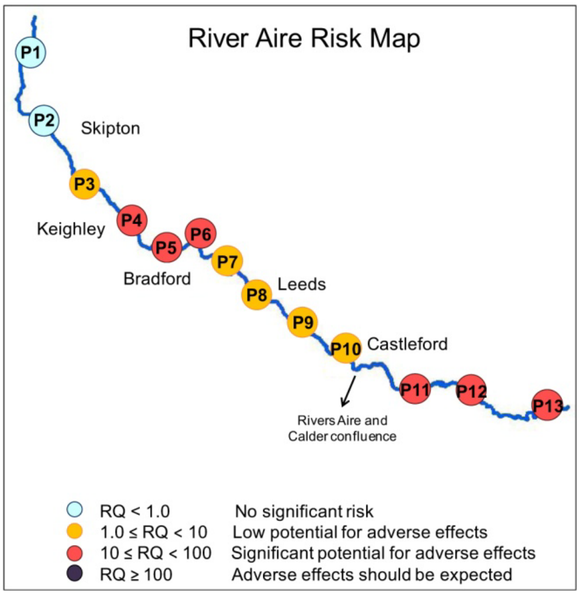

Fig. 4. Risk quotients estimated for fish, *Daphnia* and algae along the River Aire (UK).

for each sampling point (Santos et al., 2007; Sánchez-Avila et al., 2012). Fig. 4 presents the risk assessment for algae, *Daphnia* and fish at each sampling point. Since the sampling points that presented no significant, low or significant potential for adverse effects were the same for these three aquatic organisms, the same risk map was used to illustrate all these results. For the sampling points 1 and 2, located near the river source, no significant potential for adverse effects were observed. The points 3 to 13 presented low or significant risk for adverse effects, while significant risk was observed at the sampling points situated near Keighley, Bradford and Leeds (P4 to P6), and at the points situated after the Calder and Aire confluence (P11 to P13). Since BDE-209 presented RQ values about 100 to 10 000 times higher than the ones obtained for OPFRs, evidently the potential for adverse effects is attributed almost exclusively to the presence of BDE-209. No studies are available concerning the synergistic effect of BDE-209 and OPFRs for aquatic organisms.

Another important issue concerning BDE-209 in the aquatic environment is its bioavailability to organisms. BDE-209 is considered a low-bioavailable compound because its large molecule size is impeding its transport through the pores and cell membranes, and because its strong affinity to organic carbon makes it difficult to be displaced from the associated particles to cell membranes of living organisms (New York, 2011). However, many studies report BDE-209 accumulation in aquatic organisms at levels from ng g^{-1} to μg g^{-1} of lipid (Kuo et al., 2010), and BDE-209 biomagnification was observed in some ecosystems (Law et al., 2006). In this study, BDE-209 was detected in water in 11 of the 13 sampled points from River Aire, indicating that BDE-209 is transported along the river, being feasible its bioaccumulation and biomagnification in aquatic organisms.

In this study, the risk assessment indicated no risk for potential effects to aquatic organisms associated to the TCEP, TCPP, TDCP and TPhP concentration along the River Aire, considering short-term exposure. On the other hand, OPFRs are constant pollutants in River Aire. A previous study reported the integrated concentration of OPFRs in the River Aire using a passive sampling device (Cristale et al., 2013). For a three week sampled period, the derived concentrations of TCEP, TCPP, TDCP and TPhP were 181 ng L^{-1}, 4821 ng L^{-1}, 49 ng L^{-1}, and 17 ng L^{-1}, respectively. These concentrations are comparable to the present results, indicating that OPFRs occur in River Aire at constant concentrations. In addition, in agreement with this study, mixtures of OPFRs in surface waters are frequently reported, while TCEP and TCPP, together with other OPFRs such as tributyl phosphate and tris(2-butoxyethyl) phosphate, are the most abundant OPFRs in river water (García-López et al., 2010; Martínez-Carballo et al., 2007; Andresen et al., 2004). Thus, for a more reliable risk assessment, more short-term and long-term toxicological data are needed concerning the synergistic effect of OPFRs mixtures in water, at relevant environmental conditions.

Some studies have indicated that organophosphorus flame retardants can be accumulated in biota (Sundkvist et al., 2010; Kim et al., 2011). Sundkvist et al. (2010) measured OPFRs in herring, perch, mussels, eelpout and salmon, and found that TCPP and TPhP were present at the highest levels, ranging from 170 to 770 ng g^{-1} and from 21 to 180 ng g^{-1}, respectively. These results indicate that, despite TCPP presents a $\log K_{ow}$ of 2.59, its continuous input in the environment can promote its bioaccumulation in biota. Therefore, more studies are needed concerning bioaccumulation and biomagnification of OPFR in biota for a better risk evaluation of these chemicals in the water environment.

4. Conclusions

This study presented the occurrence of priority and emerging flame retardants in a river high affected by urban and industrial pressures. BDE-209 and organophosphorus flame retardants were detected along the River Aire (UK) at high concentrations, reaching 26 050 ng L^{-1} for TCPP and 295 ng L^{-1} for BDE-209. The highest concentration of these contaminants was observed near high populated areas and wastewaters discharges which were identified as an important source of these contaminants. A risk assessment was performed based on water concentration measurements and acute toxicity data. A significative risk for algae, *Daphnia* and fish was estimated for several points along the River and risk was only observed for BDE-209. However, long term and bioaccumulation studies for these pollutants in the water environment are needed to define the environmental stress produced by the high concentration of flame retardants.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.04.001.

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Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK)

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Supplementary data

Table S1 – Fish Risk Quotinent (RQ) results for TCEP, TCPP, TDCP, BDE-209, and the Sum of RQs for each sampled site in River

Puntos	TCEP	TCPP	TDCP	TPhP	BDE-209	Sum
P1		0.004				0.0042
P2		0.006				0.0056
P3	0.0015	0.042	0.061	0.015	6.5	6.7
P4	0.0015	0.59	0.090	0.049	33	34
P5	0.0013	0.87	0.083	0.039	18	19
P6	0.0023	0.28	0.12	0.051	14	15
P7	0.0031	0.26	0.080	0.042	7.0	7.4
P8	0.0025	0.094	0.054	0.029	3.8	4.0
P9	0.0026	0.050	0.090	0.043	3.9	4.1
P10	0.0025	0.046	0.053	0.036	5.5	5.6
P11	0.0020	0.082	0.057	0.028	65	65
P12	0.0025	0.088	0.058	0.036	30	30
P13	0.0020	0.057	0.061	0.034	19	19

Table S2 – *Daphnia* Risk Quotinent (RQ) results for TCEP, TCPP, TDCP, BDE-209, and the Sum of RQs for each sampled site in River.

Puntos	**TCEP**	**TCPP**	**TDCP**	**TPhP**	**BDE-209**	**Sum**
P1		0.0014				0.0014
P2		0.0018				0.0018
P3	0.00041	0.014	0.017	0.006	6.2	6.3
P4	0.00041	0.19	0.026	0.019	32	32
P5	0.00036	0.29	0.024	0.015	17	18
P6	0.00063	0.092	0.034	0.020	14	14
P7	0.00085	0.085	0.023	0.016	6.6	6.8
P8	0.00069	0.031	0.016	0.011	3.6	3.7
P9	0.00071	0.016	0.026	0.016	3.7	3.9
P10	0.00068	0.015	0.015	0.014	5.2	5.3
P11	0.00053	0.027	0.016	0.011	62	62
P12	0.00068	0.029	0.016	0.014	29	29
P13	0.00055	0.019	0.017	0.013	18	18

Table S3 – Algae Risk Quotinent (RQ) results for TCEP, TCPP, TDCP, BDE-209, and the Sum of RQs for each sampled site in River.

Puntos	**TCEP**	**TCPP**	**TDCP**	**TPhP**	**BDE-209**	**Sum**
P1		0.0028				0.0028
P2		0.0037				0.0037
P3	0.0026	0.028	0.0019	0.0013	5.7	5.8
P4	0.0027	0.39	0.0028	0.0041	29	30
P5	0.0023	0.58	0.0025	0.0033	16	16
P6	0.0041	0.19	0.0037	0.0043	13	13
P7	0.0055	0.17	0.0025	0.0035	6.1	6.3
P8	0.0045	0.062	0.0017	0.0024	3.3	3.4
P9	0.0046	0.033	0.0028	0.0036	3.4	3.5
P10	0.0044	0.031	0.0016	0.0031	4.8	4.8
P11	0.0034	0.054	0.0018	0.0024	57	57
P12	0.0044	0.059	0.0018	0.0030	26	26
P13	0.0035	0.038	0.0019	0.0029	17	17

3.3.2 Paper 5: "Priority and emerging flame retardants in rivers: Occurrence in water and sediment, *Daphnia magna* toxicity and risk assessment"

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Priority and emerging flame retardants in rivers: Occurrence in water and sediment, *Daphnia magna* toxicity and risk assessment

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The occurrence, partitioning and risk of eight polybrominated diphenyl ethers (PBDEs), nine new brominated (NBFRs) and ten organophosphorus flame retardants (OPFRs) were evaluated in three Spanish rivers suffering different anthropogenic pressures (Nalón, Arga and Besòs). OPFRs were ubiquitous contaminants in water (ΣOPFRs ranging from 0.0076 to 7.2 μg L^{-1}) and sediments (ΣOPFRs ranging 3.8 to 824 μg kg^{-1}). Brominated flame retardants were not detected in waters, whereas ΣPBDEs ranged from 88 to 812 μg kg^{-1} and decabromodiphenyl ethane (DBDPE) reached 435 μg kg^{-1} in sediments from the River Besòs, the most impacted river. The occurrence of flame retardants in river water and sediment was clearly associated with human activities, since the highest levels occurred near urban and industrial zones and after wastewater treatment plants discharge. *Daphnia magna* toxicity was carried out for OPFRs, the most ubiquitous flame retardants, considering individual compounds and mixtures. Toxicity of nine tested OPFRs differed largely among compounds, with EC_{50} values ranging over three magnitude orders (0.31–381 mg L^{-1}). Results evidenced that these compounds act by non-polar narcosis, since their toxicity was proportional to their lipophilicity (K_{ow}). Furthermore, their joint toxicity was additive, which means that single and joint toxicity can be predicted knowing their concentration levels in water using quantitative structure activity relationships (QSARs) and predictive mixture models. Based on these results, a risk assessment considering joint effect was performed calculating and summing risk quotients (RQs) for the water and sediment samples. No significant risk to *D. magna* (ΣRQs <1) was observed for any of the monitored rivers.

1. Introduction

Flame retardants are chemical substances incorporated to different materials to inhibit or slow down the growth of fire. The use of these substances has proved to be effective for saving lives and preventing injuries and property losses (EFRA, 2005). Despite these benefits, many flame retardants are toxic and potentially harmful to human health and the environment. In this context, polybrominated diphenyl ethers (PBDEs) are developmental neurotoxicants, potential endocrine disruptors (Costa et al., 2008) and bioaccumulate in biota (Wu et al., 2012). Owing to these toxic properties, PBDEs were banned in Europe (ECJ, 2008; OJEU, 2003) and are regulated by EU Water Framework Directive (OJEU, 2008). Because of these bans, PBDEs have been substituted by other compounds, such as new brominated flame retardants (NBFRs) and organophosphorus flame retardants (OPFRs) (Covaci et al., 2011; van der Veen and de Boer, 2012). These compounds are considered as emergent pollutants since they have been detected in all environmental compartments and many of them have toxic properties.

Industrial and Wastewater Treatment Plant (WWTP) discharges (Eljarrat et al., 2007; Meyer and Bester, 2004; Ricklund et al., 2009), releases from materials (Birgul et al., 2012; Brommer et al., 2012), atmospheric deposition (Melymuk et al., 2011; Regnery and Püttmann, 2010a) and runoff (Regnery and Püttmann, 2010b) are indicated as a source of flame retardants to the environment. Consequently, several flame retardants are expected to co-occur in the aquatic environment, and their relative concentration in water or sediment will depend on their production/use/discharge, their persistence and partition properties.

Few studies are available concerning the presence of PBDEs, NBFRs and OPFRs in rivers. Due to their lipophilicity, ΣPBDEs were detected in sediment from 3.67 to 2520 μg kg^{-1} and ΣNBFR ranged from 0.22 to 5270 μg kg^{-1} in the Pearl River Delta (China) (Chen et al., 2013). PBDEs have been also detected in the River Aire (UK) waters, reaching concentrations of 0.295 μg L^{-1} (only BDE-209 detected) (Cristale et al., 2013). Maximum ΣPBDEs were of 0.0043 μg L^{-1} in the River Prédecelle (France) (Labadie et al., 2010). OPFRs are reported as ubiquitous contaminants in river water, present at ng–μg L^{-1} levels (Cristale et al., 2013; Martínez-Carballo et al., 2007; Regnery and Püttmann, 2010a; Rodil et al., 2012). In addition, OPFRs were also detected in river sediments, reaching concentrations of 1300 μg kg^{-1} in the River Schwechat (Austria) (Martínez-Carballo et al., 2007).

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However, mere knowledge of pollutant concentration has only limited use, unless such data can be related to the assessment of ecological risk (Wu et al., 2008). Various tools can be used for ecological risk assessment and frequently correlate environmental concentrations (either measured environmental concentrations — MEC, or predicted environmental concentration — PEC) to a predicted no effect concentration (PNEC), that is derived from toxicological data (EC, 2003). *Daphnia magna* acute toxicity (LC_{50} or EC_{50}) is commonly used to assess the risk of a particular chemical to freshwater aquatic environment (Verbruggen et al., 2005). Nevertheless, most of the ecotoxicological studies focus on individual substances, and the effect of pollutant mixtures has not received the appropriate attention. Joint toxicity of chemicals at low concentrations can produce significant effects in mixtures (Backhaus et al., 2011; Barata et al., 2007; Payne et al., 2001; Silva et al., 2002). For this reason, combined effects have to be considered for risk assessment and water quality criteria establishment.

The objective of this study was to increase the knowledge about the presence, partitioning, and toxic effect of mixtures of flame retardants (priority and emerging) in river environment. For this purpose, water and sediment collected from source to mouth of three Spanish rivers receiving different pressures (mining, agricultural, industrial, urban) were monitored to determine the impact of a large number of flame retardants. In addition, the most ubiquitous compounds were tested for *D. magna* acute toxicity assays, and the joint effect of mixtures was studied. These results were finally applied to assess the risk of flame retardants for aquatic organisms in rivers with different pressures and anthropogenic impacts.

2. Materials and methods

2.1. Samples

One liter of water and approximately 0.5 kg of surface sediment were collected from the source to mouth in the Spanish rivers Arga (Navarra), Nalón (Asturias) and Besòs (Catalonia) during spring 2012. The source of these rivers is located in mountainous areas, generally in Natural Parks with minor human activities, and their flow through industrial, agricultural and urban areas affects water quality, which highly depends on the geographic and climatologic conditions of each basin. Fig. 1 presents the sampled points in each studied river. Industrial and urban zones, WWTPs and protected natural areas are indicated.

The source of the River Nalón (Asturias, NW Spain) is located at the "Fuente La Nalona" in "Puerto de Tarna" at 1500 m (asl) and within the Natural Park of Redes, far from anthropogenic impacts. Nalón length is of 153 km and flows to Cantabrian Sea forming the Ria de Pravia. The river basin is 3692 km^2, with an average flow of 55.18 $m^3\ s^{-1}$, although the annual maximum is of 1250 $m^3\ s^{-1}$ and the minimum of 3.4 $m^3\ s^{-1}$ (CHCANTABRICO, 2013). The use of water is evident from the source of the river. Upstream, there is a drinking water treatment plant supplying the whole community, an aquaculture factory and several hydroelectric plants. In the middle course, there is a large pharmaceutical factory. Multiple carbon mines, that historically have used Nalón's waters until their almost complete closure just recently, are spread over the basin.

The second river studied is the Arga (Navarra, N Spain), whose source is located at the Urquiaga Hill, in the north of Erro Valley, crosses Pamplona and flows into the River Aragón near Funes, which is a tributary of the River Ebro. Arga length is approximately 150 km, the river basin is 2730 km^2, with an annual average flow of 53.50 $m^3\ s^{-1}$ (CHEBRO, 2013). The river is dammed in the Eugui reservoir, close to the source, that supplies water to the Pamplona metropolitan area. Close to the city of Pamplona, the Arga receives urban wastewaters and effluents from industrial installations with metal and car factories. Through its course to the river Aragón, the basin is highly agricultural, with 275.218 ha of crops including cereals, potatoes, tobacco, beans, asparagus, fruit trees, vegetables and wines, among other less important.

The last river studied is the Besòs (Catalonia, NE Spain), a river with Mediterranean regimen highly affected by high population density. The source of the River Besòs is located in the Natural Park of Montseny, and the Besòs is formed after the confluence of rivers Congost and Mogent. The rivers Caldes, Ripoll and Tenes flow into Besòs. The Besòs discharges into the Mediterranean Sea in "Sant Adrià de Besòs" city. Besòs length is 18.4 km, the river basin is 1026 km^2 (including all the rivers) and its volume is very irregular throughout the year (average flow 4.12 $m^3\ s^{-1}$) (ACA, 2013). The historical agricultural activity carried out in this relatively small area has been totally substituted by industry, with involvement of all sectors, specifically the chemical, metallurgic, plastic, tanneries, textile, construction materials, paper, and food, with almost 10,000 factories potentially contaminating. Besides, the area is surrounded by highways. The Besòs basin is the most densely populated in Catalonia, with more than 2 million inhabitants.

2.2. Chemicals

A solution mixture of BDEs 28, 47, 99, 100, 153, 154, 183 and 209 in n-nonane at 1 $\mu g\ mL^{-1}$ was acquired from Cambridge Isotope Laboratories (Andover, USA). Individual solution of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) at 50 $\mu g\ mL^{-1}$ in 5% toluene in nonane, decabromodiphenyl ethane (DBDPE) at 25 $\mu g\ mL^{-1}$ in toluene, bis(2-ethyl-1-hexyl)tetrabromo phthalate (BEHTBP), pentabromotoluene (PBT), 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE), hexachloro cyclopentadienyl dibromooctane (HCDBCO) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) (at 50 $\mu g\ mL^{-1}$ in toluene) were acquired from Wellington Laboratories (Guelph, Canada). Pure standards of hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), tris(2-choroethyl) phosphate (TCEP), tris(2-chloro-1-methylethyl) phosphate (TCPP), tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP), triphenyl phosphate (TPhP), 2-ethylhexyl diphenyl phosphate (EHDP) and tributyl phosphate (TBP) were acquired from Sigma Aldrich (Germany). TCPP was acquired as a mixture of isomers (tris(2-chloro-1-methylethyl) phosphate, bis(1-chloro-2-propyl)-2-chloropropyl phosphate, and bis(2-chloropropyl)-1-chloro-2-propyl phosphate). Tris(2-butoxyethyl) phosphate (TBEP), tris(2-ethylhexyl) phosphate (TEHP) and tricresyl phosphate (TCP) were acquired as pure standards from Dr. Ehrenstorfer (Augsburg, Germany). Tri-iso-butyl phosphate (TiBP) at 1000 $\mu g\ mL^{-1}$ was acquired from Chiron (Trondheim, Norway).

