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

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ABSTRACT

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.

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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 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)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). 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 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 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 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 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;
$1.0 \le RQ < 10$ indicates a small potential for adverse effects;
$10 \le RQ < 100$ indicates significant potential for adverse effects;
RQ ≥ 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,
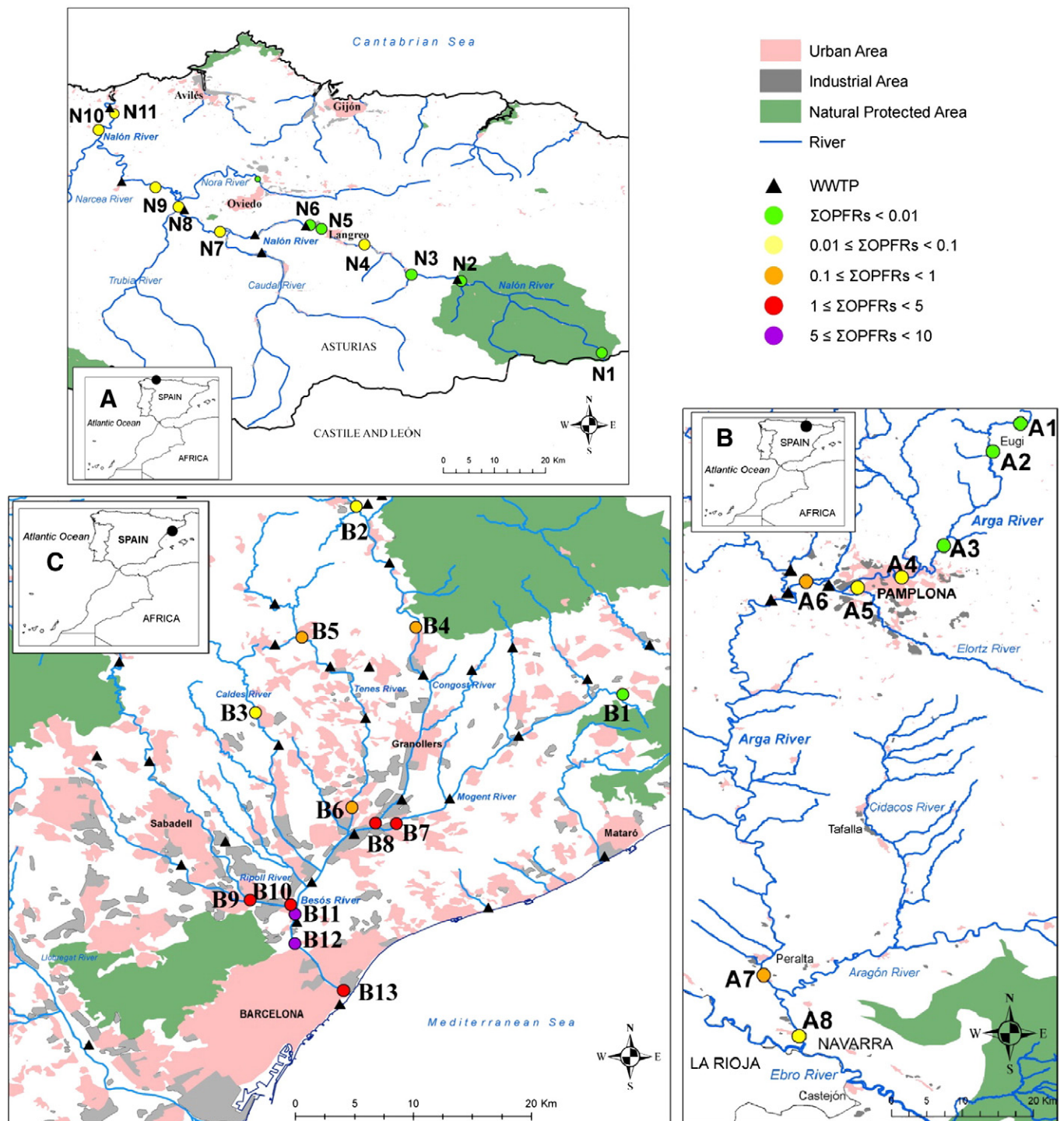
Fig. 1. Sampled sites along the Spanish rivers Nalón (A), Arga (B) and Besòs (C). ΣOPFR water concentration ranges are indicated as µg L^{-1}.

