

# HEXABROMOCYCLODODECANES, POLYBROMINATED DIPHENYL ETHERS AND NEW ORGANOBROMINATED COMPOUNDS IN MARINE MAMMALS FROM HONG KONG, CHINA

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## Abstract

Levels of polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), and three new flame retardants, namely 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), bis-(2-ethylhexyl)-tetrabromophthalate (TBPH) and hexachlorocyclopentadienyldibromocyclooctane (HCDBCO), were determined in blubber samples of two species of marine mammals in Hong Kong. Significant increases in PBDE and HBCD concentrations were observed in finless porpoises (*Neophocaena phocaenoides*) and Indo-Pacific humpback dolphins (*Sousa chinensis*), respectively, when the levels in samples collected between 1997 – 2001 and 2002 – 2008 were compared. The novel brominated flame retardant TBPH, which has only recently been identified in house dust from the US, also showed an increasing trend in porpoise samples, and was found at levels comparable to HBCDs and approximately one order of magnitude lower than those of PBDEs. These results show that concentrations of these brominated flame retardants are increasing in these marine top predators. However, the occurrence and concentration trends of these brominated flame retardants in the Asia-Pacific region, particularly in China, are still unclear, and thus additional monitoring studies on the levels and toxicology of flame retardants in the environment are urgently needed in order to provide more information for future risk assessments of these compounds.

## Introduction

Hexabromocyclododecanes (HBCDs) and polybrominated diphenyl ethers (PBDEs) are additive brominated flame retardants (BFRs) which are widely applied to household and industrial products such as textiles, plastics, furniture, and electronic products to inhibit or resist the spread of fire<sup>1,2</sup>. Since PBDEs are persistent, bioaccumulative and potentially harmful to human health and the environment, use of these compounds has been restricted or they have been voluntarily withdrawn from use in some parts of the world. Like PBDEs, HBCDs have been found to be persistent in the environment and bioaccumulative in biota<sup>2</sup>. In addition, concentrations of these compounds have been increasing in biological samples and have become a matter of concern in recent years.

In recent decades, China has been the fastest-growing country in the Asia-Pacific region in terms of population and economic development. The Pearl River Delta (PRD), in particular, has been developed into a leading industrial manufacturing area in China. To date, there is no legislation in China restricting the use of BFRs such as HBCDs. Thus, it is likely that large quantities of BFRs were previously or are currently used in manufacturing processes and consequently are being released into the environment, both in the production and disposal of BFR-containing products (i.e. e-waste). However, data on the concentrations of these contaminants in China are very limited.

Apart from these known BFRs, a novel flame retardant, hexabrominated heterocyclic tris-(2,3-dibromopropyl) isocyanurate (TBC), was recently identified in various environmental matrices near a flame retardant manufacturing plant in South China<sup>3</sup>. Other new FRs, such as hexachlorocyclopentadienyldibromocyclooctane (HCDBCO), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and bis-(2-ethylhexyl)-tetrabromophthalate (TBPH), have also been measured in house dust samples in the US<sup>4,5</sup>. These findings raised concern as to whether these novel compounds could be detected in the PRD region. Accordingly, the present study was undertaken to address the occurrence and temporal variations of these traditional and new flame retardants in the marine environment of the PRD by analyzing

blubber from two species of marine mammals, the Indo-Pacific humpback dolphin (*Sousa chinensis*) and finless porpoise (*Neophocaena phocaenoides*) from Hong Kong.

### Materials and Methods

Stranded specimens of Indo-Pacific humpback dolphins and finless porpoise were collected in Hong Kong, China, between 2002 and 2007 and between 2003 and 2008, respectively. Analyses of PBDEs and HBCDs, HCDBCO, TBB and TBPH were accomplished by use of previously established methods<sup>5-7</sup> with modifications. Briefly, each blubber sample was extracted in a Soxhlet apparatus and lipids were removed by use of gel permeation chromatography (GPC). Before being subjected to GPC for lipid removal, <sup>13</sup>C<sub>12</sub>-labeled PBDE and HBCD standards were added to the extract. The concentrated GPC fraction was then transferred and further purified by elution through activated silica gel. <sup>13</sup>C<sub>12</sub>-labeled BDE139 was added as the recovery spike and deuterized HBCDs ( $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD-*d*<sub>18</sub>) were spiked into the concentrated fraction prior to GC analysis and LC-MS/MS analysis, respectively. Procedures for sample preparation for HCDBCO, TBB and TBPH analysis were similar to those for the analysis of PBDEs with some modifications. Briefly, the concentrated GPC fraction was purified by elution through activated silica gel. HCDBCO, TBB and TBPH were eluted with hexane and dichloromethane.