The surrogates [$^{13}C_6$]hexabromobenzene (MHBB) at 50 $\mu g\ mL^{-1}$ in toluene, 3,3′,4,4′-tetrabromo[$^{13}C_{12}$]diphenyl ether (MBDE-77) at 50 $\mu g\ mL^{-1}$ in nonane and decabromo[$^{13}C_{12}$]diphenyl ether (MBDE-209) at 25 $\mu g\ mL^{-1}$ in toluene, were acquired from Wellington Laboratories (Guelph, Canada). Solid standard of triphenyl phosphate-D15 (TPhP-D15) was acquired from Sigma Aldrich (Germany). Tributyl phosphate-D27 (TBP-D27) as pure standard was acquired from Cambridge Isotope Laboratories (Andover, USA). The internal standards 2,3,5,6-tetrachlorobiphenyl (PCB-65) and decachlorobiphenyl (PCB-209), both at 10 $\mu g\ mL^{-1}$ in iso-octane, were acquired from Dr. Ehrenstorfer (Augsburg, Germany). Acetone, hexane, methanol, dichloromethane, ethyl acetate and toluene were acquired from Merck (Darmstadt, Germany). Cyclohexane was acquired from PANREAC (Castellar del Vallès, Spain).

2.3. Extraction and analysis

Extraction methods for waters and sediments were described in detail in previous studies (Cristale and Lacorte, in press; Cristale et al., 2013). For water samples, a volume of 500 mL of unfiltered water was spiked with labeled surrogate standards (100 ng of TPhP-D15, TBP-D27 and MBDE 209; 25 ng of MHBB and MBDE 77) and was extracted using OASIS HLB 200 mg cartridges (WATERS, USA). The cartridges were conditioned with 15 mL of hexane followed by 15 mL of dichloromethane, 15 mL of methanol and

15 mL of Milli-Q water. After the preconcentration step, the cartridges were dried using a vacuum manifold, eluted with 15 mL of dichloromethane/hexane (1:1) followed by 15 mL of dichloromethane/acetone (1:1), concentrated under N_2 flow to almost dryness and reconstituted in 250 μL of toluene with the internal standard PCB-65 and PCB-209 at 0.05 μg mL^{-1}.

For sediment samples, a mass of 1.5 g of freeze-dried sediment (sieved at 120 μm) was transferred to glass centrifuge tubes (30 mL), spiked with surrogate standards (200 ng of TPhP-D15, TBP-D27 and MBDE-209; 50 ng of MHBB and MBDE 77) and kept in contact overnight. After that, the sediment samples were extracted with 20 mL of ethyl acetate/cyclohexane (5:2 v/v) by vortex (1 min) followed by ultrasonic extraction (10 min). The extract was centrifuged (10 min at 3000 rpm) and transferred to 40 mL amber vials. This procedure was repeated twice, using 10 mL of ethyl acetate/cyclohexane (5:2 v/v). The extract was concentrated to 1 mL under N_2 flow in a Turbovap. Activated copper was used to minimize sulphur interference on GC–MS performance. Copper was activated using hydrochloric acid (25%) and ultrasonication for 15 min. The acid was eliminated by several Milli-Q rinses until obtaining pH 7. The water was eliminated by rinsing twice with acetone and finally the activated copper was stored in hexane at −20 °C. About 200 mg of activated copper was added to each sample and kept in contact overnight. After that, the clean-up was performed using 10 g Florisil cartridges (Phenomenex — Torrance, USA). The cartridges were conditioned with 60 mL of ethyl acetate/cyclohexane (5:2 v/v). After sample percolation, elution was performed with 60 mL of ethyl acetate/cyclohexane (5:2 v/v). Finally, the extract was concentrated under N_2 flow to almost dryness and reconstituted in 500 μL of toluene containing the internal standards PCB-65 and PCB-209 at 0.05 μg mL^{-1}.

Analysis was performed in a GC Agilent 7890A equipped with a 7000A GC–MS Triple Quadrupole. The column used was a DB-5MS with 15 m (length) × 0.250 mm (I.D.) × 0.10 μm (film) (J&W Scientific, USA). The GC-EI-MS/MS used conditions were the ones described by Cristale et al. (2012) and Cristale and Lacorte (in press). The oven program was set at 60 to 220 °C at 10 °C min^{-1} and to 315 °C at 15 °C min^{-1} (8 min).

2.4. QA/QC

In this study, six procedural blanks for water and for sediments were extracted and analyzed together with the samples. All OPFRs were detected in the procedural blanks, ranging from 0.0002 to 0.0040 μg L^{-1} for waters and from 1.2 to 26 μg kg^{-1} for sediments (no TCEP detection in sediment blanks), and so the limits of detection of the method (MDL) were calculated as the average blank concentration plus three times the standard deviation, and ranged from 0.0008 to 0.044 μg L^{-1} for waters and from 1.9 to 60 μg kg^{-1} for sediments. PBDEs and NBFRs were not detected in the procedural blanks and their limits of detection of the method (MDL) were calculated as three times the signal to noise ratio obtained with spiked samples. MDL for waters ranged from 0.0003 to 0.060 μg L^{-1} for PBDEs and from 0.0004 to 0.050 μg L^{-1} for NBFRs. MDL for sediments ranged from 0.29 to 40 μg kg^{-1} for PBDEs and from 0.21 to 80 μg kg^{-1} for NBFRs. A rigorous identification criteria was applied, where positive values were confirmed by comparing retention times and SRM transition ratio (T1/T2), and the accepted variation range among standard and samples, for these parameters, was followed as recommended by the Commission Decision 2002/657/EC (OJEC, 2002). Details about method performance are provided in previous studies (Cristale and Lacorte, in press; Cristale et al., 2012, 2013).

2.5. Daphnia assay

The toxicity test using *D. magna* was performed for OPFRs (TBP, TCEP, TCPP, TDCP, TPhP, EHDP, TBEP, TEHP and TCP), that were the most ubiquitous contaminants in the studied rivers. Two independent sets of experiments were performed, which included the toxicity study for single substances and for OPFR mixtures. For single substances, standardized 48 h acute assays were used where animals were exposed to freshly prepared solutions and their survival was monitored at 48 h. Single compound dose–responses were then fitted to the Hill regression model (Eq. (1)) to obtain accurate concentration dose–response curves.

In a second experiment, multicomponent mixtures of the nine studied compounds were assayed using the ray design, in which exposure levels were selected to include constant equitoxic (EC_{50}) mixture ratios and 10 different mixture effect levels, that allow consideration of explicit concentration–response relationships (Altenburger et al., 2003). This design is best suited to comparing responses with the concentration addition (CA) and independent action (IA) concepts. Both concepts predict non-interactive joint additive effects of similar (CA) and dissimilar (IA) acting chemicals and are widely used in aquatic toxicology (Altenburger et al., 2003).

All dilutions are reported as nominal concentrations. Stock solutions (2000×) of the individual chemicals or mixtures were prepared in acetone on the day of the experiment. Embryos exposed to 0.1 mL L^{-1} acetone were used as a vehicle control.

The concentration–response relationships of the individual substances were biometrically modeled by using a best-fit approach (Scholze et al., 2001) and the Hill model of Eq. (1):

$$E(\%inh) = \frac{100}{1 + (EC_{50}/x)^{p}} \qquad 1$$

with E = effect in %; p = slope; EC = effect concentration; and x = concentration (μM).

On the basis of the concentration–response functions of individual compounds, predictions of concentration addition were calculated for mixture containing binary combinations in a definite ratio (based on EC_{50}). A total concentration of the mixture, at which a certain effect is generated, can be calculated using CA according to Eq. (2):

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i}\right)^{-1}. \qquad 2$$

In this equation ECx_{mix} is the total concentration of the mixture provoking x% effect; ECx_i is the concentration of component i provoking the x% effect, when applied singly; and p_i denotes the fraction of component i in the mixture. The calculation of total mixture concentrations for various effect levels leads to a complete iteration of an expected concentration–effect relationship.

The prediction concept IA allows explicit calculation of combined effects according to Eq. (3):

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(c_i)). \qquad 3$$

The effect at the total concentration of the mixture, $E(c_{mix})$, is based on the effects of the components which they generate at concentration x at which they are present in the mixture ($E(c_i)$). If the latter is expressed as a fraction (p_i) of the total mixture concentration, it holds Eq. (4):

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(p_i c_{mix})). \qquad 4$$

This allows calculation of an effect expected according to the concept of response addition for any concentration of the mixture.

To determine the mode of action of the studied compounds, estimated EC_{50}s were related with reported K_{ow} following the proposed quantitative structure activity regression models (QSAR) for class I or non-polar narcotic chemicals of van Leeuwen and Hermens (1995) ($\log EC_{50}$ ($mol\ L^{-1}$) $= -0.95 \times \log K_{ow} - 1.19$); chemicals acting by non-polar narcosis or having a baseline toxicity should have a toxicity proportional to their K_{ow} with slopes between −0.85 and −1.

2.6. Risk assessment

The risk evaluation for *D. magna* along rivers Arga, Nalón, and Besòs was performed based on the concentrations of the detected flame retardants (OPFRs) in water and the EC_{50} results obtained for *D. magna*. RQ was calculated according to:

$$RQ = \frac{MEC}{PNEC} = \frac{MEC}{EC_{50}/f} \qquad 5$$

where, MEC is the measured environmental concentration and PNEC is the predicted no effect concentration, that was estimated as a quotient of the toxicological relevant concentration (EC_{50}) and a security factor (f). For sediments, it was assumed that pore water is the primary route of exposure for *D. magna*, and MEC was based on pore water concentration, that was estimated using the equilibrium partitioning approach by Di Toro et al. (1991):

$$C_{pw} = \frac{C_s}{f_{oc}K_{oc}} \qquad 6$$

where, C_{pw} is the estimated pore water concentration, C_s is the measured sediment concentration, f_{oc} is the fraction of organic carbon on sediments, and K_{oc} is the partition coefficient for sediment organic carbon.

For data interpretation, the maximum probable risk for ecological effects from contaminated water was followed as recommended by Wentsel et al. (1996):

$RQ < 1.0$ indicates no significant risk;
$1.0 \leq RQ < 10$ indicates a small potential for adverse effects;
$10 \leq RQ < 100$ indicates significant potential for adverse effects;
$RQ \geq 100$ indicates that potential adverse effects should be expected.

3. Results

3.1. Flame retardants in water

In water, OPFRs were detected in the three rivers while brominated flame retardants (PBDEs and NBFRs) were not detected. OPFR concentrations along the three studied rivers are presented at Table 1. TCPP and TBEP were the most abundant contaminants in most of the samples (ranging from 0.0083 to 4.6 μg L^{-1}), while the sum of their concentrations ([TCPP] + [TBEP]) ranged from 35 to 98% (average 68%) of the ΣOPFR concentration in each sampled site. This behavior is in agreement with Rodil et al. (2012) that indicated TCPP and TBEP among the most abundant OPFRs in effluents from Spanish WWTPs. TiBP, TBP, TCEP and TDCP presented intermediate concentrations, ranging from 0.0016 to 1.2 μg L^{-1}, while EHDP, TPhP, TEHP and TCP were detected at the lowest concentrations, ranging from 0.0010 to 0.046 μg L^{-1}.

The sampled sites, ΣOPFR water concentration ranges, WWTPs, and industrial and urban zones along each river are presented in Fig. 1. This figure clearly indicates the anthropogenic impact that industrial and urban zones pose on OPFR concentrations in river water. In all cases, no detection or low OPFR concentrations (ΣOPFRs <0.01 μg L^{-1}) were observed near the river sources. OPFR concentrations increased at points situated at urban zones and after WWTP discharges, while the highest levels were found in the proximity of industrial areas. The sampling was conducted in April–May 2012, which in north Spain corresponds to the rainy season. Therefore, under that situation, it is expected that dilution occurs and that FR is detected at the lowest concentration, especially if compared to summer where flows can decrease 10 times.

The River Nalón (Fig. 1(A)) was the less affected river by urban and industrial pressures and the one that presented the lowest OPFR concentrations, with ΣOPFRs ranging from <LOD to 0.088 μg L^{-1}. No OPFRs were detected at points situated at the river source (N1–2) and were seldom detected at points of low urban density (N3–6). At the points N7–11, situated after WWTP discharges, OPFRs ranged from 0.0018 to 0.045 μg L^{-1}. TBP, TDCP, TEHP, EHDP and TCP were not detected in this river.

The River Arga (Fig. 1(B)) also presented a very low level of OPFRs. These compounds were not detected at points situated in the mountain area (A1) nor before or after a drinking water treatment plant (A2 and A3). TiBP, TCPP and EHDP were detected at samples collected in the Pamplona urban area (A4 and A5). The highest OPFR concentrations were found in A6, situated after the Pamplona city and the industrial zones, with concentrations ranging from 0.0022 μg L^{-1} (TBP) to 0.20 μg L^{-1} (TBEP). The overall low concentrations detected in this point indicate a high dilution effect. OPFR concentrations decreased at points A7 and A8, indicating dilution/degradation of these contaminants along the river.

The highest concentrations (ΣOPFRs >1 μg L^{-1}) were observed for the River Besòs (Fig. 1(C)), which was the most affected river by urban and industrial pressures. OPFRs were detected in all the samples collected at the River Besòs and its confluence rivers (Mogent, Congost, Tenes, Caldes and Ripoll) except in B1, which is situated at the source of the River Mogent. OPFR concentrations ranged from 0.0010 to 0.45 μg L^{-1} at the points B2–B5, and WWTPs are indicated as the main source of these compounds since there are few industrial zones near these sampled sites. OPFR concentrations increased one to two orders of magnitude in B7–13 (industrial and urban areas of Montmeló, Ripollet, Montcada i Reixac, and Barcelona Metropolitan Area). In these areas there are industries of thermoplastic polyurethanes, personal care products, food, and plastic materials, among others, and ΣOPFRs ranged from 2.7 to 7.2 μg L^{-1}.

Rivers are the main vehicle for transport or mobilization of some pollutants from the continent to the sea (Sánchez-Avila et al., 2012). The River Nalón and the River Besòs discharge on the Cantabric and Mediterranean Sea, respectively. Thus, OPFR discharge from these rivers to the sea was estimated based on the ΣOPFR concentrations for the samples collected near the river mouth (B13 and N11) and the respective annual average flows. Approximately 1.3 kg day^{-1} of OPFRs reaches the Mediterranean Sea from Besòs discharges (4.33 $m^3\ s^{-1}$), while about 0.12 kg day^{-1} of OPFRs reaches the Cantabric Sea from Nalón discharges (55.18 $m^3\ s^{-1}$).

3.2. Flame retardants in sediments

Among the studied compounds, OPFRs were the most ubiquitous flame retardants detected in sediment samples from the rivers Arga, Nalón, and Besòs (Table 2). Unlike water samples, TEHP, EHDP, TCP and TPhP were frequently detected in sediment samples, ranging from 2.1 to 290 μg kg^{-1}. TCPP was detected in most of the sediment samples, with concentrations ranging from 13 to 365 μg kg^{-1}. Finally, TBP, TiBP, TDCP and TCEP were detected in few sediment samples, at concentrations from 2.2 to 13 μg kg^{-1}. TBEP was not detected in sediments.

The River Nalón presented the lowest OPFR levels, with ΣOPFRs ranging from 4.9 to 53 μg kg^{-1}. The most frequently detected OPFRs were TDCP, TPhP and TEHP, while EHDP and TCP were not detected. Soluble compounds such as TiBP, TBP and TCPP were detected in the samples N7–11. The influence of urban and industrialized zones on TDCP, TPhP, and TEHP sediment concentrations was not observed,

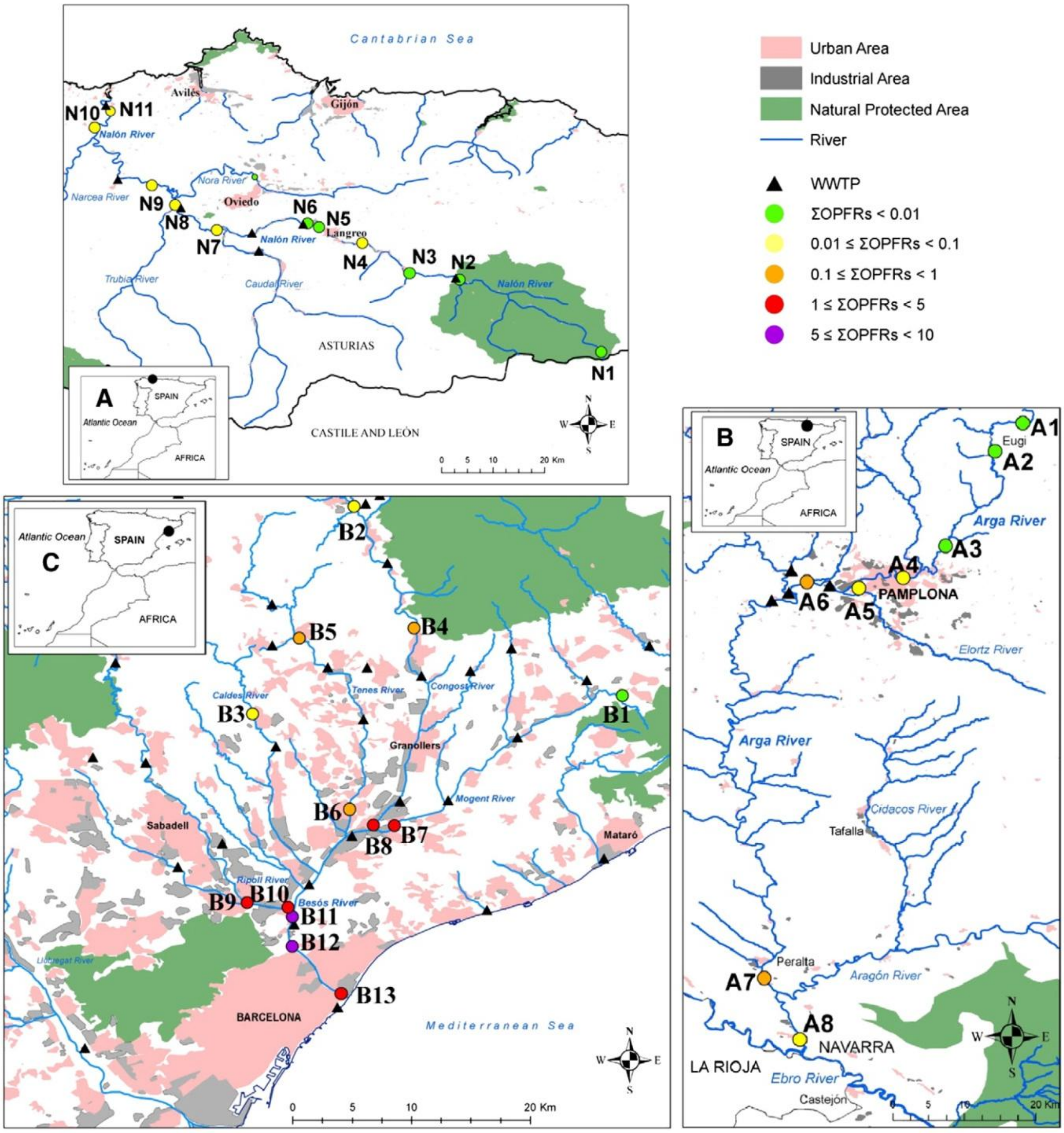

Fig. 1. Sampled sites along the Spanish rivers Nalón (A), Arga (B) and Besòs (C). ΣOPFR water concentration ranges are indicated as μg L^{-1}.

since their concentrations were at the same order of magnitude along the river.

