Environment International 75 (2015) 110–116

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

First report of pyrethroid bioaccumulation in wild river fish: A case study in Iberian river basins (Spain)

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ARTICLE INFO

Article history:
Received 2 August 2014
Accepted 11 November 2014
Available online 20 November 2014

Keywords:
Bioaccumulation
Cypermethrin
Enantioselectivity
Pyrethroids
River fish

ABSTRACT

For the first time, this work described pyrethroid bioaccumulation in edible river fish samples. We analyzed 42 whole fish samples collected in 4 different Iberian rivers. All samples were positive to these insecticides. Levels of concentration ranged from 12 to 4938 ng g^{-1} lipid weight (lw). Moreover, isomeric characterization was carried out. Our results remarked a general preference of *cis* isomers in bioaccumulation. Finally, the enantiomeric evaluation showed that there was an enantioselective bioaccumulation of some pyrethroids, depending on the studied species. Pyrethroid concentrations were compared with levels obtained for other common pollutants, such as flame retardants, personal care products, hormones and pharmaceuticals. The highest values corresponded to pyrethroid insecticides, even though, pyrethroid levels are safe for human consumption taken into account the current regulations.

1. Introduction

Pyrethroids are synthetic insecticides derived from the natural pyrethrins. Structurally they have 2 or 3 chiral centers. This means that they have 2 or 4 diastereomers and 4 or 8 enantiomers. The use of pyrethroids is extensive around the world. They are common in agronomics both on crops and directly over grain to store, in veterinary on cattle and pets, as domestic insecticides and even for health purposes against scabies, lice or vectors of some diseases such as malaria or typhus (Barr et al., 2010).

These insecticides were the alternative to other biocides, e.g. organochlorines and organophosphates, because of their low persistence and toxicity. However, even when it is known that pyrethroid environmental persistence is usually lower than 90 days (UH, 2011), it is also true that they are found in environmental samples, such as water and sediments (Feo et al., 2010b; Weston et al., 2013; Xue et al., 2005), food (Esteve-Turrillas et al., 2005; Garcia-Rodriguez et al., 2012), mammals (Alonso et al., 2012) and even human samples (Bouwman et al., 2006; Corcellas et al., 2012; Channa et al., 2012). The explanation to these findings may be the continuous, and sometimes excessive, use of these compounds.

The origin of these pyrethroids in the environment is very diverse. Agronomics should be an importance source. Despite this, some works pointed out that pyrethroid presence in river water and sediments because of the agronomic workings is punctual and it depends on how long the pesticides were applied (Feo et al., 2010b). Besides, a lot of countries have already banned some of these insecticides in agronomics (EC, 1991; EPA, 1991). However, the usage of these biocides is also very common in non-agricultural sectors such as industry, government, and home and garden. For example, the last Pesticide Industry Sales and Usage Report (EPA, 2011) estimated that in 2007, over 1500 t of pyrethroids was used only in the U.S. Home and Garden market sector. Therefore, domestic and urban uses might be other focal points of their environmental presence (Kuivila et al., 2012; Lu et al., 2013; Weston and Lydy, 2010).

Moreover, pyrethroid toxic effects in water ecosystems are not negligible. For instance, LC_{50} of bifenthrin in *Daphnia* and trout are 0.11 and 150 μg/L respectively (UH, 2011). Some authors had studied LC_{50} of some other pyrethroids in fishes. Their values ranged from 0.06 μg/L (tefluthrin on trouts) to 19 μg/L (allethrin on trouts) (UH, 2011). Even when in literature there were no studies of pyrethroid bioaccumulation in wild fish tissues, some authors had studied the bioaccumulation in exposed fishes. The main objective of most of these studies was to calculate the Bioconcentration Factor (BCF) in fishes for concrete pyrethroids. These studies demonstrated high bioaccumulation but, as well, the possibility of depuration in appropriate conditions (Devillers et al., 1996; Jackson et al., 2009; Muir et al., 1994; Schimmel et al., 1983).

Lately, pyrethroid toxicology and exposition in mammals are being further investigated (Goulding et al., 2013; Jin et al., 2012; Xu et al., 2010; Zhang et al., 2008; Zhao et al., 2010). These works included new data about isomer-toxicology, even some of them were focused on enantioselective toxicology. For instance, *cis*-isomer of permethrin

Abbreviations: dw, dry weight; LC_{50}, Lethal Concentration at 50%; lw, lipid weight; LOD, limit of detection; LOQ, limit of quantification; MRL, Maximal Residue Level; MS, mass spectrometry.

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http://dx.doi.org/10.1016/j.envint.2014.11.007
0160-4120/

seems to be less metabolized and, consequently, more toxic than *trans*-permethrin in mice (Zhang et al., 2008). Besides, one of the *cis*-enantiomers presented more toxicity than the other (Jin et al., 2012). Moreover, human exposure to pyrethroids has been widely studied by urine analysis of their metabolites and related with some diseases, such as leukemia (Ding et al., 2012). However, these analyzed metabolites are nonspecific, so it was not possible to know the contribution of each pyrethroid (Barr, 2008; Barr et al., 2010; Ding et al., 2012; Koureas et al., 2012; Olsson et al., 2004) or their isomers.

