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UV filters bioaccumulation in fish from Iberian river basins

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HIGHLIGHTS

- First evidence of UV filters in fish from Iberian rivers
- Biota-sediment accumulation factors (BSAFs) were always below 1.
- Predator species presented higher UV-F concentrations suggesting trophic magnification.

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ABSTRACT

The occurrence of eight organic UV filters (UV-Fs) was assessed in fish from four Iberian river basins. This group of compounds is extensively used in cosmetic products and other industrial goods to avoid the damaging effects of UV radiation, and has been found to be ubiquitous contaminants in the aquatic ecosystem. In particular, fish are considered by the scientific community to be the most feasible organism for contamination monitoring in aquatic ecosystems. Despite that, studies on the bioaccumulation of UV-F are scarce.

In this study fish samples from four Iberian river basins under high anthropogenic pressure were analysed by liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Benzophenone-3 (BP3), ethylhexyl methoxycinnamate (EHMC), 4-methylbenzylidene camphor (4MBC) and octocrylene (OC) were the predominant pollutants in the fish samples, with concentrations in the range of ng/g dry weight (d.w.). The results indicated that most polluted area corresponded to Guadalquivir River basin, where maximum concentrations were found for EHMC (241.7 ng/g d.w.). Sediments from this river basin were also analysed. Lower values were observed in relation to fish for OC and EHMC, ranging from below the limits of detection to 23 ng/g d.w. Accumulation levels of UV-F in the fish were used to calculate biota-sediment accumulation factors (BSAFs). These values were always below 1, in the range of 0.04–0.3, indicating that the target UV-Fs are excreted by fish only to some extent. The fact that the highest concentrations were determined in predators suggests that biomagnification of UV-F may take place along the freshwater food web.

1. Introduction

UV filters (UV-Fs) are emerging environmental pollutants of recent concern for which there is currently a lack of knowledge about their occurrence, fate and effects on the environment (Richardson, 2010). These compounds are used extensively in a variety of personal care products as well as in many industrial goods to protect products against photodegradation. UV-Fs enter the aquatic environment by direct inputs from recreational activities but mainly through the effluents of wastewater treatment plants (WWTPs) (Cuderman and Heath, 2007). These chemicals have been widely detected in surface water and wastewater at high concentrations, up to 19000 ng/L and 4000 ng/L in influent and effluent wastewater, respectively (Balmer et al., 2005; Kasprzyk-Hordern et al., 2008; Gago-Ferrero et al., 2013a) and up to 3000 ng/L in surface water (Rodil et al., 2009; Negreira et al., 2010). They are present in high concentrations in sewage sludge and sediments (Plagellat et al., 2006; Gago-Ferrero et al., 2011a; Gago-Ferrero et al., 2011b; Amine et al, 2012), due to their high lipophilicity and poor degradability. Their widespread occurrence has raised serious concern because of the known effects of these chemicals on various organisms. Several UV-Fs have an endocrine disrupting capacity, including benzophenone-3 (BP3), ethylhexyl methoxycinnamate (EHMC), octocrylene (OC) and 4-methylbenzylidene camphor (4MBC) (Schlumpf et al., 2004; Kunz and Fent, 2006; Calafat et al., 2008; Blüthgen et al., 2012). Adverse effects on fecundity and reproduction

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have been observed for BP3 and other benzophenone derivatives in fish and rodents (Calafat et al., 2008; Kunz and Fent, 2009).

Ecological factors, including aquatic species, size (weight and length), body lipid content, and sampling location, may affect bioaccumulation of chemicals (Yu et al., 2012). Aquatic organisms store chemical substances either directly from the surrounding environment or from their diet. Humans are consumers of fish and sea food. Exposure assessment currently considers fish and sea food as a potential route of human exposure to chemicals in the environment (Binelli and Provini, 2004). So far very little data is available on the bioaccumulation of UV-F in aquatic organisms from marine and fresh water, which was reviewed by Gago-Ferrero et al. (2012). Reported concentrations in fish ranged from 9 to 2400 ng/g lipid weight (l.w.) (Nagtegaal et al., 1997; Balmer et al., 2005; Fent et al., 2010; Spiric et al., 2010) in some monitoring studies conducted in different rivers and lake waters from Germany and Switzerland. Higher concentrations were found in mussels (Bachelot et al., 2012; Picot Groz et al., 2014), and relevant values of OC (89–782 ng/g l.w.) were recently determined in marine mammals (*Franciscana* dolphins (*Pontoporia blainvillei*)) along the Brazilian coast (Gago-Ferrero et al., 2013b).

