Halogenated and organophosphorus flame retardants in cetaceans from the Indian Ocean

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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.

A growing alternative to halogenated flame retardants (HFRs), are organophosphorus flame retardants (OPFRs). They comprised 20 % of the amount of FRs used in 2006 in Europe —doubling the amount of brominated FRs— and the ban on PBDEs increased their popularity. OPFRs are also released from materials and access environmental matrices through washout, infiltration, deposition, etc. Moreover, OPFRs are used as plasticisers; so they leak from the tones of plastic that reach seas and oceans. Their presence has been reported in sediments, water and fish[1-3]. OPFRs show toxic effects on the reproductive and endocrine systems, as well as systemic and carcinogenic effects[4].

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 present study assesses the occurrence of the aforementioned compounds in three species of dolphin from the Indian Ocean, including long-beaked common dolphin (*Delphinus capensis*), Indian Ocean humpback dolphin (*Sousa plumbea*) and Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). It is also the first study to analyse OPFRs in marine mammals.

Materials and methods

2.1. Sampling

A total of 13 muscle samples of three species of dolphin were collected from individuals incidentally caught in shark nets of KwaZulu-Natal (east-coast South Africa), in the Indian Ocean, between 2012 and 2015. The samples included two individuals of long-beaked common dolphin (*Delphinus capensis*), five individuals of Indian Ocean humpback dolphin (*Sousa plumbea*) and six individuals of Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). For brevity purposes, in this article the species are going to be referred to as simply common dolphin, humpback dolphin and bottlenose dolphin. The samples contained individuals of different age groups. Age groups were assigned according to the size of the dolphins[5-7]. See Table 1 for details. Samples were freeze-dried prior to shipping to the analytical laboratory. Lipid content referenced to dry weight (dw) was between 0.65-11.7%.

Table 1. Sampling information and contaminant concentrations (ng g^{-1} lw)

species	sex	maturity stage	size (cm)	∑PBDEs	Dec 602[a]	∑MeO-PBDEs	∑OPFRs
long-beaked common dolphin (*Delphinus capensis*)	female	adult	222	97.7	150	52.4	102
	male	adult	239	165	n. d.	112	107
Indian Ocean humpback dolphin (*Sousa plumbea*)	female	calf	125	494	58.0	37.6	85.9
	male	juvenile	183	244	127	64.0	202
			214	573	321	220	1266
		adult	245	667	n. d.	132	714
			249	33.3	49.1	23.1	176
Indo-Pacific bottlenose dolphin (*Tursiops aduncus*)	female	calf	146	563	81.8	76.8	193
		juvenile	154	382	132	529	156
			205	264	n. d.	65.3	1597
	male	juvenile	180	1309	2034	1.5	1531
			224	189	n. d.	51.3	401
		adult	248	424	59.5	123	310
frequency of detection (%)				100	69.2	100	100
LOD[b]				0.04	0.02	0.43	0.19
LOQ				0.12	0.07	1.42	1.03

[a]Dec 603 and *anti*-DP were detected in three samples below their L_Q (0.02 and 0.01 ng g^{-1} lw).
[b]LOD and LOQ of the compound with the lowest values of the group for PBDEs, MeO-PBDEs and OPFRs.

2.2. Sample preparation

The extraction of OPFRs from muscle tissue was carried out by ultrasound assisted extraction according to an existing method[3]. Freeze-dried sample (0.5 g) was extracted by sonication. The extract was reconstituted and centrifuged and an aliquot of 200 µl was used for the instrumental analysis. Purification was performed on-line at the beginning of the instrumental analysis. Labelled OPFRs standards were added prior to analysis by turbulent flow chromatography coupled to LC-MS/MS (TFC-LC-MS/MS).

For the other compounds, sample extraction was carried out according to previous works[8]. 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

For OPFRs, online sample purification and analysis was performed with a Thermo Scientific TurboFlow™ system[3]. CycloneTM-P (0.5×50 mm) and C18-XL (0.5×50 mm) columns were used in combination for purification. Chromatographic separation was achieved with an analytical column Purosphere Star RP-18 (125 mm × 0.2 mm). Mobile phase was a gradient of water (0.1% formic acid) and methanol (0.1% formic acid) at 0.75 ml min^{-1}. Spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source. For all compounds, selective reaction monitoring (SRM) mode was used with two transitions monitored for each one. Recoveries for individual compounds ranged 47-98% and RSDs were 2.4-16%. LOQs and LODs were, respectively, 0.97-24.8 ng g^{-1} lipid weight (lw) and 0.19-19.3 ng g^{-1} lw.

HFRs 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[9] and the spectrometric determination[10] are described in previous works. 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)[11]. The analysis of dechloranes was performed by NCI as described in a previous article[12]. Recoveries were between 51 and 99 %, RSDs were 1.1-22 %, LODs were 0.0023-10.6 ng g^{-1} lw and LOQs were

0.0077-35.4 ng g^{-1} lw. After analysis of the previous HFRs, extracts were redissolved in methanol and d_{18}-HBCD was added. HBCD was analysed using an Agilent HP 1100 binary pump LC system coupled to a hybrid triple quadrupole/linear ion trap 4000QTRAP[13]. Recoveries for α-, β- and γ-HBCD ranged 85-105 % and RSDs were 3.1-8.3 %. LOQs and LODs were, respectively, 0.4-4.4 ng g^{-1} lw and 0.2-2.0 ng g^{-1} lw.

Results and discussion:

See Table 1 for results. Mean PBDEs concentration was 416 ± 333 ng g^{-1} lw. BDE-47 was found in all samples and was almost half the total PBDEs contamination (42 ± 16 %). BDE-209, BDE-100 and BDE-99 were present in 100, 92 and 85 % of the samples, respectively, representing an average 20, 9 and 19 % of the PBDEs contamination. BDE-183 was not detected.

