PBDEs in the blubber of marine mammals from coastal areas of São Paulo, Brazil, southwestern Atlantic

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) are additive flame retardants that have been incorporated into several polymeric materials (e.g., plastics, textiles, furnishing foam) to prevent their risk of fire. There is no production of PBDEs in the southern hemisphere although technical mixtures may be imported by local industries. Manufactured goods known to contain PBDEs (e.g., TV sets, computers, automobiles) are mostly imported from overseas. PBDEs were first detected in the environment in the 1970s (Andersson and Blomkvist, 1981; DeCarlo, 1979) and have been considered of increasing concern to scientists and policy makers since the 1990s (de Wit, 2002; Yogui and Sericano, 2009b). In the northern hemisphere, temporal trends have shown increasing concentrations of PBDEs in the environment of North America and decreasing levels in Europe and Japan (Hale et al., 2003 and references therein). In contrast, limited information has been published on the levels of PBDEs in marine biota of the southern hemisphere and most of these investigations were carried out in Antarctica (Chiuchiolo et al., 2004; Corsolini et al., 2007; Corsolini et al., 2006; Dorneles et al., 2010; Leonel, 2007; Ueno et al., 2004; Yogui and Sericano, 2009a). Such a fact emphasizes the importance of measuring PBDEs in samples collected in the southern hemisphere to better understand their distribution and fate, especially in tropical and subtropical regions.

The coast of São Paulo (Brazil) is one of the most developed areas in the southwestern Atlantic (Fig. S1 in the Supplementary Material). It has been impacted by several human activities including industry, tourism and agriculture. A total of 29 cetacean species have been recorded along the coast of São Paulo (Santos et al., 2010). The Guiana dolphin (Sotalia guianensis) and the franciscana (Pontoporia blainvillei) are the most abundant species inshore (Bertozzi and Zerbini, 2002; Rosas et al., 2002; Santos et al., 2001). Their diets basically consist of fish, squid and shrimp (Santos et al., 2002; Santos and Haimovici, 2001). The Atlantic spotted (Stenella frontalis), rough-toothed (Steno bredanensis), and bottlenose (Tursiops truncatus) dolphins also occur in the area (Santos et al., 2010). However, little is known about these species as they are distributed in continental shelf waters deeper than 20 m. In this study, PBDEs were determined in archived blubber extracts belonging to the five species described above. Analysis of archived extracts is a valid approach to get more information on a particular set of samples and has been used by other authors (e.g., Batterman et al., 2007; Hassanin et al., 2005; Zhu and Hites, 2004). Contamination is discussed and compared to small cetaceans from other regions of the world. In addition, contribution of PBDEs to the total burden of persistent organic pollutants (POPs) in the evaluated dolphins is assessed through comparison with data previously published in the literature.
2. Materials and methods

2.1. Sampling and chemical analysis

Blubber samples were collected from animals found stranded on beaches or incidentally caught in drift nets between 1996 and 2003. The following samples are reported in this study: nine blubbers from *S. guianensis* (5 males and 4 females), eight from *P. blainvillei* (4 males and 4 females), two from *S. frontalis* (both males), one from *S. bredanensis* (male) and one from *T. truncatus* (male). Detailed information on these animals is provided in the Supplementary material (Table S1). Sampling procedures followed international standards for the analysis of trace organic contaminants (see Aguilar, 1985; Borrell and Aguilar, 1990; UNEP/ICES/IOC, 1991). The method used for extraction and clean up of samples is described elsewhere as these samples have been previously analyzed for chlorinated compounds (see Yogui et al., 2003, 2010). Briefly, a known amount of internal standard (PCB-103) was added to the sample prior to extraction. Approximately 1 g of blubber was extracted in a Soxhlet apparatus with 70 mL of n-hexane and methylene chloride (1:1, v/v). The extract was cleaned up with sulfuric acid (96%), concentrated to 0.5 mL, added recovery standard (TCMX) and stored at −20 °C until analysis. Lipids were gravimetrically determined using an aliquot of the extract taken before acid treatment.