With this background, we studied for the first time the potential bioaccumulation of pyrethroids in wild river fish. We analyzed 42 pooled edible fish samples from four Iberian river basins. In these samples we determined 12 different pyrethroids: *cis*-bifenthrin, cyfluthrin, cypermethrin, cyhalothrin, deltamethrin, fenvalerate, fluvalinate, permethrin, phenothrin, resmethrin, tetramethrin, and tralomethrin. In addition, given the relevance of isomerism on toxicology, we reanalyzed these samples with an enantiomeric-selective methodology.

2. Materials and methods

2.1. Standards and reagents

All analytical standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). As surrogate standards d_6-*trans*-permethrin and d_6-*trans*-cypermethrin were chosen and purchased from the same commercial firm. Organic solvents were obtained from J.T. Baker "for use in HPLC" quality (Deventer, The Netherlands). Standard solutions were prepared in ethyl acetate ("for gas chromatography" quality from Merck, Darmstadt, Germany). Calibration curves were prepared at different concentrations ranging between 0.4 and 150 ng mL^{-1}. Solid phase extraction (SPE) cartridges were obtained from Isolute Biotage (Uppsala, Sweden) (C18, 2 g 15 mL^{-1}) and from Interchim (Montluçon, France) (Basic alumina, 5 g 25 mL^{-1}).

2.2. Sampling

In the frame of the project SCARCE-Consolider-Ingenio, four Iberian river basins were sampled in 2010. Fig. 1 showed the distribution of these four basins in the Iberian territory as well as the sampling points. Only one of this sample points corresponded to a reservoir. For each river, two fish species were selected for monitoring along the river. These species used to be one carp and one barbel species, when it was possible. Other species were also sampled, e.g. trouts, gudgeons and catfishes. Fish samples were collected, homogenized for species by a meat grinder, freeze-dried and stored at −20 °C until analyses. More details of this procedure were specified in previous works (Jakimska et al., 2013; Santin et al., 2013). Table A summarizes sample details such as species, sampling point and pool composition. With some species, for example barbels, juvenile and adult samples were differentiated by fork length; in this particular case, barbels with length lower than 30 cm were considered as juvenile.

2.3. Analytical methods

Sample treatment was adapted from Feo et al. (2012). Briefly, 0.3 g of freeze-dried sample was spiked overnight with 10 μL of a solution of 0.025 ng L^{-1} and 0.0125 ng L^{-1} of d_6-*trans*-permethrin and d_6-*trans*-cypermethrin, respectively. Extraction procedure was carried out with 20 mL of hexane:dichloromethane 2:1 and assisted by ultrasound for 15 min. This extraction was repeated twice and all solvent dried by a N_2 stream. A following tandem SPE (basic alumina and C18 cartridges, 30 mL acetonitrile as eluent) cleaned up. The eluent was evaporated under N_2 and the sample reconstituted 100 μL of ethyl acetate.

Analyses were performed on an Agilent Technologies 7890A coupled to a 7000A GC–MS Triple Quad. The columns chosen were a DB5-ms (Agilent Technologies, Santa Clara, CA, USA) (15 m × 0.25 mm × 0.1 μm) for the quantitative analysis and a BGB-172 (BGB Analytik, Switzerland) (30 m × 0.25 mm × 0.25 μm) for the enantiomeric determination. Details of chromatographic conditions to both achiral and chiral analyses are found in Corcellas et al. (in press). The selected mass spectrometry (MS) mode was negative chemical ionization with ammonium as reagent gas. All MS parameters are found in Feo et al. (2011).

In parallel, 1 g of sample was extracted with an equivalent extraction procedure in order to determine the lipid content gravimetrically.

