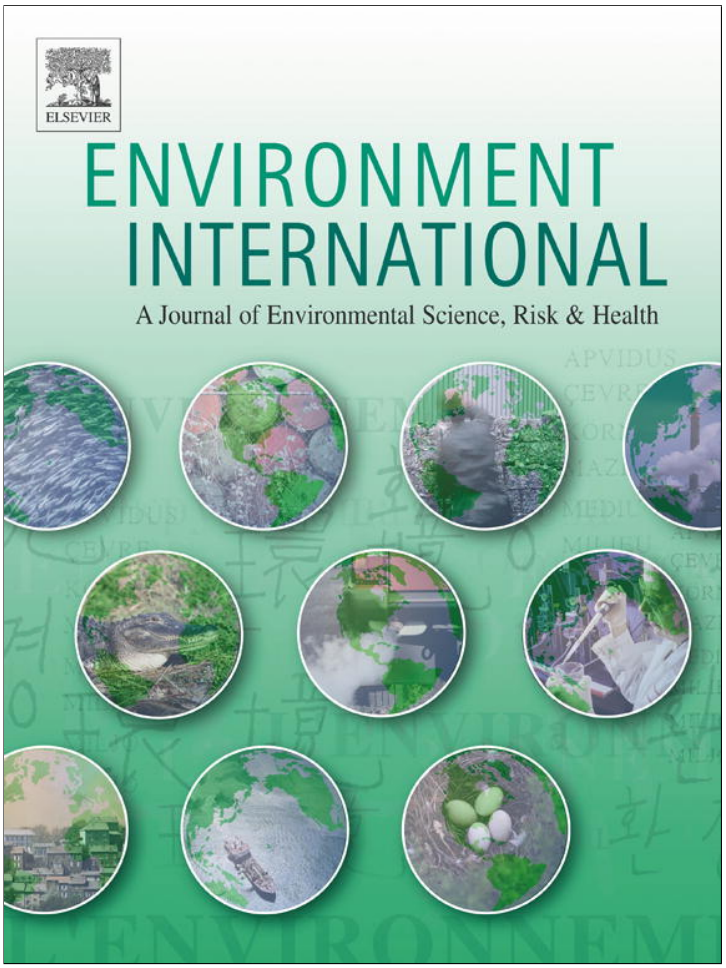

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Hydroxylated and methoxylated polybrominated diphenyl ethers in blood plasma of humans in Hong Kong

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ABSTRACT

Hydroxylated (OH-) and methoxylated (MeO-) polybrominated diphenyl ethers (PBDE) are suspected endocrine disruptors. Little is known about the accumulation or sources of these chemicals in tissues of humans, particularly those residing in Hong Kong, which is one of the most densely populated cities in the world. Seven MeO-BDEs, fifteen OH-BDEs and three bromophenols (BRPs) were analyzed in blood plasma of 116 humans that had been collected by the Hong Kong Red Cross. Total concentrations of MeO-BDEs, OH-BDEs and BRPs ranged from 3.8×10^{2} to 52×10^{3} pg g^{-1} lipid (median 4.5×10^{3} pg g^{-1}), 5.3 to 4.9×10^{2} pg g^{-1} lipid (81 pg g^{-1}) and ND to 1.1×10^{2} pg g^{-1} lipid (3.7 pg g^{-1}), respectively. 3-MeO-BDE-47, 6-OH-BDE-47 and 2, 4, 5-TBP were the predominant MeO-BDEs, OH-BDEs and BRPs, respectively. These results are consistent with accumulation of MeO-BDEs, OH-BDEs and BRPs in human plasma being primarily from natural products and inter-conversion of natural products. Coefficients of determination for some pairs of congeners such as 3-OH-BDE-100 and 6-OH-BDE-47, 6-OH-BDE-85 and 5′-OH-BDE-99, and 2, 4-DBP and 6-OH-BDE-85, were near 1.0, which is consistent with them having common sources. Patterns of relative concentrations of the target analytes were similar in the diet, particularly fish, as in blood plasma of humans, which suggests that the diet and particularly seafood might be a source of these compounds and PBDEs. Furthermore, biotransformation of natural chemicals such as OH-BDEs to BRPs might be the primary route of their elimination from humans.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as brominated flame retardants (BFRs) in a variety of consumer products such as polyurethane foams, textiles, and electric appliances (Hoh et al., 2005). Several studies have established that PBDEs are ubiquitous in the environment samples (water, sediment and air), wildlife and humans (Frederiksen et al., 2009). Concentrations of PBDE in biota have been shown to increase with a doubling time of ~5 years, although some recent studies indicated a decrease in concentrations in some environmental matrices (Vorkamp et al., 2011). Furthermore, structural analogs of PBDEs, such as hydroxylated (OH-), methoxylated (MeO-) BDEs and bromophenols (BRPs), also occur in the environment and humans, often at concentrations that are greater than those of PBDEs (Covaci et al., 2011). These structural analogs, particularly for OH-BDEs, are more potent for some endpoints, such as endocrine disruption, disturbing Ca^{2+} homeostasis and neurotransmitter release than PBDEs (Dingemans et al., 2008). There are several different theories about the sources of MeO- and OH-BDEs in biota samples: i) uptake of PBDEs and subsequent biotransformation. A small proportion of about 1% of PBDEs have been shown to be biotransformed in mammals through cytochrome P450-mediated processes which generate OH-BDEs (Stapleton et al., 2009); ii) direct uptake of natural sources: recent studies revealed that some of the OH-BDEs and MeO-BDEs congeners are natural products of marine organisms such as red algae (*Ceramium tenuicorne*) and blue mussels (*Mytilus edulis*) (Malmvarn et al., 2005); iii) the metabolic relationship between OH-BDEs and MeO-BDEs: e.g. fish fed 6-MeO-BDE-47 accumulated significant concentrations of 6-OH-BDE-47 in the liver and eggs (Wan et al., 2010b).