When a substance is not metabolized or excreted at the pace that it is ingested, it accumulates and biomagnification may occur through the food web as shown in the study by Fent et al. (2010) for some UV-F, including EHMC. For this compound, values up to 22.50 ng/g l.w. were detected in crustacean and mollusks, and values as high as 300 ng/g l.w. in fish. The highest concentrations, above 700 ng/g l.w., however, were determined in fish-eating birds (*Phalacrocorax sp.*) suggesting the trophic transfer of EHMC in the aquatic ecosystem.

In this scenario, the aim of this study was to investigate for the first time the presence and concentration of UV filters in freshwater fish from four Iberian river basins as well as the sediments of the most polluted river basin. The concentration determined allowed us to estimate the bioaccumulation factors (BAFs) for the bioconcentrated compounds.

2. Materials and methods

2.1. Chemicals

Table 1 lists the target compounds and some of their relevant physicochemical properties. BP3, OC, ethylhexyldimethyl PABA (OD-PABA), 2,4-dihydroxybenzophenone (BP1), 4-hydroxybenzophenone (4HB), 4,4′-dihydroxybenzophenone (4DHB) and the isotopically labelled compound benzophenone-C_{13} (BP-C_{13}) were of the highest purity (>99%) and were obtained from Sigma-Aldrich (Steinheim, Germany); 4MBC (99% purity) was supplied by Dr Ehrenstorfer (Augsburg, Germany); and EHMC (98%) by Merck (Darmstadt, Germany). The isotopically labelled compounds 2-hydroxy-4-methoxy-2′,3′,4′,5′,6′-d_5 (BP3-d_5) and 3-(4-methylbenzylidene-d_4)camphor, used as internal standards (>99%), were obtained from CDN isotopes (Quebec, Canada). Solvents including methanol (MeOH), acetone, dichloromethane (DCM), acetonitrile (ACN), ethyl acetate (AcEt) and HPLC grade water, as well as formic acid (98% purity), aluminium oxide and Florisil were provided by Merck. N_2 and Ar purchased from Air Liquide (Barcelona, Spain) were of 99.995% purity. Pressurized liquid extraction cellulose filters used were obtained from Dionex Corporation (Sunnyvale, CA, USA). Isolute C18 (500 mg, 3 mL) cartridges used for solid phase extraction (SPE) were obtained from Biotage (Uppsala, Sweden).

Individual stock standard solutions as well as the isotopically labelled internal stock standard solution were prepared on a weight basis in MeOH at 200 mg/L. The solutions were stored in the dark at −20 °C. A mixture standard solution at 20 mg/L in MeOH of each compound was prepared weekly and working solutions were prepared daily by appropriate dilution of the mixture stock standard solution in MeOH.

2.2. Sample collection and preparation

Fish samples analysed in this study were collected in four Iberian river basins: Llobregat, Ebro, Jucar and Guadalquivir in 2010. These rivers have a Mediterranean regime and are exposed to a high anthropogenic impact. Detailed information about each sampling point can be found in http://www.scarceconsolider.es/publica/P000Main.php. Five sampling stations were selected distributed along each river basin except for Guadalquivir River, where four were selected (see Fig. 1).

In order to obtain a representative sample of different trophic levels within the aquatic community, specific fish species were targeted (see Table 2). For each river basin two fish species were selected, i.e. carp and barbel. However, it was not always possible to find them, and then other species were considered. Altogether, 49 individuals were collected using electro-fishing, and were weighed and measured, wrapped in aluminium foil, and immediately frozen for transport to the laboratory. In the particular case of *Luciobarbus sclateri* individuals lower than 30 cm were considered as juveniles. Once in the laboratory, the fish were composited (thawed, ground, homogenized and lyophilized) according to species and sampling point. The lyophilized samples were stored in sealed containers at −20 °C until analysis.

Sediment samples were collected in the same sampling stations as the fish samples located in the Guadalquivir River basin. Around 250 g of sediment was taken using a Van Veen grab sampler (500 mL capacity); they were transferred and wrapped into an aluminium foil and were frozen at −20 °C overnight before freeze-drying for approximately one week. Then, they were ground, sieved (2 mm) and finally stored at −20 °C until analysis.