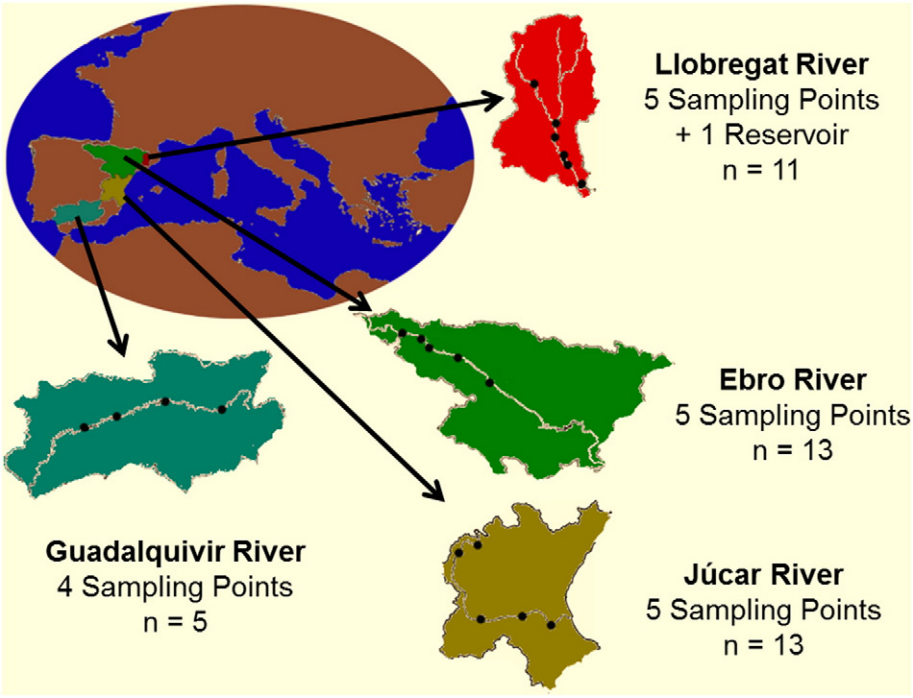

Fig. 1. Map of the four Iberian river basins, and sampling stations selected for each one (n = number of samples).

2.4. Quality assurance/control

For each batch of 12 samples, one methodological blank was carried out. Levels of blanks were always negligible (lower than 1% of the sample signals). Linearity was proven obtaining correlation coefficient higher than 0.98 in the studied concentration interval for all pyrethroids. The mean recovery was 79%, being 53% the lower value, obtained for deltamethrin. Limits of detection (LOD) ranged from 0.03 to 0.46 ng g^{-1} lw and limits of quantification (LOQ) from 0.10 to 1.54 ng g^{-1} lw.

2.5. Isomeric and enantiomeric analyses

After quantitative analysis, representative samples of each river and species were selected in order to be analyzed with the chiral column. This method allowed discerning the isomeric proportion of bifenthrin, cyhalothrin, cyfluthrin, cypermethrin, permethrin and tetramethrin. Fig. 2 showed the results obtained by both analyses for type I and type II standard pyrethroids.

Following Corcellas et al. (in press) indications, enantiomeric factors (EFs) for each enantiomeric pair were calculated with Eq. (1).

$$EF = A_i/A_T \quad (1)$$

where A_i is the area of the first eluting enantiomer and A_T is the sum of areas of both enantiomers. EF was defined for each enantiomeric pair: EF*cis* and EF*trans* for type I pyrethroids, and EF*cis1*, EF*cis2*, EF*trans1* and EF*trans2* for type II pyrethroids. A racemic mixture of an enantiomeric pair is always represented by an EF equal to 0.5.

Moreover, diastereoisomeric factors were also defined. First of all, R*cis*/*trans* was defined as the ratio between *cis* and *trans* isomers of the same pyrethroid. In the case of type II pyrethroids, this relationship was the ratio (*cis1* + *cis2*)/(*trans1* + *trans2*). For type II pyrethroids more ratios are possible. For instance, R*cis1*/*cis2* was the proportion of the isomer *cis1* with respect to the isomer *cis2*. Analogously, R*trans1*/*trans2* was defined as the proportion of the isomer *trans1* with respect to the isomer *trans2*.

3. Results and discussion

3.1. Quantification analyses

Our study is the first one reporting pyrethroid levels in wild non-treated fish tissues. Pyrethroids were detected in all the analyzed samples. Table 1 summarized the results obtained as a sum of pyrethroids in each river basin (for individual sample results see Table B).

Total pyrethroid concentrations ranged from 12 to 4938 ng g^{-1} lw. The highest value corresponded to a trout sample from a reservoir. In river course sample points, the highest concentration was of 1508 ng g^{-1} lw, corresponding to a carp sample. Generally, carps were the fishes with more pyrethroid bioaccumulation capacity.

Other studies about pyrethroids in fish samples were not about wild biota, so the comparison of our levels with previous published was difficult. In a previous work, we found median concentrations for total pyrethroids of 7.04 and 68.4 ng g^{-1} lw in adults and calves of Brazilian liver dolphins, respectively (Alonso et al., 2012). Thus, levels in dolphins were even two orders of magnitude lower than those found in the present work for river fish. The lower values obtained for dolphins could be explained by the capability of mammals to metabolize pyrethroids, with the conversion to non-toxic metabolites by hydrolysis (Demoute, 1989; Scollon et al., 2009). However, it should be also taken into account that samples were completely different (liver dolphin sample vs. whole fish sample) and came from different ecosystems (ocean vs. river) and water conditions, such as dissolved organic matter, could affect the bioaccumulation of pyrethroids (Haitzer et al., 1998). Regarding studies in fishes, the levels reported in exposed fishes where quite high, as well. For example, Muir et al. (1994) described levels of almost 30, 20, 40 and 20 ng g^{-1} ww of *cis*-cypermethrin, fenvalerate, *cis*-permethrin and deltamethrin, respectively, in rainbow trout. These levels are in the same order of magnitude of our maximum levels (taking into account the transformation from lw to ww).