Unlike PBDEs, there is little information on the presence of OH- or MeO-BDEs in humans due to a previous lack of analytical methods and authentic standards (Wan et al., 2009). The limited data indicated

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that MeO-/OH-BDEs are significantly accumulated in human tissues such as blood and milk. For example, the total concentration of OH-BDEs and BRPs ranged from 2.0×10^3 to 9.0×10^5 pg g^{-1} (mean of 79×10^3 pg g^{-1} lipid) in blood serum of people from the United States (Qiu et al., 2009), while concentrations of OH-BDEs in blood serum of people in Nicaragua ranged from 11 to 1.2×10^2 pmol g^{-1} lipid (about equal to 6.6×10^3 to 7.2×10^5 pg g^{-1} lipid) for those individuals working at or living near a waste disposal site, while concentrations in people from a more remote area ranged from 3.1 to 5.6 pmol g^{-1} lipid (about equal to 1.9×10^3 to 3.4×10^3 pg g^{-1} lipid) (Athanasiadou et al., 2008). The results of previous studies have suggested that OH-BDEs in pregnant women in Korea originated primarily from natural sources (Wan et al., 2010a).

Results of previous studies suggested that concentrations of PBDEs in blood were positively correlated with fish consumption (Thomsen et al., 2008), which contributed approximately 40 to 50% of the total intake of PBDEs from the diet (Meng et al., 2007). Previously published information revealed that concentrations of MeO-/OH-BDEs were relatively high in twenty fishes from markets in Hong Kong (Wang et al., 2011a). Due to the fact that fish is the major dietary source of protein for many Hong Kong residents with an average consumption rate of 164.4 g d^{-1} per person (Dickman and Leung, 1998), it is reasonable to assume that some of these structural analogs of PBDEs could be accumulated in humans. To date there has been no information on MeO-/OH-BDEs in blood plasma of people living in Hong Kong or an evaluation of correlations between dietary intakes of MeO-/OH-BDEs in fish and concentrations in humans.

The objectives of the present study were: (1) to indentify and quantify the MeO-/OH-BDEs and BRPs in blood plasma of people living in Hong Kong; (2) to assess potential sources and bioaccumulation of these compounds in humans; and (3) to evaluate the contribution of dietary intakes of these compounds via consumption of fish.

2. Materials and methods

2.1. Collection and preparation of samples

All studies with humans were performed in accordance with the guidelines and approval of the Human Investigation Ethics Committee of the Department of Biology, Hong Kong Baptist University. Detailed information about the collection and preparation of human blood plasma were previously reported by Wang et al. (under review). Briefly, a total of 116 participants (blood donors were Southern Han Chinese in origin, female 54, male 62) were recruited during February 2011 and classified into age groups ≤20 (n = 8), 21–30 (n = 36), 31–40 (n = 22), 41–50 (n = 28), ≥51(n = 22) years of age. All participants were determined to be eligible as blood donors based on their health history statuses and screening by nurses at the Hong Kong Red Cross before recruitment into the present study. Samples of blood were collected in heparinized tubes, maintained at 4 °C, and centrifuged at $1000\times g$ for 15 min to allow collection of the plasma fraction. All samples of plasma were kept at −20 °C until extraction.