The River Arga presented ΣOPFRs ranging from 3.8 to 292 μg kg^{-1}. TBP was detected near the source of the river at 3.8 μg kg^{-1}. The highest concentration in River Arga was observed in A5 and A6, located after the Pamplona urban center and the industrial zone, indicating that OPFR is preferably accumulated in sediment than transported by water. TCP, TEHP, EHDP and TPhP presented peak concentrations at A6, reaching 84 μg kg^{-1}, 40 μg kg^{-1}, 44 μg kg^{-1} and 8.0 μg kg^{-1}, respectively. TCPP presented the highest concentrations in A5 (142 μg kg^{-1}) followed by A6 (92 μg kg^{-1}).

The River Besòs presented the highest OPFR sediment levels, with ΣOPFR concentrations ranging from 153 to 824 μg kg^{-1}. The most abundant OPFRs were TCPP (62–365 μg kg^{-1}), TEHP (9.8–290 μg kg^{-1}), EHDP (19–63 μg kg^{-1}), TCP (11–47 μg kg^{-1}) and TPhP (6.0 to 23 μg kg^{-1}). The maximum observed concentrations were obtained at the source of Besòs, by the confluence of rivers Mogent and Congost. Two sediment samples were taken at this location, and were collected just before and after the confluence point (B8 and B8(b), respectively). The highest concentration was observed for B8(b), with TEHP and TCPP reaching 290 μg kg^{-1} and 365 μg kg^{-1}, respectively.

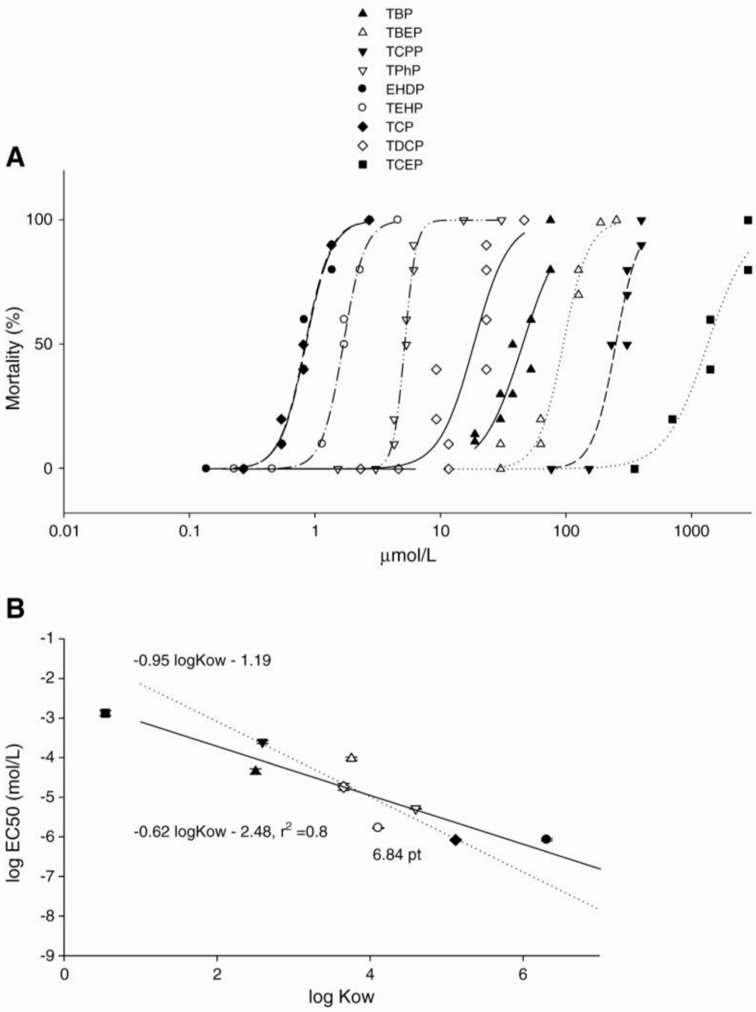

Fig. 2. Single compound *D. magna* toxicity responses of the studied organophosphorus substances: (A) concentration–mortality curves fitted to the Hill regression model; (B) estimated quantitative structure–activity regression (QSAR) curves between log octanol–water partition coefficients (log K_{ow}) and log median effect concentrations (log EC_{50}). In graph A each symbol corresponds to a single value. In graph B the dotted line is the QSAR model reported by van Leeuwen and Hermens (1995) for baseline toxicity for *D. magna* (log EC_{50} (M) $= -0.95\log K_{ow} - 1.19$). Error bars are 95% confidence intervals.

PBDEs were detected only in sediments from the Besòs River, possibly because this river is more affected by industrial and high density urban areas and because of the ten times lower flow compared to the other rivers. Table 3 presents the PBDE concentrations in sediments from the River Besòs. ΣPBDEs ranged from <LOD to 812 μg kg^{-1}. BDE-209 was detected at the highest concentrations, ranging from 196 to 807 μg kg^{-1}, while the other PBDE congeners ranged from 1.3 to 44 μg kg^{-1}. At the sample B11, collected in Santa Coloma de Gramenet, several PBDE congeners were detected, while BDE-209 was below detection limit. This behavior indicates that this zone in particular was more affected by the use of penta- and octaBDE formulations than by the more recent use of decaBDE formulations.

DBDPE, indicated as the main decaBDE substitute (Covaci et al., 2011), was detected in two sediment samples from River Besòs at 91 μg kg^{-1} (B5) and 435 μg kg^{-1} (B7). These two sediment samples also presented the highest BDE-209 levels, which could indicate BDE-209 replacement by DBDPE in industrial applications or products.

3.3. *D. magna* toxicity

D. magna was used as a model organism to evaluate aquatic toxicity. Since OPFRs were ubiquitous and the only flame retardants detected in water samples, a toxicity study was performed for these compounds, under the hypothesis of additive toxic effects. The acute toxicity using *D. magna* was firstly performed, and EC_{50} was determined. For individual OPFRs, mortality responses observed for each OPFR followed a sigmoidal curve (Fig. 2A), which could be modeled by the Hill regression function of Eq. (1). In all cases, the residuals of the regression models obtained were normally distributed (Kolmogorov–Smirnov tests $P > 0.05$) giving coefficients of determination higher than 0.8 (Table 4). The shape and steepness depicted as the Hill index (p in Table 4) of the obtained curves varied 3.7 fold across chemicals, with TPhP and TCEP having the steepest and the smoothest curves, respectively. Toxicity of the nine tested chemicals differed largely across substances, with EC_{50} values ranging over three

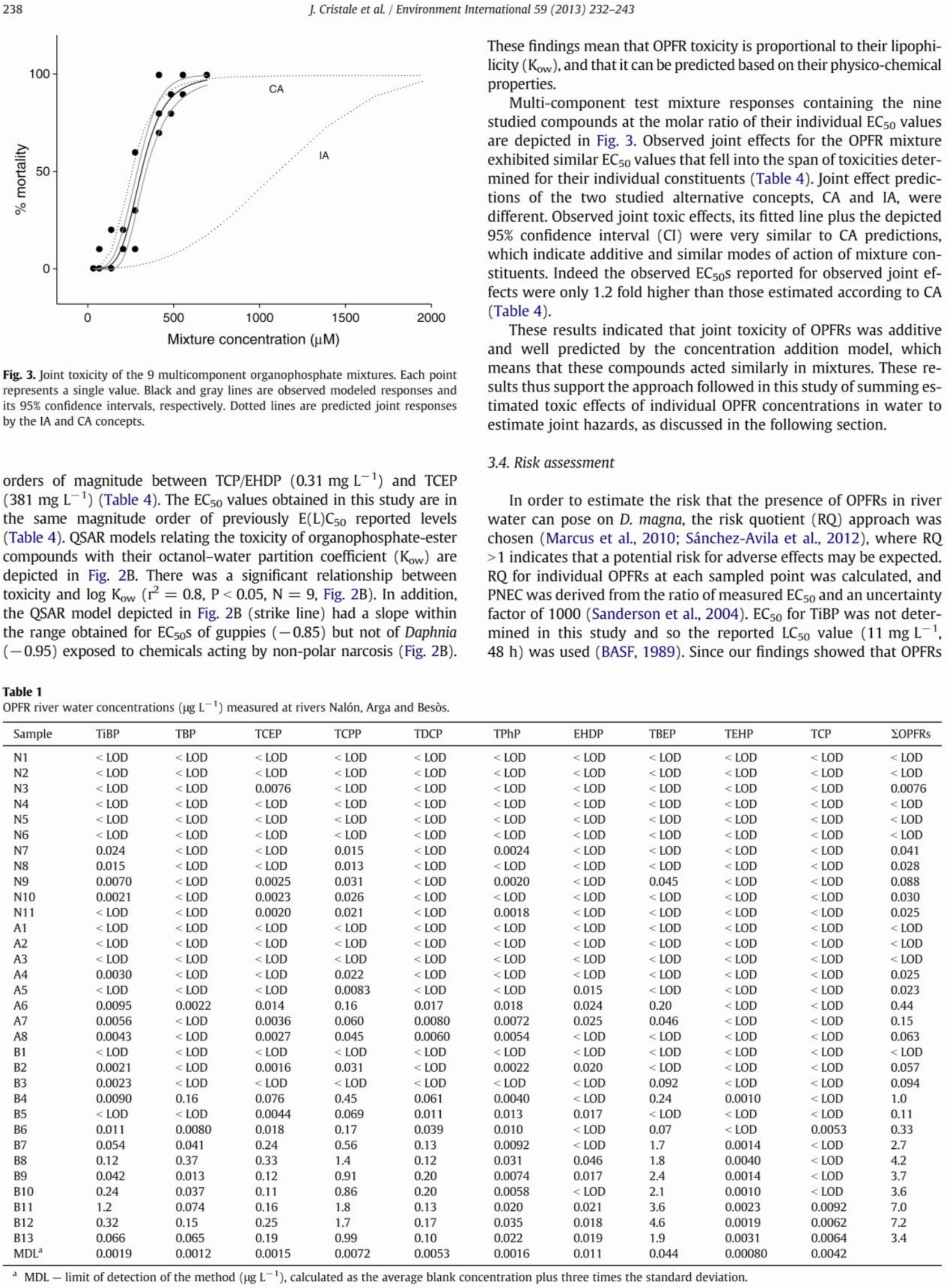

Fig. 3. Joint toxicity of the 9 multicomponent organophosphate mixtures. Each point represents a single value. Black and gray lines are observed modeled responses and its 95% confidence intervals, respectively. Dotted lines are predicted joint responses by the IA and CA concepts.

orders of magnitude between TCP/EHDP (0.31 mg L^{-1}) and TCEP (381 mg L^{-1}) (Table 4). The EC_{50} values obtained in this study are in the same magnitude order of previously $E(L)C_{50}$ reported levels (Table 4). QSAR models relating the toxicity of organophosphate-ester compounds with their octanol–water partition coefficient (K_{ow}) are depicted in Fig. 2B. There was a significant relationship between toxicity and log K_{ow} ($r^2 = 0.8$, $P < 0.05$, $N = 9$, Fig. 2B). In addition, the QSAR model depicted in Fig. 2B (strike line) had a slope within the range obtained for EC_{50}s of guppies (−0.85) but not of *Daphnia* (−0.95) exposed to chemicals acting by non-polar narcosis (Fig. 2B). These findings mean that OPFR toxicity is proportional to their lipophilicity (K_{ow}), and that it can be predicted based on their physico-chemical properties.

Multi-component test mixture responses containing the nine studied compounds at the molar ratio of their individual EC_{50} values are depicted in Fig. 3. Observed joint effects for the OPFR mixture exhibited similar EC_{50} values that fell into the span of toxicities determined for their individual constituents (Table 4). Joint effect predictions of the two studied alternative concepts, CA and IA, were different. Observed joint toxic effects, its fitted line plus the depicted 95% confidence interval (CI) were very similar to CA predictions, which indicate additive and similar modes of action of mixture constituents. Indeed the observed EC_{50}s reported for observed joint effects were only 1.2 fold higher than those estimated according to CA (Table 4).

These results indicated that joint toxicity of OPFRs was additive and well predicted by the concentration addition model, which means that these compounds acted similarly in mixtures. These results thus support the approach followed in this study of summing estimated toxic effects of individual OPFR concentrations in water to estimate joint hazards, as discussed in the following section.

3.4. Risk assessment

In order to estimate the risk that the presence of OPFRs in river water can pose on *D. magna*, the risk quotient (RQ) approach was chosen (Marcus et al., 2010; Sánchez-Avila et al., 2012), where RQ >1 indicates that a potential risk for adverse effects may be expected. RQ for individual OPFRs at each sampled point was calculated, and PNEC was derived from the ratio of measured EC_{50} and an uncertainty factor of 1000 (Sanderson et al., 2004). EC_{50} for TiBP was not determined in this study and so the reported LC_{50} value (11 mg L^{-1}, 48 h) was used (BASF, 1989). Since our findings showed that OPFRs

Table 1
OPFR river water concentrations (μg L^{-1}) measured at rivers Nalón, Arga and Besòs.

Sample	TiBP	TBP	TCEP	TCPP	TDCP	TPhP	EHDP	TBEP	TEHP	TCP	ΣOPFRs
N1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
N2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
N3	< LOD	< LOD	0.0076	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0076
N4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
N5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
N6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
N7	0.024	< LOD	< LOD	0.015	< LOD	0.0024	< LOD	< LOD	< LOD	< LOD	0.041
N8	0.015	< LOD	< LOD	0.013	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.028
N9	0.0070	< LOD	0.0025	0.031	< LOD	0.0020	< LOD	0.045	< LOD	< LOD	0.088
N10	0.0021	< LOD	0.0023	0.026	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.030
N11	< LOD	< LOD	0.0020	0.021	< LOD	0.0018	< LOD	< LOD	< LOD	< LOD	0.025
A1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
A2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
A3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
A4	0.0030	< LOD	< LOD	0.022	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.025
A5	< LOD	< LOD	< LOD	0.0083	< LOD	< LOD	0.015	< LOD	< LOD	< LOD	0.023
A6	0.0095	0.0022	0.014	0.16	0.017	0.018	0.024	0.20	< LOD	< LOD	0.44
A7	0.0056	< LOD	0.0036	0.060	0.0080	0.0072	0.025	0.046	< LOD	< LOD	0.15
A8	0.0043	< LOD	0.0027	0.045	0.0060	0.0054	< LOD	< LOD	< LOD	< LOD	0.063
B1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
B2	0.0021	< LOD	0.0016	0.031	< LOD	0.0022	0.020	< LOD	< LOD	< LOD	0.057
B3	0.0023	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.092	< LOD	< LOD	0.094
B4	0.0090	0.16	0.076	0.45	0.061	0.0040	< LOD	0.24	0.0010	< LOD	1.0
B5	< LOD	< LOD	0.0044	0.069	0.011	0.013	0.017	< LOD	< LOD	< LOD	0.11
B6	0.011	0.0080	0.018	0.17	0.039	0.010	< LOD	0.07	< LOD	0.0053	0.33
B7	0.054	0.041	0.24	0.56	0.13	0.0092	< LOD	1.7	0.0014	< LOD	2.7
B8	0.12	0.37	0.33	1.4	0.12	0.031	0.046	1.8	0.0040	< LOD	4.2
B9	0.042	0.013	0.12	0.91	0.20	0.0074	0.017	2.4	0.0014	< LOD	3.7
B10	0.24	0.037	0.11	0.86	0.20	0.0058	< LOD	2.1	0.0010	< LOD	3.6
B11	1.2	0.074	0.16	1.8	0.13	0.020	0.021	3.6	0.0023	0.0092	7.0
B12	0.32	0.15	0.25	1.7	0.17	0.035	0.018	4.6	0.0019	0.0062	7.2
B13	0.066	0.065	0.19	0.99	0.10	0.022	0.019	1.9	0.0031	0.0064	3.4
MDL[a]	0.0019	0.0012	0.0015	0.0072	0.0053	0.0016	0.011	0.044	0.00080	0.0042	

[a] MDL – limit of detection of the method (μg L^{-1}), calculated as the average blank concentration plus three times the standard deviation.

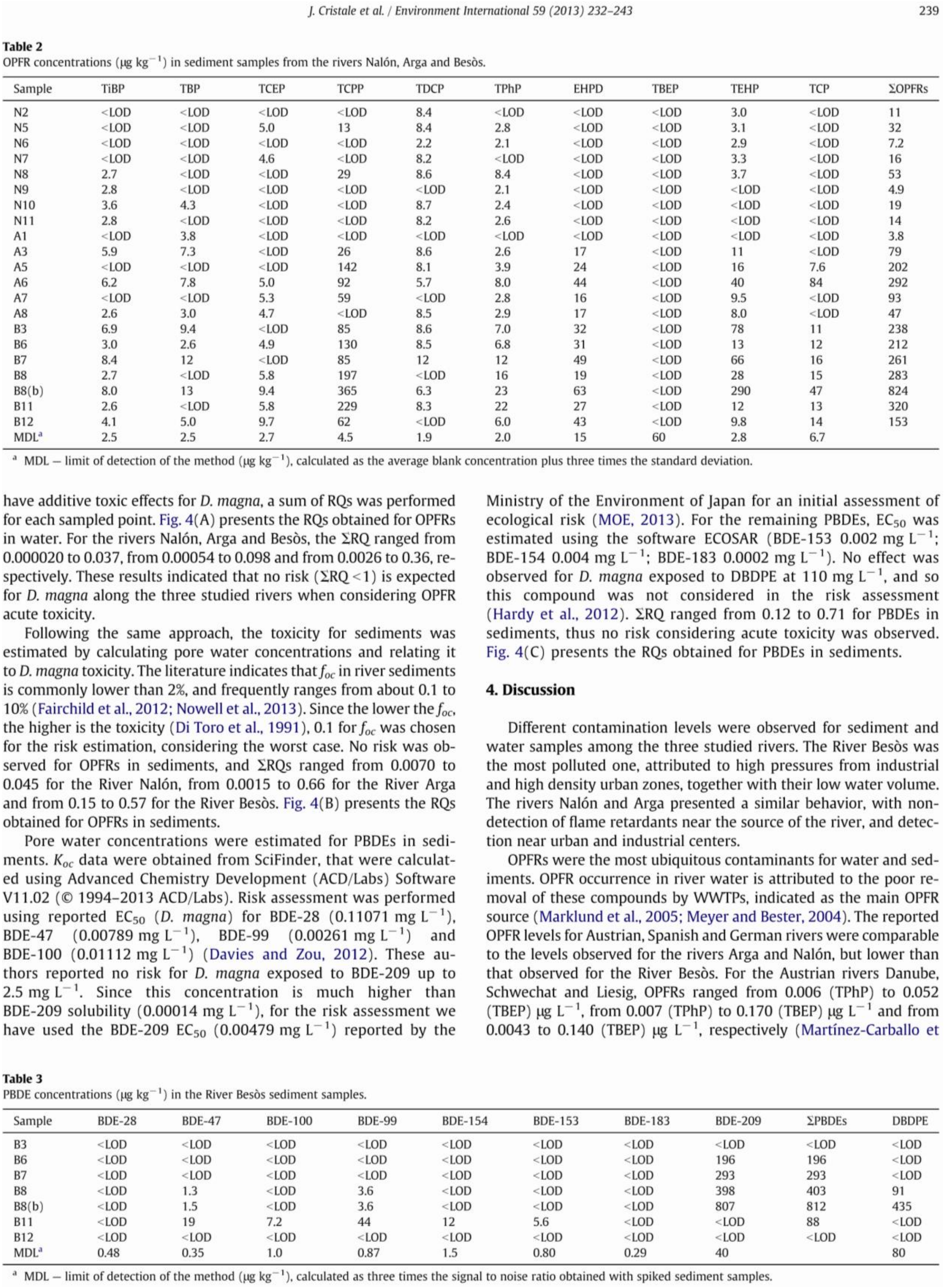

Table 2
OPFR concentrations (μg kg^{-1}) in sediment samples from the rivers Nalón, Arga and Besòs.