Nine pyrethroids out of the 12 included in the analytical work were detected. Bifenthrin, cyhalothrin and cypermethrin were ever present. Detection frequencies of the rest of the detected pyrethroids were 88%, 83%, 81%, 57% and 31% for fenvalerate, tetramethrin, permethrin, cyfluthrin and deltamethrin/tralomethrin, respectively. Tralomethrin

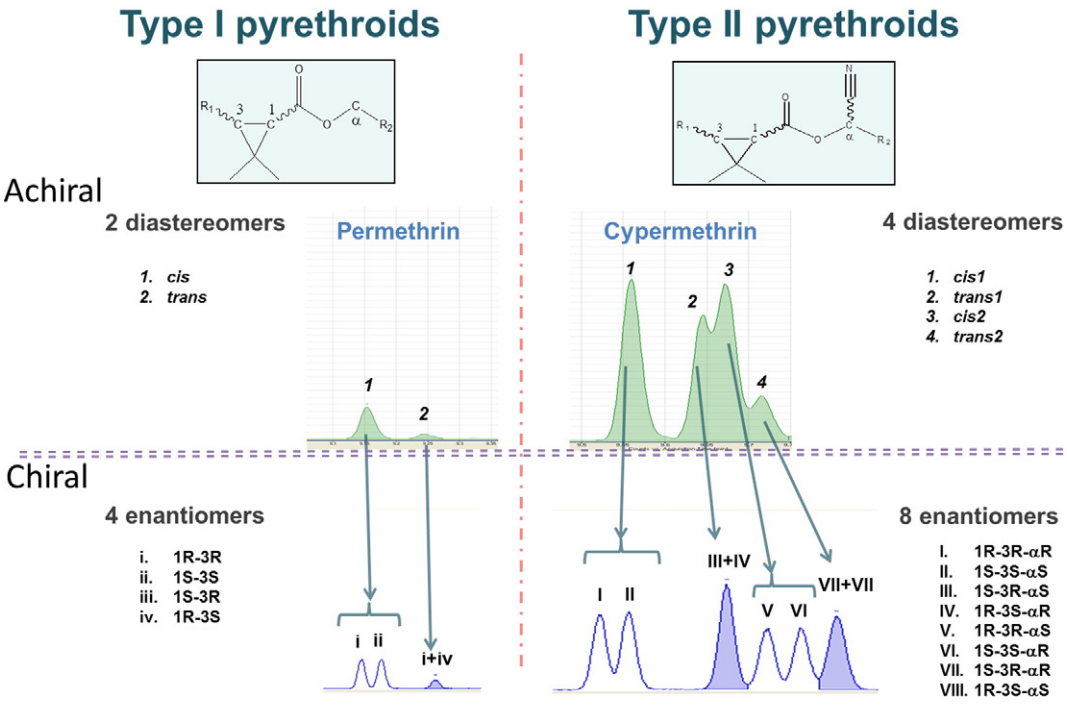

Fig. 2. Peak assignation for the chromatograms obtained in diastereomeric and enantiomeric analyses of type I and type II pyrethroids.

Table 1
Summary of pyrethroid levels (expressed in ng g^{-1} lw) obtained in fish samples collected from the four Iberian river basins (SP: sampling point).

River basin (SP)	Barbels			Carps			Trouts			Other		
	Species name	Range	Mean	Species name	Range	Mean	Species name	Range	Mean	Species name	Range	Mean
Ebro	*L. graellsii* (5)	53–154	114	*C. carpio* (4)	46–1017	307	–	–	–	*Silurus glanis* (2)	147–329	238
Llobregat	*L. graellsii* (2 + 1[a])	102–504	356	*C. carpio* (5)	152–1508	551	*Salmo trutta* (1[a])	–	4938	–	–	–
Guadalquivir	*L. sclateri* (4)	20–843	608	*C. carpio* (1)	–	140	–	–	–	–	–	–
Júcar	*B. guiraonis* (2)	12–123	68	–	–	–	*Salmo trutta* (2)	379–583	481	*Gn Lozanoi* (5)	114–670	327

[a] This sampling point is a reservoir.

is converted to deltamethrin in a GC injector (Feo et al., 2010a), which means that results of deltamethrin are always the undifferentiated sum of these two pyrethroids. Samples never presented detectable levels of fluvalinate, phenothrin and resmethrin.

Pyrethroid distribution was different depending on the sampling point. Fig. 3a shows the median contribution of each pyrethroid to the total contamination for each river basin. For instance, permethrin was the predominant pyrethroid in the Ebro and Llobregat river basins, whereas the Guadalquivir and Jucar river basins were dominated by cypermethrin and tetramethrin, respectively. This fact could be due to different local insecticide practices. A confirmation of this hypothesis would be the similarity of patterns between Llobregat and Ebro rivers, because they are the geographically closest basin rivers. More local information about the use of pyrethroids would be necessary in order to describe a relationship between its dumping and bioaccumulation. Nonetheless, the presence of some pyrethroids like bifenthrin, whose agrarian use is banned (EC, 1991), supports the hypothesis that non-agricultural sectors contribute considerably to the pyrethroid contamination of the environment.