Published data about flame retardants in dolphins from the Indian Ocean is very scarce. Total PBDEs in blubber of two species from India were below 20 ng g^{-1} lw[14,15]. Contrary to what one could expect, PBDE levels on the African coast were higher than on the Asian coast. Some studies from the last two decades on different dolphin species showed mean concentrations values between 420 and 880 ng g^{-1} lw in Europe, with the highest values at 2340 ng g^{-1} lw[16,17] and of 166 ng g^{-1} lw in Brasil, ranging from 6 to 1800 ng g^{-1} lw[18]. These results seem to be similar to the ones of the present study. A lack of published information on PBDEs in South Africa makes it hard to comment on the high levels in the present study. However, some of the available data on environmental South African samples show high levels, suggesting high environmental contamination in the area. Possible explanations for high levels of HFRs would be atmospheric transportation, air-water exchange and deposition. Another reason could be the city of Durban as a local source.

HBCD was detected in just two juvenile dolphins; α-HBCD in a female humpback dolphin (20.7 ng g^{-1} lw) and β- and γ-HBCD in a male bottlenose dolphin (173 and 158 ng g^{-1} lw).

PBEB and HBB were not detected, while DBDPE was in all samples but always below its LOQ, 0.26 ng g^{-1} lw. As DBDPE is the marketed alternative to Deca-BDE, it seems logical to detect the presence of DBDPE in modern samples where BDE-209 accounts for 20 % of the total PBDEs. Dec 602 was the only quantifiable dechlorane at 232 ± 549 ng g^{-1} lw. Dec 602 has a higher bioaccumulation potential than other dechloranes. Dec 603 was detected in one adult common dolphin and *anti*-DP was found in both adult common dolphins and an adult humpback dolphin, both compounds below their LOQs. It must be noted, however, that other species of dolphins from the Mediterranean Sea had total dechloranes levels in blubber below 60 ng g^{-1} lw[17].

Mean OPFRs concentration was 526 ± 565 ng g^{-1} lw. TBOEP was found in all samples making most of the total OPFRs contamination. TPPO and TDCPP were detected in 53.8 % of the samples, generally below 1.0 ng g^{-1} lw. IPPP, TEHP, TBP, EHDP and TMCP were detected in one to three samples at concentrations up to 90 ng g^{-1} lw; except for IPPP and TEHP in a juvenile male bottlenose dolphin with 880 and 279 ng g^{-1} lw, respectively. Since there is no published data on OPFRs in marine mammals, there are no reference values for comparison. However, it is important to note that OPFRs were at concentrations similar to PBDEs (t-test $t = 0.63$, df = 22, $p > 0.1$) and higher than dechloranes ($t = 2.49$, df = 22, $p < 0.05$), so they should be further monitored.

As for the natural compounds, MeO-PBDEs showed in all the samples with a mean concentration of 114 ± 137 ng g^{-1} lw. These levels are similar to those found in other dolphin species from Tanzania, 65 ± 43 ng g^{-1} lw[19,20]. Moreover, these compounds are present in the same order of magnitude as the other compounds, including the analogous PBDEs. Sharing levels with a POP of similar structure and properties is a reason to consider these natural compounds in routine monitoring.

This study was conducted with individuals that were incidentally caught in shark nets. Thus, the number of samples, the species, the sex and the maturity stage of the dolphins were impossible to control. POPs levels should show a characteristic trend during the lifetime of the dolphins, with males showing increasing levels with age, as POPs are bioaccumulated. Females should show a decrease after giving birth due to the mother-to-calf transfer. With the present samples, no differences between species could be assessed statistically for any family of compounds, except for MeO-PBDEs. The concentration of the natural compounds increased from juvenile to adult male dolphins ($t = 4.19$, df = 4, $p < 0.05$, Figure 1a). Female dolphins were excluded in case they had reproduced and transferred their contamination to their calves.

Additionally, although OPFRs did not show differences between maturity stages, some congeners (e.g. TBOEP) can be metabolised and they would not follow the increasing pattern with age, but their levels could decrease even in male individuals. TBEOP levels showed a statistically significant increase from calves to male juveniles ($t = 2.66$, df = 6, $p < 0.05$, Figure 1b). However, this trend did not follow into adulthood, when levels seem slightly lower, probably due to the metabolisation of this congener.

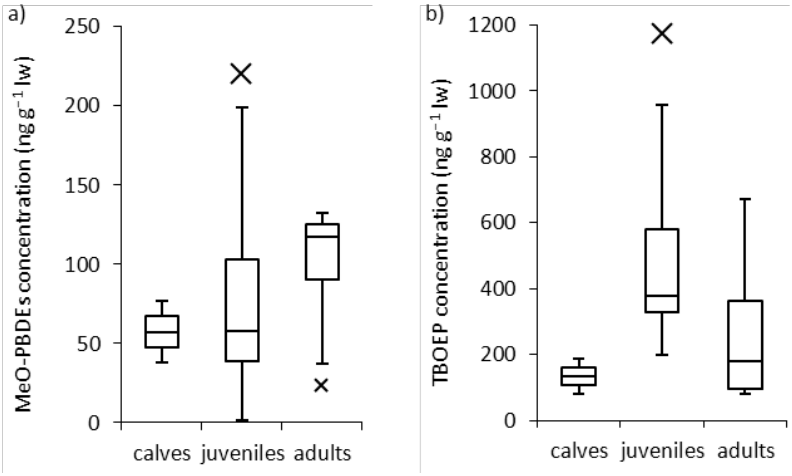

Figure 1. Box plots of MeO-PBDEs and TBOEP concentrations for all maturity stages. Juveniles and adults include only males. Outliers marked (×)

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