CONCENTRATIONS OF ORGANOBROMINATED COMPOUNDS OF NATURAL AND INDUSTRIAL ORIGIN IN TOP PREDATORS FROM BRAZILIAN WATERS

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Introduction

Determination of organic micropollutants in cetaceans is of special interest, owing to their top position in the food web, their long life-span and, for some species, their year-round presence in polluted and relatively small areas [1]. Environmental contamination by polybrominated diphenyl ethers (PBDEs) has received considerable attention due to the bioaccumulative nature of these compounds as well as their widespread use as flame retardants. In addition, brominated compounds of natural origin have been detected in marine mammals at concentrations comparable to those of industrially-produced halogenated organic compounds [2]. Therefore, the main objective of the present study was to determine the current concentrations of organobrominated compounds in odontocete species from a highly industrialized and urbanized region in the Southern Hemisphere. To generate information on the placental transfer of organobrominated compounds, foetus/mother ratios of hepatic concentrations of these molecules were calculated for two mother-foetus pairs. To the best of our knowledge, this is the first study to determine organobrominated compounds in tissues of marine mammals from the southwest Atlantic Ocean.

Material and methods

Liver samples were collected from 51 cetaceans stranded on the beaches of Rio de Janeiro state, in southeast Brazil, from 1994 to 2006. Sampled specimens (Table 1) comprised delphinid species that occupy estuarine (marine tucuxi dolphin, *Sotalia guianensis*), continental shelf (Atlantic spotted dolphin, *Stenella frontalis*; rough-toothed dolphin, *Steno bredanensis*; common dolphin, *Delphinus delphis*; bottlenose dolphin, *Tursiops truncatus*; and false killer whale, *Pseudorca crassidens*) and oceanic environments (Fraser's dolphin, *Lagenodelphis hosei*; spinner dolphin, *Stenella longirostris*; pantropical spotted dolphin, *Stenella attenuata*; and striped dolphin, *Stenella coeruleoalba*). The age of cetacean specimens was determined by counting the growth layer groups present in dentine and cementum of the teeth. However, not all the individuals had their age determined, which limited statistical treatment to male marine tucuxi dolphins and female Fraser's dolphins. The following PBDE-congeners (IUPAC numbers 28, 47, 66, 85, 99, 100, 153, 154 and 183) were targeted for analysis. Additionally, two methoxylated-PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) have also been determined in all samples. The sample preparation and analysis are described elsewhere [3,4]. BDE 77 was used as internal standard (IS) for tetra and penta-BDEs and MeO-PBDEs, and BDE 128 for hexa and hepta-BDEs. Measurements were carried out using an Agilent 6890-5973 GC-MS. Recoveries for individual PBDE congeners were between 87 and 104 % (RSD $\leq$ 12 %) during method validation. Analysis of standard reference material SRM 1945 (PBDEs in whale blubber) indicated that the method accuracy was above 90 %. For statistics, parametric (*Student's t-test* and *Pearson's correlation test*) or non-parametric tests (*Mann-Whitney U test* and *Spearman's correlation test*) were used depending on data normality (*Shapiro-Wilk's W test*). Due to the low number of individuals of some species, only data from marine tucuxi dolphins, Atlantic spotted dolphins and Fraser's dolphins were used for inter-species statistical comparison.

Results and discussion

Brominated compounds – levels and spatial distribution

Among the analyzed PBDE congeners, BDE 183 could not be detected in any of the cetacean samples. The absence of BDE 183 is probably a result of a combination of factors, such as low use of OctaBDE technical mixture, debromination of BDE 183 to BDE 154 (as observed in fish [5]), or a reduced bioavailability in aquatic

environments. Concentrations of PBDEs and MeO-PBDEs measured in the various delphinid species from the present study are given in Table 1. Covaci et al. [3] reported a similar range of PBDEs (410 to 5810 ng/g, lipid wt.) in liver samples from North Sea harbour porpoises (*Phocoena phocoena*).

Table 1. Hepatic concentrations (ng/g, lipid wt.) of PBDEs and MeO-PBDEs in dolphins from Brazilian waters.

				ΣPBDEs		ΣMeO-PBDEs	
Habitat	**Species**	**N**	**Gender**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Estuarine	*Sotalia guianensis*	13	M	670 ± 433	258 - 1624	151 ± 151	38 - 484
		6	F	158 ± 151	13 - 446	75 ± 43	26 - 156
		1	NB	242		33	
C. Shelf	*Stenella frontalis*	6	M	1151 ± 712	359 - 2439	19179 ± 19418	6326 - 55360
		1	F	96		3106	
	Pseudorca crassidens	2	NB	3585 ± 3359	1210 - 5960	148707 ± 141800	48439 - 248974
	Tursiops truncatus	3	M	957 ± 597	270 - 1353	19891 ± 10926	12458 - 32436
	Steno bredanensis	1	M	357		3896	
		2	F	1151 ± 641	697 - 1604	15171 ± 14646	4815 - 25527
	Delphinus delphis	1	M	239		3669	
		1	F	124		2936	
Ocean	*Stenella attenuata*	1	M	1216		88233	
	Stenella longirostris	1	F	153		37978	
	Stenella coeruleoalba	1	Unident.	211		6854	
	Lagenodelphis hosei	2	M	21.5 ± 9	15 - 28	2309 ± 688	1822 - 2795
		7	F	7.4 ± 4.2	3 - 14	1008 ± 658	423 - 2177