2.2. Chemicals

Twenty-three PBDE congeners (IUPAC congener numbers 3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, and 209) had been detected in our previous study (Wang et al., under review). Standards for target compounds in the present study included 7 target MeO-BDE congeners: 2′-MeO-BDE-28, 4′-MeO-BDE-49, 2′-MeO-BDE-68, 6-MeO-BDE-137, 6-MeO-BDE-47, 3-MeO-BDE-47, 5-MeO-BDE-47 were purchased from Accustandard (New Haven, CT, USA), 15 target OH-BDE congeners: 2′-OH-BDE-7 (HK), 3′-OH-BDE-7 (HK), 2′-OH-BDE-28, 4′-OH-BDE-17 (HK), 2′-OH-BDE-68 (HK), 6-OH-BDE-47, 2′-OH-BDE-66 (HK), 3-OH-BDE-47, 6′-OH-BDE-99, 4-OH-BDE-42 (HK), 6-OH-BDE-90 (HK), 5′-OH-BDE-99 (HK), 6-OH-BDE-85 (HK), 3-OH-BDE-100 (HK), and 4-OH-BDE-90 (HK) were purchased from Wellington Laboratories (Guelph, ON, Canada), except 11 OH-BDE target congeners (HK) which were synthesized in the Department of Biology and Chemistry of the City University of Hong Kong with purities > 98% (Marsh et al., 2003), 3 BRP congeners: 2, 4-dibromophenol (2, 4-DBP), 2, 4, 5-tribromophenol (2, 4, 5-TBP) and 2, 4, 6-tribromophenol (2, 4, 6-TBP) were purchased from Accustandard (New Haven, CT, USA), and ^{13}C-6-MeO-BDE-47, ^{13}C-6-OH-BDE-17, ^{13}C-6-MeO-BDE-100, and ^{13}C-6-MeO-BDE-100 were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). All solvents used were GC grade obtained from Tedia Company, Inc. (Fairfield, Ohio, USA). All other reagents were of analytical grade or HPLC grade.

2.3. Extraction and instrumental analysis

A more detailed description of the methods for extraction, clean up and quantification is given in the Supporting Information. Briefly, the blood plasma samples (about 5 g) were extracted with a hexane/MTBE mixture. Then, potassium hydroxide was added to organic extracts to ionize phenolic analytes. Phenolic compounds were separated from neutral compounds by partitioning with KOH. The neutral fraction (PBDEs and MeO-BDEs) were treated with concentrated sulfuric acid twice to remove lipids and purified by alumina column chromatography. The aqueous layer (KOH) was extracted and acidified with hydrochloric acid. Then phenolic compounds (OH-BDEs and BRPs) were extracted, purified, concentrated before derivatization with N, *O*-bis-(Trimethylsilyl) trifluoroacetamide (BSTFA) and Trimethylchlorosilane (TMCS). Samples were dissolved in DCM and purified on a column with silica gel. PBDEs and MeO-PBDEs were detected by GC–MS with EI mode. OH-BDEs and BRPs were analyzed by GC–MS Electron Capture Negative Ionization (ECNI) mode with derivatization.

2.4. Quality assurance/quality control

To ensure that the samples and the analytic process were free of contamination, for every sequence of 15 samples, a solvent blank and a procedural blank were analyzed. Several quality control criteria were used to ensure the correct identification and quantization of the target compounds: first, retention times matched with those of the authentic reference compounds; second, the ratios of the two characteristic ions were within 15% of the theoretical values; third, the signal-to-noise (S/N) ratio was greater than 3 for the selected ions; fourth, the amount of the analytes in the sample had to be at least two times that in the blank sample if there were interferences. If any of these four criteria failed, the congener was excluded. The limit of detection (LOD) defined as a signal-to-noise ratio (S/N) of 3 was 1 to 8 pg g^{-1} lipid for MeO-/OH-BDEs and BRPs in plasma (as listed in Table 1 and Table S2). Only 3-MeO-BDE-47, 2, 4-DBP, and 2, 4, 6-TBP were detected in the solvent blank samples, and the blank values were around 5%, 3%, and 7% of mean concentrations in plasma, respectively. The data in the present study were not corrected for concentrations in blanks. Recoveries of spiked ^{13}C-6-MeO-BDE-47 ranged from 69 to 120% (mean 84%), and ^{13}C-6-OH-BDE-17 ranged from 71% to 125% (mean 89%). Recoveries of all target compounds obtained by standard addition method were listed in Table 1. Due to the small rates of recovery of MeO-BDEs, OH-BDEs and BRPs, they were corrected for recovery by the use of surrogates in matrix spike samples. Concentrations were presented as pg g^{-1}, lipid, for MeO-BDEs, OH-BDEs and BRPs and reported with a precision of two significant digits (3 imputed).