However, it should be pointed out that also differences in pattern distribution were observed between species collected at the same sampling point. As an example, Fig. 3b shows the pattern distribution for different species collected in the same sampling point of the Júcar River. In this case, the contribution of cyhalothrin decreased from barbels to gudgeons and trouts. In contrast, the highest contribution of permethrin was observed in trouts, followed by gudgeons, whereas it was not detected in barbels. It was supposed that each species bioaccumulates distinctly pyrethroids. For instance, Jackson et al. (2009) estimated the BCF of cyhalothrin for *Lepomis macrochirus* in 19 while the IUPAC database of agrochemicals (IUPAC, 2011) assumed that this value reach 1950 for the whole fish samples. In the case of fenvalerate, Schimmel et al. (1983) estimated its BCF with carps in 1100 but Devillers et al.'s (1996) estimation with sheepshead minnow (*Cyprinodon variegatus*) was of 570. These divergences could be due to different parameters like the dissolved organic carbon and the pyrethroid exposition (Muir et al., 1994). Given that our comparison is among individuals equally exposed, more studies are necessary to identify the exact causes of our results. One potential explanation could be the different metabolism of these species. Even when pyrethroids are structurally similar, small differences in metabolism could make some of them more bioavailable, more bioaccumulative or even more metabolizable. Other reasoning is about fish habits. Species monitored had different diet preferences and various strata. This could mean divergent direct exposure even when they came from the same river zone.

No significant differences were found between juvenile and adult samples, in both levels (ANOVA test, $\alpha > 0.01$) and profiles. No general trends on concentration were described against physical characteristics such as weight, longitude or even Fulton's condition factor. However, given that finding these trends was not the main objective of this first study, to check these results should need a more exhaustive sampling with a larger number of samples specifically selected in order to study these potential trends.

3.2. Pyrethroids versus other emerging pollutants

Levels of pyrethroids were compared with those of other pollutants analyzed in the same fish samples. Fig. 4 represented the comparison with levels of flame retardants, personal care products, hormones and pharmaceuticals. In this figure pyrethroid levels are recalculated in ng g^{-1} dry weight (dw) in order to be compared with the rest of the published values. The first important difference among pyrethroid and most of other families was the detection frequency. Pyrethroids were

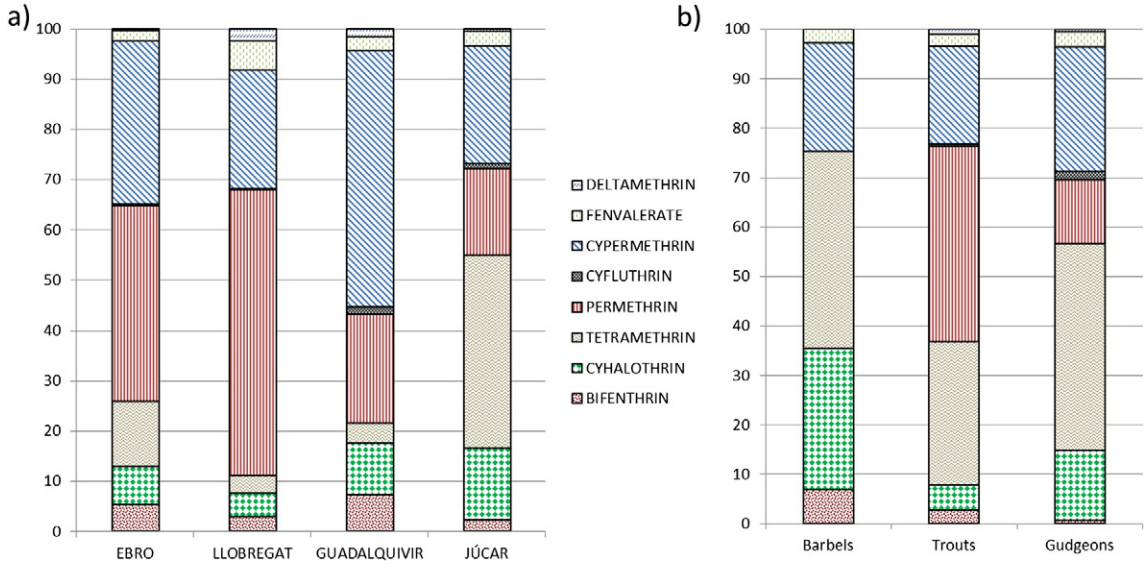

Fig. 3. Percentage (%) contribution of each pyrethroid by different a) river basins, and b) fish species (fishes collected at the same sampling point, JUC6).

