ANALYSIS OF ANTHROPOGENIC ORGANOBROMINATED COMPOUNDS (PBDEs) IN SOUTHERN ELEPHANT SEALS (*MIROUNGA LEONINA*) FROM ANTARCTICA

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Introduction

In the past decades, the environment has been affected by various anthropogenic sources. The human population growth, the large food production, and the demand of new materials to modernize civilization have resulted in thedevelopment of new chemical compounds - many of which are harmful to organisms[1]. Environmental contamination appears as one of the biggest current, and future challenges to the balance of continental and coastal systems[2].

Persistent organic pollutants (POPs) are a group of organic compounds that present physicochemical properties, exhibit persistence, bioaccumulate,and are highly toxic. They can be transported over long distances in the environment (e.g. marine environment and atmosphere). In marine environment, itacts as the final reservoir and therefore it contains a major portion of these compounds[3]. The Stockholm Convention classifies compounds such as,organochlorine, organofluorine, and organobromine as POPs.In the past, some of these were largely used as pesticides in agriculture and as flame-retardantsin the industry[4].

Brominated flame retardants (BFRs) are used in many combustible materials to reduce the risk of fire in products such as plastics, textiles, furniture, electronics[5,6]. The BFRs are divided into various classes of compounds including organic phosphorus halogenated, nitrogenated, and other inorganic ones. Among the halogens, brominated compounds have the highest sales in the market because of their low decomposition temperature, high efficiency, and low cost[6]. The most used brominated flame retardants are polybrominated diphenyl ethers (PBDEs).

PBDEs are structurally similar to polychlorinated biphenyls (PCBs) including the nomenclature and numbers of congeners. Theoretically, there are 209 congeners of PBDEs, mainly produced in the form of three technical mixtures: penta- (Penta-BDE), octa- (Octa-BDE), and decabromodiphenyl ether (Deca-BDE)[7]. They tend to be persistently stable in nature, having high hydrophobicity, and low relative volatility[8]. They are persistent compounds that bioaccumulate and biomagnify through the food chain. Despite the large size of the molecule, they have bioaccumulation factors higher than PCBs of similar hydrophobicity[9].

The potential risk of PBDE to wildlife is still unclear, but experimental studies have shown different effects to human and animal health. A variety of biologicaleffectshave been reported for PBDEs,as neuro and immunotoxicity, and endocrine disruptor activity[5,12,13].

Large differences in the concentration of contaminants can be observed when comparing males and females in organisms of different ages due to different detoxification/excretion processes and bioaccumulation of contaminants. As well, because of the transfer of these compounds from females to their offspring[1]. Thus, the aim of this study is to determine the concentrations of anthropogenic organobrominated compounds (PBDEs) in marine mammal pups(*Mirounga leonina*) from South Shetland Islands, Antarctic Peninsula.

Materials and methods

Biological samples of Southern elephant seal (*Mirounga leonina)* pups (*n*=7) were collected from dead individuals found stranded at South Shetlands Islands, during Brazilian NavyOPERANTAR XXXI in November-December, 2012. Samples were kept frozen in the Radioisotopes Laboratory Eduardo Penna Franca (UFRJ), Rio de Janeiro, Brazil until analysis. The tissue analyzed in this study was the muscle from the posteriorflipper due to being well preserved in these samples. The samples were lyophilized to perform the analysis. The methodology followed Alonso et al. (2012) with modifications after optimization tests[14].Twenty µL of the internal standard BDE-181was added to six grams of dry tissue and extracted by Soxhlet with hexane/ diclorometane (DCM) (1:1).

