Anthropogenic and naturally-produced organobrominated compounds in Antarctic humpback whales, *Megaptera novaeangliae*

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Introduction

Taking into account the absence of industrial or agricultural activities in Antarctica, the continent was considered to be free of contamination by anthropogenically-produced organohalogen compounds until the late 1960s, when this type of contamination was first scientifically recorded [1]. Since then a number of investigations have shown trace levels of these pollutants [2]. With the exception of small areas of significant environmental pollution from military and scientific activities [3], Antarctica remains relatively protected from widespread human disturbance. This relative protection is a consequence of its remoteness and extreme climate conditions, which turns the continent into a region of high ecotoxicological interest.

Organohalogen compounds have been detected in Antarctic marine biota and some animal groups have been used for monitoring residual contamination, due to the bioaccumulation and biomagnification processes [1]. Antarctic waters are feeding grounds for several marine mammals, including great whales. These animals are long-lived top marine predators that are regarded as ideal repository for lipophilic halogenated hydrocarbons, due to their large lipid reserves.

Environmental contamination by polybrominated diphenyl ethers (PBDEs) has received considerable attention due to the toxicity, environmental persistency and bioaccumulative nature of these compounds, as well as their widespread use as flame retardants. The high long-range transport potential (LRTP) of these compounds turned possible for them to reach remote polar areas [4].

Methoxylated polybrominated diphenyl ethers (MeO-PBDEs) have been evidenced in marine environments, with the tetrabrominated 2'-MeO-BDE 68 and 6-MeO-BDE 47 being the most abundant. MeO-PBDEs have been reported as having natural origin, being formed by sponges or algae. These naturally-produced compounds have been detected in marine mammals at concentrations comparable to halogenated organic compounds of anthropogenic origin [5].

The humpback whale (*Megaptera novaeangliae*) occurs from polar zones to equatorial regions, since it performs long latitudinal migration. During the end of spring and summer, whales can be observed in high latitudinal waters, where they migrate for feeding purposes [6].

This study presents results of organobrominated compound bioaccumulation in humpback whales that feed in Antarctic waters.

Materials and methods

Samples were obtained during the Brazilian Antarctic Operations XIX, XX and XXI (2000/2001, 2001/2002 and 2002/2003 summers, respectively), which are part of the Brazilian Antarctic Programme (PROANTAR). During these surveys, skin/blubber biopsy samples were obtained from humpback whales in the western Antarctic Peninsula waters. Samples were collected from a 4 m long inflatable boat (eventually from the ship), using a 120-150 lb crossbow with arrows and tips M8X40mm appropriated to great cetaceans. In order to reduce the chances of analyzing biopsy samples from same individuals, sampled whales were photo-identified whenever possible.

For gender determination, DNA was extracted from skin samples. Subsequently, DNA was used for PCR amplification of a fragment of the *SRY* gene, multiplexed with fragments of the *ZFY/ZFX* genes as positive control, as described by Cunha & Solé-Cava [7].

The following PBDE-congeners (IUPAC numbers) were targeted for analysis: 28 (2,4,4'-triBDE), 47 (2,2',4,4'-tetraBDE), 99 (2,2',4,4',5-pentaBDE), 100 (2,2',4,4',6-pentaBDE), 153 (2,2',4,4',5,5'-hexaBDE), 154 (2,2',4,4',5,6'-hexaBDE) and 183 (2,2',3',4,4',5,6'-heptaBDE). Additionally, two methoxylated-PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) have also been determined. The analytical procedure was detailed elsewhere [5]. Briefly, 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, operated in electron capture negative ionization (ECNI) mode, equipped with a 20 m × 0.18 mm × 0.20 µm AT-5 capillary column (Alltech, Lokeren, Belgium). Recoveries for individual PBDE congeners were between 87 and 104 % (RSD ≤ 12 %) during method validation. Analysis of standard reference material SRM 1945 (PBDEs in whale blubber) indicated that the method accuracy was above 90 %. Method LOQs ranged between 0.1 and 0.2 ng/g lipid weight (lw) for individual PBDE and MeOPBDE congeners.

For statistics, depending on data normality (Shapiro-Wilk's W test), a parametric (Student's t-test) or a non-parametric test (Mann–Whitney U test) was used.

Results and discussion:

Concentrations of PBDEs and MeO-PBDEs measured in blubber samples from humpback whales biopsied during the Brazilian Antarctic Operations XIX, XX and XXI (2000/2001, 2001/2002 and 2002/2003 summers, respectively) are given in Table 1.