2.3. Analytical methods

2.3.1. Quality assurance and quality control

Background contamination is a common problem in the determination of UV filters at environmental levels. To avoid it, all glassware used was washed and heated overnight at 380 °C, and further sequentially rinsed with different organic solvents and HPLC grade water. Furthermore, gloves were worn during sample preparation; separate solvents and only previously unopened packages of solvents, chemicals and other supplies were used. Many of the compounds analysed undergo photodegradation. Therefore, stock standard solutions and samples were always covered with aluminium foil and stored in the dark.

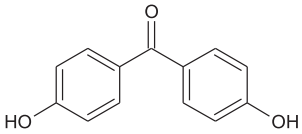
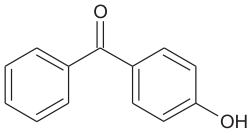
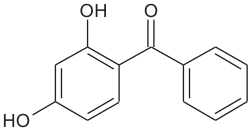
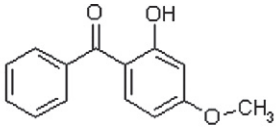
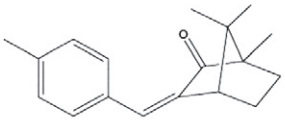
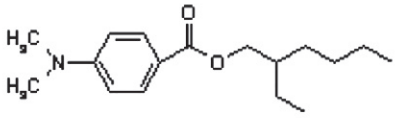
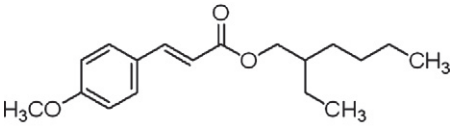
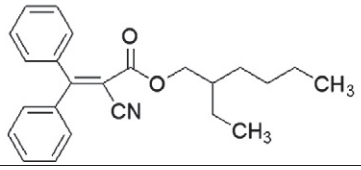
With every six samples, a methodological blank was analysed. Concentration of the target UV-F in the blanks was always <LOD. Linearity was satisfactory ($r^2 > 0.9$) for all the compounds. Recoveries in fish were >66% for all the compounds except for OD-PABA (36%). In sediment samples, recoveries were >58% for all the analytes. More details on QA/QC can be found in the Supporting Information in Appendix A.

The method performances of the methodologies for the analysis of UV-F in fish and in sediments are summarized in Table S1 and Table S2, respectively of the Supporting Information.

2.3.2. Analysis of fish

The analysis of UV-F and derivatives in the fish samples was carried out following a previously developed analytical methodology based on pressurized liquid extraction (PLE) and LC–MS/MS described elsewhere (Gago-Ferrero et al., 2013c). Briefly, the extraction of the analytes was performed using an ASE 350 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA, USA). One cellulose filter followed by 1 g of Florisil was placed at the bottom of the cells. Aliquots of 1 g of freeze-dried fish (spiked with the surrogate standard mix solution) was mixed in the extraction cells with Florisil. Extraction was implemented in 4 cycles of 5 min of static time each at 100 °C and 1500 psi using AcEt/DCM (1:1, v/v) as extracting solvent. The PLE extract obtained (~25 mL) was diluted to 200 mL with HPLC grade water (MeOH < 5%), and further purified by solid phase extraction (SPE)

Table 1
Target compounds: Names, abbreviations, CAS numbers, structures and Log Kow.

Name (INCI nomenclature)[a]	Abbreviation	CAS no.	Structure	Log K_{ow}
4,4´–Dihydroxy benzophenone	4DHB	611–99–4		2.19[b]
4–Hydroxybenzophenone	4HB	1137–42–4		2.92[c]
Benzophenone–1	BP1	131–56–6		3.15[c]
Benzophenone–3	BP3	131–57–7		3.79[b]
4–Methylbenzylidene camphor	4MBC	36861–47–9		4.95[b]
Ethylhexyl dimethyl PABA	OD–PABA	21245–02–3		5.412[c]
Ethylexyl methoxycinnamate	EHMC	5466–77–3		5.8[b]
Octocrylene	OC	6197–30–4		6.88[b]

[a]INCI (International Nomenclature for Cosmetic Ingredient) elaborated by CTFA and Cosmetics Europe (former COLIPA).
[b]Experimental values, from database of physicochemical properties. Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm.
[c]Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (©1999–2011 ACD/Labs).

using Isolute C18 (500 mg, 3 mL) cartridges from Biotage. The compounds were eluted sequentially with AcEt/DCM (1:1, v/v) and 2 mL of DCM at 1 mL/min flow rate. Finally, the SPE extracts were evaporated and reconstituted with 1 mL of ACN containing the isotopically labelled internal standards.

