DISTRIBUTION OF PYRETHROID INSECTICIDES IN MEDITERRANEAN COMMON DOLPHIN TISSUES

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Introduction

Pyrethroids are semi-synthetic insecticides derived from pyrethrins. They are structurally related with the chrysanthemic acid and their esters. Depending on their chemical structure, they could be classified in type I and II pyrethroids. The main difference between them is the cyano group that type II pyrethorids have in the Cα' and type I ones have not (see Figure 1). Generally, these types of pyrethroids present different chiral properties. Briefly, those of type I usually comprise 2 diastereomers or enantiomeric pairs while type II commonly adds up to 3 enantiomeric pairs.

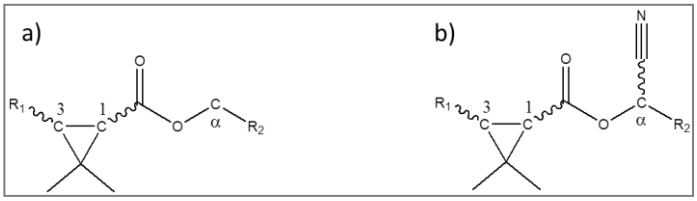

Figure 1. a) type I and b) type II pyrethroid structures.

Given the high toxicity and bioaccumulation of other insecticide families, pyrethorids were a good alternative to organophosphatated and organochlorinated pesticides. Besides, pyrethroid stability in the environment was estimated in less than 90 days. Finally, mammal metabolism is able to degrade this family of xenobiotic and excrete their metabolites. Because of all these advantages, the usage of pyrethroids has increased widely indoors as household insecticides, insect-control products, pet shampoos and lice treatments, and outdoors as agrarian and aquaculture pesticides and for pest control. For example, last Pesticide Industry Sales and Usage Report[1] estimated that in 2007, over 1500 tons of pyrethroids were used only in the U.S. Home and Garden market sector.

Nevertheless, the continuous and excessive dumping of these biocides made them ubiquitous in environment. Different studies in river waters and sediments demonstrated the presence of some pyrethroids from both urban[2] and agrarian[3] sources. Moreover, some recent works exposed the accumulation of pyrethroids in biota[4,5]. Even in human samples, such as blood[6] and breast milk[7], some authors have determined the presence of pyrethroids. It seems that an over-exposition to pyrethroids could hinder their complete metabolization[8].

Regarding pyrethroid toxicology, they are supposed to be low toxic for non-target organisms. However, it is known their high toxicity to aquatic environments. Moreover, nowadays they are been studied because of their potential capacity to decrease fertility in mice and some authors even have described pyrethroids as carcinogenic and endocrine disruptors[9]. Furthermore, they have been related with some other diseases at sub-lethal levels[10]. In addition, their enantiomeric properties might make important to take into account the potential differences in enantiotoxicology.

In previous works[4,5], our group found levels of pyrethroids in Brazilian dolphins and even in Iberian river fishes. With that background, our goal was to evaluate the presence of 12 pyrethroids (*cis*-bifenthrin, cyfluthrin, cypermethrin, cyhalothrin, deltamethrin, fenvalerate, fluvalinate, permethrin, phenothrin, resmethrin, tetramethrin and tralomethrin) in common dolphins (*Delphinus delphis*) from the Mediterranean Sea. These subpopulation is considered as endangered by the IUCN (International Union for Conservation of Nature), so special consideration should be done to understand the threats that they face and the decline in this area. .

Nonetheless, we want to study the distribution of pyrethroids in different dolphin tissues. We analyzed blubber, muscle, liver, brain and kidneys from 11 stranded individuals. Additionally, we evaluate the isomeric proportion of pyrethroids.

Materials and methods

Standards and reagents.

All analytical standards were purchased to Dr. Ehrenstorfer (Augsburg, Germany). As surrogate standards d_6-*trans*-permethrin and d_6-*trans*-cypermethrin were chosen and purchased to the same commercial firm. Organic solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions were prepared in ethyl acetate. 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 (C18, 2 g / 15 mL) and from Interchim (Basic alumina, 5g / 25 mL).

Sampling.

11 individuals were analyzed in order to determine levels of pyrethroids in their tissues. A total of 38 samples were analyzed including 10 samples of both muscle and kidney, 7 of liver, 6 of blubber and 5 of brain tissues (See Table 1). Samples were collected from 2004 to 2009 in the Spanish coast of the *Alboran Sea* (western Mediterranean Sea). These individuals were found stranded and samples were recollected and kept frozen at -20ºC until the analyses were carried out.

Table1. Analyzed tissues from each individual and total number of samples..

Individual	Tissues				
	Blubber	**Brain**	**Kidney**	**Liver**	**Muscle**
1	X	X			
2	X	X	X		X
3			X		X
4			X	X	X
5			X		X
6	X	X	X	X	X
7	X		X	X	X
8	X		X	X	X
9			X	X	X
10		X	X	X	X
11	X	X	X	X	X
TOTAL	6	5	10	7	10

Analytical methods.

Sample treatment was adapted from Feo *et al.*[11]. Briefly, 0.1 g of sample was spiked over night with 0.25 ng and 0.125 ng 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 ultrasounds during 15 minutes. 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 in 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 (15 m x 0.25 mm x 0.1 µm) for the quantitative analysis and a BGB-172 (30 m x 0.25 mm x 0.25 µm) for the enantiomeric determination. Details of chromatographic conditions to both achiral and chiral analyses are found in Corcellas *et al.*[12]. The selected mass spectrometry (MS) mode was the negative chemical ionization, with ammonium as reagent gas. All MS parameters are found in Feo *et al.* [11].

