FIRST ASSESSMENT OF THE PBDE CONTAMINATION IN RESIDENT CETACEANS FROM THE GULF OF CALIFORNIA, MX

Muñoz-Arnanz J[1], Fossi MC[2],Urban J[3],Maltese S[2],Coppola D[2],Panti C[4], Rojas-Bracho L[5], Marsili L[2], Jiménez B[1]

[1]Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC. Juan de la Cierva 3, 28006 Madrid, Spain. email: juan.ma@iqog.csic.es
[2]Department of Environmental Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy
[3]Departamento de Biología Marina, Universidad Autónoma de Baja California Sur, La Paz, Mexico
[4]Department of Evolutionary Biology, University of Siena, Via A. Moro 2, 53100 Siena, Italy
[5]Programa de Mamíferos Marinos - Instituto Nacional de Ecologia, C/o CICESE, Ensenada, Mexico

Introduction

Due to its widespread use as flame retardants, polybrominated diphenyl ethers (PBDEs) are today ubiquitous environmental contaminants. Structurally related to polychlorinated biphenyls (PCBs), they exhibit persistence in the environment, long-range atmospheric transportation and ability to bioaccumulate and biomagnify through terrestrial and aquatic food webs. Additionally, there is growing information about PBDE toxicological effects on organisms and their potential as endocrine disruptors. The specific toxicity of these chemicals is not well established yet. However, they seem to pose endocrine-disrupting properties that lead to very similar toxic effects to that of PCBs. Furthermore, both POP families seem to bind to the same cellular receptors and to show synergistic deleterious effects[1]. As a result, since 2009 some of them are regarded as persistent organic pollutants (POPs) under the Stockholm Convention. Production and use of penta- and octa-commercial formulations were banned or voluntarily phased out in Europe and the USA as of 2004. However, currently the deca-BDE mixture remains mostly unregulated save for the exceptions of the European Union and Canada. BDE-209 is the main product of the deca-BDE mixture. Based on its high molecular weight and its tendency to strongly bind to particulate matter, it was firstly thought not to be bioavailable and not to degrade in the environment. However, increasing data about this congener tendency to bioaccumulate in organisms and to degrade into more toxic and available BDE congeners seem to prove otherwise[2].

Increasing concentrations of these flame retardants are reported in aquatic organisms, including fish and marine mammals worldwide. Previous studies with PBDEs in marine mammals from the Sea of Cortez (Mexico) have reported high levels of low-brominated PBDEs compared to other areas such as the Mediterranean Sea which could be of particular concern[3].

The Gulf of California is located in the Pacific Ocean between the lower Californian Peninsula (Baja California) and the state of Sonora (on mainland Mexico). Regarded as an UNESCO world heritage site, it is one of the most diverse and rich water masses of the world in terms of wildlife. Thus, the gulf is home to one-third of the world's marine mammal species, and over 170 and 900 seabird species and fish species, respectively. Regrettably, the increasing of human activity (pollution from industrial and human wastes, aquaculture residues, and agricultural run-off) is beginning to transform it[4].

The objective of this study was to provide a wide assessment of the contamination by PBDEs in cetaceans from the Sea of Cortez considering 4 different species of cetaceans all permanent residents of the Gulf of California, i.e., long-beak common dolphins, bottlenose dolphins, sperm whales and killer whales. This work constitutes part of a much wider and ambitious research project focused on the evaluation of the toxicological status of cetacean populations in the Gulf of California. In this sense, contamination status regarding emerging contaminants such as BFRs and legacy POPs will be evaluated in combination with a ‘‘multi-trial diagnostic tool" (based on field and in vitro studies), combining molecular biomarkers (western blot of CYP1A1, CYP2B) and gene expression (qRT-PCR of HSP70, ERa, AHR, E2F-1) as a set of sensitive non-lethal biomarkers in skin biopsies of different cetacean species in order to evaluate their toxicological status in the Gulf of California[3].

Materials and methods

Sampling

A total of 39 integument biopsies (epidermis, dermis and blubber) were obtained in the Gulf of California during the winter of 2008 using biopsy darts launched with a crossbow/biopsy pole. The samples species were free-ranging long-beak common dolphins (*Delphinus capensis*, n=12, 9 females (f) and 3 males (m)), bottlenose dolphins (*Tursiops truncates*, n=11, 8f and 3m), sperm whales (*Physeter macrocephalus*, n=13, 9f and 4m) and killer whales (*Orcinus orca*, n=2m). Samples were immediately stored in liquid nitrogen and keep in the laboratory stored at -80°C until analysis. Sex was determined according to Bérubé and Palsbøl (1996)[5].

