PYRETHROIDS: AN OLD CONTAMINANT MAY REPRESENT A NEW CONCERN TO MARINE MAMMALS

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Introduction

Pyrethroids are organic contaminants with high hydrophobicity (log Kow ranging between 5.7 and 7.6) and very low water solubility (of a few μg/L)[1]. Historically, concern has existed regarding aquatic organism exposure to pyrethroids, particularly arthropods and fish, because of the high degree of toxicity observed in standard laboratory studies[2]. Moreover, a number of recent studies have also suggested the carcinogenic, neurotoxic, immunosuppressive and reproductive potential toxicity of pyrethroids in mammals[3]. However, pyrethroids are considered to be safe because they are converted to non- toxic metabolites in fish and mammals[4]. Most studies on exposure to pyrethroids are based on the determination in urine samples. Most studies are based on the determination of pyrethroid concentration in urine samples, but recent works have indicated the presence of pyrethroids in human breast milk[5].

Cetaceans have long been used as sentinel species for environmental contamination by organic pollutants. The Franciscana dolphin (*Pontoporia blainvillei*) is a small cetacean that occurs exclusively in the western Atlantic and has limited movement and a small home range[6]. Due to its nearshore distribution, this species is especially vulnerable to the effects of human activities.

Twelve pyrethroids were determined in liver samples from 23 male Franciscana dolphins from Brazil. In order to assess the mother-to-calf transfer of pyrethroids, three samples of breast milk and three other of placenta were also analyzed. The present investigation is the first attempt to determine pyrethroid insecticide levels in marine mammal tissues to identify their bioaccumulation.

Materials and methods

A total of 23 liver samples from male dolphins were collected from two locations along the Brazilian Coast (São Paulo State, SP - n = 12, from 2004 to 2008 and Rio Grande do Sul State, RS - n = 11, from 1994 to 2000). Sampling locations ranged from a highly urbanized area (e.g. SP Coast) to a more agricultural zone (e.g. RS coast). The collected samples came from individual dolphins incidentally caught in fishing nets. Only male dolphins were analyzed in this study in order to investigate potential differences in pyrethroid concentrations between locations and to analyze the metabolic imbalance during life cycle. In order to assess the mother-to-calf transfer of pyrethroids, three samples of milk and three of placenta were collected from dead females of SP.

Pyrethroid analyses were performed using previously described methods[5]. The samples were fortified with surrogate standards and extracted by sonication. Extracts were cleaned up by elution through C18 coupled to basic alumina cartridges. GC–MS–MS analysis was performed in negative chemical ionization mode in a DB-5ms capillary column. Quality parameters of the method had been evaluated. Recovery tests were carried out by addition of each pyrethroid to a sample. These samples were previously analyzed in order to determine pyrethroid presence before spiking (blank). Three replicates were prepared for the evaluation of the reproducibility of the method. The limits of detection of the method (mLOD), defined as 3 times the noise level, and the limit of quantification of the method (mLOQ), defined as 10 times the noise level, were calculated. Statistical analyses were conducted using non parametric tests (Shapiro Wilk´s W) followed by individual one-way analysis of variances (ANOVAs) and post hoc by Tukey´s Honestly Significant Difference (HSD).

Results and discussion

Recoveries ranged between 53 and 116%, with relative standard deviation (RSD) values lower than 20%. mLODs ranged between 0.02 to 0.71 ng/g lw, and mLOQs ranged between 0.08 and 2.38 ng/g lw. The GC-MS-MS identification of pyrethroids was based on the following criteria: (i) simultaneous responses for the two monitored transitions (SRM1 and SRM2) must be obtained at the same retention time as that of available standards; (ii) signal-to- noise ratios must be >3; and (iii) relative peak intensity ratio must be within ±20% of the theoretical values obtained with standard solutions.

Hepatic pyrethroid concentrations in Franciscana dolphins from two Brazilian states (SP and RS) are presented in **Table 1**. All targeted pyrethroid compounds were detected in liver samples, with the exception of resmethrin, which was not found in any sample. The concentrations for total pyrethroids were 7.04 and 68.4 ng/g lw in an adult and a calf, respectively. Permethrin was the predominant pyrethroid in all samples, with mean percentage contribution of 73%, 58% and 56% in calf, juvenile and adult dolphins, respectively. The next contributing compounds are tetramethrin and cypermethrin, with contribution values between 5-15% and 8-11%, respectively.

Table 1. Median values of pyrethroids concentrations (ng/g lw) in livers from Franciscana dolphin males from two Brazilian locations.