Table 1
Subcutaneous adipose tissue concentrations (ng/g, lipid wt.) of PBDEs and MeO-PBDEs in humpback whales from western Antarctic Peninsula waters.

BAO	Period Summer	Sex	ΣPBDEs (Industrial) Mean ±S.D.	Range	ΣPBDEs (Natural) Mean ±S.D.	Range	*n*
XIX	2000/2001	M	2.5 ±3.8	0.5 - 10.9	0.3 ±0.5	<LOQ - 1.4	7
		F	5.8 ±12.6	0.4 - 50.8	0.2 ±0.3	<LOQ - 1.4	15
XX	2001/2002	M	1.1 ±0.5	0.4 - 1.8	1.6 ±3.3	0.1 - 10.4	9
		F	1.6 ±1.4	0.3 - 4.8	0.2 ±0.3	<LOQ - 0.7	10
XXI	2002/2003	M	1.3 ±1.4	0.1 - 4.5	0.4 ±0.2	0.2 - 0.9	8
		F	1.8 ±1.1	0.9 - 5.3	0.2 ±0.2	0.1 - 0.8	16

BAO, Brazilian Antarctic Operation

BDE 99 was the predominant PBDE congener for both sexes and the three Antarctic Operations, followed by BDE 47 (Figure 1). The presence of BDE 183 is noteworthy, since the congener was not detected in dolphins that inhabit waters close to a region of high urban and industrial activity in South America, the Rio de Janeiro state [5]. Therefore, the latter finding indicates that technical formulations other than the Penta-BDE mixture have found their way into the Antarctic marine ecosystems. The sum of BDEs 47, 99 and 100 represented over 60% of the total PBDEs. This percentage seems to compare favorably with those of other biological matrices from Antarctica [2].

In order to investigate possible temporal variation in the humpback whale exposure, pairwise group comparisons were performed (BAO XIX versus BAO XX, BAO XX versus BAO XXI, BAO XXI versus BAO XIX), for males and females independently, regarding ΣPBDE, BDE 99, BDE 47, ΣPBDE/ΣMeO-PBDE ratio, as well as BDE 47/BDE 99 ratio. Significant difference was only found for the BDE 47/BDE 99 ratio, when females of AO-XIX and AO-XX were compared. The BDE 47/BDE 99 ratio rendered significantly higher values in female humpback whales sampled during AO-XIX (2000/2001 summer) than in those sampled during AO-XX

(2001/2002 summer) (p=0.001). Debromination of BDE 99 to BDE 47 was demonstrated [8] and a model applied to experimental laboratory data indicated the occurrence of debromination of BDE 47 to BDE 28 [9]. The BDE 28/BDE 47 ratio was not calculated since only eight whales presented detectable levels of BDE 28. The latter finding would indicate, at first, a more intense debromination of BDE 99 to BDE 47 in a previous period. However, assumptions should be drawn with great caution from this finding. Local sources [10] and long range transport [11] of PBDEs are contributing to levels and profiles in Antarctic biota and knowledge on temporal trends for the compounds originated from these different sources needs additional investigation.