Sample	TiBP	TBP	TCEP	TCPP	TDCP	TPhP	EHPD	TBEP	TEHP	TCP	ΣOPFRs
N2	<LOD	<LOD	<LOD	<LOD	8.4	<LOD	<LOD	<LOD	3.0	<LOD	11
N5	<LOD	<LOD	5.0	13	8.4	2.8	<LOD	<LOD	3.1	<LOD	32
N6	<LOD	<LOD	<LOD	<LOD	2.2	2.1	<LOD	<LOD	2.9	<LOD	7.2
N7	<LOD	<LOD	4.6	<LOD	8.2	<LOD	<LOD	<LOD	3.3	<LOD	16
N8	2.7	<LOD	<LOD	29	8.6	8.4	<LOD	<LOD	3.7	<LOD	53
N9	2.8	<LOD	<LOD	<LOD	<LOD	2.1	<LOD	<LOD	<LOD	<LOD	4.9
N10	3.6	4.3	<LOD	<LOD	8.7	2.4	<LOD	<LOD	<LOD	<LOD	19
N11	2.8	<LOD	<LOD	<LOD	8.2	2.6	<LOD	<LOD	<LOD	<LOD	14
A1	<LOD	3.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.8
A3	5.9	7.3	<LOD	26	8.6	2.6	17	<LOD	11	<LOD	79
A5	<LOD	<LOD	<LOD	142	8.1	3.9	24	<LOD	16	7.6	202
A6	6.2	7.8	5.0	92	5.7	8.0	44	<LOD	40	84	292
A7	<LOD	<LOD	5.3	59	<LOD	2.8	16	<LOD	9.5	<LOD	93
A8	2.6	3.0	4.7	<LOD	8.5	2.9	17	<LOD	8.0	<LOD	47
B3	6.9	9.4	<LOD	85	8.6	7.0	32	<LOD	78	11	238
B6	3.0	2.6	4.9	130	8.5	6.8	31	<LOD	13	12	212
B7	8.4	12	<LOD	85	12	12	49	<LOD	66	16	261
B8	2.7	<LOD	5.8	197	<LOD	16	19	<LOD	28	15	283
B8(b)	8.0	13	9.4	365	6.3	23	63	<LOD	290	47	824
B11	2.6	<LOD	5.8	229	8.3	22	27	<LOD	12	13	320
B12	4.1	5.0	9.7	62	<LOD	6.0	43	<LOD	9.8	14	153
MDL[a]	2.5	2.5	2.7	4.5	1.9	2.0	15	60	2.8	6.7	

[a] MDL — limit of detection of the method (μg kg^{-1}), calculated as the average blank concentration plus three times the standard deviation.

have additive toxic effects for *D. magna*, a sum of RQs was performed for each sampled point. Fig. 4(A) presents the RQs obtained for OPFRs in water. For the rivers Nalón, Arga and Besòs, the ΣRQ ranged from 0.000020 to 0.037, from 0.00054 to 0.098 and from 0.0026 to 0.36, respectively. These results indicated that no risk ($\Sigma RQ < 1$) is expected for *D. magna* along the three studied rivers when considering OPFR acute toxicity.

Following the same approach, the toxicity for sediments was estimated by calculating pore water concentrations and relating it to *D. magna* toxicity. The literature indicates that f_{oc} in river sediments is commonly lower than 2%, and frequently ranges from about 0.1 to 10% (Fairchild et al., 2012; Nowell et al., 2013). Since the lower the f_{oc}, the higher is the toxicity (Di Toro et al., 1991), 0.1 for f_{oc} was chosen for the risk estimation, considering the worst case. No risk was observed for OPFRs in sediments, and ΣRQs ranged from 0.0070 to 0.045 for the River Nalón, from 0.0015 to 0.66 for the River Arga and from 0.15 to 0.57 for the River Besòs. Fig. 4(B) presents the RQs obtained for OPFRs in sediments.

Pore water concentrations were estimated for PBDEs in sediments. K_{oc} data were obtained from SciFinder, that were calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994–2013 ACD/Labs). Risk assessment was performed using reported EC_{50} (*D. magna*) for BDE-28 (0.11071 mg L^{-1}), BDE-47 (0.00789 mg L^{-1}), BDE-99 (0.00261 mg L^{-1}) and BDE-100 (0.01112 mg L^{-1}) (Davies and Zou, 2012). These authors reported no risk for *D. magna* exposed to BDE-209 up to 2.5 mg L^{-1}. Since this concentration is much higher than BDE-209 solubility (0.00014 mg L^{-1}), for the risk assessment we have used the BDE-209 EC_{50} (0.00479 mg L^{-1}) reported by the Ministry of the Environment of Japan for an initial assessment of ecological risk (MOE, 2013). For the remaining PBDEs, EC_{50} was estimated using the software ECOSAR (BDE-153 0.002 mg L^{-1}; BDE-154 0.004 mg L^{-1}; BDE-183 0.0002 mg L^{-1}). No effect was observed for *D. magna* exposed to DBDPE at 110 mg L^{-1}, and so this compound was not considered in the risk assessment (Hardy et al., 2012). ΣRQ ranged from 0.12 to 0.71 for PBDEs in sediments, thus no risk considering acute toxicity was observed. Fig. 4(C) presents the RQs obtained for PBDEs in sediments.

4. Discussion

Different contamination levels were observed for sediment and water samples among the three studied rivers. The River Besòs was the most polluted one, attributed to high pressures from industrial and high density urban zones, together with their low water volume. The rivers Nalón and Arga presented a similar behavior, with non-detection of flame retardants near the source of the river, and detection near urban and industrial centers.

OPFRs were the most ubiquitous contaminants for water and sediments. OPFR occurrence in river water is attributed to the poor removal of these compounds by WWTPs, indicated as the main OPFR source (Marklund et al., 2005; Meyer and Bester, 2004). The reported OPFR levels for Austrian, Spanish and German rivers were comparable to the levels observed for the rivers Arga and Nalón, but lower than that observed for the River Besòs. For the Austrian rivers Danube, Schwechat and Liesig, OPFRs ranged from 0.006 (TPhP) to 0.052 (TBEP) μg L^{-1}, from 0.007 (TPhP) to 0.170 (TBEP) μg L^{-1} and from 0.0043 to 0.140 (TBEP) μg L^{-1}, respectively (Martínez-Carballo et

Table 3
PBDE concentrations (μg kg^{-1}) in the River Besòs sediment samples.

Sample	BDE-28	BDE-47	BDE-100	BDE-99	BDE-154	BDE-153	BDE-183	BDE-209	ΣPBDEs	DBDPE
B3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
B6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	196	196	<LOD
B7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	293	293	<LOD
B8	<LOD	1.3	<LOD	3.6	<LOD	<LOD	<LOD	398	403	91
B8(b)	<LOD	1.5	<LOD	3.6	<LOD	<LOD	<LOD	807	812	435
B11	<LOD	19	7.2	44	12	5.6	<LOD	<LOD	88	<LOD
B12	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
MDL[a]	0.48	0.35	1.0	0.87	1.5	0.80	0.29	40		80

[a] MDL — limit of detection of the method (μg kg^{-1}), calculated as three times the signal to noise ratio obtained with spiked sediment samples.

Table 4
Hill regression models fitted to the single and studied mixtures. All regressions were significant at $P < 0.001$.

Single	EC_{50} (mg L^{-1})	EC_{50} (μmol L^{-1})	SE_1	p	SE_2	r^2	N	Reported $E(L)C_{50}$ (mg L^{-1}, 24 h and 48 h)[a]
TCEP	381	1334	94	2.6	0.4	0.9549	10	235–451
TCPP	81	248	10	4.7	0.9	0.9282	12	63–131
TBEP	38	95	4	4.3	0.5	0.9847	10	75–84
TBP	12	45	3	2.7	0.6	0.8381	10	5.8–35
TDCP	7.9	18	2	3.2	0.8	0.8664	16	3.8–4.6
TPhP	1.70	5.20	0.05	9.7	0.8	0.9925	14	1–1.35
TEHP	0.74	1.69	0.02	5.1	0.3	0.9949	12	
TCP	0.31	0.84	0.02	4.3	0.4	0.9917	10	0.27–5.6
EHDP	0.31	0.85	0.03	4.3	0.6	0.9821	12	

	Observed response						Predicted response	
	EC_{50} (μmol L^{-1})	SE	p	SE	r^2	N	EC_{50}-IA (μmol L^{-1})	EC_{50}-CA (μmol L^{-1})
Mixture	314	12	4.6	0.6	0.9418	27	1112	258

p and EC_{50} are the regression coefficients for the model depicted in Eq. (1); r^2, coefficient of determination; N, sample size; SE_1 standard error for EC_{50} (μmol L^{-1}). SE_2 standard error for p. Differences in N values are due to missing values. Predicted joint effects (EC_{50}s) of the IA and CA models are also included.

[a] Acute toxicity data (range values) revised by Verbruggen et al. (2005).

al., 2007). In a previous Spanish study, OPFRs were reported as the dominant emerging pollutants occurring in surface water (Mero river basin) and drinking water (private homes at A Coruña), ranging from 0.020 to 0.200 μg L^{-1} (Rodil et al., 2012). At the River Ruhr (Germany), OPFRs ranged from 0.010 (TPhP) to 0.87 (TBEP) μg L^{-1} (Andresen et al., 2004). The River Besòs presented concentrations lower than the reported ones for the River Aire (United Kingdom), where TCPP was detected up to concentrations of 26.050 μg L^{-1} (Cristale et al., 2013).

Few studies are available concerning OPFR concentrations in river sediments for data comparison. OPFRs were determined in sediment from the Austrian rivers Danube, Schwechat and Liesig, and low OPFR

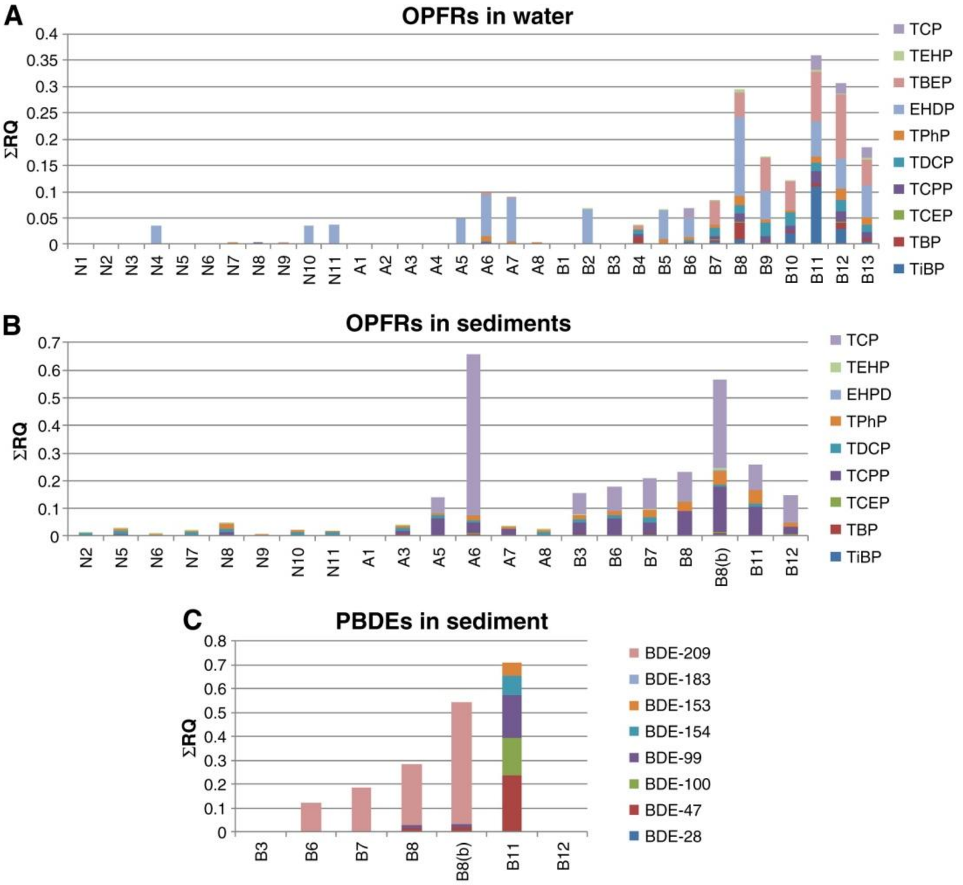

Fig. 4. Risk quotient (RQ) for detected flame retardants in water and sediments. In some sites, the river bed had no sediments and therefore there is no data.

contamination was found for the River Danube (up to 50 μg kg^{-1} for TBP), while the small rivers Schwechat and Liesig presented concentrations up to 1300 μg kg^{-1} for TCPP and up to 160 μg kg^{-1} for TCEP and TPhP (Martínez-Carballo et al., 2007). Ricking et al. (2003) reported TBP and TCP concentrations ranging from 10 to 390 μg kg^{-1} for sediment from the Havel and Spree rivers (Germany). Lower levels were reported in sediment collected from the rivers around Chung-Li city (Taiwan), which presented TCEP, TCPP, TDCP, and TPhP concentrations ranging from <LOD to 9.5 μg kg^{-1} (TCPP) (Chung and Ding, 2009). In a previous study, TBP and TCP were indicated as anthropogenic markers for sediments (Ricking et al., 2003), due to their exceeding background concentrations at sites near industrial zones. In this study, TCPP, TCP, TEHP, EHDP and TPhP presented the peak concentrations at sites near industrial zones (A6 and B8(b)) and are also proposed as anthropogenic markers.

PBDEs were detected in sediments from the River Besòs, while DBDPE was the only detected NBFR. Despite the PBDE ban in Europe, these compounds were detected in sediment samples, probably as a result of their environmental persistence and release from materials. PBDE levels herein obtained are higher than the ones reported for rivers in Spain, France, United States, and China, whereas lower than the levels in the Pearl River Delta (China). In accordance with our results, all of these studies report BDE-209 as the main PBDE congener in river sediments. At the River Cinca (East, Spain), ΣPBDEs ranged from 2 to 42 μg kg^{-1} (Eljarrat et al., 2004). At the River Prédecelle (France), ΣPBDEs ranged from 3.1 to 15.1 μg kg^{-1} (Labadie et al., 2010). ΣPBDE varied from 0.05 to 49.44 μg kg^{-1} in surface sediments and from 0.02 to 55.1 μg kg^{-1} dw in floodplain soils from the Saginaw River Watershed (Michigan, USA) (Yun et al., 2008). Li et al. (2012) reported ΣPBDE (BDE-209 excluded) levels ranging from 1.2 to 12.1 μg kg^{-1} and BDE-209 ranging from 2.4 to 30.5 μg kg^{-1} in sediments from urban rivers in Chaohu City (China). In sediments from the Pearl River Delta (South China), ΣPBDE congeners (BDE-209 excluded) ranged from 0.04 to 94.7 μg kg^{-1}, while BDE-209 ranged from 0.4 to 7340 μg kg^{-1} (Mai et al., 2005). For DBDPE, few studies are available concerning their presence in river sediments. DBDPE concentrations for riverine and estuarine surface sediments ranged from <LOD to 1730 μg kg^{-1} (Chen et al., 2013). DBDPE was detected in river sediment from a metal recycling site at 1.2 μg kg^{-1} in Norway (Nyholm et al., 2013).

PBDE profile and DBDPE presence in the River Besòs are in agreement with a recent Spanish study that reported BDE-209 as the main detected PBDE congener in sewage sludge samples from Catalan WWTPs (Spain), with ΣPBDEs ranging from 20.7 to 2326 μg kg^{-1} and BDE-209 ranging from <LOD to 2303 μg kg^{-1} (Gorga et al., 2013), while the reported DBDPE concentrations ranged from <LOD to 257 μg kg^{-1}. Also, the non-detection of HBB and PBEB in sediments is also in accordance with Gorga et al. (2013), since these compounds were only detected in 2 of 17 sludge samples, and were at low concentrations (from 1.78 to 5.71 μg kg^{-1}). The non-detection of the other NBFRs can be associated to their low production volume (LPV) and/or their degradation in the environment. PBEB, PBT, BTBPE and BEHTBP are LPV chemicals in Europe (production below 1000 tons/year), while no information about European production of HBB, EHTBB, DPTE and HCDBCO is available (ESIS, 2013). Photodegradation experiments showed sequential reductive debromination for EHTBB and BEHTBP, possibly generating non-brominated degradation products (Davis and Stapleton, 2009). Even though there are few data about the presence of NBFR in European aquatic environment, most of them are in accordance with this study, reporting NBFRs at low concentrations (or not detected) and PBDEs at levels from one to two orders of magnitude higher. For sediment samples collected near suspected sources in Tromsø, Drammensfjorden and Lake Mjøsa (Norway), BTBPE ranged from <LOD to 1.0 ± 1.7 μg kg^{-1}, DBDPE was not detected and PBDEs ranged from <LOD to 62 ± 18 μg kg^{-1} (Nyholm et al., 2013).

Toxicity responses of the studied OPFRs in *D. magna* were proportional to their log K_{ow} and the fitted QSAR model was close to the ones obtained in fish and *D. magna* for non-polar narcotic compounds (van Leeuwen and Hermens, 1995). These results indicate that the studied OPFR compounds acted similarly by non-polar narcosis. Mixture toxicity results support the previous argument indicating that joint toxicity of OPFRs was additive and well predicted by CA, which means that mixture constituents acted by the same mode of action in a mixture. Translating the above mentioned results into risk assessment means that it's possible to predict the toxicity of complex mixtures of OPFRs from their K_{ow} using QSAR models for non-polar narcotic chemicals and the CA or related approaches such as the sum of the risk quotients of mixture constituents (ΣRQs) proposed in this study and in many others (see for example Damásio et al. (2011)).

The risk assessment for OPFRs in water and sediments indicated no risks. However, ΣRQs were higher for sediments than for water, mainly attributed to the presence of more lipophilic and toxic OPFRs such as TEHP, TPhP, TCP and EHDP. Since these compound have high log K_{ow} (from 4.22 to 5.73), they tend to associate to suspended particles and accumulate on sediments (Martínez-Carballo et al., 2007). These results point out the need of more studies concerning the presence of OPFRs on sediments, especially considering the lipophilic ones, which are more toxic and bioaccumulative (van der Veen and de Boer, 2012; Verbruggen et al., 2005). On the other hand, because OPFRs showed additive toxicity, the risk assessment results based on ΣRQ for 10 OPFRs, are possibly sub-estimated, since other organophosphate flame retardants/plasticizers, not included in this study, could also be present in river water. In addition, once organophosphate flame retardants are in water environment, these chemicals can undergo biotransformation, photodegradation and hydrolysis, that could generate transformation products with additive toxic effect for *D. magna* as well.

For brominated flame retardants in sediments, the risk assessment showed no risk for *D. magna*. Despite that PBDEs are much more toxic to *D. magna* than OPFRs, PBDEs are very lipophilic (log K_{ow} from 6.3 to 9.5), tending to remain absorbed on sediment organic carbon, and so the estimated pore water concentrations did not reach toxic levels. On the other hand, PBDEs are bioaccumulative, and their presence in sediment can promote the continuous intake of these pollutants by water organisms and their accumulation in the food chain (Vonderheide et al., 2008).