HPLC–MS/MS analyses were carried out in a system consisting of an Agilent HP 1100 pump (Agilent Technologies, Palo Alto, CA, USA) connected to a 4000 Q TRAP™ MS/MS system from Applied Biosystems-Sciex (Foster City, California, USA). The chromatographic separation was achieved on a Hibar Purospher® STAR® HR R-18 ec. (50 mm × 2.0 mm, 5 μm) from Merck, preceded by a guard column of the same packaging material. A gradient using a mixture of HPLC grade water and ACN, both 0.15% formic acid, at a flow rate of 0.3 mL/min was used as the mobile phase.

The MS/MS detection of UV-F was performed in positive (PI) electrospray ionization (ESI) mode under selected reaction monitoring (SRM) mode. Two major characteristic fragments of the protonated molecular ion $[M + H]^+$ were monitored per analyte for improved sensitivity and selectivity. The most abundant transition was used for quantification, whereas the second most abundant was used for

Fig. 1. Sampling locations of fish and surface sediments in the four river basins evaluated.

confirmation. Other experimental conditions can be found elsewhere (Gago-Ferrero et al., 2013c).

Determination of lipid contents of fish was based on the method described by Spiric et al. (2010).

2.3.3. Analysis of sediments

The analysis of UV-F in the sediments of the Guadalquivir River basin was carried out using the method previously developed by Gago-Ferrero et al. (2011a). The extraction and in-cell purification was performed by PLE. One gram of freeze-dried and sieved sediment was mixed in the extraction cells with aluminium oxide. PLE extraction was carried out using MeOH and a mixture MeOH/water (1:1 *v*/*v*). The PLE extract (≈20 mL) was brought to 25 mL with MeOH. Two milliliters of this solution was passed through 0.45 μm filters to LC-vials, evaporated and finally were reconstituted in 250 μL ACN.

Instrumental analysis was performed by ultra high resolution liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS), using an Acquity UHPLC chromatograph coupled to a TQD mass spectrometer (Waters) according to a previously developed methodology (Gago-Ferrero et al., 2011a).

In order to investigate the distribution of the selected UV-F between the biota and the sediment, the biota-sediment accumulation factors (BSAFs) were calculated in the most polluted basin, Guadalquivir River, using the following equation (Jia et al., 2011):

$$BSAF = \left(C_b / f_{lip}\right) / \left(C_s / f_{oc}\right) \quad (1)$$

where C_b is the UV-F concentration (ng/g wet weight) in fish, f_{lip} is the lipid content in fish (g lipids/g wet weight), C_s is the UV-F concentration (ng/g d.w.) in surficial sediment, and f_{oc} is the organic carbon content in sediment (g organic carbon/g sediment d.w.).

3. Results and discussion

3.1. Levels and distribution profiles of UV-F in fish and sediments

3.1.1. Spatial distribution

Table 2 summarizes the UV-F concentrations in different fish species collected at each sampling site. Method limits of detection (LOD, lowest analyte concentration with a signal to noise (S/N) ratio of 3) and method limits of quantification (LOQ, concentration with S/N ratio of 10 and imprecision lower than 20%) ranged from 0.1 to 6.0 ng/g d.w. and from 0.3 to 20.0 ng/g d.w., respectively (see Table 3).

Of the eight compounds analysed, four, i.e. BP3, EHMC, 4MBC and OC (the most lipophilic ones except OD-PABA) were detected with frequencies ranging from 5.6% to 80%. Table 3 shows the detection frequencies, ranges and median concentrations for each compound. The total detection frequencies were under 21.3% except for EHMC and BP3 in Guadalquivir River that attained 60% and 80%, respectively, showing big variations depending on the river basin. The total UV-F concentrations ranged from not detected (<LOD) to 363 ng/g d.w.

Guadalquivir River, with a length of 657 km, was by far the most polluted of the four basins investigated. This river basin is of particular ecological value because of the Doñana National Park, an important and protected wetland area. The river is navigable up as far as Seville (about 90 km upstream), a major inland port, which leads to a serious environmental problem due to erosion and pollution. The lower Guadalquivir River basin is also impacted by reservoirs and dams and its regime is rather artificial. Highest levels were observed in fish of the species *L. sclateri*, endemic of the Iberian Peninsula, where UV-F concentrations above 290 ng/g d.w. were observed. For this river basin, sediments collected in the same sampling points as the fish were also analysed and positive results for the compounds EHMC and OC were found. EHMC was detected at concentrations of 7.5, 22.9 and 18.9 ng/g d.w. at the sampling points GUA3, GUA4 and GUA5, respectively. OC was detected at 22.5 ng/g d.w. at the sampling point GUA4 and under the limit of quantification at GUA5. UV-F residues were not

Table 2
Locations and number of fish samples, fish species and concentration of the detected UV filters (ng/g d.w.) of target UV F along the four studied river basins.