BDE 47 was the predominant PBDE congener in all species and it contributed by 32-80 % to the ΣPBDEs, except for the Fraser's dolphin, a delphinid species of extreme oceanic habit [1]. BDE 47 was the only PBDE congener detected in two out of the nine analyzed Fraser's dolphins. For the marine tucuxi dolphins, BDE 47 was followed by BDE 100, BDE 99, BDE 154, BDE 153, BDE 28, and BDE 85 (Fig. 1). The PBDE profile in this species differs from that in the oceanic Fraser's dolphin, indicating a skewing of PBDEs in oceanic waters off southeast Brazil in favour of more volatile components. BDE 28 was the second most abundant congener in tissues of the latter species, while BDE 66 was measured in delphinids living on continental shelf and oceanic environments and it was not detected in estuarine dolphins. This finding constitutes additional observation of increased contribution of more volatile PBDEs in a region far away from the coast. It also suggests that differentiation among estuarine, continental shelf and oceanic food webs plays an important role. Longer food chains, such as those characteristic of oligotrophic oceanic environments, may favour the occurrence of higher micropollutant levels in nektonic predators [6]. The presence of BDEs-47, 99, and 100 in liver of the estuarine dolphins indicates the possible use of the PentaBDE mixture in Brazil. A similar congener distribution was reported in marine mammals from North America, where the PentaBDE mixture has been extensively used [7].

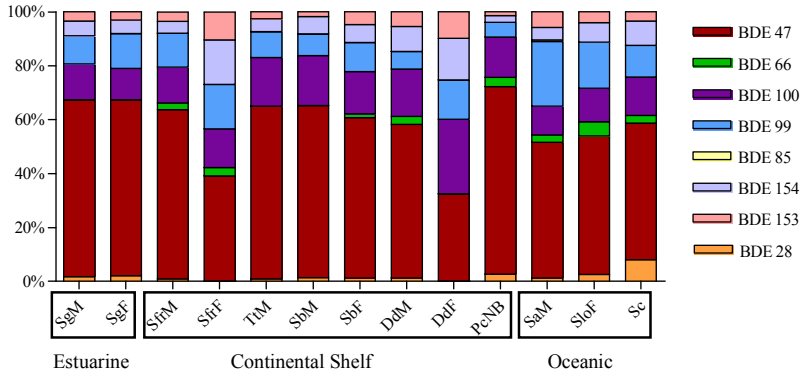

Fig. 1. Contribution of each PBDE congener to the ΣPBDE (SgM and SgF, male and female *S.guianensis*; SfrM and SfrF, male and female *S. frontalis*; TtM, male *T. truncatus*; DdM and DdF, male and female *D. delphis*; PcNB, newborn *P. crassidens*; SaM, male *S. attenuata*; SloF, female *S.longirostris*; Sc, *S. coeruleoalba* of unidentified sex).

To the best of our knowledge, the concentrations of naturally-produced brominated compounds, such as MeO-PBDEs, determined in cetaceans from Brazilian waters are among the highest detected to date in marine mammals (Table 1). The highest concentration of 2'-MeO-BDE 68 previously reported for marine mammal tissues was 3760 ng/g wet weight found in the blubber of a pygmy sperm whale from Queensland, northeast Australia [8]. Blubber lipid percentage in marine mammals range between 30 to 90 % [9]. Considering the lower bound lipid percentage (30%) a maximum value of 12 500 ng/g lipids can be calculated for 2'-MeO-BDE 68 in the pygmy sperm whale. The magnitude of the MeO-PBDE concentrations measured in Brazilian cetaceans is indicated by the high number of dolphins (n=8) in the present study which exceed this value. Significant higher ΣMeO-PBDE concentrations were measured in male Atlantic spotted dolphins than in male marine tucuxi dolphins (p=0.0006). Similarly, higher concentrations of these compounds were determined in female Fraser's dolphin than in female marine tucuxi dolphins (p=0.006). MeO-PBDEs have been isolated from sponges of the genus *Dysidea* [2] and the presence of these organisms has already been reported in "Região dos Lagos" area (Rio de Janeiro state) [10], a region strongly influenced by the upwelling phenomenon [6]. Interestingly, 11 out of the 17 delphinids that inhabit the continental shelf were found in that region, while marine tucuxi dolphins were found elsewhere. Advection by upwelling may be contributing to the transport of MeO-PBDEs from the benthic to the pelagic food chain in the region. Squids constitute important prey for the analyzed continental shelf delphinids [6]. Since these nektonic invertebrates carry out vertical diel migrations [6], they may also play a role on the benthic-pelagic transportation of MeO-PBDEs and hence contribute to the high concentrations found in the present study. The fact that the highest concentrations reported for cetaceans were found in samples from Queensland, Australia (~19°S) [8] and Rio de Janeiro state, Brazil (~22°S) draws attention to a possible more intense biosynthesis of MeO-PBDEs and/or bioavailability to nektonic organisms in tropical areas of the globe.

