INSECTICIDE PYRETHROIDS IN LIVER OF STRIPED DOLPHIN FROM THE MEDITERRANEAN SEA

Aznar-Alemany Ò[1]*, Giménez J[2], de Stephanis R[2], Eljarrat E[1], Barceló D[1,3]

[1]Water and Soil Quality Research Group, Dept. of Environmental Chemistry (IDAEA-CSIC), Barcelona, Spain; [2]Department of Conservation Biology (EBD-CSIC), Sevilla, Spain; [3]Catalan Institute for Water Research (ICRA), Girona, Spain.

Introduction

Pyrethroids are organic pollutants with high hydrophobicity (log K_{ow} between 5.7 and 7.6) and a very low water solubility (few $\mu g\,l^{-1}$) used as insecticides[1]. Therefore, these insecticides tend to rapidly bind to suspended particulate matter or sediments and low concentration is generally present in water. Applied to land or for domestic purposes as vector control, they can enter the aquatic environment through processes such as atmospheric deposition, river runoff and municipal treatment discharges. Once associated with sediments, benthic organism exposure of pyrethroids can be by ingestion or contact of sediment particles or from interstitial water. In fish, exposure to pyrethroids can be through diet or gill absorption due to their lipophilicity.

Concern has existed about aquatic organisms' exposure to pyrethroids because of their high toxicity[2]. More recent studies suggest carcinogenic, neurotoxic, immunosuppressive and reproductive potential toxicity of pyrethroids in mammals[3,4]. Despite the assumption that pyrethroids are converted to non-toxic metabolites by hydrolysis in mammals, possible evidence of their bioaccumulation has recently been found in marine mammals from Brazil[5]. Marine mammals are at the top of the food chain, which results in high exposure to a number of toxic compounds. The present study investigated the occurrence of ten pyrethroid compounds in liver samples from striped dolphins along the Mediterranean coast of Andalusia (south of Spain). The present investigation is the first attempt to determine the occurrence and bioaccumulation of pyrethroid insecticides in marine mammal tissues from the Mediterranean Sea.

Materials and method

Samples of dolphin liver (37 samples corresponding to 27 males and 10 females of different ages) were collected from striped dolphin (*Stenella coeruleoalba*) from the Mediterranean coast of Andalusia (Alboran Sea), south of Spain (Table 1). From these samples, 7 belong to dolphins found stranded between 2003 and 2005 and the remaining 30 were found between 2008 and 2010.

Table 1. Sampling information, including number of specimens, sex, maturity and total length

Sex	*n*	Age	Length (cm)
Males	8	Calves	90-128
	12	Juveniles	137-184
	7	Adults	197-225
Females	2	Calves	94- 98
	6	Juveniles	158-180
	2	Adults	214-231

Sample preparation was carried out according to Feo *et al.*[6]. Dolphin liver (0.1 g) was spiked with deuterated internal standards (d_6-*trans*-permethrin and d_6-*trans*-cypermethrin). The sample was stirred and extracted by sonication with 20 ml of hexane:dichloromethane (2 : 1) for 15 min and centrifuged for 6 min. The organic phase was transferred to a vial and evaporated with nitrogen. The remaining fat was re-dissolved with 20 ml of acetonitrile and underwent a cleanup with alumina and C_{18} SPE cartridges. The eluate was evaporated with nitrogen and re-dissolved with 100 µl of ethyl acetate to be analysed by GC-NICI-MS/MS[7]. Chromatographic conditions were as follows: injection volume was 3 µl; inlet temperature was 27 °C; DB-5ms capillary column (15 m × 0.25 mm, 0.1 film thickness) containing 5 % methyl phenyl siloxane; carrier gas was He at 1 ml min^{-1}, and temperature was 100 °C

for the first minute, then raised from 100 to 230 °C for 8 min, then from 230 to 310 °C for 8 min and, finally, was constant for 2 min. The ion source temperature for NICI-MS/MS was 250 °C and the reagent gas was ammonia at 2×10^{-4} torr. Table 2 shows the transitions and other parameters of the instrumental method.

The analytical method was selected to monitor 10 different pyrethroids, which are bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, detamethrin, fenvalerate, fluvalinate, permethrin, resmethrin and tetramethrin. Method recoveries ranged from 53 to 116 % and method LODs and LOQs are 0.02-0.46 ng g^{-1} in lipid weight (lw) and 0.08-1.54 ng g^{-1} lw, respectively.