Sampling station	Fish lipid content (%)	Common name	Scientific name	BP3	EHMC	4MBC	OC
Llobregat							
LLO3 (n = 3)	13.6	Ebro barbel (juvenile)	*Luciobarbus graellsii*	n.d.	n.d.	n.d.	n.d.
LLO4 (n = 3)	14.1			n.d.	n.d.	n.d.	n.d.
LLO6 (n = 3)	15.6			n.d.	n.d.	n.d.	n.d.
LLO3 (n = 3)	19.9	Ebro barbel (adult)	*Luciobarbus graellsii*	n.d.	n.d.	n.d.	n.d.
LLO4 (n = 2)	26.9			n.d.	n.d.	n.d.	n.d.
LLO6 (n = 3)	20.8			n.d.	n.d.	n.d.	n.d.
LLO3 (n = 3)	26.6	Common carp	*Cyprinus carpio*	n.d.	n.d.	n.d.	n.d.
LLO4 (n = 1)	n.a.			n.d.	n.d.	n.d.	n.d.
LLO5 (n = 3)	20.7			n.d.	n.d.	n.d.	n.d.
LLO6 (n = 3)	25.5			n.d.	n.d.	n.d.	n.d.
LLO7 (n = 3)	22.9			n.d.	n.d.	n.d.	<LOQ
Ebro							
OCAn (n = 4)	12.1	Ebro barbel (juvenile)	*Barbus graellsii*	n.d.	n.d.	n.d.	n.d.
EBR2 (n = 3)	11.8			n.d.	n.d.	n.d.	n.d.
EBR3 (n = 3)	12.3			n.d.	n.d.	n.d.	n.d.
EBR4 (n = 3)	12.6			n.d.	n.d.	n.d.	n.d.
EBR5 (n = 3)	n.a.			n.d.	n.d.	n.d.	n.d.
OCA (n = 3)	17.1	Ebro barbel (adult)	*Barbus graellsii*	n.d.	n.d.	n.d.	n.d.
EBR2 (n = 3)	24.1			n.d.	n.d.	n.d.	n.d.
EBR3 (n = 3)	n.a.			2.2	n.d.	2.7	n.d.
EBR4 (n = 3)	n.a.			n.d.	n.d.	n.d.	n.d.
EBR5 (n = 2)	15.4			n.d.	n.d.	n.d.	n.d.
EBR2 (n = 1)	11.2	Common carp	*Cyprinus carpio*	n.d.	n.d.	n.d.	n.d.
EBR3 (n = 3)	9.4			n.d.	n.d.	n.d.	n.d.
EBR4 (n = 3)	8.3			n.d.	n.d.	n.d.	n.d.
EBR5 (n = 3)	12.6			n.d.	n.d.	n.d.	<LOQ
EBR4 (n = 2)	24.8	Wels catfish	*Silurus glanis*	n.d.	12.2	n.d.	<LOQ
EBR5 (n = 2)	26.6			<LOQ	30.4	<LOQ	25.7
Guadalquivir							
GUA1 (n = 1)	27.1	Andalusian barbel (adult)	*Luciobarbus sclateri*	n.d.	n.d.	n.d.	n.d.
GUA3 (n = 9)	29.3			n.d.	19.0	n.d.	<LOQ
GUA4 (n = 9)	40.6			24.3	241.7	n.d.	30.4
GUA5 (n = 9)	34.5			16.5	63.0	n.d.	n.d.
GUA3 (n = 9)	9.0	Common carp	*Cyprinus carpio*	11.2	<LOQ	n.d.	n.d.
Jucar							
JUC1 (n = 3)	47.7	Brown trout (adult)	*Salmo trutta*	4.6	n.d.	n.d.	n.d.
JUC2 (n = 2)	19.3	Iberian nase	*Pseudochondrostoma polylepis*	n.d.	n.d.	n.d.	n.d.
JUC2 (n = 13)	n.a.	Iberian gudgeon (juvenile)	*Gobio lozanoi*	n.d.	n.d.	n.d.	n.d.
JUC4 (n = 10)	n.a.			n.d.	n.d.	n.d.	n.d.
JUC4 (n = 4)	n.a.	Iberian gudgeon (adult)	*Gobio lozanoi*	n.d.	n.d.	n.d.	n.d.
JUC6 (n = 4)	n.a.			n.d.	n.d.	<LOQ	n.d.
JUC4 (n = 6)	13.0	Black bass	*Micropterus salmoides*	n.d.	n.d.	n.d.	n.d.
JUC5 (n = 5)	n.a.			n.d.	n.d.	n.d.	<LOQ
JUC6 (n = 2)	18.9			n.d.	n.d.	n.d.	n.d.
JUC5 (n = 6)	n.a.	Bleak	*Alburnus alburnus*	n.d.	n.d.	n.d.	n.d.
JUC6 (n = 16)	n.a.			n.d.	n.d.	n.d.	n.d.
JUC5 (n = 3)	11.3	European eel	*Anguila anguila*	n.d.	n.d.	n.d.	n.d.
JUC6 (n = 3)	44.6			n.d.	<LOQ	n.d.	30.0
JUC6 (n = 1)	n.a.	Pumpkinseed	*Leponis gibbosus*	n.d.	n.d.	n.d.	n.d.
JUC6 (n = 2)	12.3	Mediterranean barbel (juvenile)	*Barbus guiraonis*	n.d.	n.d.	n.d.	n.d.
JUC6 (n = 1)	14.6	Mediterranean barbel (adult)	*Barbus guiraonis*	n.d.	n.d.	n.d.	n.d.
JUC6 (n = 1)	8.4	Pike	Esox lucius	n.d.	n.d.	n.d.	n.d.