Quantification of PBDEs was performed using a GC (Agilent 7890A) equipped with a mass-selective detector (Agilent 5975c) for mono- to deca- BDEs, using electron impact (EI) mode. Fourteen major PBDE congeners were quantified using the isotope dilution method to their corresponding <sup>13</sup>C<sub>12</sub>-labeled congeners. Instrumental analysis of HBCDs was performed based on the analytical method by Tomy et al.<sup>8,9</sup>. Quantification of HBCDs was carried out using an Agilent HP1100 liquid chromatograph (Agilent, Palo Alto, CA, USA) coupled with an Applied Biosystems API 2000 triple quadrupole tandem mass spectrometer equipped with a Turbo IonSpray source operated in negative mode. Identification and quantification of POPs, HCDBCO, TBB and TBPH was conducted using a GC (Agilent 7890A) equipped with a mass-selective detector (Agilent 5975C) operated in electron capture negative ionization mode (GC/ECNI-MS), similar to the analysis carried out by Stapleton et al.<sup>5</sup> with some modifications. Recoveries of <sup>13</sup>C<sub>12</sub>-labeled HBCDs and PBDEs in all samples were within 60 – 120% and recoveries of spiked HCDBCO, TBB and TBPH ranged from 70 – 120%.

### Results and Discussion

#### *Concentrations and congener and stereoisomeric profiles of flame retardants in marine mammals*

In the present study, PBDEs were predominant among all the flame retardants analyzed, followed by HBCDs, TBPH and TBB. HCDBCO was undetectable in all of the samples (LOQ: 0.04 ng/g, lipid wt.). PBDEs and HBCDs were detected in all the samples analyzed and the highest concentrations were found in Indo-Pacific humpback dolphins (51100 and 519 ng/g, lipid wt., respectively). Concentrations of HBCDs were comparable to those of TBPH, which is one of the major constituents of the flame retardant mixture Firemaster® 550 (FM 550)<sup>5</sup>. Levels of these two compounds were approximately one order of magnitude lower than those of PBDEs in the marine mammals (Figure 1; mean  $\pm$  SD). The other major constituent of FM 550, TBB, was only detected in finless porpoise samples.

Comparisons of flame retardant concentrations between the two species of marine mammal showed that significantly higher levels of PBDEs and HBCDs were present in dolphins (Mann-Whitney Rank

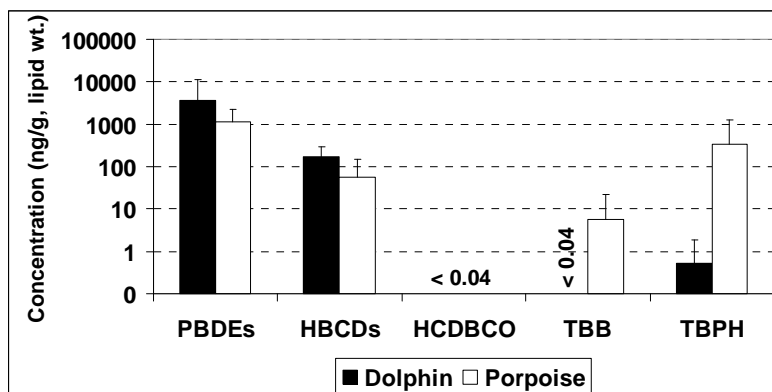


Figure 1 Concentrations of flame retardants in HK cetaceans

Sum Test,  $p < 0.001$  and  $p < 0.01$ , respectively). In contrast, levels of TBPH were significantly greater in porpoises (Mann-Whitney Rank Sum Test,  $p = 0.001$ ). The distribution patterns of dolphins and porpoises tend not to overlap; the dolphin is generally restricted to the estuarine northwestern waters of Hong Kong, whereas the porpoise is found in eastern waters. Regardless of potential species-specific differences, contaminant concentrations in the blubber of dolphins indicate that northwestern waters of Hong Kong are more contaminated by HBCDs and PBDEs than the eastern waters. In contrast, TBB and TBPH were mostly detected in porpoise samples, which may indicate the presence of a potential emission source of these new flame retardants in their habitat.