2.2. Instrumental parameters

Sample extracts were injected into a gas chromatography/mass spectrometry system (GC/MS, Agilent Technologies, models 6890 and 5973, respectively) in the selected ion monitoring (SIM) mode. A DB-5ms capillary column (30 m length × 0.25 mm id × 0.25 μm film thickness) was used for separation of compounds. Splitless injections were done using an autosampler. Helium was used as carrier gas under constant flow (0.8 mL min⁻¹) into the column while the purge flow rate was adjusted to 20 mL min⁻¹. The injector temperature was set at 270 °C. The oven was programmed as follows: 130 °C for 1 min, ramped at 12 °C min⁻¹ to 154 °C, at 2 °C min⁻¹ to 210 °C, and at 3 °C min⁻¹ to 300 °C with a final hold time of 5 min. The mass spectrometer was operated in electron ionization (EI) mode. The interface, ion source and quadrupole temperatures were set at 290 °C, 230 °C, and 150 °C, respectively. Three ions representing a typical bromine cluster were monitored for each target analyte, except for mono-BDEs (monitored using two ions). The primary m/z for mono- through hepta-BDEs were 248.00, 327.90, 405.80, 485.70, 563.60, 643.55, and 721.45.

### Table 1

Concentration of polybrominated diphenyl ethers (PBDEs) in the blubber of small cetaceans from the coast of São Paulo, southwestern Atlantic.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>n</th>
<th>Lipid (%)</th>
<th>PBDEs (ng g⁻¹ lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guiana dolphin (<em>Sotalia guianensis</em>)</td>
<td>M</td>
<td>5</td>
<td>67.8 ± 6.31¹</td>
<td>59.5 ± 47.1¹</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4</td>
<td>70.1 ± 9.19</td>
<td>73.2 ± 79.1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9</td>
<td>68.8 ± 7.27</td>
<td>65.6 ± 59.3</td>
</tr>
<tr>
<td>Franciscana (<em>Pontoporia blainvillei</em>)</td>
<td>M</td>
<td>4</td>
<td>83.7 ± 2.79</td>
<td>101 ± 91.0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4</td>
<td>85.2 ± 6.82</td>
<td>196.6 ± 18.8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>8</td>
<td>84.5 ± 4.89</td>
<td>60.3 ± 74.8</td>
</tr>
<tr>
<td>Atlantic spotted dolphin (<em>Stenella frontalis</em>)</td>
<td>M</td>
<td>2</td>
<td>54.8 ± 9.97</td>
<td>770 ± 86.4</td>
</tr>
<tr>
<td>Rough-toothed dolphin (<em>Steno bredanensis</em>)</td>
<td>M</td>
<td>1</td>
<td>70.2</td>
<td>475</td>
</tr>
<tr>
<td>Bottlenose dolphin (<em>Tursiops truncatus</em>)</td>
<td>M</td>
<td>1</td>
<td>73.6</td>
<td>64.2</td>
</tr>
</tbody>
</table>

¹ Average ± standard deviation.

2.3. Quality control

No analytes were detected in laboratory blanks. The limits of detection (LD) for individual congeners were 5 ng g⁻¹ lipid (BDEs 1–77), 7.5 ng g⁻¹ lipid (BDEs 85–126), 10 ng g⁻¹ lipid (BDEs 153–166), 12.5 ng g⁻¹ lipid (BDEs 181–190), and 15 ng g⁻¹ lipid (BDE-138). Quality assurance/quality control (QA/QC) criteria were based on Wade and Cantillo (1994). Internal standard recoveries were 75 ± 14% (mean ± standard deviation). The only congener detected above the LD in the SRM 1588a (organics in cod liver oil) was BDE-47 with an average concentration of 9.69 ng g⁻¹ lipid (SD = 0.905 ng g⁻¹; n = 2). This represents 54% of recovery when comparing to the reference value in the certificate of analysis. Such a recovery is reasonable considering that the LD for BDE-47 was 5 ng g⁻¹ lipid. Laboratory check solutions were routinely injected into the GC/MS to confirm instrument accuracy and precision. Calibration of the instrument was performed using a four-level calibration curve.