LOQ values (ng/L d.w): 4.0 (BP3), 16.7 (EHMC), 2.3 (4MBC), 20.0 (OC). n.a.: not available

detected at GUA1, located in the upper river. Fig. 2 shows the total concentration of UV-F in fish (species: *L. sclateri*) and in sediments detected in the Guadalquivir River basin. The location of fish samples with high UV-F levels corresponded to the sites where the highest UV-F values were determined in sediments. The highest concentrations were found for both fish and sediments in the sample point GUA4, which is located downstream the Cordoba city WWTP (serving 350,000 inhabitants), from which it receives large volumes of wastewater. Wastewater discharge is considered to be an important source of UV-F for aquatic environments and aquatic organisms. Important loads of these contaminants have been directly associated with dense populations and proximity to wastewater effluent discharges (Buser et al., 2006) and it seems that the concentrations detected in the sample point GUA4 constitutes an example in this regard. The second most polluted sampling location was GUA5, which is located in the main stream in Peñaflor, a municipality of around 4000 inhabitants. In GUA3 only EHMC was determined, whereas GUA1 appeared not to be contaminated by the target UV F. These two sampling sites were located in the main stream close to two small villages, Marmolejo and Mogón. The last one is included into the Sierra de Cazorla National Park.

The levels detected in fish samples are significantly higher than the ones in the corresponding sediments, showing an increased accumulation of these lipophilic compounds in fish over sediment. However, when normalizing the respective concentrations to lipid content and TOC (from 0.7 to 1.2%), the calculated BSAFs were always below 1, in the range of 0.04–0.3, which suggests that estimates based on bioavailability of the contaminant by the fish are lower than those based on the adsorption onto the sediments. This may be explained by the metabolization and elimination in the fish (Rüdel et al, 2006). These results suggest a positive correlation between UV-F concentration and

Table 3
Summary of UV filter results in fish from the selected Iberian rivers.

			Llobregat (11 samples)				Ebro (16 samples)				Guadalquivir (5 samples)				Jucar (17 samples)			
UV-F	LOD (ng/g d.w.)	LOQ (ng/g d.w.)	Freq. (%)	Positive Samples (LOQ)	Range (ng/g d.w.)	Median[a] (ng/g d.w.)	Freq. (%)	Positive Samples (LOQ)	Range (ng/g d.w.)	Median[a] (ng/g d.w.)	Freq. (%)	Positive Samples (LOQ)	Range (ng/g d.w.)	Median[a] (ng/g d.w.)	Freq. (%)	Positive Samples (LOQ)	Range (ng/g d.w.)	Median[a] (ng/g d.w.)
BP3	1.2	4.0	0.0	0	–	.	12.5	2 (1)	LOD–2.2	LOQ	60.0	3 (3)	11.2–24.3	16.5	5.6	1 (1)	LOD–4.6	4.6
EHMC	5.0	16.7	0.0	0	–	.	12.5	2 (2)	LOD–30.4	LOQ	80.0	4 (3)	LOD–241.7	41.0	5.6	1 (0)	LOD–LOQ	LOQ
4MBC	0.7	2.3	0.0	0	–	.	12.5	2 (1)	LOD–2.7	1.75	0.0	0	–	–	5.6	1 (0)	LOD–LOQ	LOQ
OC	6.0	20.0	9.1	1 (0)	LOD–LOQ	LOQ	18.8	3 (2)	LOD–25.7	LOQ	40.0	2 (1)	LOD–30.4	LOQ	11.1	1 (0)	LOD–30.0	LOQ
Total UV-F[a]			9.1	1		7	25	4	4.9–66.3[a]	13.1	80.0	4	17.1–296.4[a]	52.7	22.2	4	0.8–35.9[b]	5.8