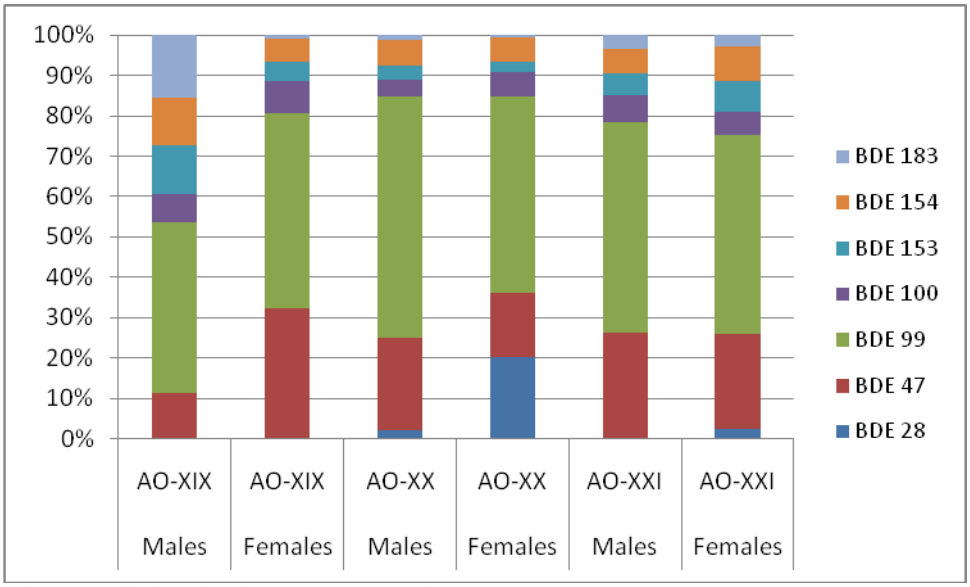

Fig. 1. Contribution of each PBDE congener to the ΣPBDE (AO-XIX, AO-XX and AO-XX - Antarctic Operations XIX, XX and XXI, respectively).

For investigating possible gender-related differences in the bioaccumulation of anthropogenic and naturally-produced organobrominated compounds by humpback whales, comparisons between sexes were performed using samples obtained during the same Antarctic Operation, regading ΣPBDE, BDE 99, BDE 47, ΣMeO-PBDE and the ΣPBDE/ΣMeO-PBDE ratio. Significantly higher ΣPBDE concentrations were verified in females than in males of the AO-XXI (p=0.04). Still concerning this Antarctic Operation (AO-XXI), significantly higher ΣMeO-PBDE concentrations were found in males than in females (p=0.02), as well as, significantly higher values of the ΣPBDE/ΣMeO-PBDE ratio were verified in females than in males (p=0.006). In fact, the same finding (significantly higher values of the ΣPBDE/ΣMeO-PBDE ratio in females) was also verified for AO-XIX (p=0.03). Figure 2 illustrates the differences between males and females, regarding the contribution of natural (ΣMeO-PBDE) and anthropogenic (ΣPBDE) brominated compounds to the sum of both classes of substances.

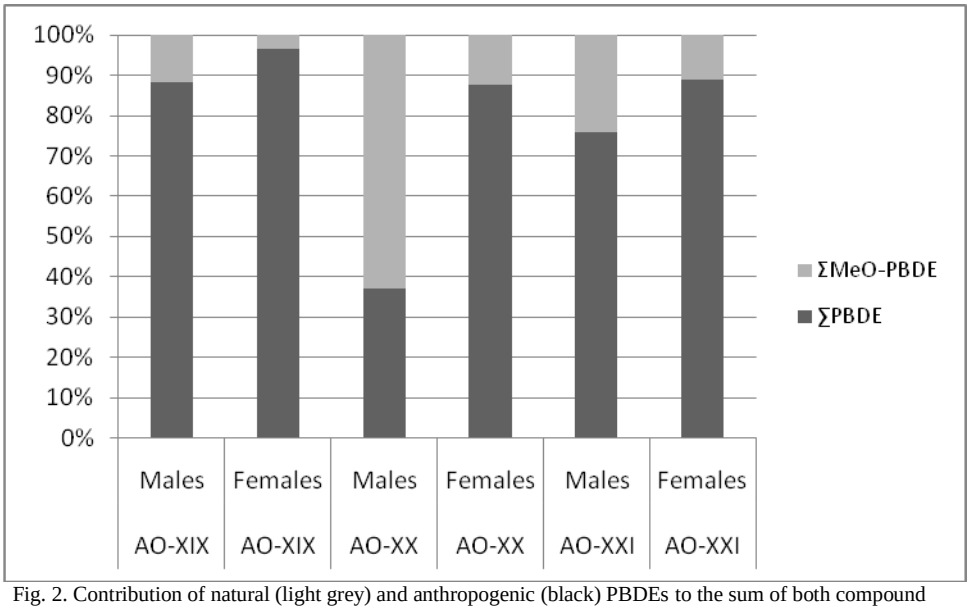

Fig. 2. Contribution of natural (light grey) and anthropogenic (black) PBDEs to the sum of both compound classes (AO-XIX, AO-XX and AO-XX - Antarctic Operations XIX, XX and XXI, respectively).

A recent study from our team [5] raised the hypothesis of using the ΣPBDE/ΣMeO-PBDE ratio for investigating possible differences in feeding areas / prey species. The ratio could be used, for instance, as an indication of inshore versus offshore contribution to food intake, for small cetaceans [5]. Therefore, the observed significant differences in the ΣPBDE/ΣMeO-PBDE ratios between males and females would point out gender-related differences in feeding areas / prey species. However, males and females humpback whales were biopsied at the same feeding area, where they prey on krill [6]. Additional studies are required for shedding further light on these differences.

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