5. Conclusions

OPFRs, PBDEs and NBFRs were analyzed in 3 rivers in the north of Spain which suffer different anthropogenic pressures and are representative Mediterranean or Atlantic rivers. OPFRs were the most ubiquitous compounds detected, and accumulated in sediments whereas the water concentrations were overall low. Arga and Nalón rivers, with high water flows, presented the lowest level of contamination, while the Besòs, affected by urban and industrial activities, had the highest levels in both water and sediment. PBDEs were only detected in this river. When assessing the risk of OPFRs, it was found that the concentrations detected in water or in sediment did not produce any risk using *D. magna* toxicity model. Although individual OPFRs produce an effect to *D. magna* only when present in water at mg L^{-1} levels, these organophosphate-ester compounds showed additive toxicity, and so the monitoring of several OPFRs is needed for an appropriated risk assessment considering this flame retardant family. On the other hand, European bans towards the use of PBDEs have resulted in low concentration of these compounds, however, an increased presence of new brominated flame retardants was not observed. Overall, it is demonstrated that OPFRs are a re-emerging family of flame retardants which in the long term could affect the aquatic ecosystems, especially if released constantly even at low quantities.

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3.4 Discussion

3.4.1 Removal of FRs in Spanish WWTPs

The occurrence of OPFRs, NBFRs and PBDEs in five Spanish WWTPs was evaluated. Table 11 presents the concentration of detected compounds in the influents, the effluents and the removal efficiencies for each compound in each WWTP. All OPFRs were detected in WWTP influents and most of them in the effluents. All the chloroalkyl phosphates were resistant to the treatment, since concentrations in influent were similar to, or somewhat higher than, the effluent concentrations in most of the WWTPs. These results are in agreement with previous studies which reported that TCPP, TCEP and TDCP were poorly removed during the treatment [34, 147]. Also in accordance with these previous studies, a higher removal was observed for alkyl phosphates and aryl phosphates, which presented a removal ranging from 28% to 95% for TBEP, TBP, TiBP and TPhP, while TEHP, EHDP and TCP were below MDL in most of the effluent samples. The most abundant OPFRs were TBEP and TCPP, followed by TiBP, TBP, TCEP and TDCP. The observed OPFR profile in influents was similar to the observed by Rodil et al. (2012) [34] for WWTPs in Galicia (Northwest Spain). However, the authors reported a poor elimination of TBEP in Galician WWTPs, while a removal from 58 to 97% was observed in this study. In addition, maximum observed concentration in the influents for TBEP (up to 46 µg L^{-1}) and TiBP (up to 166 µg L^{-1}) were higher in this study than the observed by those authors. Also, TEHP, EHDP, TCP and TPhP were not detected in the Galician WWTPs, but detected in most of the Catalan WWTPs. The differences in terms of OPFR concentrations in WWTP influents of these two Spanish regions are probably due to differences in industrial and urban density among study areas. BEHTBP was the only brominated FR detected in water, and was present in the influents of four WWTPs, but was below MDL in all the effluents, which indicate that this compound is removed during the treatment. $WWTP_3$ and $WWTP_4$ presented high levels of TiBP and TBEP in the influents, respectively. Since for both WWTPs the concentration of other OPFRs were in the same order of magnitude than the observed for the other studied WWTPs, the high concentration of TiBP in $WWTP_3$ and TBEP in $WWTP_4$ suggest specific industrial sources.

Sludge samples collected in these 5 Spanish WWTPs were analyzed, and results were presented in Paper 3 (Chapter 2). Accumulation of OPFRs (except TCEP), PBDEs (BDE-47, BDE-99 and BDE-209), DBDPE and BEHTBP was observed in most of sludge samples.

Table 11 – Influent and effluent concentration (μg L^{-1}) of detected FRs in five Spanish WWTPs, and the estimated removal efficiency.

	TCPP	TCEP	TDCP	TBEP	TBP	TiBP	TEHP[d]	TPhP	EHDP	TCP	BEHTBP
WWTP$_1$	1.7±0.2[a]	0.18±0.01[a]	0.129±0.003[a]	1.56±0.04[a]	<MDL[a]	<MDL[a]	<MDL[a]	0.101±0.009[a]	<MDL[a]	<MDL[a]	<MDL[a]
(n=2)	2.4±0.2[b]	0.25±0.04[b]	0.136±0.004[b]	0.207±0.004[b]	0.022±0.001[b]	<MDL[b]	<MDL[b]	0.040±0.007[b]	<MDL[b]	<MDL[b]	<MDL[b]
	none[c]	none[c]	none[c]	87%[c]	none[c]			60%[c]			
WWTP$_2$	6.75±0.04[a]	0.32±0.02[a]	0.29±0.03[a]	12±3[a]	0.21±0.02[a]	1.34±0.03[a]	0.131±0.008[a]	0.25±0.02[a]	0.27±0.01[a]	0.153±0.050[a]	0.13±0.01[a]
(n=2)	3.0±0.6[b]	0.373±0.004[b]	0.354±0.00[b]	0.62±0.05[b]	0.09±0.02[b]	0.74±0.07[b]	<MDL[b]	0.064±0.003[b]	<MDL[b]	<MDL[b]	<MDL[b]
	56%[c]	none[c]	none[c]	95%[c]	58%[c]	44%[c]	removed[c]	75%[c]	removed[c]	removed[c]	removed[c]
WWTP$_3$	3.6±0.2[a]	0.295±0.008[a]	0.111±0.001[a]	7±2[a]	0.9±0.3[a]	138±28[a]	0.12±0.01[a]	0.177±0.007[a]	0.44±0.07[a]	0.046±0.013[a]	0.052±0.003[a]
(n=2)	3.7±0.3[b]	0.57±0.04[b]	0.319±0.009[b]	1.97±0.08[b]	0.174±0.004[b]	23±5[b]	<MDL[b]	0.037±0.007[b]	MDL[b]	0.008±0.002[b]	<MDL[b]
	none[c]	none[c]	none[c]	71%[c]	80%[c]	83%[c]	removed[c]	79%[c]	removed[c]	removed[c]	removed[c]
WWTP$_4$	3.2[a]	0.22[a]	0.22[a]	46[a]	0.305[a]	0.32[a]	0.012[a]	0.095[a]	0.084[a]	0.021[a]	0.058[a]
(n=1)	2.8[b]	0.24[b]	0.21[b]	1.5[b]	0.136[b]	0.23[b]	<MDL[b]	0.055[b]	<MDL[b]	<MDL[b]	<MDL[b]
	none[c]	none[c]	none[c]	97%[c]	55%[c]	28%[c]	removed[c]	42%[c]	removed[c]	removed[c]	removed[c]
WWTP$_5$	3.71±0.09[a]	0.32±0.02[a]	0.067±0.001[a]	8.6±0.4[a]	0.135±0.004[a]	0.26±0.04[a]	0.035±0.003[a]	0.124±0.001[a]	0.24±0.04[a]	0.0300±0.0001[a]	0.053±0.009[a]
(n=2)	3.1±0.1[b]	0.33±0.02	0.174±0.002[b]	3.6±0.3[b]	0.065±0.001[b]	0.19±0.03[b]	<MDL[b]	0.070±0.004[b]	<MDL[b]	<MDL[b]	<MDL[b]
	15%[c]	none[c]	none[c]	58%[c]	52%[c]	28%[c]	removed[c]	44%[c]	removed[c]	removed[c]	removed[c]

[a] influent; [b] effluent; [c] removal;

[d] Recoveries for TEHP in influents were low (29±2%, see Chapter 2), and so these reported values are underestimated.

3.4.2 Comparing FR profiles among analyzed samples

Water samples

As discussed in the Chapter 1, effluents of WWTPs are considered as one of the main sources of contaminants to surface waters in developed countries. To evaluate the presence of FRs in WWTPs, including influents, effluents and sewage sludge gives a qualitative indication of compounds that can potentially be present in surface waters and sediments. The presence and elimination efficiency of OPFRs, NBFRs and PBDEs in five WWTPs (Catalonia, Spain) were studied. Despite that only WWTPs from Catalonia were included, literature research indicates that FR profiles in WWTPs tend to be similar among other locations of Spain and Europe [30, 34-35, 245]. This occurs because FRs are present in products (automobiles, electric and electronics, PU foam, plastics, textiles, etc) that are frequently commercialized and available in all European countries. However, differences in terms of concentrations and profiles are expected for WWTPs that receive effluents from industries that produce or use FRs for application in specific products.

As discussed above, all OPFRs were detected in the influents of the studied WWTPs, and most of them were not totally removed, and so, they were detected in the effluents. These results are in agreement with the detection of these FRs in river waters from Spain (Paper 5) and UK (Paper 4), since in all studied rivers, OPFR concentrations in water increased in locations downstream of the WWTPs. In addition, the most abundant OPFRs in effluents and in river water were similar, with TBEP and TCPP at the highest concentrations. TCEP, TDCP, and TBP were detected in effluents and in river water at intermediate levels, while TPhP, TCP, TEHP, and TCP were detected at the lowest concentrations. The lower observed levels of TPhP, TCP, TEHP, and TCP in effluents and in surface water is partly due their lipophilicity, since these compounds tend to accumulate and in river sediments and in WWTP sludge. In addition, another important factor affecting the fate of aryl phosphate esters is that they are more susceptible to photodegradation, hydrolysis and biodegradation processes than the other studied OPFRs [87], which affect their persistence in the aquatic environment.

Since TCEP, TCPP and TDCP pass through WWTPs unaffected, their effluents can also impact the raw water supplied for DWTFs. The evolution of OPFRs in a Spanish DWTF (see Paper 1, Chapter 2) indicated that the treatment was effective for the removal of these contaminants but their detection in the final water (TCEP and TCPP) indicated that a small percentage of these compounds remains in water.

The River Aire (UK) and River Besòs (Spain) are urban rivers highly affected by anthropogenic pressures, passing through urbanizations and receiving effluents of several WWTPs. Figure 18 presents a comparison of TCEP, TCPP, TDCP and TPhP levels in the rivers Aire and Besòs. Water samples were collected in 13 sampling points in each river. In the case of the river Besòs, five rivers that discharge in the River Besòs were included, which explain the high variation of OPFR concentrations for this river. The profile of OPFRs was the same for both rivers, with decreasing concentration as following: [TCPP] > [TCEP] > [TDCP] > [TPhP]. Except for TCPP, OPFR levels were also very similar among the rivers, with median and maximum concentrations at the same order of magnitude. TCPP presented much higher concentration in the River Aire (up to 26.05 µg L^{-1}) than in the River Besòs (up to 1.8 µg L^{-1}). In fact, this maximum TCPP concentration in the River Aire was about 4 to 15 times higher than the observed in influents from the studied WWTPs (Spain). TCPP profile in the River Aire indicated an important punctual source, which could be, discharges of treated or untreated industrial effluents as the UK has specific fire safety regulations for furniture [81]. TCPP is applied in flexible PU foam by UK foam manufactures in direct response to those regulations covering these goods. On the other hand, there are no European Directive concerning the flame retardancy of furniture, and so TCPP and other flame retardants tend to not be used in furniture and there is a much lower use of TCPP in Europe than in UK [81]. Concerning PBDEs, BDE-209 was detected in water column in the River Aire but not in the Spanish rivers, and its presence at high concentrations in water can also be related to the stricter fire safety regulations of the UK.

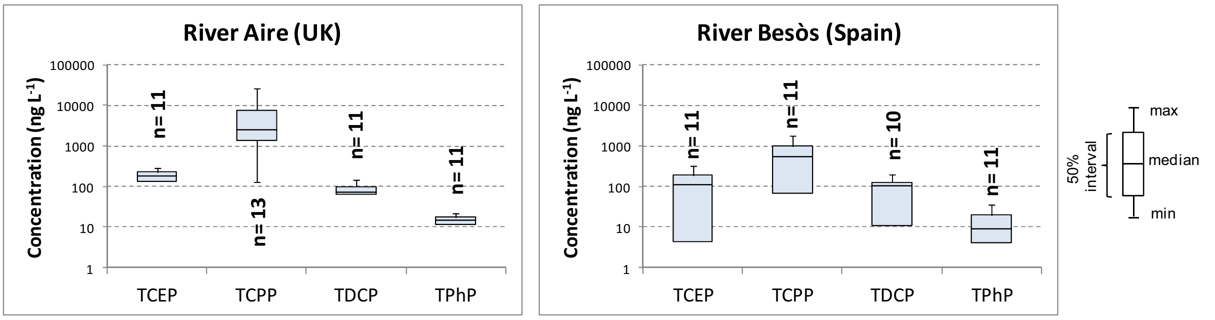


Figure 18 – TCEP, TCPP, TDCP and TPhP boxplot distribution for the rivers Aire and Besòs. n = number of observed detection. Thirteen sampled sites were monitored in each river.

Among the nine NBFRs studied in this thesis, few compounds were detected in environmental samples, and a low frequency of detection was observed. HBB and PBEB were seldom detected in the River Aire, at pg L^{-1} levels. No NBFRs were detected in water from the Spanish rivers. BEHTBP was the only NBFR detected in influents of studied WWTPs, but was not detected in effluents, which is in agreement with non-detection of BEHTBP in neither surface waters nor in sediments. The minor presence of NBFR in the aquatic environment points to the impression that either these compounds have not been used in Spain or the UK or either that they have not been used long enough or at sufficient amounts to pose an environmental problem.

Solid samples

When lipophilic flame retardants reach the aquatic environment they tend to associate to suspended particles and accumulate onto bottom sediments, and so the analysis of sediments is essential to evaluate their occurrence and fate. Besides sediments, the analysis of sewage sludge and dust allow the identification of the FRs that can potentially reach the aquatic environment. Domestic dust containing high amounts of FRs [21, 64-65] is a mobilization route of these compounds from houses. Once present in WWTPs, the most lipophilic flame retardants tend to accumulate in sewage sludge [107-108], and a small fraction can be released via effluents. During this study, sediments samples collected along the three studied Spanish Rivers, dust samples collected in five houses (Barcelona, Spain) and sewage sludge from five Spanish WWTPs (Catalonia) were analyzed in order to identify the most ubiquitous FRs and the ones that can pose a threat to the aquatic environment. A comparison among levels of flame retardants in river sediment, sewage sludge and domestic dust was presented in Paper 3 (Chapter 2). Interestingly, many flame retardants were present in dust and sludge samples at similar levels, while levels in river sediments, collected near high urbanized and industrial zones (River Besòs), where generally lower. The detection of FRs in sewage sludge at μg – mg kg^{-1} levels indicated that high amounts of these flame retardants reached WWTPs.

OPFRs were the most frequently detected FRs in sediment, sludge and dust. These compounds were the most abundant FRs in dust samples, which indicate the high use of these flame retardants/plasticizers in house products. Accumulation of OPFRs was observed in sewage sludge and sediments, where the most abundant compounds included water soluble compounds such as TiBP and TCPP, present at high concentration in river water (River Besòs) and wastewater, and lipophilic compounds such as TPhP, EHDP, TCP and TEHP, which are present in water (rivers and WWTPs) at low concentration due their higher log K_{ow}. TBEP accumulation was observed only in few sludge samples, and was not observed in sediments.

BDE-209 was detected in sediments in the River Besòs in Catalonia, and other PBDE congeners, such as BDE-99 and BDE-47, were detected at concentrations about 100 times lower. BDE-209 was also the most abundant congener in sewage sludge and dust. The predominance of BDE-209 over other PBDE congeners was not surprising, since the production volume of Deca-BDE was about 10 times higher than for Penta- and Octa-BDE formulations (estimated for 2001 [47]). In addition, Deca-BDE formulation is probably still used in the EU for applications other than in electric and electronic equipments. This profile in sediment, and sewage sludge was also reported in several studies in Europe, North America and China [192, 245-246].

Different profile in sediment, dust and sludge was observed for NBFRs. DBDPE was the most abundant compound (followed by BEHTBP) and the only NBFR detected in river sediments. BEHTBP was detected in most of sludge and dust samples, but a lower frequency of detection (40%) was observed for EHTBP (sludge and dust). BTBPE was detected in most of dust samples, but was detected in only one sludge sample. PBT and HBB were seldom detected in dust samples. These results indicate that, among the nine studied NBFRs, DBDPE and BEHTBP deserve special attention due their higher frequency of detection, and their high concentrations.

3.4.3 Risk assessment for aquatic organisms

In order to assess the risk that detected FRs in rivers can pose on aquatic organisms, the MEC (water and sediments) were compared to their estimated PNEC. Although OPFRs were detected in all studied rivers (UK and Spain) reaching μg L^{-1} levels, their concentrations were much lower than their respective PNEC, and in no case their RQ was higher than 1, which means that MEC was never higher than PNEC. On the other hand, RQ > 1 was obtained for BDE-209 in the River Aire (UK), indicating a potential risk for adverse effects to aquatic organisms.

The study performed with *D. magna* showed that OPFRs have similar mode of action (non-polar narcosis) and that their joint toxicity was additive. This means that no toxic concentrations of several OPFRs can reach toxic levels due the sum of their individual effects. These results are of concern considering that several organophosphates, including compounds not herein studied, can be simultaneously present in the aquatic environment of urban rivers. For example, Rodil et al. (2012) [34] reported the presence of another organophosphate triester (triethyl phosphate) and three organophosphate diesters (diethylhexyl phosphate, diphenyl phosphate and di-*n*-butyl phosphate) in WWTP effluents and in surface waters from Galicia (Spain). Small differences in the chemical structure can change the modes of action of compounds [247]. Thus, since only organophosphate triesters were included in the toxicity tests with *D. magna*, more experiments are necessary in order to evaluate if other organophosphate esters (di- and monoesters) present similar mode of action and if they also follow a concentration-addition toxicity pattern.

MEC of OPFRs in influents and effluents of studied WWTPs were compared to their respective PNEC in order to scale their environmental relevance in wastewater. For this purpose, the experimentally obtained EC_{50} for *D. magna* (Paper 5) were used for PNEC calculation, using $f = 1000$. Figure 19 presents the RQs calculated for OPFRs detected in influents and effluents of five Spanish WWTPs. Considering these nine OPFRs, RQ > 1 was observed for most WWTP influents and the highest value was observed in $WWTP_3$, due its high TiBP concentration. These results indicate that potential risks would be expected for *D. magna* if these organisms would be exposed to OPFR concentrations similar to the observed ones in WWTP influents. On the other hand, $\Sigma RQ < 1$ was observed for all effluents (except in $WWTP_3$) which indicated no risk. The reduction in the potential risk to *D. magna* in WWTP effluents is mainly attributed to the removal of EHDP, TCP and TEHP, the most toxic OPFRs. On the other hand, TiBP concentration in the effluent of $WWTP_3$ was high enough to exceed its PNEC (*D. magna*).