Congener analysis indicated that BDE-47 was predominant and contributed over 60% of the total PBDEs in both species. There were no obvious changes among the congeners detected in samples collected between 1997 – 2001 (reported by Isobe et al.<sup>7</sup>) and 2002 – 2008 with the exception of BDE-209, the major constituent of the DecaBDE technical mixture, which is the last PBDE commercial mixture to be phased out of use; DecaBDE has been banned in some European countries, but is still used in the US (Figure 2; mean concentrations). This result suggests changes in the use of different PBDE commercial formulations over time.

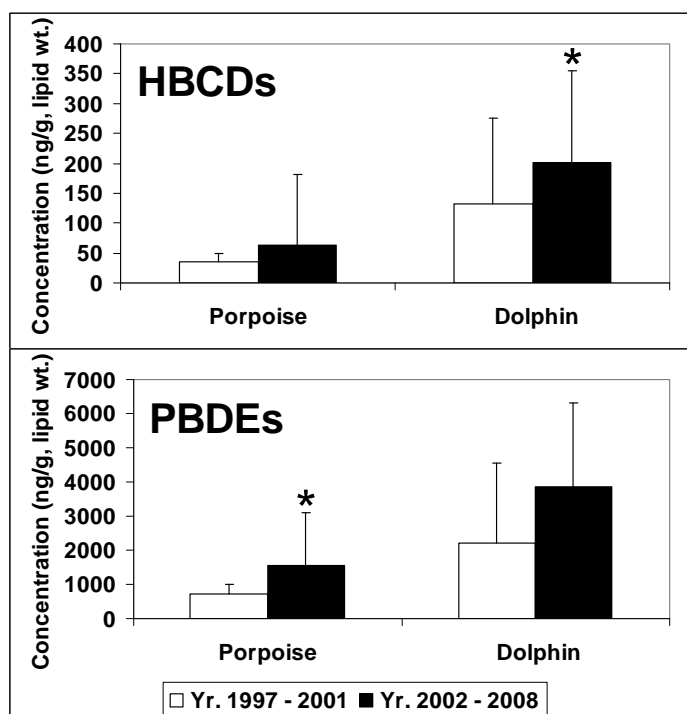


Figure 3 Temporal variations of HBCDs and PBDEs in HK cetaceans

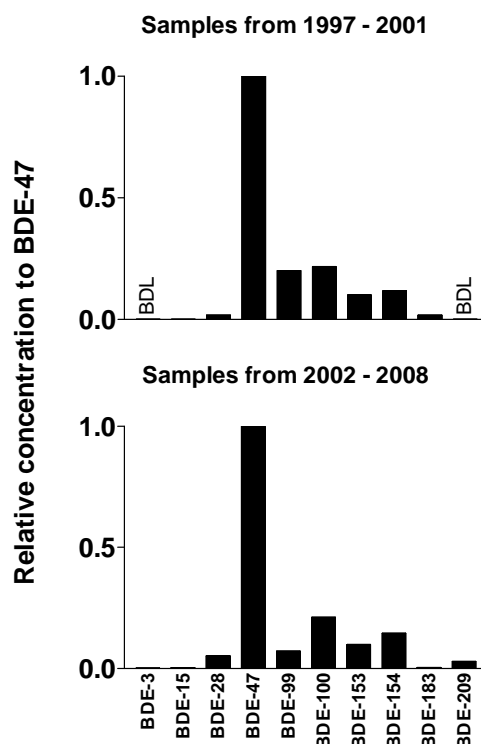


Figure 2 Congener profiles of PBDEs in dolphins

Three stereoisomers of HBCD were detected in the samples:  $\alpha$ -HBCD was detected at the greatest concentrations in the marine mammals, followed by  $\gamma$ -HBCD and  $\beta$ -HBCD; these patterns are similar to those reported for cetacean studies conducted elsewhere<sup>2</sup>.

