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Changes of accumulation profiles from PBDEs to brominated and chlorinated alternatives in marine mammals from the South China Sea

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ABSTRACT

The present study investigated the composition profiles and levels of polybrominated diphenyl ethers (PBDEs) and five PBDE alternatives in the blubber of two species of marine mammals, Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) from the South China Sea. Despite the fact that PBDEs were the most predominant brominated flame retardants in the samples analyzed, decabromodiphenyl ethane (DBDPE), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), bis- (2-ethylhexyl) -tetrabromophthalate (TBPH), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and Dechlorane Plus (DP) were all detected in both cetacean species. In addition, significantly increasing temporal shifting trends of Deca-BDE to DBDPE, Octa-BDE to BTBPE, and Deca-BDE to DP were observed in porpoise samples between 2003 and 2012 and dolphin samples between 2003 and 2011. These patterns may be attributed to the replacement of PBDEs by alternative halogenated flame retardants (HFRs) and the increasing usage of these alternatives following the restriction/voluntary withdrawal of the production and use of PBDE commercial mixtures. Our findings suggest that the study region may be a source of contamination by PBDE alternative flame retardants due to the high detection frequencies and levels of these compounds in marine mammals.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants and extensively applied in a variety of consumer products in order to inhibit or reduce the spread of fire (Alaee et al., 2003). Due to their persistence, bioaccumulative tendencies and potential adverse effects on organisms, as well as their large scale usage, the Penta-BDE and Octa-BDE commercial mixtures have been banned in the European Union EU in 2004 and were included in the Stockholm Convention of Persistent Organic Pollutants (POPs) (http://chm.pops.int/Convention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx). In addition, Deca-BDE has also been exempted or phased out in the production of electronic applications in Europe and the United States (European Court of Justice, 2008; Wäger et al., 2012). Since the worldwide restriction on the production and use of PBDEs (Covaci et al., 2011), market demand for its replacements is projected to increase.

There are several representative halogenated flame retardants (HFRs) used as alternatives for the banned formulations of PBDEs such as 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), bis- (2-ethylhexyl) -tetrabromophthalate (TBPH), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and Dechlorane Plus (DP) (Covaci et al., 2011; de Wit et al., 2010; European Chemicals Bureau, ECB, 2007). BTBPE has been produced since the mid-1970s and used as a replacement for technical Octa-BDE (Hoh et al., 2005), whereas both TBPH and TBB have been produced since 2003 as replacements for Penta-BDE in polyurethane foam applications (Stapleton et al., 2008). Production of BTBPE can be found in the United States (Hoh et al., 2005) and China (Shi et al., 2009), but little is known about its use in China. BTBPE has been detected in air, fish and bird samples from China (Shi et al., 2009), however, very little data are available for TBB and TBPH in China. Lam et al. (2009) reported the presence of these compounds in the blubber of marine mammals from South China. DBDPE, which is structurally similar to decabromodiphenyl ether (BDE209), has been produced and used for more than 20 years and is marketed as an alternative for technical Deca-BDE (de Wit et al., 2010). Both DBDPE and BDE209 are used as additive flame retardants in textile and plastic applications (Ricklund et al., 2010). The estimated DBDPE production volume in China in 2006 was 12,000 metric tons which was similar to that of Deca-BDE formulations (Shi et al., 2009). The presence of DBDPE has been reported in a wide range of environmental samples, although its levels were lower

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compared to levels of ubiquitous PBDEs in the environment (Covaci et al., 2011; de Wit et al., 2010). Recently, Deca-BDE has also been phased out in the production of electronic applications in the United States and Europe (European Court of Justice, 2008; Wäger et al., 2012). Thus, the restriction will lead to an increasing use of its alternative. Like DBDPE, DP has been recently regarded as a possible replacement for the Deca-BDE technical mixture in the European Union (European Chemicals Bureau, ECB, 2007). Technical DP consisting of *syn*- and *anti*-stereoisomers, is an additive chlorinated flame retardant (Sverko et al., 2011). The global estimated annual production of DP was approximately 5000 tons (Ren et al., 2008) whereas annual production amounts in China were estimated to be 300–1000 tons (Wang et al., 2010) and this has been recognized as one of the significant sources of DP in China. At least 75 non-PBDE flame retardants have been commercially produced, and for most of these, the environmental occurrences and potential health risks to humans and wildlife have not been adequately assessed (Covaci et al., 2011).

Previous studies indicated that marine mammals can be used as sentinel species of ocean and human health because they are able to integrate and reflect the environmental changes across large spatial and over long temporal scales (Fair et al., 2010; Fossi et al., 2003; Moore, 2008; Wells et al., 2004). Two resident cetacean species, the finless porpoise (*Neophocaena phocaenoides*) and Indo-Pacific humpback dolphin (*Sousa chinensis*) are the top predators in the marine food chain of South China. In general, the porpoises are found in the southern and eastern waters of Hong Kong, whereas dolphins inhabit northwestern waters of Hong Kong adjacent to the mouth of the Pearl River estuary (Barros et al., 2004). These two species of marine mammals have been the subjects of our previous monitoring studies (Hung et al., 2006a, 2006b; Lam et al., 2009) which indicated that these two species were susceptible to exposure to and bioaccumulation of environmental contaminants due to their high trophic levels and high body fat content. Our previous study also reported the elevated concentrations of conventional brominated flame retardants (PBDEs and HBCDs) in the blubber samples of these cetaceans (Lam et al., 2009). However, a knowledge gap exists for the other potential replacements for PBDEs including BTBPE, DBDPE, TBB, TBPH and DP.