2.4. Statistical analysis and data interpretation

The analysis of variance (ANOVA, Model I) was performed to compare the mean contamination between genders of *S. guianensis* and *P. blainvillei*. The ANOVA assumptions of data normality and equal variances were checked using the Kolmogorov–Smirnov and Levene’s tests, respectively. The Spearman’s rank order correlation analysis was used to assess the degree of association.
between contaminants. The critical level of significance of the tests was set at \( \alpha = 0.05 \).

A suite of 39 mono- through hepta-BDEs was investigated including the following congeners: 1, 2, 3, 7, 8, 10, 11, 12, 13, 15, 17, 25, 28, 30, 32, 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, 166, 181, 183, and 190. All congeners detected below the LD were set as zero for calculation purposes. Concentration of PBDEs in the blubber of dolphins is expressed on a lipid weight basis. For comparison purposes, data expressed as wet weight in the literature were converted to lipid weight whenever possible.

### 3. Results and discussion

Average concentration of PBDEs in *S. guianensis* was 59.5 ng g\(^{-1}\) lipid (SD = 47.1 ng g\(^{-1}\)) and 73.2 ng g\(^{-1}\) lipid (SD = 79.1 ng g\(^{-1}\)) for males and females, respectively (Table 1). Although females showed higher mean contamination, the ANOVA revealed no significant differences between genders (\( F = 0.11, \ df = 1, p > 0.05 \)). Similarly, concentration of PBDEs in males of *P. blainvillei* did not differ from females (ANOVA, \( F = 3.07, \ df = 1, p > 0.05 \)). Other studies have not observed gender differences on the levels of organochlorines in *S. guianensis* and *P. blainvillei* from Brazilian coastal waters (see Leonel et al., 2010; Yogui et al., 2003). According to Thron et al. (2004), factors such as age, sex and body condition play a role in the variability of contaminants in marine mammals. A larger sample size containing different age classes of both genders should render a more detailed investigation on the possible influence of such factors on the burden of POPs in both species.

Concentration of PBDEs was highest in *S. frontalis* (770 ng g\(^{-1}\) lipid) followed by *S. bredanensis* (475 ng g\(^{-1}\) lipid), *S. guianensis* (65.6 ng g\(^{-1}\) lipid), *T. truncatus* (64.2 ng g\(^{-1}\) lipid), and *P. blainvillei* (60.3 ng g\(^{-1}\) lipid) (Table 1). Overall, higher levels of contamination were observed in dolphins that inhabit continental shelf waters such as *S. frontalis* and *S. bredanensis*. The concentrations of PBDEs in these species were around an order of magnitude higher than in *S. guianensis* and *P. blainvillei* – inshore species that occupy areas closer to potential sources of pollution. Feeding habits might be a possible explanation for such differences. The investigated small cetaceans feed basically on fish, squid and shrimp, and share some prey species (Melo et al., 2010; Santos et al., 2002; Santos and Haimovici, 2001). In the southwestern Atlantic, *S. guianensis*, *P. blainvillei* and *S. bredanensis* feed on squids (*Loligo plei*) with mean total mass of 61 g, 69 g, and 119 g, respectively (Santos and Haimovici, 2001). Lopes (2009) found larger prey items in stomach contents of *S. frontalis* when compared to *S. guianensis* from southeastern Brazil. Larger preys might accumulate larger amount of contaminants in their bodies. For instance, Karl and Lahrssen-Wiederholt (2009) found a positive association between concentration of pollutants and size of cods (*Gadus morhua*) in the northeastern Atlantic. Likewise, anadromous fishes – species that migrate from saltwater to spawn in brackish/freshwater – might also contribute for differences in contamination since non-recruited, juvenile individuals would be less contaminated than adults. Conversely, it should be noted that *T. truncatus* was observed to feed on larger fish than *S. frontalis* along the coast of Rio de Janeiro, Brazil (Melo et al., 2010). Such a finding is not in agreement with a positive association between contamination and size of preys since *S. frontalis* exhibited higher levels of PBDEs than *T. truncatus* in this study. Other factors such as age might also contribute to explain differences in levels of PBDEs. Unfortunately, age information is available for only 5 out of 21 individuals investigated in this study (see Yogui et al., 2003).

