Antarctic marine mammals as indicators of long-range transport of emerging pollutants

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Introduction

The effects of flame retardants (FRs) on human health and the environment have been a growing concern. Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are included in the Stockholm Convention on Persistent Organic Pollutants (POPs). POPs are often halogenated and have high lipid solubility, leading to their bioaccumulation in fatty tissues. The use of PBDEs and HBCD has also been restricted or banned by several European directives and regulations: Regulation (EC) No 1907/2006 REACH; Directive 2011/65/EU; Directive 2013/39/EU.

New FRs act as substitutes for the banned compounds due to their health and environmental concerns. Some of them are decabromodiphenyl ethane (DBDPE), pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB). DBDPE is the marketed alternative to Deca-BDE as their structures are similar; therefore their properties are also expected to be. Additionally, Dechlorane Plus (DP) and dechloranes 602, 603 and 604 (Dec 602, Dec 603, Dec 604) are chlorinated alternatives to Mirex, which was banned in the United States of America due to its toxicity. Research groups around the world are currently studying emerging FRs to assess their behaviour and occurrence in the environment.

Finally, methoxylated PBDEs (MeO-PBDEs) are natural analogues to PBDEs that are synthesized by some marine sponges, algae and their associated cyanobacteria. They have been found in cetaceans and seafood and can be detected in marine mammals at similar levels to manufactured halogenated organic compounds.

The Stockholm Convention includes compounds that are characterized by their persistence, bioaccumulation through the food web, adverse effects to human health and the environment and transport capacity over long distances, far from their sources, even to regions where they have never been used or produced. The ecosystems of the poles are at risk because of this.

PBDEs are ubiquitous compounds that can be found anywhere in the world, including the Arctic and the Antarctic. Long-range atmospheric transport and ocean current transport could be the main pathways of PBDEs to remote areas although anthropogenic influence (e.g. research stations, tourism) could also influence the spatial distribution of PBDEs in the Antarctic Peninsula.

On the other hand, few publications show data about dechloranes in remote regions. Although there are studies on dechloranes in environmental matrices from the Arctic and the Antarctic regions, only very recent publications address the presence of these compounds in biota, more specifically in the South Shetland Islands.

The present study reports the presence of some aforementioned compounds in southern elephant seal (*Mirounga leonina*) and Antarctic fur seal (*Arctocephalus gazella*) from the South Shetland Islands. Each sample corresponded to muscular tissue from the fins, nervous system tissue, adipose tissue or fur. The specific aim of this study was to prove the long-range transport capacity of FRs. Furthermore, the diversity of the tissues collected allowed to evaluate if fur samples —more accessible and available— could substitute other lipid-rich tissues traditionally used for biomonitoring and to compare adipose and nervous system tissues samples of the same individual to judge the ability of these pollutants to cross the blood-brain barrier (BBB), which is relevant due to their neurological effects.

Materials and methods

2.1. Sampling

A total of 31 samples including muscle tissue from hind flippers (n = 5), nervous system tissue (n = 6), adipose tissue (n = 9) and fur (n = 11) of southern elephant seal pups (*Mirounga leonina*) and adult Antarctic fur seal (*Arctocephalus gazella*) were collected in summer of 2012 during an Antarctic expedition in the framework of the Brazilian Antarctic Program (PROANTAR) in the South Shetland Islands, Antarctic Peninsula (Figure 1). The samples were collected only from animals found dead and under Antarctic and Brazilian Environmental Agency permissions. The carcasses were in early decomposition stages. The nervous system tissue was collected inside the skull in the junction with the medulla.