It is hypothesized that with recent regulations/phasing-out of the Penta- and Octa-BDE and Deca-BDE commercial mixtures, several current use HFRs would be used to replace these “conventional” flame retardants in the products, and consequently, would be present in the environment. Little has yet been reported on the concentrations of PBDE alternatives in the tissues of marine mammals. To better understand the current status of these compounds in the marine environment, the present study aims (1) to identify and quantify PBDEs and PBDE alternatives in the two species of marine mammals from the South China Sea; (2) to assess whether there have been any changes in composition profiles/accumulation patterns in terms of the ratio of concentrations of several PBDE alternatives and PBDEs in the blubber samples over the past ten years (2003–2012), after the phasing out of PBDEs in China.

2. Materials and methods

2.1. Materials

PBDEs (BDE28, BDE47, BDE99, BDE100, BDE153, BDE154, BDE183, BDE196, BDE197, BDE206, BDE207, BDE209), BTBPE, DBDPE, DP (*syn*- and *anti*-DPs), TBB, TBPH, internal standard ($^{13}C_{12}$-labeled BDE139) and surrogate standards ($^{13}C_{12}$-labeled BDE28, BDE47, BDE99, BDE153, BDE154, BDE183, BDE197, BDE207, and BDE209; $^{13}C_{12}$-labeled DBDPE, and BTBPE) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). All organic solvents used were HPLC grade purchased from Aldrich (Milwaukee, WI, USA) and Tedia (Fairfield, OH, USA). Anhydrous sodium sulfate, copper powder (<75 μm, 99%), silica gel (particle size 0.063–0.200 mm) and aluminum oxide were all purchased from Sigma-Aldrich.

2.2. Sample collection

The blubber of adult Indo-Pacific humpback dolphins ($n = 23$) and finless porpoises ($n = 38$) were sampled from stranded animals collected by the Agriculture, Fisheries and Conservation Department (AFCD) in Hong Kong, China, between 2003 and 2012 (shown in Table S1 of the Supplementary information). The samples were dissected with stainless steel tools and then wrapped with aluminum foil before transferring to the laboratory for storage. All the samples were stored at −20 °C before chemical analysis.

2.3. Extraction and cleanup

Analysis of blubber samples were accomplished by use of previously established methods (Lam et al., 2009), with modifications. Briefly, approximately 1 g blubber sample was ground with sodium sulfate and spiked with surrogate standards ($^{13}C_{12}$-labeled BDE28, BDE47, BDE99, BDE153, BDE154, BDE183, BDE197, BDE207, and BDE209; $^{13}C_{12}$-labeled DBDPE and BTBPE). Samples were then extracted by accelerated solvent extractor (ASE) with hexane/DCM (1:9, v/v) and the lipid was then removed from each extract by gel permeation chromatography (GPC) with 5 ml/min hexane/DCM (1:1, v/v) mobile phase, prior to GPC, and a 10% portion by volume of the lipid extract was taken for gravimetric determination of lipid. Further purification was carried out using a multilayer silica/alumina column packed with activated silica gel (5 g), aluminum oxide (5 g), and anhydrous sodium sulfate (1 g) (from the bottom to the top). Both silica gel and aluminum were activated at a temperature of 110 °C. The target compounds were eluted with 150 ml hexane:DCM (1:2, v/v), and an internal standard ($^{13}C_{12}$-labeled BDE139) was added before concentrating to dryness under a gentle stream of nitrogen and finally reconstituted in 100 μl of methanol.

2.4. Instrumental analysis

Identification and quantification of PBDEs and other PBDE alternatives were performed based on the previous method (Zhu et al., 2013) using a liquid chromatography–tandem mass spectrometer system (LC–MS/MS) consisting of an Agilent 1290 Infinity LC (Agilent Technologies, Palo Alto, CA) coupled to AB DIEX QTRAP® 5500 LC–MS–MS system with an atmospheric pressure chemical ionization (APCI) interface. A Zorbax SB-C18 column (2.1 mm × 100 mm, 1.8 μm; Agilent Technologies, Palo Alto, CA) was used for the chromatography separation. The mobile phase consisted of (A) methanol/water (85:15) and (B) methanol. Liquid chromatography separation was carried out at ambient temperature with a linear gradient elution at a flow rate of 200 μl/min from 100% A to 5% A with 95% B for the first 4 min, and then increased to 100% B with 300 μl/min at 6 min before reverting to original conditions at 17 min. The injection volume was 2 μl. Data was acquired and processed using AB Sciex Analyst software (version 1.6). Concentrations of analytes were expressed as ng/g^{-} lipid weight.

2.5. QA/QC

Procedural blanks were processed with every batch of five samples to check for interferences or contamination. The detection limit (DL) was defined as the mean level in the procedural blanks plus three times of the standard deviation. The DL was 0.0014 to 0.26 ng/g of lipid for BDE congeners, 0.25 and 0.33 ng/g of lipid for DP isomers, 0.019, 0.16, 0.020 and 0.041 ng/g of lipid for BTBPE, DBDPE, TBPH and TBB, respectively. Values below DL were represented as half of the DL for data treatment in calculation and statistical analysis. Recoveries of $^{13}C_{12}$-labeled PBDE congeners in the cetacean blubber samples were