Only 9 out of 39 investigated congeners were detected in *S. frontalis* – the most contaminated species reported in this study.
S. guianensis (3) and T. truncatus (3). BDEs 47, 99, and 100 were detected in all five species, confirming their accumulative nature in marine biota (Law et al., 2002; Tuerk et al., 2005; Yogui and Sericano, 2009b). Tri- through hexa-BDEs were detected in dolphins from the coast of São Paulo (Fig. 1). This pattern of contamination resembles the one found in dolphins from the coast of Rio de Janeiro, Brazil (see Dorneles et al., 2010). The distribution of homolog groups suggests that Penta-BDE mixtures are a major source of contamination to the animals (Fig. 1). Hepta-BDEs (IUPAC Nos. 181, 183, and 190) were not detected in the blubber samples. BDE-183 – often used as a marker for Octa-BDE mixtures – was also not detected in dolphins from the coast of Rio de Janeiro, and skipjack tuna (Katsuwonus pelamis) caught in fishing areas off Brazil (Dorneles et al., 2010; Ueno et al., 2004). These findings might be suggesting a minor use of Octa-BDE formulations in Brazil.

Based on previously published data (Yogui et al., 2003, 2010), PBDEs comprise only 0.2–1.5% of the total burden of POPs in small cetaceans from the coast of São Paulo (Fig. 2). The fact that PCBs and DDTs represent 96% of the burden is in agreement with their historical use in Brazil. In the southern hemisphere, PBDEs were found to represent around 1% of POPs in Antarctic penguins (Corsiolini et al., 2007; Yogui and Sericano, 2009a). In the northern hemisphere, PBDEs also accounted for a small percentage (<4%) of POPs in dolphins from North America and Asia (see Fair et al., 2010; Kajiwara et al., 2006; Yordy et al., 2010). The Spearman’s rank order correlation analysis showed significant correlations between PBDEs and PCBs, DDTs, HCHs, HCB, and mirex (p = 21, r_{s} = 0.652, p ≤ 0.001). The positive association of PBDEs with most POPs may be a consequence of their high hydrophobicity (i.e., log_{Koc} = 4–10) and tendency to partition into the blubber of marine mammals.

Levels of PBDEs in the blubber of S. frontalis and S. bredanensis reported in this study are comparable to those in the liver of the same species sampled along the coast of Rio de Janeiro, Brazil (Dorneles et al., 2010). However, S. guianensis and T. truncatus from Rio de Janeiro are an order of magnitude more contaminated with PBDEs. In the case of S. guianensis, higher concentrations in animals from Rio de Janeiro are likely reflecting pollution of Guanabara Bay – the most impacted area along the coast of Brazil (Dorneles et al., 2010; Kjerfve et al., 1997). In general, concentration of PBDEs in the blubber of small cetaceans from the South Atlantic is lower than in dolphins from the North Atlantic (Table 2). For instance, levels of PBDEs in T. truncatus from the coast of São Paulo is 1–2 orders of magnitude lower than those in individuals from the United States and United Kingdom (Fair et al., 2010; Johnson-Restrepo et al., 2005; Law et al., 2005; Yordy et al., 2010). The mean contamination of T. truncatus ranked as follows: southwestern Atlantic (Brazil) < northwestern Atlantic (USA) < northeastern Atlantic (UK). In comparison to small cetaceans from Asian waters, levels of PBDEs in dolphins from the southwestern Atlantic are usually lower than those reported for industrialized areas such as Hong Kong and Japan (Kajiwara et al., 2006; Lam et al., 2009). Conversely, PBDEs in dolphins from Brazil are 1–2 orders of magnitude higher than in animals from developing countries such as the Philippines and India (Kajiwara et al., 2006; Kannan et al., 2005).

This study shows that PBDEs have reached higher trophic levels in food webs far away from production sites. Source of pollution to the southwestern Atlantic may be highly urbanized areas along the eastern coast of South America and/or long-range atmospheric transport. Studies on brominated flame retardants in the southern hemisphere are well behind those in North America, Europe and Asia. Thus, continuing efforts are necessary to investigate the distribution, transport and fate of PBDEs in environments south of the equator. Time trend studies should also be carried out in order to compare with available data elsewhere.

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Appendix A. Supplementary data


References