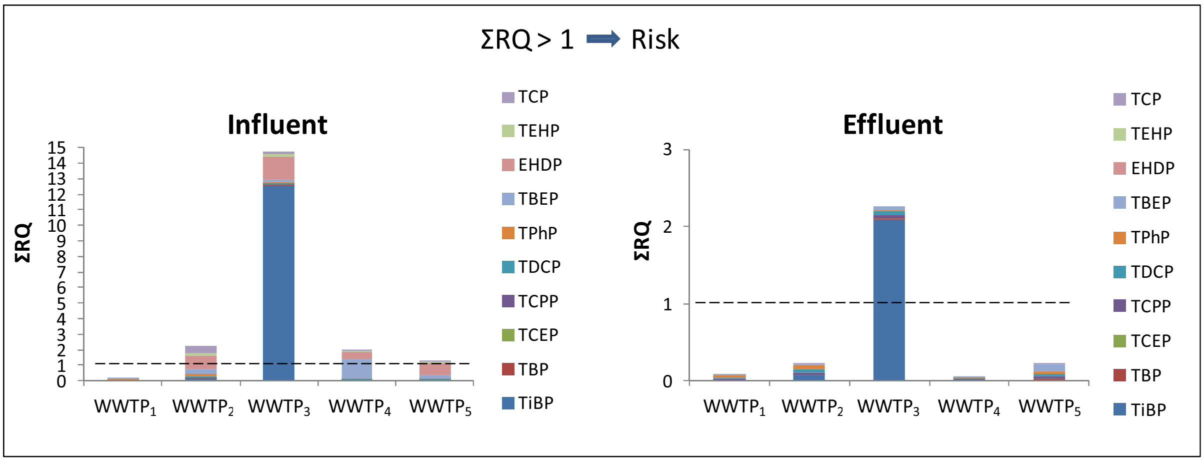


Figure 19 – Risk Quotient (RQ) obtained for OPFRs detected in influents and in effluents of five Spanish WWTPs, considering *D. magna* toxicity (EC_{50}).

The most important issue regarding discharges of WWTP effluents to receiving waters is the potential impact of toxic substances not eliminated (or generated) during treatment on the aquatic organisms. Even though dilution of effluents discharged to rivers, lakes and sea many times results in non toxic concentrations to aquatic organisms, the continuous input of persistent substances can produce negative effects in the ecosystem, such as accumulation and long term effects. An estimation of the impact of WWTP effluents on the receiving water was performed taking into account the water influent flow of each studied WWTP and the average concentration of OPFRs in the effluents. $WWTP_1$ and $WWTP_2$ discharge to rivers while the other three WWTPs discharge to the sea. Table 12 presents the estimated annual amount of OPFRs discharged to Catalan rivers (Segre and Sec) and to the Mediterranean Sea. These results indicate that compounds such as TCPP, TBEP and TiBP can reach an annual discharge of about half ton per year in some of studied WWTPs, while the observed annual emission for the other OPFRs ranged from 0.17 to 63 kg $year^{-1}$. About 3 tonnes $year^{-1}$ of ΣOPFRs were estimated to be annually discharged to waters only considering these five WWTPs. Taking into account OPFRs joint toxicity and that some of these substances, especially the chloroalkyl phosphates, are not quickly photo- or biodegraded [81, 83, 153], the continuous input of OPFRs to the aquatic environment needs be taken into account in environmental risk assessment studies in order to evaluate their potential long term impact to the aquatic ecosystem.

Table 12 – Annual discharge (kg $year^{-1}$) of OPFRs to waters receiving WWTP effluents.

	TCPP	TCEP	TDCP	TBEP	TBP	TiBP	TPhP	TCP	ΣOPFRs
$WWTP_1$	77	8.0	4.3	6.6	0.70	–	1.3	–	98
$WWTP_2$	36	4.5	4.3	7.5	1.1	8.9	0.77	–	63
$WWTP_3$	77	12	6.6	41	3.6	479	0.77	0.17	620
$WWTP_4$	429	37	32	230	21	35	8.4	–	792
$WWTP_5$	594	63	33	690	12	36	13	–	1441
$\Sigma WWTP_{1\text{-}5}$	1213	125	80	975	38	559	24	0.17	3015

4 Conclusions

The major conclusions that can be drawn from the studies presented in this thesis can be summarized as follows:

1. A comparison of the instrumental performances of GC-EI-MS/SIM, GC-EI-MS/MS and GC-ECNI-MS/SIM methods revealed that both ionization techniques (EI or ECNI) and both acquisition modes (SIM and SRM) can be applied for the multiresidue determination of flame retardants in environmental samples. GC-ECNI-MS/SIM presented the lowest instrumental detection limits but provides low spectral information for many NBFRs and PBDEs. GC-EI-MS/MS allow simultaneous detection of OPFRs, NBFRs and PBDEs and proved to be very advantageous in terms of selectivity.

2. The developed method for the simultaneous extraction of OPFRs, NBFRs and PBDEs in river water and wastewater (influents and effluents) using SPE with HLB cartridges was efficient for the multiresidue analysis of flame retardants differing in polarity and log K_{ow}. Using GC-EI-MS/SIM and GC-EI-MS/MS, target compounds can be identified and quantified with good accuracy.

3. The ceramic dosimeter passive samplers proved to be promising for the analysis of OPFRs in river water, allowing the estimation of integrated water concentrations for a deployment time of about 3 weeks.

4. A method based on ultrasound extraction with ethyl acetate/cychlohexane (5:2, v/v) and clean-up using florisil cartridges, and determination by GC-EI-MS/MS was efficient and robust and allowed the determination of OPFRs, NBFRs and PBDEs in river sediment, sewage sludge and dust.

5. OPFRs and BEHTBP were detected in influents of WWTPs, and most of these compounds were detected in the effluents, except for BEHTBP, TCP, EHDP and TEHP. No removal was observed for TCEP, TCPP and TDCP, while TBEP, TBP and TPhP were partially eliminated from waters. Accumulation of OPFRs, PBDEs, BEHTBP, and DBDPE was observed in sewage sludge.

6. Among all FR studied, OPFRs were the most ubiquitous contaminants in surface waters. Their presence was correlated to anthropogenic sources, and their levels increased near urban and industrial areas. TCPP and TBEP were the most abundant compounds, followed by TBP, TiBP, TCEP, and TDCP, while the minor compounds

in water were TPhP, TCP, TEHP and EHDP. WWTPs were identified as the main source of these contaminants to receiving waters. Accumulation of lipophilic OPFRs (TCP, EHDP, TPhP and TCP) was observed in river sediments at sites impacted by anthropogenic pressures.

7. PBDEs were detected in the aquatic system of Spain and UK. BDE-209 was detected in water in the River Aire (UK). No PBDEs were detected in waters from Spanish rivers, but were present in sediments from the River Besòs (Catalonia), where BDE-209 was the most abundant congener.

8. Low frequency of detection was observed for NBFRs in the studied rivers. HBB and PBEB were detected in waters from the River Aire. DBDPE was the only NBFR detected in river sediments (River Besòs). The minor presence of NBFR in the aquatic environment points to the impression that either these compounds have not been used in Spain or the UK or either that they have not been used long enough or at sufficient amounts to pose an environmental problem.

9. Comparing Spanish and English river waters, OPFRs were the most abundant contaminants in both countries, while differences in concentrations of NBFRs and PBDEs were observed, probably due different applications of FRs in finished products in each internal market.

10. The toxicity test performed with *D. magna* allowed the determination of lethal concentrations for each individual OPFRs. Obtained EC_{50} were well correlated with their lipophilicity (log K_{ow}), which indicate that they may act by non-polar narcosis. The study of joint effects indicated that these compounds present similar mode of action and the results were well correlated to the concentration-addition model, which means that their toxicity is additive. Thus joint toxicity of OPFRs at low concentrations can produce toxic effects in the aquatic environment.

11. A risk assessment performed for the studied river basins indicated no risk associated to observed OPFR levels in waters. A potential risk for adverse effects was estimated for the River Aire (UK) associated to the concentration of BDE-209 in water.

5 Resumen de la Tesis

5.1 Introducción y objetivos

Los retardantes de llama son compuestos químicos utilizados para disminuir la inflamabilidad de materiales varios, tales como polímeros, tejidos y espumas utilizados en equipos eléctricos y electrónicos, ordenadores, vehículos, muebles, materiales para la construcción, etc. Estos compuestos se aplican a los productos para cumplir las normas de seguridad y así prevenir los incendios accidentales y proteger a los seres humanos.

Se emplean como retardantes de llama compuestos orgánicos o inorgánicos que contienen bromo, cloro, fósforo, nitrógeno y boro, además de los hidróxidos metálicos. Estos compuestos están disponibles comercialmente para su utilización en materiales de distintas características. Los retardantes de llama se pueden clasificar en dos grupos, los aditivos (no ligados químicamente al polímero) y los reactivos (añadidos durante el proceso de polimerización y modificando en consecuencia la estructura del polímero). Hoy en día se consumen en el mundo unos 2 millones de toneladas al año de retardantes de llama, siendo el hidróxido de aluminio el compuesto más utilizado (40,4%), seguido de los compuestos bromados (19,7%), los organofosforados (OPFRs) (14,6%) y los clorados (11,3%). Los retardantes de llama actúan en las diferentes etapas del ciclo del fuego (calentamiento, descomposición, ignición y propagación de la llama) e interrumpen química o físicamente el proceso de combustión. Según su composición química, los retardantes de llama pueden actuar en la fase gaseosa secuestrando a los radicales de elevada energía ($H^•$ y $OH^•$) e interrumpiendo las reacciones en cadena altamente exotérmicas (actúan así por ejemplo, los compuestos halogenados). Otra posible vía de actuación es sobre la fase condensada, y en este caso forman una capa carbonosa en la superficie del polímero la cual absorbe parte del calor y reduce el flujo de energía que promueve la emisión de gases inflamables (los compuestos organofosforados son un ejemplo de este tipo de comportamiento).

A pesar de los beneficios asociados al uso de retardantes de llama, que han permitido una disminución del número de muertes y de lesiones debidas a los incendios, algunos de estos compuestos son tóxicos y bioacumulables. Los retardantes de llama halogenados (especialmente los bromados) y los retardantes de llama organofosforados (OPFRs) son considerados como una amenaza para el medio ambiente, dado que son persistentes y están presentes en todos los compartimentos ambientales. Los retardantes de llama son emitidos por los materiales que los contienen, y por los efluentes de las industrias que los fabrican o que los utilizan en sus productos. Una vez al medio, pueden ser transportados por el aire y llegar a zonas remotas, acumularse en suelos y en el medio acuático. Además, los efluentes de las depuradoras son importantes fuentes de estos contaminantes hacia el medio acuático, aunque los compuestos más lipofílicos se acumulan en los lodos.

Los éteres de polibromodifenilo (PBDEs) son hasta la fecha los compuestos más estudiados debido a su elevada utilización y toxicidad. Actualmente, el uso de formulaciones de PBDEs están prohibidas o reguladas en diversos países. Además, los PBDEs están incluidos en el segundo listado de contaminantes orgánicos persistentes de la Convención de Estocolmo. En consecuencia, el uso de nuevos retardantes de llama bromados (NBFRs) y de OPFRs ha aumentado. Por esta razón, actualmente existe una gran preocupación en relación a la presencia de NBFRs y los OPFRs en el medio ambiente debido a que son compuestos tóxicos y varios estudios demuestran que algunos de estos compuestos se bioacumulan y se pueden biomagnificar en los seres vivos, y además son transportados por la atmosfera a larga distancias.

Los ríos que fluyen por los centros urbanos e industriales reciben grandes cantidades de contaminantes que llegan a sus aguas principalmente por medio de los efluentes, por el transporte atmosférico y por las escorrentías. Dependiendo de la intensidad y la duración de la exposición a una sustancia tóxica, se pueden producir efectos directos para los organismos acuáticos (por ejemplo, aumento de la mortalidad, la reducción de la fecundidad, el estrés fisiológico, etc.), lo que puede iniciar efectos tróficos en cascada. Por lo tanto, los estudios relacionados con la presencia, la persistencia, el destino, la toxicidad y el riesgo asociado a los retardantes de llama en el medio acuático permitirán identificar los compuestos más peligrosos y contribuir a la protección y gestión medioambiental.

El objetivo general de esta tesis doctoral es estudiar la presencia e impacto de los retardantes de llama prioritarios y emergentes en el medio acuático. Para alcanzar este objetivo principal, se han desarrollado métodos multiresiduo para el análisis de agua de río y agua residual, sedimentos, polvo y lodos de depuradora. Se han optimizado las condiciones de extracción y análisis con el fin de determinar de forma simultánea las diferentes familias de retardantes de llama en muestras ambientales. En segunda instancia se ha estudiado la presencia y distribución de los PBDE, NBFRs y OPFRs en los ríos españoles y del Reino Unido con el propósito de identificar las zonas más contaminadas a lo largo de los ríos, las fuentes de contaminación, y evaluar los riesgos potenciales para los organismos acuáticos. Además, se ha estudiado la presencia y la eliminación de estos compuestos en las plantas de tratamiento de aguas residuales.

5.2 Desarrollo analítico

La primera etapa de este estudio consistió en optimizar las condiciones instrumentales para la determinación simultánea de todos o la mayor parte de los compuestos propuestos. Este primer estudio incluyó 4 OPFRs (tris(2-choroetil) fosfato (TCEP), tris(2-cloro-1-metiletil) fosfato (TCPP), tris[2-cloro-1-(clorometil)etil] fosfato (TDCP), trifenilfosfato (TPhP)), 9 NBFRs (pentabromotolueno (PBT), pentabromoetilbenceno (PBEB), 2,3-dibromopropil 2,4,6 tribromofenil éter (DPTE), hexabromobenceno (HBB), hexacloro ciclopentadienil dibromooctano (HCDBCO), 1,2-bis(2,4,6-tribromofenoxi)etano (BTBPE), 2-etilhexil-2,3,4,5-tetrabromobenzoato (EHTBB), bis(2-etilhexil)tetrabromo ftalato (BEHTBP), decabromodifeniletano (DBDPE)), 8 PBDEs (BDE-28, 47, 99, 100, 153, 154, 183, 209), 3 bromotoluenes, 4 bromoanilinas, 2 bromoanisoles, 8 bromofenoles y estándares marcados isotópicamente. Teniendo en cuenta la complejidad de las muestras ambientales y la baja concentración prevista de los analitos de interés, este estudio tuvo como objetivo obtener el mejor equilibrio entre los límites de detección y la selectividad.

Se eligió la cromatografía de gases (GC) como técnica de separación. Se utilizó una columna DB5-MS de 15 m (longitud) x 0,250 mm (ID) x 0,10 m (película) dado que esta columna reduce la degradación térmica del BDE-209 y del DBDPE durante el análisis. Se optimizó la rampa de temperatura del horno para obtener una separación satisfactoria y una resolución cromatográfica adecuada. Además, se optimizaron las condiciones de detección por espectrometría de masas (MS), utilizando un detector de triple cuadrupolo. Se compararon dos modos de ionización: ionización electrónica (EI) e ionización negativa por captura de electrones (ECNI). Para ello, se realizó un estudio del perfil de fragmentación de cada compuesto, utilizando cada modo de ionización, que se llevó a cabo utilizando soluciones estándar. Además, se compararon dos modos de adquisición de datos: monitoreo de iones selectivos (SIM) y monitoreo de reacciones selectivas (SRM), utilizando la espectrometría de masas en modo tándem (MS/MS). Los tres métodos desarrollados, GC-EI-MS/SIM, GC-EI-MS/MS y GC-ECNI-MS/SIM, se compararon en relación a los límites de detección instrumentales (iLOD), selectividad, reproducibilidad y repetitividad. Para los compuestos bromados, los límites de detección más bajos se obtuvieron por medio de GC-ECNI-MS/SIM, monitorizando los iones *m/z* 79 y 81 $[Br]^-$. Sin embargo, muchos retardantes de llama bromados no presentan ningún otro ión característico con una abundancia superior a un 20% para su uso como ión de confirmación. La adquisición del ión $[Br]^-$ como único ión para los PBDEs y los NBFRs puede dar lugar a falsos positivos, ya que la co-elución de cualquier compuesto conteniendo átomos de bromo en su estructura puede interferir en los resultados. Además, el método GC-ECNI-MS/SIM no permitió la detección del TCEP y TCPP con las condiciones utilizadas. Sin embargo, los métodos basados en EI permitieron obtener una mayor selectividad, ya que se utilizaron tres iones selectivos para cada compuesto utilizando GC-EI-MS/SIM, o dos transiciones específicas para el método GC-EI-MS/MS. La selectividad se mejora cuando se trabaja en el modo SRM, dado que los iones más abundantes sufren una fragmentación adicional que generan iones característicos. Además esta técnica disminuye mucho el ruido de fondo, y como consecuencia se disminuyen los límites de detección. Por lo tanto, GC-EI-MS/MS fue el método elegido para el análisis de las distintas familias de retardantes de llama en muestras ambientales, ya que esta técnica permitió una determinación simultánea de todos los compuestos seleccionados con alta selectividad y ha permitido la identificación de los compuestos a niveles de pg. Mencionar también que fue posible analizar los bromofenoles utilizando GC-MS sin derivatización previa, pero los límites de detección fueron superiores a los publicados en la literatura para bromofenoles analizados con GC-MS previa derivatización.

En cuanto a la extracción de la muestra, se llevó a cabo la optimización de un método para la preconcentración simultánea de las diferentes familias de retardantes de llama en agua. Para este propósito, se utilizaron los cartuchos de extracción en fase solida (SPE) conteniendo el adsorbente hidrófilo-lipófilo (HLB), que son adecuados para análisis multiresiduos de compuestos orgánicos de distintas polaridades. Teniendo en cuenta que los PBDEs y los NBFRs están preferiblemente asociados a las partículas en suspensión, las muestras no fueron filtradas para evitar la subestimación de su concentración. Se utilizaron cartuchos HLB 60 mg (Oasis, Waters) que se acondicionaron con 10 mL de hexano seguido de 10 mL de diclorometano, 10 mL de metanol y 15 mL de agua HPLC. Utilizando un manifold, se preconcentraron 100 mL de la muestra de agua previamente fortificada con los patrones de recuperación (MHBB, MBDE-77 y MBDE-209), a un flujo aproximado de 5 mL min^{-1}. A continuación, se secaron a los cartuchos utilizando un sistema de vacío. La elución se realizó con 10 mL de diclorometano/hexano (1:1, v/v) seguido de 10 mL de diclorometano/acetona (1:1, v/v). Se concentraron los extractos bajo un flujo de nitrógeno hasta un volumen a 250 µL. Se estudió la eficiencia del método utilizando agua Milli-Q enriquecida con los patrones a niveles de 0,4 hasta 2,5 µg L^{-1}. El método fue satisfactorio para los OPFRs, PBDEs y NBFRs, con recuperaciones entre un 70 y un 120% para la mayoría de los compuestos. Sin embargo, la mayoría de los bromofenoles, bromoanilines y bromoanisoles analizados presentaron una recuperación entre un 50 y un 70%. El método propuesto se aplicó al monitoreo de retardantes de llama en una planta de tratamiento de agua potable. Los OPFRs fueron los únicos compuestos detectados en los influentes, a niveles de 0,32 a 0,03 µg L^{-1}. Se observó su eliminación durante el tratamiento, pero se detectaron el TCEP y el TCPP a niveles de ng L^{-1} en el agua final. Estos resultados, y los resultados del desarrollo de método instrumental descrito anteriormente, se incluyen en el Artículo 1 del Capítulo 2 de esta memoria.

Una vez optimizado el método analítico para la determinación de los OPFRs, NBFRs y PBDEs, y teniendo en cuenta que no se detectaron los bromofenoles, bromoanilines, bromotoluenos y bromoanisoles en los influentes de la potabilizadora, no se incluyeron estos compuestos en los demás estudios llevados a cabo en esta tesis. Sin embargo, se incluyeron otros seis OPFRs debido a su frecuente detección en el medio ambiente acuático según la literatura. Los OPFRs incluidos fueron: tributil fosfato (TBP), tri-*iso*-butil fosfato (TiBP), tricresil fosfato (TCP), tris(2-butoxietil) fosfato (TBEP), 2-etil-hexil-difenil fosfato (EHDP), tris-2-etilhexil fosfato (TEHP).