The lipid content was determined gravimetrically in parallel with an equivalent extraction procedure of 1 g of sample.

Results and discussion

Levels of pyrethroids

All the samples were positive to any pyrethroid. Levels ranged from 0.73 to 218 ng/g wet weight (ww), or between 20.5 and 6730 ng/g lipid weight (lw) (Table 2). Permethrin was the pyrethroid with the highest detection frequency (100%) followed by tetramethrin and cypermethrin with a 56% of positive samples. Bifenthrin, fluvalinate, phenothrin and resmethrin were never detected while cyfluthrin was detected in one single sample of liver.

Table2. Basic statistics of pyrethroid concentrations in dolphin tissues.

	Wet weight			Lipid weight		
	Arithmetic mean	Geometric mean	Range	Arithmetic mean	Geometric mean	Range
Blubber	65.3	42.9	11.7-218	145	99	20.5-437
Brain	10.1	5.69	0.73-18.3	253	160	28.0-551
Kidney	17.6	11.3	1.42-37.2	822	531	67.2-2460
Liver	23.6	18.3	6.39-48.9	942	628	68.8-2310
Muscle	18.6	10.1	1.49-51.9	2550	1420	192-6730

There was only one published work in bibliography about levels of pyrethroids in dolphins[5]. In this study, it was described a maximum level of pyrethroids of 64.4 ng/g lw in Brazilian franciscana dolphin (*Pontoporia blainvillei*) livers. In spite of that, in a recent study on Iberian fishes[4], levels of pirethroids calculated up to 4938 ng/g lw. The most concentrated pyrethroid in Brazilian dolphins was permethrin followed by cpermethrin and tetramethrin, the same behavior observed in the present work.

Pyrethroid distribution

As expected because of the lipophilic behavior of pyrethorids, blubber was the most contaminated tissue with a mean value of 65.3 ng/g ww. Following, liver, kidney and muscle presented very similar levels with means of 23.6, 17.6 and 18.6 ng/g ww respectively. The less concentration of pyrethroids was found in brain with a mean value of 10.1 ng/g ww in this tissue.

The most contributing pyrethroid in all the tested tissues was permethrin, with contribution ranging between 85 and 95 % of the total pyrethroids. In blubber samples, permethrin and tetramethrin were the unique detected pyrethroids. In addition to permehtrin and tetramethrin, cypermethrin was also found in brain, but not either fenvalerate or deltamethrin.

Lipid discussion

Figure 2 shows the distribution of pyrethroids when the levels were expressed in ww or in lw. When concentration of pyrethroids was normalized to the lipid content, the highest value corresponded to muscle, by far. This fact implies that there was a preference for some tissues in pyrethroid accumulation. In the cases where the pollutant trend is dominated by lipophilic properties, normalized concentration is similar in all tissues[13]. However, in our study, normalized pyrethroid level in muscle was even 8 times bigger than in kidney. Tissues like brain, which is very fatty, seemed to present lower concentration of pyrethroids while leaner tissues, like muscle, displayed a tendency of accumulating more pyrethroids per unit of fat. On the other hand, the typical detoxificant organs presented similar levels between them. Further studies are necessary to understand this behavior.

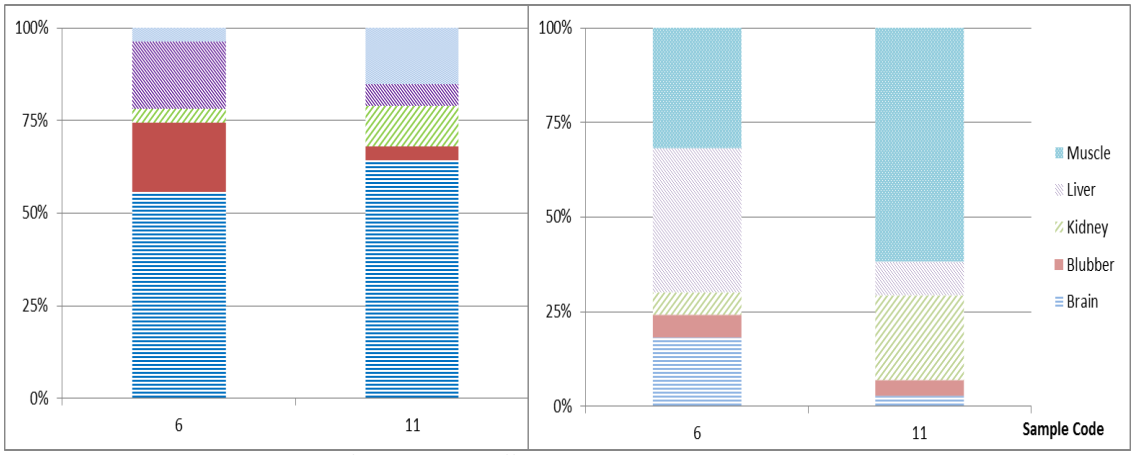

Figure 2. Distribution of pyrethroids in different tissues a) levels in ww b) levels normalized to lw.

Conclusion

Pyrethroid insecticides are present in biota samples. In this work we determined pyrethroid concentrations in Mediterranean dolphins for the first time. Besides, the selected species is considered endangered in this sea, so special attention should be paid due to the presence of the highest amount of this contaminant ever found in dolphins, with levels up to 6725 ng/g lw. All the analyzed samples were positive to pyrethroids. Permethrin was ever present but bifenthrin, fluvalinate, phenothrin and resmethrin were not found in any sample.

Pyrethroid distribution in tissues demonstrated a preference of these pollutants to be accumulated in specific tissues. This accumulation not seemed to be related with the fat content. Thus, the most polluted samples were blubber and the less were from brain. This results need to be further studied to be well understood.

Acknowledgements

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