	São Paulo (SP)			Rio Grande do Sul (RS)		
	Calves	Juveniles	Adults	Calves	Juveniles	Adults
Tetramethrin	2.59	0.96	3.56	6.67	1.82	4.75
Bifenthrin	0.85	0.64	1.01	0.10	nq	0.10
λ-Cyhalothrin	0.35	2.90	0.31	nq	nq	0.51
Deltamethrin/Tralomethrin	0.96	0.16	2.68	5.36	nq	0.96
Fluvalinate	nq	1.02	nq	nq	nq	nq
Esfenvalerate/Fenvalerate	0.57	0.48	nq	3.64	0.48	nq
Permethrin	31.2	11.0	18.7	11.1	9.04	7.66
Cyfluthrin	1.61	0.23	0.56	3.45	1.33	nq
Cypermethrin	3.28	1.58	4.34	4.93	2.54	2.47
TOTAL	41.2	17.5	31.5	35.3	18.0	16.8

nq – below the limit of quantification

Comparing the concentrations obtained in these two locations, higher pyrethroid levels were found in dolphin samples from SP. This fact may indicate a greater use of pyrethroids in urban areas (SP) compared with their application for agricultural purposes (RS). Similar findings were observed in California, where the urban runoff contributes more to pyrethroid input than the discharge of irrigation runoff[7]. While pyrethroid input may be greater as a result of urban runoff, the colder temperatures at RS, the temperate zone of Brazilian coast, could make the pyrethroids more toxic[7], putting the Franciscanas from Southern Brazil at risk, even at lower concentrations.

Total pyrethroid concentrations had a peculiar pattern of distribution according to the total length of the sampled dolphins (**Figure 1**). In dolphins from SP, the highest concentrations occurred in the smallest individuals (calves, 91 to 98 cm in length). Concentration decreased with calf length, demonstrated an increase in concentration with length in juveniles (ranging in length from 103 to 108 cm), and again showed a decrease in concentration with length in adults (length from 116 to 124 cm). With the growth of the animal, pyrethroid concentrations could become diluted in the dolphin’s body and then begin to accumulate due to predation on squids, shrimps and fish until reaching sexual maturity. As adults, these mammals seem to be capable of metabolizing/degrading pyrethroid compounds. This would be the explanation for the fact that the lowest concentrations were found in the lengthier Franciscana dolphins. However, it should be noted that the observed pattern is based on a very limited sample size. Therefore, future studies involving a larger number of individuals are required.

Based on these results, we proceeded to evaluate the potential maternal transfer of these compounds through the analysis of breast milk and placenta samples. Breast milk reflects maternal body burden and the post-natal transfer of pyrethroids from mothers to calves, whereas placenta provides a good indication for pre-natal exposure. Three milk and three placenta samples of Franciscanas from SP were analyzed. Pyrethroids were detected in both matrices, with values between 2.53-4.77 ng/g lw and 331-1812 ng/g lw, for breast milk and placenta, respectively (**Table 2**). Therefore, for the first time, a study shows mother-to-calf transfer of pyrethroids by both gestational and lactation pathways in dolphins. It is interesting to note that pyrethroids distribution pattern presented some differences in milk in relation to placenta samples (**Figure 2**).

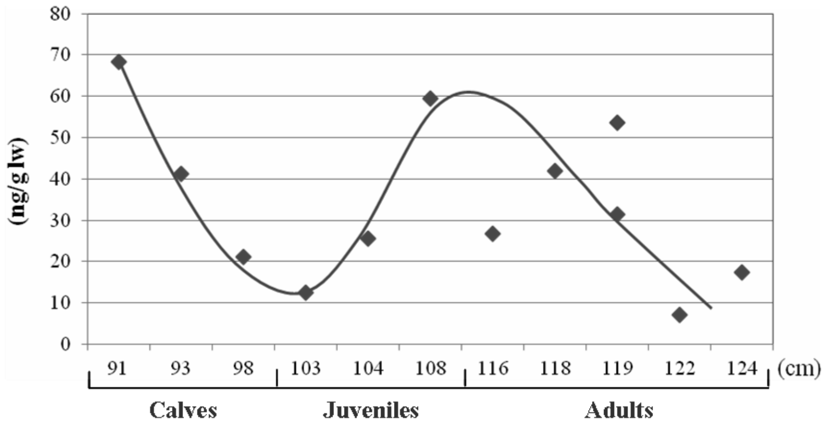

Figure 1. Pyrethroid concentration (ng/g lw) according to total length (cm) of male Franciscana dolphins from São Paulo coast, Brazil.

Similar to the distribution observed in liver samples, permethrin is the dominant pyrethroid in milk samples, with a mean contribution of 40% of the total. In placenta samples the contribution of permethrin decreased to 25%. Also for bifenthrin, the contribution decreased from milk (17%) to placenta (6%) samples. Similar behavior was also observed for tetramethrin (from 10% to 5%), deltamethrin/tralomethrin (from 13% to 3%), esfenvalerate/fenvalerate (from 8% to 1%) and cyfluthrin (from 5% to 2%). In contrast, other compounds presented higher contributions in placenta compared with those of milk samples. The most prominent was cypermethrin, which was the dominant pyrethroid in placenta with a mean contribution of 55%, whereas their milk samples contribution was only 8%. Moreover, λ-cyhalothrin and fluvalinate were not detected in milk samples, and their contributions in placenta were 3% and 1%, respectively. Analyzing this scenario we can assume that some pyrethroids, such as permethrin, bifenthrin, tetramethrin, deltamethrin/tralomethrin were transferred maternally through both gestational and lactation pathways, but mainly through the breast milk. In contrast, other pyrethroids, such as cypermethrin were basically transferred through gestational pathways by the placenta. The different pyrethroid behavior may be due to their physico-chemical properties.