Para el análisis de OPFRs (incluyendo los 6 nuevos compuestos), NBFRs y PBDEs en agua de río y agua residual, se utilizaron cartuchos SPE HLB 200 mg (OASIS, Waters), que poseen una capacidad mayor que los utilizados en el estudio para aguas potables (HLB, 60 mg), dado que esta vez se propuso extraer una cantidad superior de muestra. Se evaluaron la eficiencia del método y el efecto de la matriz utilizando agua de río (500 mL) y agua residual (influente - 100 mL, efluente - 250 mL) fortificadas con los patrones (de 0,02 hasta 0,2 µg L^{-1} para agua de río, de 0,1 hasta 2,5 µg L^{-1} para los influentes, de 0,04 hasta 1 µg L^{-1} para los efluentes) y con los patrones de recuperación isotópicamente marcados (TPhP-D15, TBP-D27, MHBB, MBDE-77 y MBDE-209). Se obtuvieron recuperaciones entre un 80 y un 120%, con una variación estándar relativa (RSD) menor que 20% en la mayoría de los casos, lo que indica que el método fue eficiente para el análisis de las diferentes familias de retardantes de llama en los distintos tipos de agua. Sin embargo, el TEHP presentó una recuperación pobre para los influentes de depuradoras (29 ± 2%), mientras que la recuperación para el agua de río y para los efluentes fueron aceptables. Teniendo en cuenta la complejidad de los diferentes tipos de agua estudiados, que difieren en la concentración de partículas en suspensión y el contenido de carbono orgánico disuelto (DOC), el uso de patrones de recuperación fue mandatorio para controlar el comportamiento del método y corregir las variaciones en el rendimiento del método SPE, y también para corregir efectos de la matriz sobre la respuesta cromatográfica.

Además, se desarrolló un método para la determinación simultánea de las diferentes familias de retardantes de llama estudiadas en sedimentos de río, en lodos de depuradora y en polvo. Se realizó la extracción utilizando un baño de ultrasonidos y una mezcla de acetato de etilo/ciclohexano (5:2, v/v), que proporcionó mejores rendimientos que el uso de hexano/acetona. La purificación de los extractos se llevó a cabo utilizando cartuchos de florisil (10 g o 5 g). Se utilizaron diferentes estrategias para el desarrollo del método con el fin de obtener la exactitud y la precisión adecuadas. Para ello, se utilizaron muestras enriquecidas con los compuestos objeto de estudio a dos niveles de concentración y por triplicado. Para los OPFRs en sedimentos y polvo, la validación se llevó a cabo mediante la participación en un estudio interlaboratorio. Además, se evaluó la precisión del método para PBDE en polvo usando un material de referencia certificado (SRM 2585, NIST). El método de extracción y la determinación utilizando GC-EI-MS/MS permitieron el análisis simultáneo de todos los compuestos propuestos. Además, debido a la elevada selectividad del método MS/MS, fue posible el análisis de las distintas familias de retardantes de llama en matrices complejas, como son los sedimentos, lodos y polvo, utilizando únicamente una etapa de purificación de los extractos (cartuchos florisil). Las recuperaciones obtenidas para muestras de distinto tipo, sedimentos, lodos y polvo enriquecidas, fueron similares (entre un 70 y un 120%, con una RSD hasta un 20% para la gran mayoría de los compuestos) lo que indica la robustez del método. Cabe señalar sin embargo que el TBEP presentó menores recuperaciones, que variaron entre un 48 y un 69% en sedimentos y lodos, y que no fue posible calcular la recuperación de este compuesto en el polvo debido a las elevadas cantidades de este compuesto presente en la matriz. Además, se obtuvieron resultados satisfactorios en el ejercicio interlaboratorio, con errores entre un 0 y 18% en relación al valor asignado para la solución patrón, entre un 0 y 30% para los sedimentos (excepto TCEP que presentó un error de 49%) y entre un 0,5 y 11% para la muestra de polvo, excepto para el TBEP que presentó error de un 80%. Para los PBDEs en el material de referencia certificado (polvo), se obtuvieron errores entre un 1,5 y 18% y por lo tanto el rendimiento se consideró satisfactorio. En resumen los resultados permiten proponer un método robusto y eficaz para el análisis multiresiduo de OPFRs, PBDEs y NBFRs en sedimentos, lodos y polvo a niveles traza. En cuanto a la aplicabilidad del método, esta se evaluó analizando muestras de sedimento de río, lodo de depuradora y polvo de las casas. El método permitió la detección de 24 de los 27 compuestos propuestos en las muestras analizadas. Los resultados del desarrollo del método se incluyen en el Artículo 3 del Capítulo 2.

Muchos trabajos en la literatura indican la detección de los OPFRs en los blancos de laboratorio por lo que en esta tesis se han analizado siempre blancos de laboratorio juntamente con las muestras analizadas. Además, se han tomado precauciones para minimizar la contaminación de los blancos, que consisten calentar en la mufla a 350 ºC durante 8 horas todo el material de vidrio (tubos de centrífuga, viales y pipetas), lavar a el material de vidrio aforado con disolvente antes de usarlo, y evitar el uso de materiales plásticos. Además, las muestras y el material de vidrio se taparon con papel de aluminio durante las etapas de extracción, para evitar la deposición de partículas de polvo. Sin embargo, y a pesar de tomar estas precauciones, se detectaron sistemáticamente los OPFRs en los blancos de laboratorio. Por lo tanto, se tuvo en cuenta la contribución de los blancos para calcular los limites de detección del método, que fueron calculados como la media de los compuestos en las réplicas de los blancos más tres veces la desviación estándar.

Se desarrolló un método de muestreo pasivo para la determinación de 4 OPFRs y 2 NBFRs en agua de río. Este estudio se realizó durante una estancia en la Universidad de Lancaster (Reino Unido). Las ventajas de esta tecnología de muestreo incluyen: una pre concentración "*in situ*" de los analitos en el dispositivo de muestreo pasivo, la reducción y/o eliminación de las interferencias de la matriz, la rentabilidad, la no utilización de fuentes de energía para la operación, y la estimación de la concentración media en el agua durante tiempo de muestreo. Este último punto proporciona una información valiosa para los programas de vigilancia ambiental dado que la concentración de los contaminantes en el agua puede variar de forma continua. El muestreador pasivo utilizado fue el "*ceramic dosimeter*", que es un muestreador cerámico en formato cilíndrico donde en el interior se introduce un adsorbente capaz de preconcentrar los analitos. La membrana cerámica actúa como barrera de difusión y además separa el medio acuoso de la fase receptora (contenida dentro del muestreador pasivo). Los compuestos estudiados utilizando el muestreador pasivo fueron el TCEP, TCPP, TDCP, TPhP, PBEB y HBB. La principal ventaja de este dispositivo de muestreo es que la tasa de absorción de los compuestos en el dispositivo de muestreo es independiente del flujo del agua, simplificando los experimentos de calibración. Se utilizó el adsorbente HLB como fase receptora debido a su buen rendimiento de extracción para los retardantes de llama polares y apolares, tal como se había comprobado anteriormente. Se llevó a cabo la calibración del muestreador pasivo en condiciones de laboratorio con el fin de determinar la recuperación analítica, la capacidad del muestreador, su estabilidad y la tasa de muestreo para cada analito. Para realizar la calibración, se utilizaron 5 contenedores, cada uno conteniendo 1.8 L de agua enriquecida con los compuestos de interés (TCEP, TCPP, TDCP y TPhP – 20 $\mu g\ L^{-1}$, PBEB y HBB – 1 $\mu g\ L^{-1}$) y un par de muestreadores Los muestreadores se recogieron pasados 2, 4, 6, 8 y 10 días. Al final de cada etapa del experimento, se extrajo el adsorbente del interior de cada muestreador y se analizó. Se determinaron las prestaciones del muestreador mediante el cálculo de la tasa de muestreo de los compuestos en relación al tiempo de exposición, y los resultados obtenidos fueron: TCEP – 3,7 mL dia^{-1}, TCPP – 2,7 mL dia^{-1}, TDCP – 2,6 mL dia^{-1}, TPhP – 2,4 mL dia^{-1}, HBB – 0,39 mL dia^{-1}, PBEB – 0,66 mL dia^{-1}. Después de la etapa de calibración, se implementaron seis muestreadores pasivos en un río, los cuales se retiraron del agua pasadas 1 (n=1), 3 (n=2) y 5 (n=3) semanas. Asimismo, se tomaron diversas muestras puntuales durante el tiempo de exposición de los muestreadores pasivos, con el fin de comparar los resultados obtenidos con el "*ceramic dosimeter*" y los resultados obtenidos para las muestras de agua puntuales extraídas usando SPE. El muestreador pasivo permitió la detección de todos los OPFRs estudiados (TCEP, TCPP, TDCP y TPhP) en el agua del río, y las concentraciones estimadas utilizando el dispositivo de muestreo pasivo estaban de acuerdo con las concentraciones obtenidas para las muestras de agua puntuales. Los resultados mostraron la eficacia del diseño del "*ceramic dosimeter*" para su implementación para el seguimiento de OPFR en agua superficiales, hasta un periodo de muestreo de 3 semanas. Tiempos de exposición superiores producían un efecto de "*fouling*" que afectaba la adsorción de los compuestos, disminuyendo las tasas de muestreo. Sin embargo, el efecto del "*fouling*" debe ser estudiado con más profundidad para poder controlar los fenómenos de superficie que pueden afectar la eficiencia del muestreador. Estos efectos incluyen la formación de biopelículas, la biodegradación en la biopelícula y la precipitación de minerales que taponan los poros de la pared cerámica del muestreador. Ese trabajo y sus resultados se describen en el Artículo 2.

Los métodos desarrollados para agua (SPE) y para sedimentos y lodos fueron utilizados para la vigilancia ambiental de OPFRs, NBFRs y PBDEs en ríos y depuradoras.

5.3 Estudio de vigilancia ambiental

El primer estudio de vigilancia ambiental se llevó a cabo en el río Aire, Reino Unido, durante una estancia en la Universidad de Lancaster (UK). El Aire es un río importante en Yorkshire, tiene una extensión de usos 114 kilómetros. Históricamente, este río sufrió elevadas presiones antropogénicas, y hace unos veinte años fue considerado muerto ya que ningún pez podía sobrevivir en las zonas donde se descargaban los efluentes de aguas residuales (no tratados). Actualmente se encuentran a lo largo del río varios puntos de descarga de depuradora. Además, el río fluye por una de las zonas más pobladas del Reino Unido, Oeste de Yorshire (2.709.211 habitantes), y atraviesa ciudades tales como Leeds

(443.247 habitantes), Keighley (49.453 habitantes) y Castleford (37.525 habitantes), entre otras. Para el estudio de vigilancia ambiental, se colectaron muestras de agua en 13 puntos distribuidos a lo largo del río Aire. El muestreo se realizó durante la primavera de 2011. Los puntos de muestreo incluyeron un sitio cerca del nacimiento del río, y otros en las cercanías de las descargas de las plantas de tratamiento de agua residual, cubriendo un tramo de > 100 km, desde Malhan a Eggborough. Las muestras de agua se extrajeron utilizando el método SPE para agua de río descrito anteriormente para PBDEs, NBFRs y OPFRs (no se incluyeron en este estudio el TBEP, TEHP, TCP, TBP, TiBP y EHDP). Los extractos de las muestras se analizaron mediante GC-EI-MS/SIM desarrollado previamente.

Se detectaron los OPFRs en todos los puntos de muestreo. El TCPP fue el compuesto más abundante, seguido por el TCEP, TDCP y el TPhP. Las concentraciones del TCPP variaron entre 0,113 y 26,05 μg L^{-1}, y fue el único compuesto detectado en el nacimiento del río Aire. Se observó que la concentración del TCPP fue muy elevada en un cierto tramo, seguido de un descenso de las concentraciones a lo largo del río. Este comportamiento se atribuyó a una posible fuente puntual de este compuesto, como por ejemplo las actividades industriales en las que se utilizan a los retardantes de llama en la fabricación de productos. Además, las concentraciones máximas observadas para el TCPP en el río Aire fueron más elevadas que las observadas en ríos de Alemania, Austria y España. Sin embargo, las concentraciones de TCEP (<MDL – 0,28 μg L^{-1}), TDCP (<MDL – 0,14 μg L^{-1}) y TPhP (<MDL – 0,021 μg L^{-1}) fueron comparables a los niveles observados en otros ríos Europeos. Las descargas de las plantas de tratamiento de agua residual se atribuyeron como las principales fuentes de OPFRs, ya que se observó un aumento de las concentraciones en los puntos de muestreo situados después de las descargas de los efluentes de las depuradoras, a lo largo del río. Además, el BDE-209 fue el único PBDE detectado a lo largo del río Aire. Las concentraciones del BDE-209 en agua (fase disuelta + fase de particulada) oscilaban entre 0,017 y 0,295 μg L^{-1}. Estas concentraciones son de 10 a 100 veces más elevadas que los niveles publicados para este mismo compuesto en el río Sena (París, Francia). Las concentraciones más elevadas del BDE-209 se detectaron en los puntos situados después de la confluencia del río Aire con el río Calder. Se detectaron el PBEB y el HBB en algunas de las muestras, y sus concentraciones variaron de 0,00016 - 0,0004 μg L^{-1} (n = 5) y 0,00076 μg L^{-1} (n = 1), respectivamente. El resto de NBFRs no se detectaron en el río Aire.

Se llevó a cabo una evaluación del riesgo para los organismos acuáticos, teniendo en cuenta la concentración de los retardantes de llama más detectados en el agua (TCEP, TCPP, TDCP, TPhP y BDE-209) a lo largo del río Aire. La evaluación de riesgo se realizó considerando las concentraciones de toxicidad aguda (LC_{50} o EC_{50}) para peces, *Daphnia* y algas, según los datos de toxicidad disponibles en la literatura. Se calcularon los coeficientes de riesgo (RQ) para cada compuesto y para cada punto de muestreo. El RQ se obtuvo por medio de la relación entre la concentración medida (MEC) y la concentración sin efecto previsible (PNEC). Los PNECs, a su vez, se obtuvieron utilizando el valor de toxicidad aguda dividido por un factor de seguridad. En este trabajo se utilizó un factor de seguridad de 1000, según la recomendación de la Unión Europea. Un $RQ > 1$ indica posibles efectos negativos para los organismos acuáticos, dado que el MEC supera PNEC. Se obtuvieron $RQ < 1$ para todos los OPFRs y en todos los puntos de muestreo, lo que indicó que no se espera un riesgo elevado relacionado con la presencia de estos compuestos en agua. Sin embargo, se obtuvo un $RQ > 1$ para el BDE-209 en la mayoría de los puntos de muestreo, lo que indica que sus concentraciones en el río Aire pueden afectar negativamente a los organismos acuáticos. El trabajo de vigilancia ambiental en el río Aire (EU) se incluye en el Artículo 4 del Capítulo 3 de esta memoria.

El segundo estudio de vigilancia ambiental se llevó a cabo en tres ríos de España, el río Arga (Navarra), Nalón (Asturias) y Besós (Catalunya). El nacimiento de estos ríos se encuentra en las zonas montañosas, en general, en zonas de baja actividad humana o Parques Naturales. Estos ríos circulan a través de áreas industriales, agrícolas y urbanas, afectando a la calidad del agua, lo que depende en gran medida de las condiciones geográficas y climatológicas de cada cuenca. Para llevar a cabo el estudio se colectaron aguas y sedimentos desde el nacimiento hasta la desembocadura de cada uno de estos ríos. Las muestras de agua y los sedimentos se analizaron utilizando el método GC-EI-MS/MS desarrollado.

Los OPFR se detectaron en las aguas de los tres ríos estudiados, mientras que los PBDE y NBFRs no se detectaron. El TCPP y TBEP fueron los contaminantes más abundantes en la mayoría de las muestras, con concentraciones de 0,0083 hasta 4,6 $\mu g\ L^{-1}$. El TiBP, TBP, TCEP y TDCP presentaron concentraciones intermedias, que variaron desde 0,0016 hasta 1,2 $\mu g\ L^{-1}$, mientras que EHDP, TPhP, TEHP y TCP se detectaron en las concentraciones más bajas, entre 0,0010 y 0,046 $\mu g\ L^{-1}$. Considerando los tres ríos estudiados, el Besós fue el más contaminado, seguido por el Arga, mientras que se observaron concentraciones menores en el río Nalón. El perfil de concentraciones observados a lo largo de cada río indicó claramente el impacto antropogénico que las zonas industriales y urbanas representan. En todos los casos, se observaron bajas concentraciones (o no detección) de los OPFRs cerca del nacimiento de los ríos. Sin embargo, las concentraciones aumentaron en los puntos situados en zonas urbanas y después de las descargas de las depuradoras, mientras que se encontraron los niveles más altos en las proximidades de zonas industriales.

Los OPFRs fueron los retardantes de llama más ubicuos detectados en las muestras de sedimentos de los tres ríos estudiados. A diferencia de las muestras de agua, el TEHP, EHDP, TCP y TPhP se detectaron con elevada frecuencia en los sedimentos, atribuido a su carácter lipofílico. Se detectaron a niveles que variaron desde 2,1 hasta 290 µg kg^{-1}. El TCPP se detectó en la mayoría de las muestras de sedimento, con concentraciones desde 13 hasta 365 µg kg^{-1}. Por último, el TBP, TiBP, TDCP y TCEP se detectaron en algunas de las muestras a concentraciones que variaron desde 2,2 hasta 13 µg kg^{-1}. Sin embargo, el TBEP no se detectó en sedimentos. Siguiendo la misma pauta que las muestras de agua, los niveles más elevados de contaminación se observaron en el río Besós, seguido del río Arga.

Los PBDEs se detectaron solamente en los sedimentos del río Besós, lo que se atribuye al mayor impacto antropogénico que sufre este río debido a su paso por zonas urbanas y industriales, y debido a su volumen de agua diez veces menor en comparación con los otros ríos. El total de PBDEs (ΣPBDEs) osciló entre <MDL a 812 µg kg^{-1}. El BDE-209 se detectó a las concentraciones más elevadas, que variaron desde 196 hasta 807 µg kg^{-1}, mientras que las concentraciones de los otros congéneres de PBDEs variaron entre 1,3 y 44 µg kg^{-1}. El único NBFR detectado fue el DBDPE, presente en dos muestras de sedimentos del río Besós (91 µg kg^{-1} y 435 µg kg^{-1}). Estas dos muestras de sedimentos también presentan los niveles más altos de BDE-209, lo que podría indicar el uso de ambos compuestos o el reemplazo del BDE-209 por el DBDPE en las aplicaciones industriales o productos que se producen en la zona.

Teniendo en cuenta que los OPFRs fueron los contaminantes más ubicuos en todos los ríos, y que las mezclas de diferentes OPFRs coexisten en el agua, se llevó a cabo un estudio de la toxicidad de estos compuestos, bajo la hipótesis de que sus efectos tóxicos son aditivos. Para este propósito, se realizaron ensayos de toxicidad aguda con *Daphnia magna*, que fue elegido como un organismo modelo para evaluar la toxicidad acuática. Los tests de toxicidad aguda con *D. magna* se realizaron según de la Directriz OECD 202. En una primera fase, se determinaron los EC_{50} para cada compuesto. Para esto, se llevó a cabo un ensayo de toxicidad aguda, donde los animales fueron expuestos a soluciones recién preparadas de los compuestos individuales, y se monitorizó su supervivencia a las 48 h. En un segundo experimento, se expusieron los organismos a soluciones conteniendo una mezcla de los nueve compuestos estudiados, a 10 concentraciones diferentes. Este experimento fue adecuado para comparar las respuestas según los conceptos de la adición de concentración (CA) y de acción independiente (IA).