Table 2. GC-MS/MS parameters for pyrethroid determination and quantification.

Pyrethroid	Internal standard	t_{R1}* (min)	t_{R2}* (min)	Transitions
Resmethrin	d_6-*trans*-Permethrin	7.27	7.34	337→149 337→187
Tetramethrin	d_6-*trans*-Permethrin	7.48	7.57	331→167
Bifenthrin	d_6-*trans*-Permethrin	7.45	-	205→141 205→121
Cyhalothrin	d_6-*trans*-Permethrin	7.99	8.09	205→141 205→121
Permethrin	d_6-*trans*-Permethrin	8.49	8.58	207→ 35 209→ 35
Cyfluthrin	d_6-*trans*-Cypermethrin	8.87	8.98	207→ 35 209→ 35
Cypermethrin	d_6-*trans*-Cypermethrin	9.07	9.18	207→ 35 209→ 35
Fenvalerate	d_6-*trans*-Cypermethrin	9.57	9.82	211→163 213→169
Fluvalinate	d_6-*trans*-Cypermethrin	9.81	9.88	294→250 294→194
Deltamethrin	d_6-*trans*-Cypermethrin	10.23	-	297→ 79 297→ 81
d_6-*trans*-Permethrin		8.56	-	213→ 35 215→ 35
d_6-*trans*-Cypermethrin		9.13	9.20	213→ 35 215→ 35

*Retention times for the different isomers or groups of isomers

Results and discussion

Insecticide pyrethroids were detected in 87 % of the samples, with bifenthrin, cyhalothrin, detamethrin, permethrin and tetramethrin being found. Total pyrethroid concentrations ranged from nd to 141 ng g^{-1} lw; whereas five samples presented higher levels, between 345 and 5210 ng g^{-1} lw. These levels were compared with those reported in the first and unique investigation of pyrethroid bioaccumulation in dolphins from the Brazilian coast (between 7.0 and 68 ng g^{-1} lw)[5] and proved to be higher. However, a new study of dolphins of another species (*Delphinus delphis*) also from the south of Spain shows similar levels in liver (69-2036 ng g^{-1} lw) if our five outliers are taken into account[8].

Figure 1 shows the average pyrethroid profile for Spanish dolphin livers, with a major contribution of permethrin and tetramethrin. These profiles were compared to that observed for Spanish human breast milk samples[9]. Also in human breast milk, tetramethrin and permethrin were the most abundant pyrethroids. This may account for a specific use of these pyrethroids in Spain. In contrast, profile corresponding to Brazilian dolphins was dominated by permethrin and cypermethrin[5].

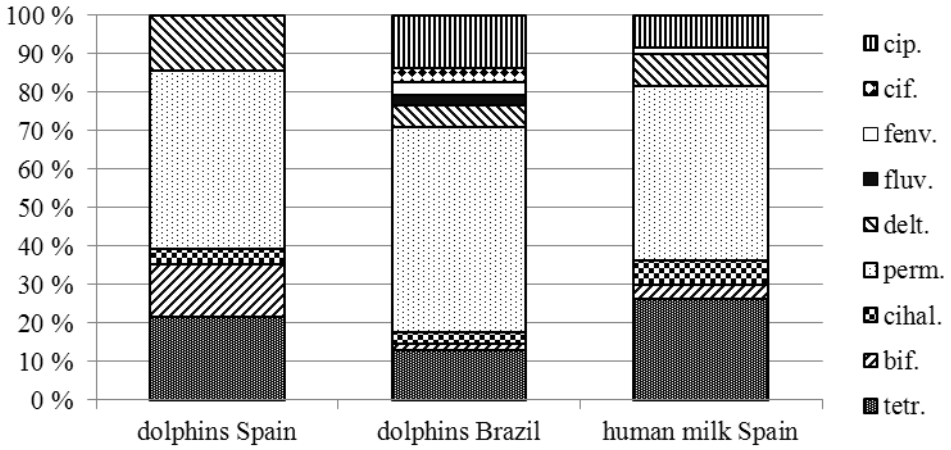

Figure 1. Pyrethroid profiles for dolphin livers and human breast milk.

The published work on pyrethroids in Brazilian dolphins showed the presence of these insecticides in breast milk and placenta samples, showing a mother-to-calf transfer of pyrethroids by both gestational and lactation pathways. Due to these findings, the present study aims to compare concentration levels in adult males and females. However, the insufficient number of adult female samples ($n = 2$) prevented the comparison between levels of adult male and female dolphins.