#### *Temporal variations of flame retardants in marine mammals*

$\Sigma$ HBCD and  $\Sigma$ PBDE concentrations in Indo-Pacific humpback dolphins and finless porpoises collected from 2002 to 2008 (2000s) were compared with the contaminant dataset for blubber samples collected from 1997 to 2001 (1990s) reported by Isobe et al.<sup>7</sup> To minimize the influence of differences between the sexes caused by maternal transfer and possible age-related differences, adult males with body lengths greater than 200 cm and 120 cm for dolphins and porpoises, respectively, were selected for

comparison (Figure 3; mean  $\pm$  SD). Concentrations of  $\Sigma$ PBDEs and  $\Sigma$ HBCDs detected in the two species of marine mammals collected from the 2000s were higher than those from the 1990s and were significantly higher in porpoises (Mann-Whitney Rank Sum Test,  $p = 0.033$ ) and dolphins (Mann-Whitney Rank Sum Test,  $p = 0.048$ ), respectively, suggesting that levels of these two BFRs are increasing in the Asia-Pacific region. It is also important to note that  $\Sigma$ PBDEs in porpoises and  $\Sigma$ HBCDs in dolphin increased by two- and 1.5-fold, respectively, in recent years compared with levels in the 1990s samples.

Apart from these two BFRs, an increasing trend in TBPH levels was also observed. TBPH concentrations detected in individual porpoises collected from 2004 to 2007 were ranked and are shown in Figure 4. Most of the samples from 2004 contained very low concentrations of TBPH; however, the concentrations sharply increased after 2005 and the greatest concentration (3800 ng/g, lipid wt.) was found in a sample collected in 2007. These increasing concentrations of TBPH are possibly due to high demand for FM 550, which consists of TBB and TBPH<sup>5</sup>. However, the exposure pathways and accumulation patterns of these contaminants in the marine environment are still unclear, and these topics deserve further investigation. The production and usage of BFRs and other new FRs are anticipated to increase in the coming years, but information on the levels of HBCDs and other new flame retardants such as TBB and TBPH in China are very limited. Recommendations for future work include research on the fate and effects of these FRs. Trend analyses need to be carried out in more detail and more data on various environmental matrices such as sediments are urgently needed.

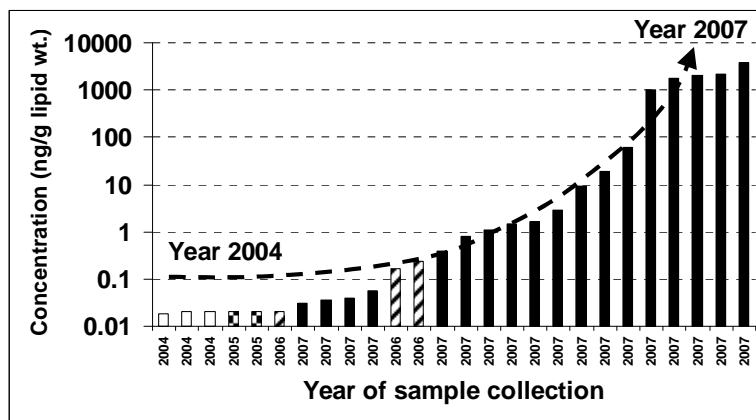


Figure 4 Temporal variation of TBPH levels in porpoises

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### References

1. de Wit C.A.. *Chemosphere* 2002; 46: 583.
2. Covaci A., Gerecke A.C., Law R.J., Voorspoels S., Kohler M., Heeb N.V., Leslie H., Allchin C.R., de Boer J. *Environ Sci Technol* 2006; 40: 3679.
3. Ruan T., Wang Y., Wang C., Wang P., Fu J., Yin Y., Qu G., Wang T., Jiang G. *Environ Sci Technol* 2009; 43: 3080.
4. Zhu J., Hou Y., Feng Y. L., Shoeib M., Harner T. *Environ Sci Technol* 2008; 42: 386.
5. Stapleton H. M., Allen J. G., Kelly S. M., Konstantinov A., Klosterhaus S., Watkins D., Mcclean M. D., Webster T. F. *Environ Sci Technol* 2008; 42: 6910.
6. Lam J. C. W., Murphy M. B., Wang Y., Tanabe S., Giesy J. P., Lam P. K. S. *Environ Sci Technol* 2008; 42: 6296.
7. Isobe T., Ramu K., Kajiwara N., Takahashi S., Lam P. K. S., Jefferson T. A., Zhou K., Tanabe S. *Mar Pollut Bull* 2007; 54: 1139.
8. Tomy G. T., Budakowski W., Halldorson T., Whittle D.M., Keir M.J., Marvin C., MacInnis G., Alae M. *Environ Sci Technol* 2004; 38: 2298.
9. Tomy G. T., Halldorson T., Danell R., Law K., Arsenault G., Alae M., MacInnis G., Marvin C. H. *Rapid Commun Mass Spectrum* 2005; 19: 2819.