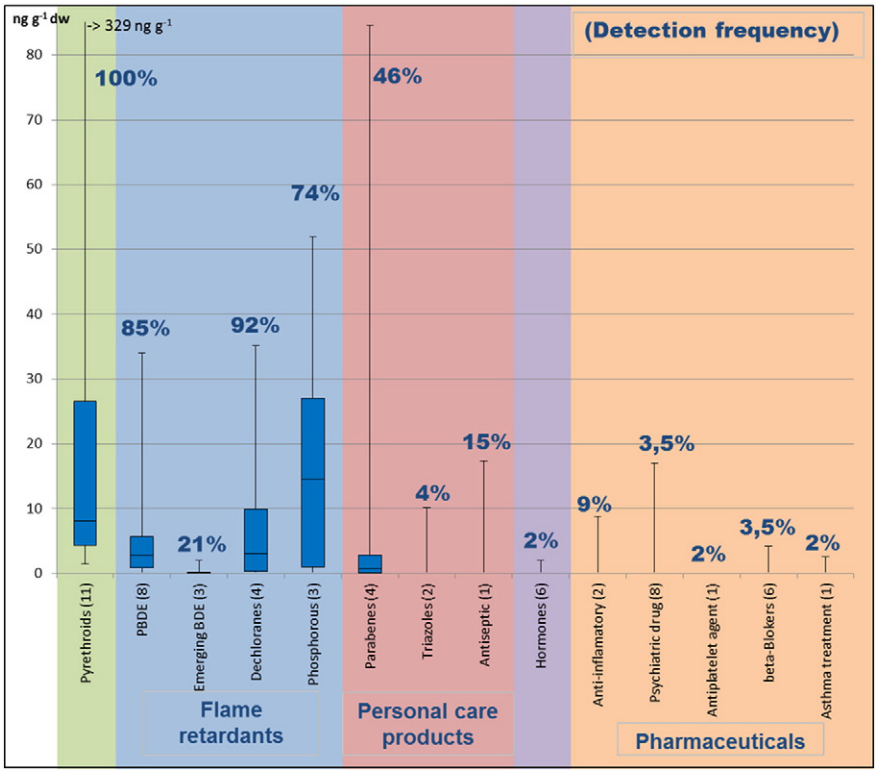

Fig. 4. Levels of different emerging pollutants analyzed in the same fish samples. Pyrethroid data corresponded to the present work, and data for the rest of the compounds were adapted from ref. Santin et al. (2013), Huerta et al. (2013), and Jakimska et al. (2013). Number of analytes of each family in parenthesis.

detected in all the samples. Only some of the flame retardants such as polybrominated diphenyl ethers (PBDEs) and dechloranes had frequencies near to 100% (Santin et al., 2013). The rest of the contaminants presented frequencies lower than 50% (Huerta et al., 2013; Jakimska et al., 2013). Moreover, pyrethroid concentrations were the highest, followed by parabens and organophosphorous flame retardants, whose maximum levels were third and sixth of pyrethroid's, respectively. All these results remark the importance of including pyrethroids in environmental quality and monitoring studies, given that, even at non-lethal doses, pyrethroids are known as stressors (Forsgren et al., 2013).

3.3. Chiral analysis

Results obtained with the chiral column are summarized in Table 2. This method is less sensitive than the isomeric one, so, even when samples presented quantitative levels of pyrethroids, sometimes it was not possible to evaluate the enantiomeric contribution. This was the case of bifenthrin and tetramethrin.

3.3.1. Diastereomeric evaluation

Almost all the samples showed accumulation preference of the *cis* pyrethroid isomers. Thus, ratios among *cis* and *trans* isomers (Rcis/

Table 2
Diastereomeric ratios and enantiomeric fractions obtained for different pyrethroids in different fish species.

Pyrethroid	Species	EF*cis1* (EF*cis*)		EF*cis2*		R*cis1*/*cis2*		R*cis*/*trans*	
		Range	Enhanced enantiomer	Range	Enhanced enantiomer	Range	Enhanced diastereomer	Range	Enhanced diastereomer
Permethrin	Barbels	0.27–0.47	SPD	–	–	–	–	2.65–11.1	cis
	Carps	–	–	–	–	–	–	–	–
	Trouts	0.41–0.45	Rac	–	–	–	–	0.60–2.20	SPD
	Gudgeons	0.58–0.76	SPD	–	–	–	–	1.18–4.08	cis
	Catfishes	–	–	–	–	–	–	–	–
Cyhalothrin	Barbels	0.20–0.38	II	0.46–0.49	Rac	0.38–0.57	SPD	–	–
	Carps	–	–	–	–	–	–	–	–
	Trouts	–	–	–	–	–	–	–	–
	Gudgeons	0.42–0.46	Rac	0.29–0.40	VI	0.29–0.35	cis2	–	–
	Catfishes	0.38	II	0.46–0.47	Rac	0.64–0.65	cis2	–	–
Cyfluthrin	Barbels	0.2	II	–	–	–	cis1	–	cis
	Carps	–	–	–	–	–	–	–	–
	Trouts	0.39	II	0.60	V	1.61	cis1	1.31	cis
	Gudgeons	0.20–0.37	Rac	0.12–0.23	VI	–	–	–	–
	Catfishes	–	–	–	–	–	–	–	–
Cypermethrin	Barbels	0.21–0.47	SPD	0.25–0.43	SPD	0.90–1.32	SPD	3.01–29.7	cis
	Carps	0.44	Rac	0.40	VI	0.92	–	3.16	cis
	Trouts	0.38–0.45	SPD	0.25–0.45	SPD	0.78–0.87	cis2	2.27–4.23	cis
	Gudgeons	0.36–0.37	II	0.11–0.14	VI	0.39–0.42	cis2	12.0–15.0	cis
	Catfishes	0.48–0.49	Rac	0.22–0.24	VI	1.14–1.15	cis1	11.5–16.7	cis