Comparison between organobrominated compounds of natural and industrial origin

A clear shift on the contribution of naturally-occurring compounds (MeO-PBDEs) and the anthropogenically produced PBDEs to the sum of organobrominated contaminants was observed between estuarine and continental shelf dolphins (Fig. 2). A ratio between these two groups of brominated compounds (ΣPBDEs/ ΣMeO-PBDEs) was calculated for each individual. For the marine tucuxi dolphin, the ratio had a mean value of 7.12 (SD = 8.31) and ranged from 0.23 to 29.4. Remarkably different results were obtained for the other cetacean species. For the Atlantic spotted dolphin, values varying from 0.02 to 0.16 (mean ± SD = 0.08 ± 0.06) were found, while for the oceanic Fraser's dolphin, all the individuals had a mean value of 0.01. A trend pointing towards a higher proportion of naturally-occurring brominated compounds in female than in male marine tucuxi dolphins was observed (p=0.066), which could be explained by the more efficient placental transfer of BDE 47.

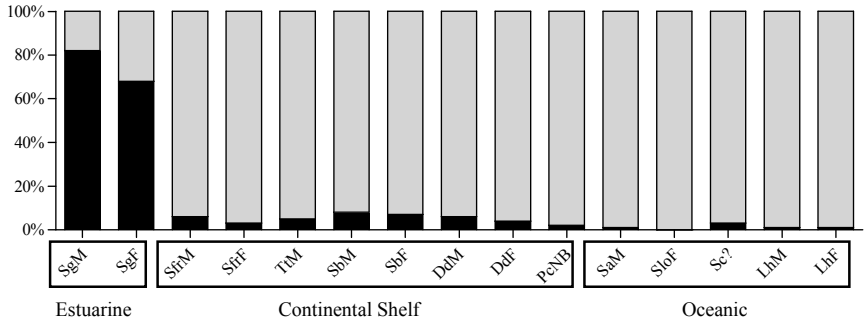

Fig 2. Contribution of natural (light grey) and industrial (black) PBDEs to the ΣPBDE. Codes are given in Fig. 1. (LhM and LhF, male and female *Lagenodelphis hosei*)

Levels of brominated compounds and dolphin age, with special reference to temporal variation.

No correlation was found between ΣPBDE concentrations and the age of male marine tucuxi dolphins (p=0.997). A positive correlation was observed between concentrations and the year of stranding (p=0.002), indicating a temporal variation (from 1994 to 2006) in marine tucuxi dolphin exposure, which may have hampered the existence of an age-related correlation. Significant negative correlations were found between brominated compound concentrations and the age of female Fraser's dolphins for both ΣPBDEs (p=0.005) and ΣMeO-

PBDEs (p=0.006). Since there was only a four-day interval between strandings of these females, it is plausible to assume that these negative correlations are a consequence of similar reproductive stages at the time of death, suggesting a strict seasonality in the reproduction of Fraser's dolphins in southwest Atlantic Ocean.

Transfer of brominated compounds from mother to foetus

The foetus/mother ratios were calculated for two marine tucuxi pairs (Fig. 3). These ratios compare favourably with Law et al.[11], who found ratios of 0.78 for BDE 47, 0.49 for BDE 99, 0.65 for BDE 100 and 0.34 for BDE 153 in blubber of a harbour porpoise foetus/mother pair. Lower ratios and therefore reduced placental transfer were found for higher brominated compounds (Fig. 3). A significant negative correlation was observed between the number of bromine atoms of each congener and the mean values of foetus/mother ratios (p=0.008). This can be due to increased K_{ow} values and molecular sizes [7]. Both factors influence the movement of lipophilic compounds from the mother's lipid storage sites into the mother's blood, thereby indirectly influencing placental transfer. Differences between both foetus/mother pairs in the present study are possibly due to a combination of factors, such as the number of previous pregnancies, the age of the mother, and stage of development of the foetus. Because of the transplacental movement, male and female marine tucuxi dolphins differed significantly in ΣPBDE concentrations (p=0.003), but not in ΣMeO-PBDE concentrations (p=0.661). This might be a reflection of an adaptation to compounds of natural origin during the evolution process, although it needs further research.

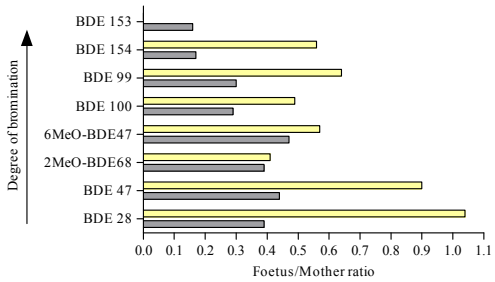

Fig. 3. Foetus/mother ratios of two pairs (*S. guianensis*). □ mother 23 years old, ■ mother 30 years old.

Acknowledgments

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