En los ensayos de toxicidad se observó que la toxicidad variaba en gran medida para los nueve OPFRs estudiados. Se obtuvieron valores de EC_{50} que oscilaban más de tres órdenes de magnitud (0,31 a 381 mg L^{-1}), siendo el TCP y EHDP los compuestos más tóxicos y el TCEP el menos tóxico. Los resultados evidenciaron que estos compuestos actúan por narcosis no polar, ya que su toxicidad es proporcional a su lipofilicidad (K_{ow}). Los ensayos de toxicidad para las mezclas de OPFRs indicaron que la toxicidad conjunta de estos compuestos es aditiva y que se predice según el modelo de adición de concentración, lo que significa que actúan de manera similar en mezclas. Por tanto, es posible estimar el riesgo para *D. magna* para una mezcla de OPFRs a través de la suma de los RQs de cada componente de la mezcla.

Se llevó a cabo la evaluación del riesgo a lo largo de los tres ríos españoles estudiados. Se calculó la suma de los RQs para cada punto de muestreo, relacionando la concentración individual de cada OPFRs detectado en el agua con los EC_{50} obtenidos para *D. magna*, y utilizando un factor de seguridad de 1000. Para los sedimentos, la evaluación del riesgo se llevó a cabo estimando las concentraciones en los poros de agua, las cuales se relacionaron con los EC_{50} obtenidos para los OPFRs, mientras que para los PBDEs se utilizaron los datos de toxicidad de la literatura. Se encontraron $\Sigma RQs < 1$ en todos los casos, por lo tanto no se puso de manifiesto un riesgo para *D. magna* para las concentraciones ambientales detectadas en este estudio. El estudio de vigilancia ambiental para los ríos españoles, así como el estudio de la toxicidad utilizando *D. magna*, se incluyen en el Artículo 5 del Capítulo 3.

Comparando los resultados del río Aire (Reino Unido) y del río Besós (España), se observa que el perfil de los OPFRs es el mismo para ambos los ríos, con concentraciones decrecientes como se sigue: [TCPP] > [TCEP] > [TDCP] > [TPHP]. Exceptuando el TCPP, los niveles para cada compuesto son similares entre los ríos, con concentraciones medias y máximas en el mismo orden de magnitud. Sin embargo, el TCPP presentó una concentración mucho mayor en el río Aire que en el río Besós. Este comportamiento se puede atribuir al mayor uso de TCPP en el Reino Unido que en los demás países de la Unión Europea, debido a reglamentos anti-fuego estrictos para los muebles por parte del Reino Unido.

Para determinar las fuentes de contaminación de los ríos y determinar si los efluentes de depuradora contribuyen a la contaminación de los ríos, se evaluó la presencia y la eliminación de OPFRs, NBFRs y PBDEs en cinco depuradoras de Cataluña. Se detectaron todos los OPFRs en el influyente, y la mayoría de ellos se detectaron en los efluentes. El TCEP, TCPP y TDCP fueron resistentes al tratamiento, ya que las concentraciones en los influentes fueron similares a las concentraciones en los efluentes para la mayoría los casos. Se observó una eliminación del 28% al 95% para TBEP, TBP y TiBP TPHP, mientras que el TEHP, EHDP y TCP no se detectaron en los efluentes. Los compuestos más abundantes fueron el TBEP (1,6 – 46 µg L^{-1}), TCPP (1,7 – 6,8 µg L^{-1}) y el TiBP (0,26 – 138 µg L^{-1}), seguido por el TBP (0,14 – 0,90 µg L^{-1}), el TCEP (0,18 – 0,32 µg L^{-1}) y el TDCP (0,067 – 0,29 µg L^{-1}). El BEHTBP fue el único retardante de llama bromados detectado en los influentes de las depuradoras (0,052 – 0,13 µg L^{-1}), pero no se detectó en los efluentes, lo que indicó que este compuesto se elimina durante el tratamiento. Además, se analizaron muestras de lodos de las depuradoras. Se observó la acumulación de OPFRs (excepto TCEP), de los PBDE (BDE-47, BDE-99 y BDE-209), el DBDPE y del BEHTBP en la mayoría de las muestras de lodo, a niveles de µg – mg kg^{-1}.

Los resultados observados en los efluentes de las depuradoras están de acuerdo con la detección de los OPFRs en las aguas de los ríos de España y del Reino Unido. Los perfiles de concentración de los OPFRs en los efluentes de las depuradoras y en agua de río fueron similares, siendo el TBEP y el TCPP los compuestos presentes a concentraciones más altas. El TCEP, TDCP y TBP fueron detectados en los efluentes y en el agua de los ríos a niveles intermedios, mientras que el TPhP, EHDP, TEHP y TCP se detectaron a concentraciones más bajas.

5.4 Conclusiones

Los estudios llevados a cabo en la presente tesis han permitido llegar a las siguientes conclusiones principales:

1. La comparación de las prestaciones de los métodos GC-EI-MS/SIM, GC-EI-MS/MS y GC-ECNI-MS/SIM ha puesto de manifiesto que ambas técnicas de ionización (EI o ECNI) y ambos modos de adquisición de datos (SIM y SRM) pueden ser adecuados para la determinación multiresiduo de los retardantes de llama en muestras ambientales. La GC-ECNI-MS/SIM ha presentado los límites de detección instrumentales más bajos, pero con baja selectividad para muchos de los NBFRs y PBDEs. El método GC-EI-MS/MS ha permitido la detección simultánea de OPFRs, NBFRs y PBDEs, y ha demostrado ser muy ventajoso en términos de selectividad.

2. El método desarrollado para la extracción simultánea de OPFRs, NBFRs y PBDEs en agua de río y agua residual (influentes y efluentes), basado en extracción en fase solida con cartuchos HLB, fue eficiente para el análisis multiresiduos de retardantes de llama de diferentes polaridades y log K_{ow}. Además, ambas técnicas de GC-EI-MS/SIM y GC-EI-MS/MS han permitido la identificación y cuantificación de los compuestos de interés con una buena precisión.

3. Los muestreadores pasivos "*ceramic dosimeter*" son unos sistemas prometedores para el análisis de OPFRs en agua de río y son adecuados para estimar las concentraciones medias durante un tiempo de colección de unas 3 semanas.

4. El método basado en la extracción con una mezcla de acetato de etilo/ciclohexano (5:2, v/v) utilizando un baño de ultrasonido, purificación de los extractos utilizando cartuchos de florisil, y análisis por GC-EI-MS/MS, ha demostrado ser eficiente, robusto y apto para la determinación de OPFRs, NBFRs y PBDE en sedimentos, lodos de depuradora y polvo.

5. Los OPFRs y el BEHTBP se detectaron en los influentes de las plantas de depuradoras, y la mayoría de estos compuestos se detectaron también en los efluentes, excepto el BEHTBP, TCP, y EHDP TEHP. Esto indica que muchos OPFR son recalcitrantes a los tratamientos de tratamiento de aguas residuales y por lo tanto, los efluentes contribuyen a la contaminación de los ríos. Además, se observó la acumulación de OPFRs, PBDEs, BEHTBP y DBDPE en los lodos de depuradora. Esto tiene graves implicaciones ambientales ya que los lodos se utilizan en grandes cantidades como fertilizantes agrícolas.

6. Los OPFRs fueron los contaminantes más ubicuos en las aguas superficiales. Su presencia se correlacionó con las fuentes antropogénicas ya que sus niveles fueron más elevados en la proximidad de las zonas urbanas e industriales. El TCPP y TBEP fueron los compuestos más abundantes, seguido de TBP, TiBP, TCEP, TDCP. Los efluentes de las depuradoras representan la principal fuente de estos contaminantes en el medio acuático. Además, se observó la acumulación del TCP, EHDP, TPHP y TCP en los sedimentos del río, especialmente en las zonas más impactados por las actividades humanas.

7. Los PBDEs se detectaron en aguas de ríos de España y del Reino Unido. El BDE-209 fue el único PBDE detectado en el agua en el río Aire (Reino Unido), mientras los PBDEs de 4 a 6 bromos fueron detectados en los sedimentos del río Besós (Cataluña), y el BDE-209 fue el congénere más abundante.

8. Se ha observado una baja frecuencia de detección para los NBFRs en los ríos. El PBEB y el HBB se han detectado en las aguas del río Aire. El DBDPE ha sido el único NBFR detectado en los sedimentos de los ríos españoles (río Besós). Esto implica que estos compuestos o bien no se han introduzido en el mercado, o bien no han tenido tiempo de accumularse, o bien se degradan en el medio acuatico.

9. Comparando los ríos españoles y el de Inglaterra, los OPFRs fueron los contaminantes más abundantes en ambos países, mientras que las diferencias de concentraciones de PBDE y NBFRs fueron probablemente debidas a las diferentes aplicaciones de los retardantes de llama en productos comercializados en cada país.

10. Los ensayos de toxicidad con *D. magna* han permitido la determinación de las concentraciones letales para cada uno de los OPFRs individuales. Los EC_{50} obtenidos se correlacionan con su lipofilicidad (log K_{ow}), indicando que actúan mediante narcosis no polar. Además, los ensayos indicaron que la toxicidad de los OPFRs en una mezcla es aditiva. Por lo tanto, la presencia conjunta de varios OPFRs a bajas concentraciones pueden resultar nocivas para el medio acuático.

11. La evaluación del riesgo realizada para los diferentes ríos indicó que no hay un riesgo asociado con los niveles de los OPFRs presente en las aguas. Sin embargo, se observó un riesgo potencial asociado a la presencia del BDE-209 en las aguas del río Aire (Reino Unido).

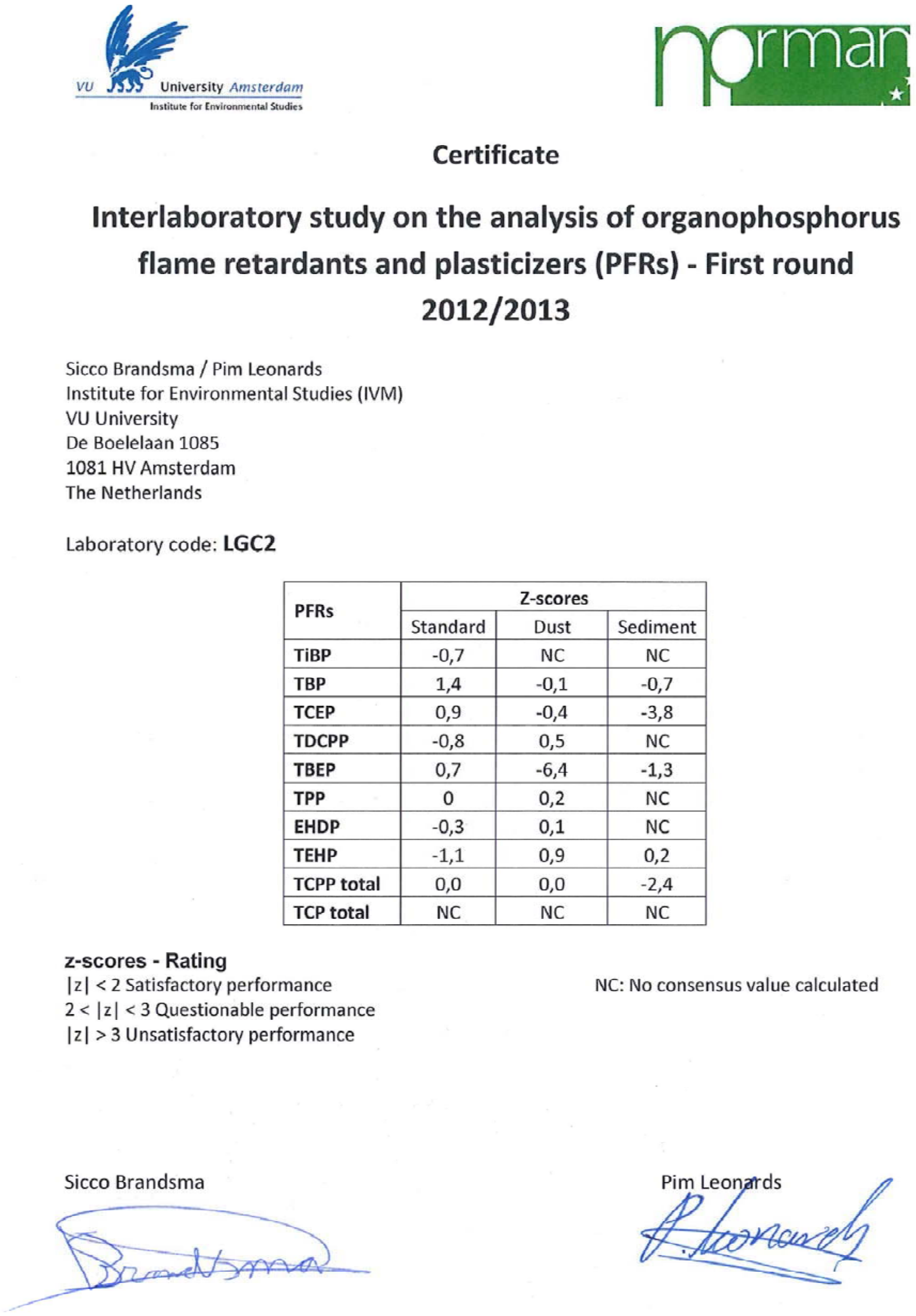
5.5 Líneas futuras de investigación

Basado en la revisión bibliográfica y en los resultados obtenidos en los estudios llevados a cabo en esta tesis, se proponen las siguientes líneas de investigaciones para trabajos futuros, con el fin de generar conocimiento con respecto del destino de los retardantes de llama en el medio ambiente:

- Evaluar la degradabilidad de OPFRs y NBFRs en el medio acuático.
- Desarrollar métodos para la total eliminación de la carga de retardantes de llama en efluentes de depuradora.
- Estudiar la adsorción de retardantes de llama en lodos y sedimentos.
- Estudiar la acumulación de retardantes de llama en suelos enmendados con lodos de depuradora y evaluar su capacidad de lixiviación.
- Desarrollar métodos de extracción para otras matrices ambientales: aire, nieve, aguas costeras y marinas, muestras biológicas, etc.
- Estudiar en profundidad el efecto del "*fouling*" utilizando el "*ceramic dosimeter*" para el muestreo pasivo de retardantes de llama en agua superficial.
- Modelizar la distribución global de los retardantes de llama.

6 Appendix

6.1 Appendix I: Certificate – Interlaboratory study (ILS)



VU University Amsterdam
Institute for Environmental Studies

norman

Certificate

Interlaboratory study on the analysis of organophosphorus flame retardants and plasticizers (PFRs) - First round 2012/2013

Sicco Brandsma / Pim Leonards
Institute for Environmental Studies (IVM)
VU University
De Boelelaan 1085
1081 HV Amsterdam
The Netherlands

Laboratory code: **LGC2**

PFRs	Z-scores		
	Standard	Dust	Sediment
TiBP	-0,7	NC	NC
TBP	1,4	-0,1	-0,7
TCEP	0,9	-0,4	-3,8
TDCPP	-0,8	0,5	NC
TBEP	0,7	-6,4	-1,3
TPP	0	0,2	NC
EHDP	-0,3	0,1	NC
TEHP	-1,1	0,9	0,2
TCPP total	0,0	0,0	-2,4
TCP total	NC	NC	NC

z-scores - Rating
|z| < 2 Satisfactory performance
2 < |z| < 3 Questionable performance
|z| > 3 Unsatisfactory performance

NC: No consensus value calculated

Sicco Brandsma

Pim Leonards

6.2 Appendix II: List of publications

1. Cristale J, Quintana J, Chaler R, Ventura F, Lacorte S. Gas chromatography/mass spectrometry comprehensive analysis of organophosphorus, brominated flame retardants, by-products and formulation intermediates in water. Journal of Chromatography A 1241 (2012) 1-12.
   Impact factor: 4.612

2. Cristale J, Katsoyiannis A, Chen C, Jones KC, Lacorte S. Assessment of flame retardants in river water using a ceramic dosimeter passive sampler. Environmental Pollution 172 (2013) 163-169.
   Impact factor: 3.730

3. Cristale J, Lacorte S. Development and validation of a multiresidue method for the analysis of polybrominated diphenyl ethers, new brominated and organophosphorus flame retardants in sediment, sludge and dust. Journal of Chromatography A 1305 (2013) 267-275.
   Impact factor: 4.612

4. Cristale J, Katsoyiannis A, Sweetman AJ, Jones KC, Lacorte S. Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK). Environmental Pollution 179 (2013) 194-200.
   Impact factor: 3.730

5. Cristale J, García Vázquez A, Barata C, Lacorte S. Priority and emerging flame retardants in rivers: Occurrence in water and sediment, Daphnia magna toxicity and risk assessment. Environment International 59 (2013) 232-243.
   Impact factor: 6.248

6.3 Appendix III: Other publications

I. Cristale J, Silva FS, Zocolo GJ, Marchi MRR. Influence of sugarcane burning on indoor/outdoor PAH air pollution in Brazil. Environmental Pollution 169 (2012) 210-216.

II. Cristale J, Dos Santos DM, Sant'Anna BS, Sandron DC, Cardoso S, Turra A, De Marchi MRR. Tributyltin in crustacean tissues: Analytical performance and validation of method. Journal of the Brazilian Chemical Society 23 (2012) 39-45.

III. Silva FS, Cristale J, Ribeiro ML, Marchi MRR. Polycyclic aromatic hydrocarbons (PAHs) in raw cane sugar (rapadura) in Brazil. Journal of Food Composition and Analysis 24 (2011) 346-350.

IV. Silva FS, Cristale J, André PA, Saldiva PHN, Marchi MRR. PM2.5 and PM10: The influence of sugarcane burning on potential cancer risk. Atmospheric Environment 44 (2010) 5133-5138.

V. de Andrade SJ, Cristale J, Silva FS, Julião Zocolo G, Marchi MRR, 2010. Contribution of sugar-cane harvesting season to atmospheric contamination by polycyclic aromatic hydrocarbons (PAHs) in Araraquara city, Southeast Brazil. Atmospheric Environment 44 (2010) 2913-2919.

VI. Santos DMd, Sant'Anna BS, Sandron DC, Cardoso de Souza S, Cristale J, Marchi MRRd, Turra A. Occurrence and behavior of butyltins in intertidal and shallow subtidal surface sediments of an estuarine beach under different sampling conditions. Estuarine, Coastal and Shelf Science 88 (2010) 322-328.

VII. Cristale J, Silva FS, Marchi MRR. Development and application of GC-MS/MS method for simultaneous analysis of 17 PAHs in airborne particulate matter. Desenvolvimento e aplicação de método GC-MS/MS para análise simultânea de 17 HPAs em material particulado atmosférico 33 (2008) 69-78.

7 References

1. SpecialChem - Flame Retardants Center. In: <http://www.specialchem4polymers.com/tc/flame-retardants/index.aspx>. Accessed 06/05/2013.

2. OJEC - Official Journal of the European Communities. 2000/147/EC. Commission Decision of 8 February 2000 implementing Council Directive 89/106/EEC as regards the classification of the reaction to fire performance of construction products.

3. BSI - British Standards Institution, 1982. British Standards 5852, part 2. Fire tests for furniture, methods of test for the ignitability of upholstered composites for seating by flaming sources.

4. OJEC - Official Journal of the European Communities. Directive 95/28/EC of The European Parliament and of the Council of 24 of october of 1995 relating to the burning behavior of materials used in the interior construction of certain categories of motor vehicle.

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