Afterwards, samples were concentrated under nitrogen flow. The cleanup procedure was performed in two stages. Firstwas made a sulfuric acid attack and the extract was centrifuged for 20 minutes. This operation was performed five times to obtain a translucent extract. The second step comprised a chromatographic column filled with 1 cm of sodium sulfate, 6g of Florisil (Sigma-Aldrich, USA), 6 g of alumina (6%), 10g of acid silica (44%) and 1 cm of sodium sulfate eluted with 120 ml of hexane/dichloromethane (1:2). Then, samples were concentrated under nitrogen,transferred to a vial, evaporated to dryness and re-suspended to 20 µL of toluene.The quantification of organobrominated compounds was conducted in an Agilent7890 A gas chromatograph coupled to mass spectrometry working with negative ion chemical ionization (GC-NCI-MS)connected to a triple axis quadrupole mass spectrometer(5975 C inert XL). in SIM mode. A DB-5 ms capillary column was used(30m x 0.25mm i.d. x 0.25 mm)with helium as thecarrier gas at a flow 1.2 mL/minanda temperature at 150°C. The SCAN mode was applied to confirm compounds identification.Chromatographic conditions applied are shown in Table 1.

Table 1: Chromatographic conditions for the analysis of organobromine compounds

	Climb rate(ºC/min)	Value (ºC)	Retention Time (min)	Run Time (min)
	initial	110	1	1
	8	180	1	10.75
	2	240	5	45.75
	2	300	6	81.75

Lipid quantification was performed by gravimetric analysis. An aliquot of 0.5 mL of the initial extract was transferred to vials previously weighed on a precision scale, and after evaporation of the solvent, the lipid content is obtained by weighing until constant weight.

Results and discussion

PBDEs were investigated in seven samples of *Mirounga leonina* from Antarctic (Table 2). Two main congeners were found (2,4,4'-TriBDE 28 and 2,2',4,4',6-PentaBDE 100).The results are presented in Table 2.

Table 2: Lipid content, concentrations of PBDEs(pg/g of lipid) of analyzed *Mirounga leonina* samples

Samples	Lipid content (%)	2,4,4'-TriBDE 28 (pg/g of lipid)	2,2',4,4',6-PentaBDE 100 (pg/g of lipid)	TriBDE 28/Penta BDE 100 ratio	∑ of PBDEs
ILH 01	29.20	n.d.	0.47	n.d	0.47
ILE 02	16.97	2.85	0.15	19.00	3.00
ILE 03	20.36	14.45	0.90	16.06	15.35
ILE 04	21.03	11.57	0.82	14.11	12.39
ILB 01	12.54	28.73	0.89	32.28	29.62
ILB 03	32.34	27.97	2.69	10.40	30.66
ILB 06	21.07	16.23	1.60	10.14	17.83

Individual concentrations of BDE-28 were higher than BDE-100,with a ratio between both compounds ranging from 10.14 to 32.28. This corroborates with the fact that lower-brominated compounds bioaccumulate easier than higher-brominated ones in higher latitudes[15].Since samples were from pups animals, with total length up to 1.20 m, they were considered probably lactating individuals. Hence, the contamination reflects the maternal transfer during pregnancy and lactation. This corroborates to a study that reports certain organochlorine compounds in females, milk, and pupsof *Mirounga leonina*, in sub-Antarctic waters[16]. A previous study found no PBDEs in water, sediment, and/or soil from the Shetland Islands[17]. However, present data are the first one to reveal PBDEs contamination in mammals of this region.Based on its environmental persistence and bioaccumulation potential, the organobrominated compounds pose a threat to marine organisms in general, but especially to those occupying the top of the food chain, such as marine mammals[18]. The potential risk of PBDEs in wildlife is still unclear, but experimental studies using different commercial mixtures of PBDEs as well as individual BDE congeners have shown different effects to human and animal health. Therefore, the consent to these compounds as potential changes of endocrine systems in wild animals have increased in recent years[18].
The analysis of this study, performed in an integrated and complementary way, will help increase the knowledge concerning the ecology of the pinniped population studied. Specifically, in regard to contamination levels and biomagnification in the Antarctica food chain. Also, these new results will contribute to the knowledge about the persistence and global distillation of halogenated organic contaminants, including emerging compounds such as brominated flame retardants.