Table 2. Pyrethroids concentrations (ng/g lw) in milk and placenta from five Franciscana dolphin of São Paulo, Southeastern Brazil.

	Breast Milk			Placenta		
	#1 (BP106)	#2 (BP132)	#3 (BP153)	#1 (BP106)	#2 (BP62)	#3 (BP71)
Tetramethrin	0.6	0.2	0.1	37	25	96
Bifenthrin	0.7	0.4	0.6	38	51	86
λ-Cyhalothrin	nq	nq	nq	22	9.3	49
Deltamethrin/Tralomethrin	0.3	0.4	0.6	nd	13	39
Fluvalinate	nq	nq	nq	20	7.1	nq
Esfenvalerate/Fenvalerate	0.4	0.2	0.2	6.9	12	10
Permethrin	2.3	0.9	0.8	259	103	385
Cyfluthrin	0.2	0.1	0.1	14	15	42
Cypermethrin	0.3	0.2	0.3	459	96	1106
TOTAL	4.8	2.5	2.7	856	331	1812

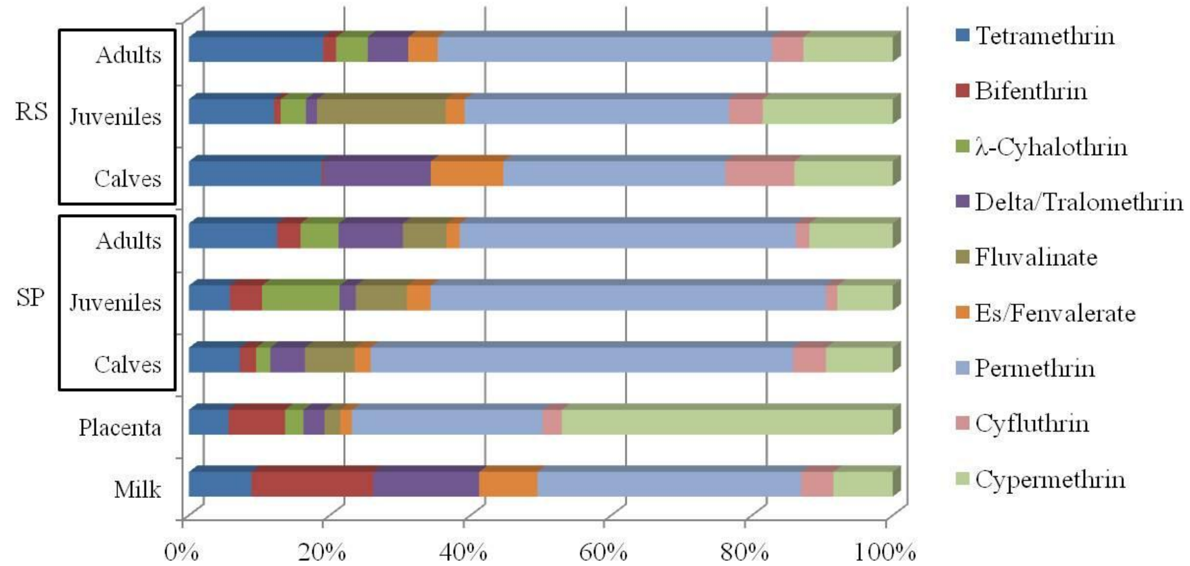

Figure 2. Percentage contribution of each pyrethroid to total contamination in liver (calves, juveniles and adults), milk and placenta samples from Franciscana dolphins of Rio Grande do Sul and São Paulo coasts, Brazil.

Contrary to the assumption that pyrethroid insecticides converted to non-toxic metabolites by hydrolysis in mammals, the present study showed for the first time the presence of different pyrethroids in marine mammal tissues, supporting their bioaccumulation. The effects of these compounds are still unknown, but cypermethrin, permethrin and biphenthrin were classified by EPA as possible human carcinogens[8]. In addition, it is known that cypermethrin is harmful to aquatic life even in low levels[9].

Taking into account the potential toxic effects of pyrethroids and the exposure to these pollutants in an early period of life, the need for further studies related to marine mammal exposure to these compounds becomes clear. Some recent works have also raised a similar concern in humans, as the presence of pyrethroids was found in human breast milk samples from different geographical areas[5].

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