Rac: racemic mixture; SPD: sampling point depending.

trans) were from 2.3 to 30 for cypermethrin, 1.3 for cyfluthrin, and from 0.6 to 11 for permethrin. The only exception was the case of tetramethrin. However, it is known that commercial mixtures are usually enriched in *trans*-tetramethrin. Concretely, it is habitual to find the d-*trans*-tetramethrin enantiomer (1R–3S-isomer) enhanced in some common domestic insecticides because it is the enantiomer with more insecticide activity (Corcellas et al., in press). Therefore, it is supposed that *trans*-tetramethrin could be more dumped to the environment.

Regarding permethrin, in previous works with biological matrices (dolphin liver and human breast milk) (Alonso et al., 2012; Corcellas et al., 2012), it was found always enriched in *cis*-isomer, which is also consistent with our findings in fish samples. In fact, some works revealed that in the case of mice, the *cis*-permethrin isomer was less metabolized than *trans*-permethrin, and it was also more accumulative and more toxic (Jin et al., 2012; Zhang et al., 2008). This selective accumulation of *cis*-permethrin we have described in our study, needs to be confirmed with further studies. More studies must be carried out in order to fill the gap of knowledge regarding potential selective bioavailability and metabolism of permethrin in fishes.

Isomers of cyhalothrin, cyfluthrin and cypermethrin were evaluated in this work for the first time in not exposed biota samples. Again, results showed that *cis* isomers were more abundant than *trans* isomers. However, even when generally *cis* isomers were enhanced, it should be taken into account that commercial mixtures could be enriched in *cis* isomers having, for example, Rcis/trans ≈ 2 (Corcellas et al., in press). In this case, we cannot confirm a *cis*/*trans* selectivity in bioaccumulation of pyrethroids, without prior knowledge about the origin of the pyrethroids to which fishes are exposed and their Rcis/trans.

Moreover, the Rcis1/cis2 for these 3 pyrethroids was also calculated. This parameter ranged from 0.29 to 0.65 for cyhalothrin, indicating a higher contribution of the *cis*2 isomer (commonly known as λ-cyhalothrin). It is important to remark that this isomer has the greatest insecticide power and it is enhanced in some commercial mixtures. In contrast, for cyfluthrin and cypermethrin, the preferential accumulation of *cis1* or *cis2* seemed to depend on the species. For instance, the *cis*1-cypermethrin was enhanced in catfish samples whereas the *cis*2-isomer was enriched in trout and gudgeon species. Nevertheless, given that this is the first study to describe diastereomeric behavior, differences in this ratio among species need to be further studied and those trends confirmed.

3.3.2. Enantiomeric interpretation

In order to analyze the enantiomeric accumulation, different EF values were calculated (Table 2). EFs could get values between 0 (there is no presence of the first eluting enantiomer) and 1 (there is no presence of the second eluting enantiomer). The medium value of 0.5 corresponds to the racemic mixture, indicating that both enantiomers are in the same proportion. Even when the standard deviation of EF ranged from 0.001 to 0.016 in standards (Corcellas et al., in press), for further discussions, we generally consider racemic mixtures to those with EF values of 0.5 ± 0.1 (a medium deviation value of 0.01 multiplied by a factor of 10 in order to include all those variables out of control that could affect differently in real samples).

Looking at the permethrin, calculated EF*cis* values were very dissimilar. They ranged from 0.27 to 0.76. Values for trouts seemed to suggest racemic mixtures. In some sampling points, both gudgeons and barbels presented values too close to 0.5 to clarify any enantioselectivity. Notwithstanding, in other sampling points, these two species showed EF*cis* values significantly higher (gudgeons) and lower (barbels) than 0.5, revealing clear enrichments of first eluting enantiomer and second, respectively. Those general differences among sampling points could show different commercial mixture uses. Additionally, in previous works (Corcellas et al., in press), we had described an enrichment of (1S–3S)-enantiomer in domestic permethrin mixtures. Results of gudgeon samples seemed to suggest either that the origin of their contamination was not the domestic use, or that the (1R–3R)-enantiomer was enhanced in the environment. Otherwise, the enantioselective trend depending on the species needs to be proved. More studies involving higher number of samples should be carried out in order to corroborate these tendencies.