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References:

1. Tanabe, S., Iwata, H., Tatsukawa, R. (1994). Global Contamination by Persistent Organochlorines and Their Ecotoxicological Impact on Marine Mammals. *Science of Total Environment,* 154:163-177.
2. Windom, H.L. (1992). Contamination of the marine environment from land-based sources. *Marine Pollution Bulletin*, 25(1-4): 32-36.
3. Tanabe, SW., S., Kan, H., Tatsukawa, R. (1988). Capacity and Mode of PCB Metabolism in Small Cetaceans. Marine Mammal Science, 4: 103-124.
4. Baird, Colin; Cann, Michael; Química Ambiental. 4º Edition. São Paulo, Bookman, 2011. 844 p.
5. De Wit, CA. (2002). An overview of brominated flame retardants in the environment. *Chemosphere*, 46: 583–624.
6. Alaee, M., Arias, P., Sjodin, A., Bergman, A. (2003). An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environment International,* 29: 683–689.
7. Rahman F, LK.., Scrimshaw, MD., Lester, JN. (2001). Polybrominated diphenyl ether (PBDE) flame retardants. *Science of the Total Environment,* 275:1-17.
8. WHO. (1994). Brominated Diphenyl Ethers.
9. Gustafsson, KB., M., Burreau, S., Gilek, M. (1999). Bioacumulation kinectics of brominated flame retardants (polybrominated diphenyl ethers) in blue mussels (Mytilus edulis). . *Environmental Toxicology and Chemistryv.* 18, n. 6: 1218-1224.
10. Yogui, GT. & Sericano, JL. (2009). Polybrominated diphenyl ether flame retardants in the U.S. marine environment: A review. *Environment International*, 35: 655-666.
11. Nylund K, AL., Jansson, B., Jonsson, P., Litzen, K., Sellstrom, U. (1992). Analysis of some polyhalogenated organic pollutants in sediment and sewage sludge. *Chemosphere*, 24: 1721–1730.
12. Darnerud, PO., Eriksen, GS., Johannesson, T., Larsen, PB., Viluksela, M. (2001). Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environmental Health Perspectives,* 109: 49–68.
13. Marsilli, L., Casini, S., Bucalossi, D., Porcelloni, S., Maltese, S., Fossi, M.C. (2008). Use of immunofluorescence technique in cultured fibroblasts from Mediterranean cetaceans as new ‘‘in vitro” tool to investigate effects of environmental contaminants. *Marine Environmental Research*, 66: 151-153.

14. Alonso, MB., Feo, ML., Corcellas, C., Vidal, LG., Bertozzi, CP., Marigo, J., Secchi, ER., Bassoi, M., Azevedo, A., Dorneles, PR., Torres, JPM., Lailson-Brito, J., Malm, O., Eljarrat, E., Barceló, D. (2012). Pyrethroids: An Old Contaminant May Represent A New Concern To Marine Mammals. *Organohalogen Compounds*, 74: 911-914.
15. Betts KS. (2002) Rapidly rising PBDE levels in North America.*Environ Sci Technol*. 1;36(3):50A-52A.
16. Miranda Filho, KC., Metcalfe, CD., Metcalfe, TL., Muelbert, MM., Robaldo, RB., Martinez, PE., Lailson-Brito, J., Dirtu, AC., Weijs, L., Azevedo, AF., Torres, JPM., Malm, O., Neels, H., Blust, R., Das, K., Covaci, A. (2009). Anthropogenic and naturally-produced organobrominated compounds in marine mammals from Brazil. *Environment International,* 36: 60-67.
17. Vecchiato, M., Zambon, S., Argiriadis, E., Barbante, C., Gambaro, A., Piazza, R. (2015). Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in Antarctic ice-free areas: Influence of local sources on lakes and soils. *Microchemical Journal,* 120: 26–33.
18. Tanabe, S. (2008). Temporal trends of brominated flame retardants in coastal waters of Japan and South China: Retrospective monitoring study using archived samples from es-Bank, Ehime University, Japan. *Marine Pollution Bulletin*, 57: 267–274.