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.
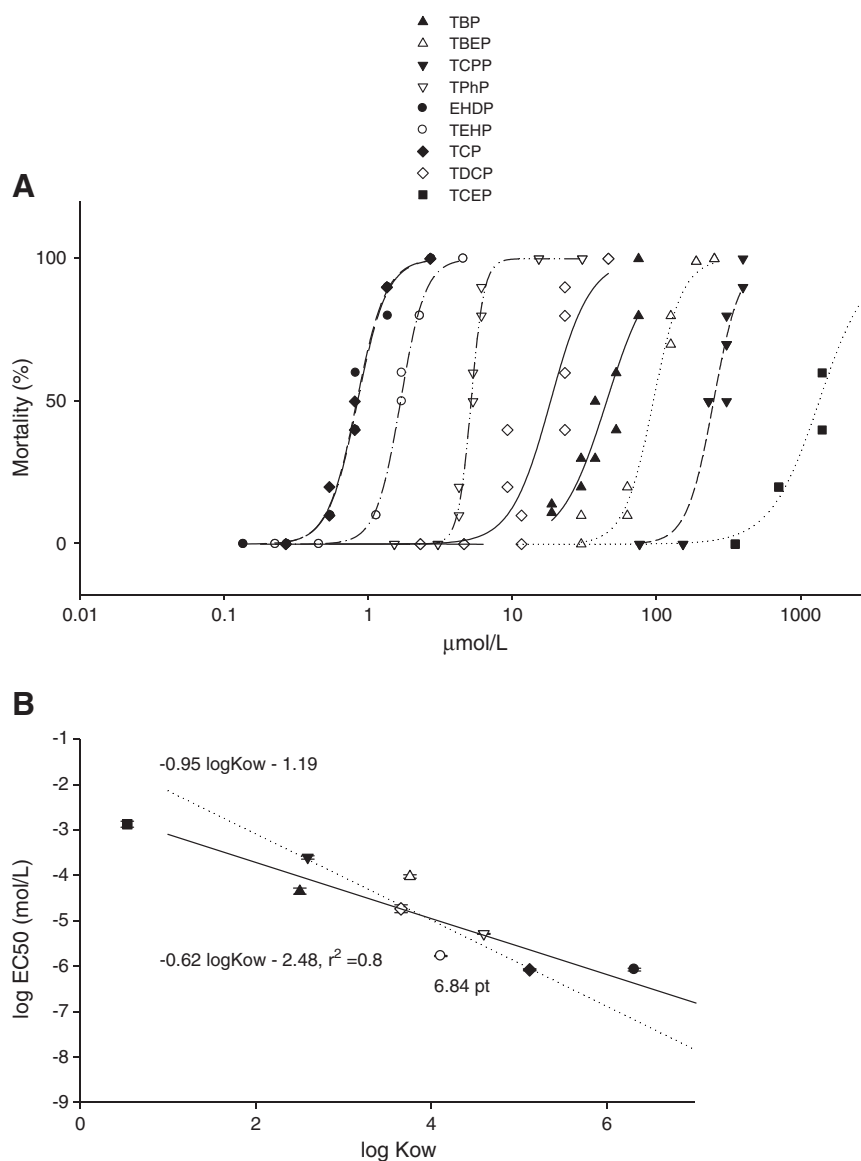
Fig. 2. Single compound *D. magna* toxicity responses of the studied organophosphorus substances: (A) concentration–mortality curves fitted to the Hill regression model; (B) estimated quantitative structure–activity regression (QSAR) curves between log octanol–water partition coefficients (log K_{ow}) 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) = −0.95log 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
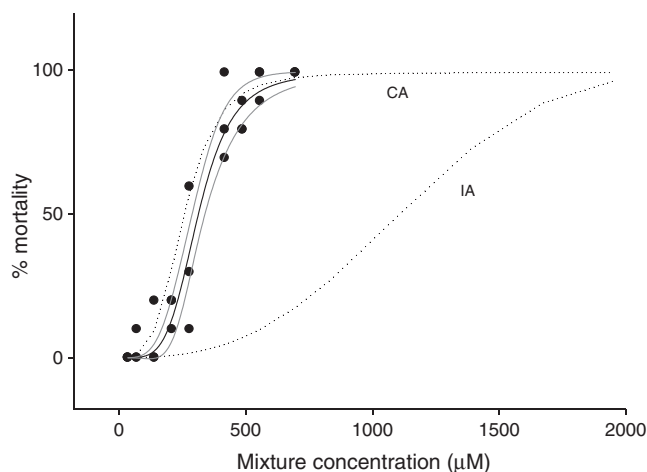
Fig. 3. Joint toxicity of the 9 multicomponent organophosphate mixtures. Each point represents a single value. Black and gray lines are observed modeled responses and its 95% confidence intervals, respectively. Dotted lines are predicted joint responses by the IA and CA concepts.