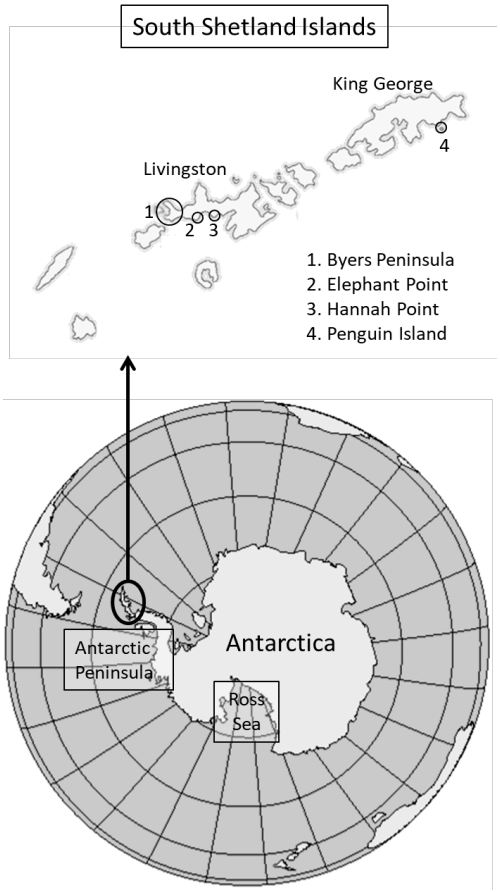

Figure 1. Map of the 4 sampling points

2.2. Sample preparation

The extraction of FRs was carried out according to published methods[1-2]. Freeze-dried sample (1.5 g) was spiked with the labelled standards. Pressurized liquid extraction (PLE) was used, lipid content was determined gravimetrically, the extract underwent an acid attack to remove the fat, the organic phase was cleaned by solid phase extraction (SPE) and the extract was reconstituted with toluene.

2.3. Instrumental analysis

FRs were analysed by GC-MS/MS using an Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer and a DB-5ms column. Brominated compounds were analysed using electronic ionization (EI). The instrumental conditions[3-4] and the spectrometric determination[5] are described in previous publications. Due to their low sensibility with GC-EI-MS/MS, BDE-209 and DBDPE were analysed by GC-MS with the same chromatographic conditions in an Agilent 5975A mass spectrometer using negative chemical ionization (NCI)[6]. The analysis of dechloranes was performed by NCI as described in a previous article[7]. Recoveries were between 51 and 99 %, RSDs were 1.1-22 %, LODs were 0.002-10.6 ng g^{-1} lw and LOQs were 0.008-35.4 ng g^{-1} lw.

Results and discussion:

3.1. Brominated flame retardants in Antarctic seals

PBDEs were detected in all sample types except for the nervous system and they were below the LOQ in fur (Figure 2). BDE-183 and -209 were not detected. Only BDE-28, -47 and -99 were above their LOQs —the latter in just one sample. Average total PBDEs concentrations were 1.00 ± 0.92 ng g^{-1} lw (range 0.27-2.21 ng g^{-1} lw) in muscle and 2.09 ± 2.64 ng g^{-1} lw (range n.d.-5.13 ng g^{-1} lw) in adipose tissue of the southern elephant seal and 1.37 ± 0.96 ng g^{-1} lw (range n.d.-2.74 ng g^{-1} lw) in adipose tissue of the Antarctic fur seal. No emerging brominated FRs were detected. 6-MeO-BDE-47 was found in muscle, fur and adipose tissue of both species, while 5′-MeO-BDE-100 was only found in adipose tissue of Antarctic fur seal.

No hexa-BDEs, hepta-BDEs or deca-BDEs were found in the samples. Concentrations of tri-BDE-28 were higher than those of tetra-BDE-47 in adipose tissue of southern elephant seal. Conversely, tetra-BDE-47 was predominant in fin muscle of the same species and in adipose tissue of Antarctic fur seal. BDE-47, -99 and -100, being the predominant congeners in commercial mixtures of PBDEs, are reported to be more abundant in biota samples from areas that are not remote. In this study, BDE-47 and BDE-99 were also detected, but concentrations of BDE-28 were unusually high in some samples in relation to the other congeners. On average, it

accounted for over a 20 % of the total PBDEs contamination. The lower degree of bromination of BDE-28 would make it more volatile thus easier to be transported over long distances. Additionally, both in aqueous solutions and in gas phase the photodegradation of PBDEs is less important for low brominated congeners, which are also relatively more soluble in water and have a higher vapour pressure. These observations would explain the greater contribution of BDE-28 in Antarctic samples.

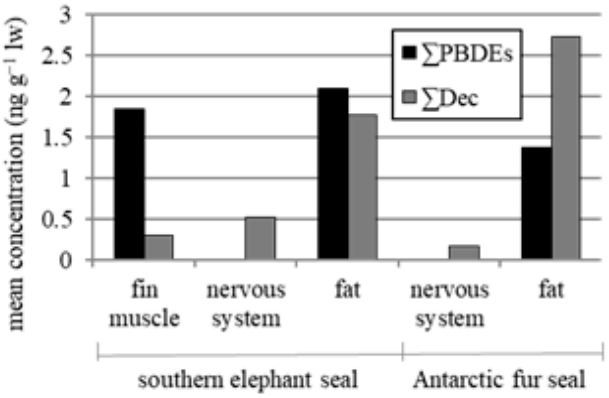

Figure 2. Mean total concentrations in tissues of seals

Some information about PBDEs in Antarctic biota is available in the literature, including different species of fish, as well as penguins and skuas and two species of seal. BDE-47 was the dominant congener in different penguin species from the Antarctic Peninsula[8]. On the other hand, BDE-100 was the dominant congener for some fishes[9-10]. The published data show that BDE-47 is the predominant congener in the food web of seals, consisting of krill, fish and cephalopods. The PBDEs profiles in the seals of this study may reflect the pattern in their sources of exposure, primarily their diet. Based on the previous data, BDE-28 would lack strong input from the food web, supporting the idea of long-range transportation.