LOD: Method limit of detection; LOQ: Method limit of quantification, Freq: Frequency of detection; LOD: Below limit of detection; LOQ: limit of quantification. LOQ: Below limit of quantification.
[a] Only positive values were used for this calculation. For positive results below LOQ, calculations were performed by assigning a value corresponding to [(LOQ–LOD)/2].

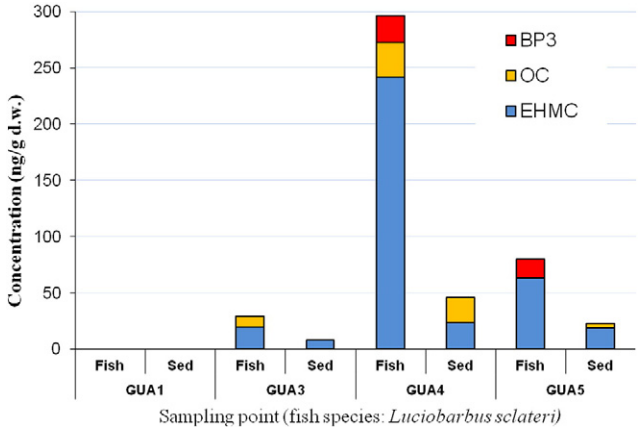


Fig. 2. Comparison of UV-F levels obtained in samples of sediment and fish in the Guadalquivir River basin. For results below the limit of quantification a value corresponding to [(LOQ–LOD)/2] was assigned. Sediment total organic carbon (TOC) values: GUA1 (0.66%), GUA3 (0.69%), GUA4 (1.20%) and GUA5 (0.98%).

lipid weight (see Table 2), and also between UV-F concentration and TOC.

The Llobregat River was found to be the lowest contaminated basin among the rivers studied. Only one out of the eleven samples taken along the basin showed OC, but at low concentration (between 6 and 20 ng/g d.w.) in the sampling station close to the large city of Barcelona. This low detection draws attention considering that Llobregat is a high industrialized river basin with a low average flow (19 m^3/s) (data from Agencia Catalana de l'Aigua) and high values of UV F have been previously detected in this river basin (Gago-Ferrero et al., 2013a), although the sampling points were not the same. However, as the Llobregat River shows a Mediterranean hydrological pattern, its flow can fluctuate considerably from dry to rainy periods. At high flow contaminants dilution occurs.

Similar findings were also observed in Jucar river basin (20% detection frequency) where only OC, the most lipophilic one, was found at a concentration above the LOQ in one *Anguila anguila* sample (30.0 ng/g d.w.). According to previous studies, this species tend to bioaccumulate more substances than the other species due to the high percentage of lipids in its body (Sancho et al., 1998).

Ebro River is regulated by dams and channels, which have altered its hydrological and sedimentary regime. Abstraction of ground and surface water, irrigation and industrial activities concentrated close to the main cities in the basin have also deteriorated soil and water quality. This river shows the highest average flow (600 m^3/s) (data from Confederación Hidrográfica del Ebro, CHE) among the rivers studied which contribute to the dilution of the contamination. UV-Fs were observed in 25% of the samples from the Ebro River. The samples of the species *Silurus glanis* showed the highest concentrations for EHMC and OC at the sampling points EBR4 and EBR5. Both sampling sites are located downstream the WWTP close to the cities of Logroño (154,000 inhabitants) and Tudela (36,000 inhabitants), respectively. BP3 and 4MBC were also determined in *Barbus graellsii* in EBR3 in La Rioja, a well-known vineyard region.