On the other hand, the samples of male dolphins where used to assess bioaccumulation as there were enough samples of all age groups. Previous studies showed that an increase of pollutant levels with the length of the specimen (which is directly related to age) is an indication of bioaccumulation. Figure 2 shows the measured total pyrethroid concentrations versus dolphin length. According to these results, there is no evidence of pyrethroid bioaccumulation in male dolphins.

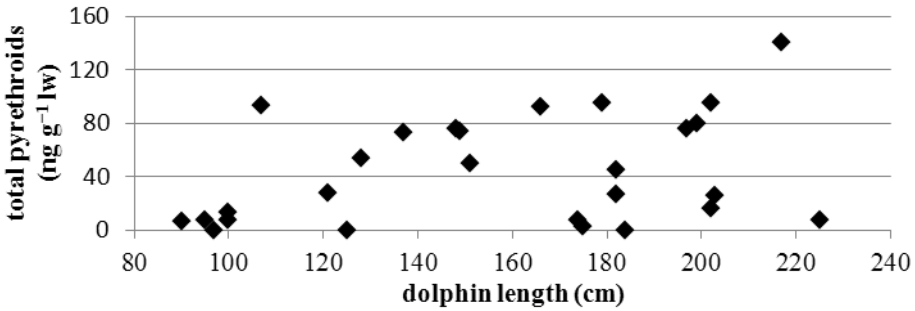

Figure 2. Individual total pyrethroid concentrations (ng g^{-1} lw) for male dolphin livers according to dolphin length (cm).

Figure 3a shows the box plot comparing concentration levels for male calves, juveniles and adults. There are no significant differences between the three maturity stages (K-W $X^2 = 3.6855$, $p = 0.1584$). It seems, nevertheless, that concentration levels increase from calves to juvenile, whereas juvenile present similar levels to adults. Metabolization of pyrethroids after achieving sexual maturity might account for this lesser and non-significant growth.

In order to study temporal trends, data from samples collected in 2003-05 were compared with those collected 5 years later, in 2008-2010 (Figure 3b). No significant differences were observed between both sampling periods (K-W $X^2 = 0.0031$, $p = 0.9558$). Typically, studies of temporal trends are carried out with a minimum difference of 10 years. Therefore, we believe that it would be necessary to do this type of study within five years from now in order to detect any clear trend.

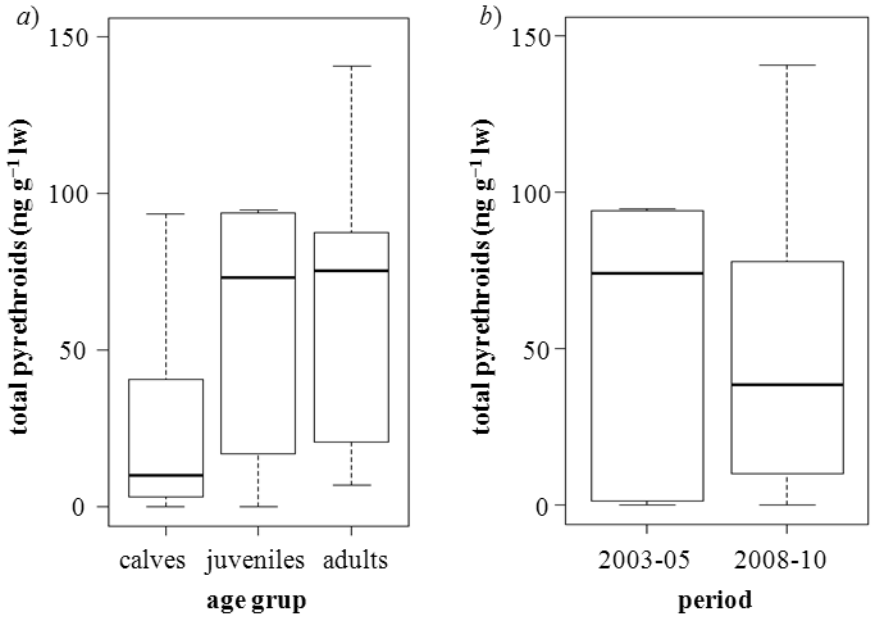

Figure 3. Box plots for total pyrethroid concentrations (ng g^{-1} lw) for dolphin livers according to (*a*) age group and (*b*) collection period.

Acknowledgements

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