Regarding concentration levels, an average of 11 ± 18 ng g^{-1} lw of PBDEs in blubber of Antarctic fur seal pup from Livingston Island has been previously reported[11]. Antarctic fur seals studied in the present work were from the same island and contained 1.37 ± 0.96 ng g^{-1} lw of PBDEs in fat, one order of magnitude lower. If the low fat content of some individuals in the present study was a consequence of a loss of fat due to nutritional stress, then contaminants might have been concentrated, thus reinforcing the observation that the present levels are lower than those published before. The bans in Europe and North America before 2010 and the inclusion of PBDEs in the Stockholm Convention in 2011 may be accountable for a decrease in the emission of these pollutants and, therefore, in the amount transported to the Antarctica. This trend has already been observed in seals from the Arctic[12]. These findings could validate the success of the prohibition measures if the tendency is confirmed in future studies.

3.2. Dechloranes in Antarctic seals

This is the first report of dechloranes in marine mammals from a remote area. Dechloranes were detected and quantifiable in samples of all tissues but fur, where they were detected in just two samples and below their LOQs (Figure 2). Only Dec 602 and *anti*-DP were found. Dec 602 appeared in greater concentrations, especially in adipose tissue, i.e. 1.48 ± 0.35 ng g^{-1} lw (range n.d.-1.72 ng g^{-1} lw) in fat of southern elephant seal and 2.63 ± 1.25 ng g^{-1} lw (range n.d.-3.19 ng g^{-1} lw) in fat of Antarctic fur seal. For adipose tissue in the same species, the concentrations of *anti*-DP were 0.60 ng g^{-1} lw and 0.16 ± 0.16 ng g^{-1} lw, respectively. In other tissues of both species, concentrations of Dec 602 were always below 0.55 ng g^{-1} lw and *anti*-DP was below its LOQ. Dec 603, Dec 604 and *syn*-DP were not detected in any sample.

Dec 602 has been reported to have a greater bioaccumulation potential than Dec 604 and DP according to their biota to sediment accumulation factors (BSAFs). Coherently, Dec 602 is the dominant compound of the dechloranes profile in other recent studies[13]. Dec 602 has a lower octanol-water partition coefficient and also higher vapor pressure and water solubility than Dec 604 and DP. These properties would make Dec 602 easier to be transported to remote areas. DP concentrations in our Antarctic seals are lower than Dec 602 concentrations, which is consistent with both the usual reported levels in biota and the mentioned properties that enable long-range transportation.

3.3. Comparison between tissues

Total dechloranes concentrations are in the same order of magnitude as total PBDEs concentrations, between n.d. and 6 ng g^{-1} lw (Figure 2). However, dechloranes were found in the nervous system of both seal species while

PBDEs were not detected in any sample of that tissue. Regarding the other tissues, southern elephant seals showed slightly higher averages for PBDEs whereas Antarctic fur seals showed otherwise. On the other hand, greater differences and a bigger number of samples are needed to draw a significant conclusion on a hypothetical selective accumulation.

It is noteworthy that Dec 602 was present in nervous system samples of both species, while none of the brominated compounds was. Looking into detail, there were four pairs of adipose and nervous tissue samples belonging to four individuals of Antarctic fur seal. In three cases, Dec 602 was detected in both samples. Dec 602 was detected neither in adipose tissue nor in the nervous system of the other individual. There was a correlation between the presence of Dec 602 in both tissues. On the other hand, concentration of Dec 602 in adipose tissue (2.05 ± 1.73 ng g^{-1} lw) was higher than in nervous system tissue (0.13 ± 0.09 ng g^{-1} lw) according to a t-test ($t = 2.48$, df = 7, $p < 0.05$). This has been observed before for cetaceans and other animals[14-16]. Most of the total POP contamination in cetaceans is found in blubber because of its high lipid content. However, it is important to note that dechloranes did cross the BBB, which is highly relevant for compounds with potential neurological toxicity.

It is also important to stress that PBDEs were not detected in the nervous system samples despite being at similar levels as dechloranes. This suggests that PBDEs might need higher concentrations than dechloranes to cross the BBB.

Finally, while the analysis of fur allowed to detect the presence of the same compounds that were found in fin muscle and fat, all concentrations were lower than in those other tissues and below their LOQs. Therefore, if fur were to be used as a biomonitoring tissue, greater amount of sample should be used for the analyses to allow quantification of the compounds, i.e. at least 4 or 5 g, instead of the 1.5 g used in the present study.

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