Residue analysis

Contaminant analysis was carried out on freeze-dried blubber biopsy samples. Briefly, analytes, including PBDEs, were Soxhlet extracted with n-hexane during 9 hours. A first purification step was achieved using H_2SO_4. The organic extracts were subsequently purified by low pressure chromatography on florisil columns. Recovery control of the extraction and purification processes was carried out spiking the samples with PCBs 30 and 209 as surrogates.

Fifteen brominated BDE congeners, from tri- to deca-substituted (# 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197, 209), were analyzed by high resolution gas chromatography low resolution mass spectrometry (HRGC-LRMS) using a 6890N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) operated in selected ion monitoring mode (SIM) with negative chemical ionization (NCI). The GC injection port was configured for 1µL pulsed hot splitless injections (4 min) at a temperature of 260ºC. Gas chromatographic separation prior to MS was achieved using a 15 m x 0.20 mm x 0.20 µm DB-5MS low bleed column (J&W Scientific, USA). The GC column was set at 120ºC for 4.2 min, then ramped at 30ºC/min to 200ºC, ramped again at 5ºC/min to 275ºC, ramped once again at 40ºC/min to 300ºC and maintained for 10 min, and finally ramped at 10ºC/min to 310ºC and held for 2 min. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. Methane was used as reaction gas. The temperatures of the transfer line, source and quadrupole were set at 300ºC, 150ºC and 150ºC, respectively. The identification of each BDE was based on detection, at the corresponding retention time, of the m/z 79 and 81 (corresponding to bromine atoms) plus two more ions corresponding to the cluster of $[M\text{-}H_xBr_y]^-$ which are specific of each congener. PBDE concentrations were quantified in all the samples analyzed and expressed as ng/g lipid weight (l.w.). Further details on the analytical procedure could be found at Muñoz-Arnanz et al, 2011[2].

Results and discussion

The highest concentrations were found in the two killer whales specimens (997 and 2200 ng/g), which reflects their top position at the food web. Average concentrations of total PBDE for the rest of the studied species were 104 ng/g in long-beaked common dolphins, 54.0 ng/g in bottlenose dolphins and 26.0 ng/g in sperm whales (Table 1). Albeit the four cetacean species are considered top predators, their average PBDEs burdens follow a rough proportion of ~15:4:2:1. This might partially reflect differences in their Gulf of California's food web position along with differences in their detoxification capacities and metabolic rates.

Table 1. Concentrations (ng/g l.w.) of PBDEs in the four cetacean species

	Killer whales	**Long-beaked common dolphins**			**Bottlenose dolphins**			**Sperm whales**		
PBDEs	males n=2	males n=3	females n=9	total n=12	males n=3	females n=8	total n=11	males n=4	females n=9	total n=13
mean	1600	155	86.5	104	48.2	56.1	54.0	30.8	23.8	26.0
median	1600	111	80.1	91.1	59.1	51.0	52.0	22.0	19.2	19.2
min	997	81	32.6	32.6	5.97	18.2	5.97	6.03	6.31	6.03
max	2200	273	171	273	79.6	130	130	73.1	57.2	73.1

After log-transformation to meet normality (Shapiro-Wilk test, $p>0.05$), no significant differences between sexes were found for any species ($p>0.05$). A Mann-Whitney U non-parametric test on the non log-transformed data yielded the same results ($p>0.05$). However, based on the little set of samples and the unequal number of males and females within each group, the power of the statistical tests was below than desired. Therefore caution should be exerted while interpreting these results.

After performing a one-way Anova test, significant differences ($p<0.05$) in the total PBDE burden was only found between sperm whale and the two dolphin species. Thus, it seems likely that aside metabolic differences, the four species studied, being permanent residents of the Gulf of California, are at three distinct positions of the food web.

The concentrations found in killer whales even though were the highest of all measured in this study, were relatively low in comparison to those reported in resident killer whales from the US and Canada (2500-15000 ng/g l.w.) [6]. Nevertheless, the detected values were in the range or above of those reported to be correlated to alterations in thyroid hormone levels in other marine mammals like grey seals[7].

As for dolphins, PBDE levels found for both species are at least one order of magnitude lower than those reported in more contaminated areas. For instance, average BDE concentrations in long-beaked common dolphins from Korean coastal waters were found at 1700 ng/g l.w. and 1600 ng/g l.w. for males and females, respectively[8]. In the case of bottlenose dolphins, concentrations from 581 to 5917 ng/g l.w. (geometric means depending on the sex and age of the animals) were detected in animals from the coast of two southeastern US areas[9].