Summarizing, the highest frequency of detection was observed for EHMC in Guadalquivir River (found in 80% of the samples), whereas OC was the most frequently found compound in the whole study, being present in all four river basins. EHMC is extensively used in several personal care products and has shown an estrogenic activity (Kunz and Fent, 2006) and effects on the global gene expression in fish (Zucchi et al., 2011) at relatively low concentration (2.2 μg/L).

The highest mean concentration was determined for the compound EHMC (82.4 ng/g d.w.). BP3 showed a mean concentration of 17.3 ng/g d.w. and in the case of OC and 4MBC this parameter was <LOQ. The contamination level (accumulated mean concentrations

of total UV F) order between the four river basins was: Guadalquivir (104.7 ng/g d.w.) > Ebro (26.3 ng/g d.w.) > Jucar (12.1 ng/g d.w.) > Llobregat (7 ng/g d.w.). For positive results < LOQ, calculations were performed by assigning a value corresponding to [(LOQ–LOD)/2].

3.1.2. Fish species distribution

In the present study, the influence of fish size, i.e. between juvenile and adults on the UV-F fish concentration was not observed.

EHMC occurred in several fish species (*L. sclateri*, *S. glanis*, *Anguila anguila* and in *Cyprinus carpio*) having different diet and strata preferences. All these fish species, except for *S. glanis*, which is a predator, are bottom feeding omnivorous species. Taking into account these data, it is not clear whether biomagnification might play a role in the concentration of UV-F in fish. However, in the Ebro River, only *S. glanis* (trophic levels of 4.3–4.7) (Encina and Granado-Lorencio, 1991), a predator at the top of the food chain in that ecosystem, showed detectable UV-F concentrations. In Guadalquivir River, EHMC accumulation was also most pronounced in *L. sclateri* (trophic level of 2.64) (Syvaranta et al., 2010) than in *C. carpio* (trophic level of 2.79) (Yu et al., 2012). In a previous study, Fent et al. suggested that biomagnification occurs for this compound in the aquatic environment (Fent et al., 2010). In that study biomagnification was suggested in the predator/prey pair cormorant and fish (barb, chub and brown trout) and between the omnivorous barb feeding on *Gammarus*. For a reliable correlation data between UV-F concentrations and for instance morphometric data of analysed fish (length, weight, gender or maturity level), a more extensive sampling in each site following a different strategy should be carried out.

The herein reported results are in agreement with those of previous studies performed in other European river basins studying fish and other fresh water organisms (Balmer et al., 2005; Mottaleb et al., 2009; Fent et al., 2010; Vela-Soria, 2011; Gago-Ferrero et al., 2013d). The concentrations needed to induce known adverse effects on organisms are higher than those observed in this study and typically reported in surface waters.

The analysis of vitellogenin (VTG) in rainbow trout (*Oncorhynchus mykiss*) and Japanese medaka (*Oricias latipes*) after aqueous exposure to BP3 indicated that high effective concentrations in the range of 620–749 μg/L were needed for its induction (Coronado et al., 2008). In male Japanese medaka, the levels of VTG and choriogenin, another known estrogen-responsive gene product, were found to increase after exposure of the fish to 4-MBC and EHMC (Inui et al., 2003), with high estrogenic potency being displayed by 4-MBC.

4. Conclusions

The present findings revealed that several fish species from four Iberian rivers contained detectable concentrations of UV-F. However, the target compounds were detected with low frequencies of detection. These results constitute the first data on bioaccumulation of UV-F in fish from Iberian rivers. Among the eight target sunscreens, only the lipophilic compounds (Log Kow > 3.5) were accumulated. The detected levels are comparable with the values reported in previous studies conducted in few European rivers and lakes indicating a similar pattern of use of these compounds. The highest concentrations were detected in fish from the Guadalquivir River, which accumulated BP3, EHMC and OC. The sediments corresponding to the same sampling locations where the fish were collected were contaminated only with the two most lipophilic compounds EHMC (Log Kow 5.8) and OC (Log Kow 6.88). In general, the highest UV-F contamination level in fish was observed downstream WWTPs close to populated urban areas along the basins. The BSAF values estimated for OC and EHMC (always < 1) indicated that the target UV-F tend to bioaccumulate in fish but are also eliminated to some extent. Predator species occupying a higher position in the trophic chain showed higher levels of UV-F, which suggests that biomagnification may play a certain role in the accumulation of these chemicals in fish. Nevertheless, due to the short food chain available in the present study, further investigation at longer food chains is still needed to clearly identify the trophic magnification potential of UV filters.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.03.026.

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