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 ($\mu g\ kg^{-1}$) 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 ($\mu g\ kg^{-1}$), 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 (ΣRQ <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) $\mu g\ L^{-1}$, from 0.007 (TPhP) to 0.170 (TBEP) $\mu g\ L^{-1}$ and from 0.0043 to 0.140 (TBEP) $\mu g\ L^{-1}$, respectively (Martínez-Carballo et

Table 3
PBDE concentrations ($\mu g\ kg^{-1}$) 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 ($\mu g\ kg^{-1}$), 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
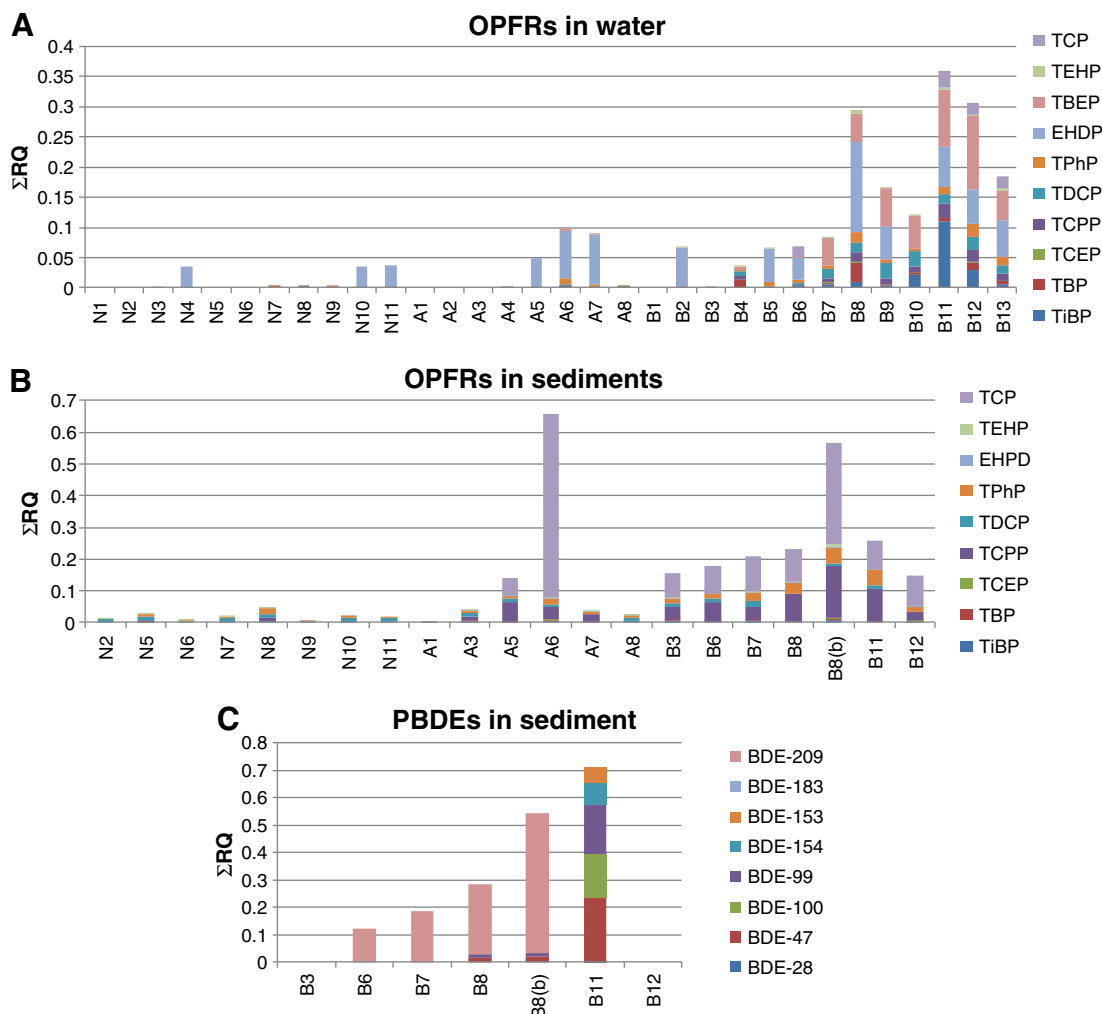

Fig. 4. Risk quotient (RQ) for detected flame retardants in water and sediments. In some sites, the river bed had no sediments and therefore there is no data.

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.

Acknowledgements

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