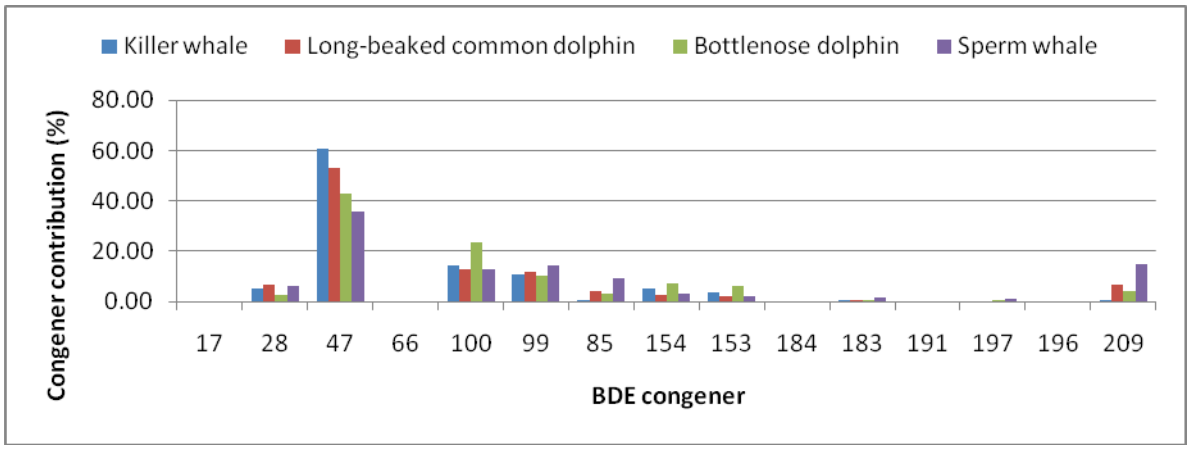

Figure 1. PBDE congener profiles in the four cetacean species

PBDE profiles were relatively similar in all the species under study (Figure 1). BDE-47 was always the predominant congener, followed by BDE-100, -99, -154, -153 and -85. The presence of these congeners was expected and it is consistent with their relative abundance in aquatic food webs. More interesting, however, was to find a relatively high contribution of BDE-209. The presence of this congener in killer whales is almost negligible while they present the highest contribution from BDE-47. The opposite situation occurs in the case of sperm whales. This fact may be related to different metabolic rates rather than to differences in diet, highlighting an increased ability in the case of killer whales to metabolize the higher brominated congeners into lower brominated BDEs like BDE-47.

As preliminary results concerning POPs contamination in the Gulf of California, the present study highlights the existence of PBDE burdens affecting resident cetaceans from this area. Levels of these brominated flame retardants seem to be lower than those reported in cetaceans from more contaminated areas. However, given the protected nature of the Gulf of California a more extended study of the contamination by

POPs in the cetacean populations of this area should be encouraged. Data from this work will be used in a comprehensive study of the toxicological stress under which cetaceans from the Gulf of California are currently coping.

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References:
1. Ross PS, Couillard CM, Ikonomou M, Johannessen SC, Lebeuf M, Macdonald RW, Tomy GT. (2009); Mar. Poll. Bull. 58: 7-10
2. Muñoz-Arnanz J, Sáez M, Aguirre JI, Hiraldo F, Baos R, Pacepavicius G, Alaee M, Jiménez B. (2011); Environ. Int. 37: 572-576
3. Fossi MC, Urban J, Casini S, Maltese S, Spinsanti G, Panti C, Porcelloni S, Panigada S, et al. (2010); Mar. Poll. Bull. 69: S17-S20
4. Lluch-Cota SE, Aragón-Noriega EA, Arreguín-Sáchez F, Aurioles-Gamboa D, Bautista-Romero JJ, Brusca RC, Cervantes-Duarte R, Cortés-Altamirano R, et al. (1997); Prog. Oceanogr. 73:1-26
5. Bérubé M and Palsbøl P. (1996); Mol. Ecol. 5: 283-287
6. Krahn MM, Hanson MB, Baird RW, Boyer RH, Burrows DG, Emmons CK, Ford JKB, Jones LL, et al. (2007); Mar. Poll. Bull. 54:1903-1911
7. Hall AJ, Kalantzi OI, Thomas GO. (2003); Environ. Poll. 126:29-37
8. Moon H-B, Kannan K, Choi M, Yu J, Choi H-G, An Y-R, Choi S-G, Park J-Y, Kim Z-G. (2010); J. Hazard. Mater. 179:735-741
9. Fair PA, Adams J, Mitchum G, Hulsey TC, Reif JS, Houde M, Muir D, Wirth E, et al. (2010); Sci. Total Environ. 408:1577-1597