EF values of both *cis* enantiomeric pairs of cyhalothrin were lower than 0.5, with mean rates of 0.37 and 0.43 respectively. In this case, behaviors in barbel and catfish samples were similar. For EF*cis1*, these species showed the first eluting enantiomer (1R–3R–αR-isomer) enhanced but EF*cis2* showed a racemic enantiomeric mixture. On the other hand, gudgeon samples presented the opposite behavior: EF*cis1* showed no enantiomeric selectivity but EF*cis2* could indicate an (1R–3R–αS)-cyhalothrin enrichment. Knowing that, the gudgeon samples came from other rivers, different exposures are most probably the explanation to their different cyhalothrin patterns.

For cyfluthrin, EF*cis1* was always lower than 0.39. EF*cis2* of this pyrethroid could be calculated only for one sample, being 0.60. On the other hand, all samples presented concentrations of cypermethrin higher than the LOQ of the chiral method and all their EF values were lower than 0.5. Thus, *cis1* cypermethrin enantiomeric pair was enriched in the second eluting enantiomer (1S–3S–αS). Though, in the case of catfishes, this value was clearly near to racemic mixture. This indicated different enantio-accumulation of the same pyrethroid depending on the species, even in the same sampling point. Fig. 5 shows the chromatograms of cypermethrin for a barbel and a catfish in order to show graphically those results. Nonetheless, the *cis2* enantiomeric pair always presented an enhancement of the second eluting enantiomer (1S–3S–αR), as well for the catfish samples. Therefore, there was no correlation between enantioselectivity of one enantiomeric pair and the other. In commercial mixtures, only racemic patterns have been described (Corcellas et al., in press).

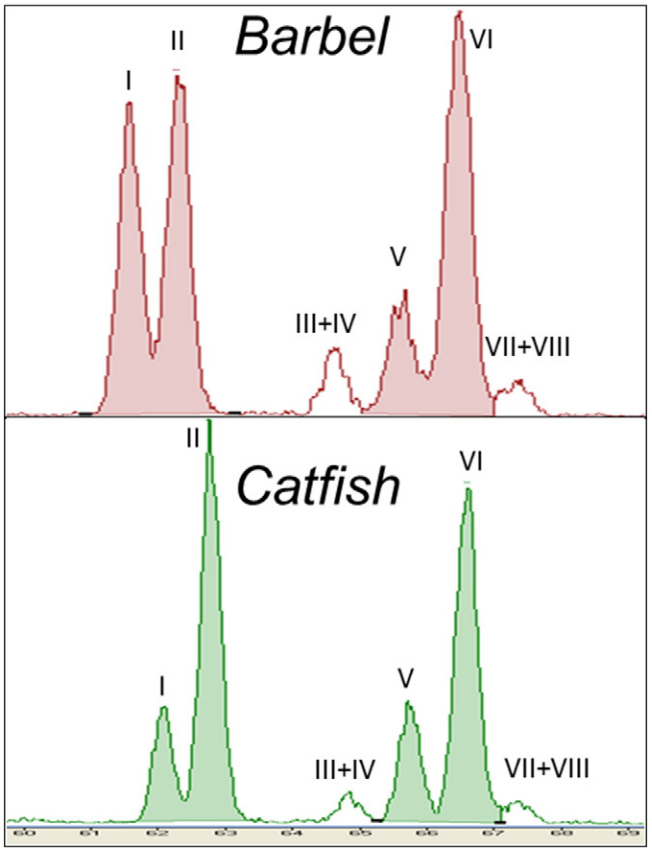

Fig. 5. Chromatograms obtained for the chiral determination of cypermethrin of barbel and catfish samples. Peak assignation following Fig. 2. (*cis* isomers shadowed).

4. Conclusions

Our work represents the first study showing the real bioaccumulation of pyrethroids in river wild fishes. Thus, the assumption of the safety of these insecticides is questioned. Nowadays, both European and American legislations establish maximum residue levels (MRL) of these pesticides for wild terrestrial animal products (EC, 2014; FAS, 2014). However, there is no legislation for fish. For example, the European Council laid down a MRL of 0.05 mg kg^{-1} of cypermethrin for wild game food. After doing the required unit conversion, in this study, the sum of pyrethroid concentrations in wild fishes is near to 0.03 mg kg^{-1} wet weight. Consequently, our study shows the importance of establishing new insecticide controls and the extension of their coverage to include edible fish groups. Besides, more investigations are necessary to evaluate this contamination in other sampling areas around the world, in which pyrethroids are extensively applied, as well.

At the same time, our results show that some enantioselective accumulation might be given in biota samples. Moreover, even if a concrete fish species showed enantioselective bioaccumulation for one specific pyrethroid, it did not mean that the fish presented the enantioselective bioaccumulation for other pyrethroids. Therefore, more research regarding enantioselective accumulation and enantiomeric toxicology is needed in order to establish which enantiomers cause a greater environmental risk.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.11.007.

Acknowledgment

This research was funded by the Spanish Ministry of Science and Innovation through the project SCARCE (Consolider Ingenio 2010 CSD2009-00065). This work has also been partly funded by the Generalitat de Catalunya (Consolidated Research Groups 2014 SGR 418 — Water and Soil Quality Unit). Biotage is acknowledged for SPE cartridges.

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