2.5. Data analysis

The concentrations of PBDE dominant congeners and $\sum$PBDEs were cited from our previous study (Wang et al., under review). Statistical analyses were performed using SPSS 17.0 for Windows. The data were log-transformed prior to conducting statistical tests. If the

Table 1
Concentrations of PBDE dominant congeners, MeO-BDEs, OH-BDEs and BRPs in blood plasma of humans from Hong Kong (pg g^{-1}, lipid).

	LOD	RR	Female (n = 54)				Male (n = 62)			
			Mean ± SD	Median	Range (min to max)	%>LOD	Mean ± SD	Median	Range (min to max)	%>LOD
Lipid content (%)			9.5 ± 4.5	7.6	4.5–22	–	8.1 ± 3.5	6.8	4.0–18	–
PBDEs										
BDE-47	10	89	$(2.4 \pm 3.2) \times 10^3$	1.7×10^3	$(0.43–18) \times 10^3$	100%	$(1.4 \pm 0.86) \times 10^3$	1.1×10^3	ND–4.0×10^3	94%
BDE-28	10	92	$(0.9 \pm 1.1) \times 10^3$	0.6×10^3	$(0.11–4.3) \times 10^3$	100%	$(2.6 \pm 8.5) \times 10^3$	0.49×10^3	ND–41×10^3	94%
BDE-99	10	95	$(1.5 \pm 1.8) \times 10^3$	1.0×10^3	ND–9.7×10^3	82%	$(0.97 \pm 0.73) \times 10^3$	0.93×10^3	ND–2.6×10^3	68%
∑PBDEs	–		$(9.2 \pm 17) \times 10^3$	5.5×10^3	$(1.5–92) \times 10^3$	–	$(9.8 \pm 13) \times 10^3$	5.3×10^3	$(0.56–57) \times 10^3$	–
MeO-BDEs										
3-MeO-BDE-47	5	97	$(5.7 \pm 9.0) \times 10^3$	2.9×10^3	$(0.5–47) \times 10^3$	100%	$(3.1 \pm 4.0) \times 10^3$	1.6×10^3	ND–16.4×10^3	74%
2′-MeO-BDE-28	5	105	$(7.8 \pm 7.5) \times 10^2$	6.7×10^2	ND–3.6×10^3	67%	$(1.0 \pm 0.9) \times 10^3$	9.8×10^2	ND–3.3×10^3	58%
2′-MeO-BDE-68	8	104	$(3.1 \pm 2.5) \times 10^2$	2.4×10^2	ND–1.1×10^3	93%	$(2.1 \pm 2.2) \times 10^2$	1.2×10^2	ND–0.9×10^3	71%
4′-MeO-BDE-49	5	86	$(3.7 \pm 8.7) \times 10^2$	1.6×10^2	ND–4.4×10^3	78%	$(1.4 \pm 1.1) \times 10^2$	1.6×10^2	ND–0.4×10^3	71%
5-MeO-BDE-47	5	74	$(1.4 \pm 1.6) \times 10^2$	93	ND–0.6×10^3	56%	$(1.5 \pm 1.5) \times 10^2$	1.0×10^2	ND–0.6×10^3	78%
6-MeO-BDE-47	5	119	$(0.95 \pm 1.1) \times 10^2$	73	ND–0.5×10^3	56%	$(1.0 \pm 1.1) \times 10^2$	90	ND–0.6×10^3	65%
∑MeO-PBDEs	–		$(7.8 \pm 9.7) \times 10^3$	5.4×10^3	$(1.6–52) \times 10^3$	–	$(4.9 \pm 4.7) \times 10^3$	3.5×10^3	$(0.4–19) \times 10^3$	–
OH-BDEs										
6-OH-BDE-47	5	86	56 ± 61	29	ND–219	89%	60 ± 73	31	ND–299	74%
5′-OH-BDE-99	5	110	34 ± 33	29	ND–102	56%	16 ± 24	4.9	ND–66	52%
3-OH-BDE-100	2	91	7.1 ± 9.1	ND	ND–14	7.4%	27 ± 44	ND	ND–78	9.7%
3-OH-BDE-7	1	73	2.2 ± 5.1	ND	ND–15	30%	23 ± 68	ND	ND–217	32%
6-OH-BDE-85	1	106	3.8 ± 4.4	ND	ND–12	19%	16 ± 31	ND	ND–62	13%
∑OH-PBDEs	–		83 ± 77	58	9.8–333	–	86 ± 105	27	4.8–473	–
BRPs										
2,4,5-TBP	2	79	30 ± 18	ND	ND–65	37%	17 ± 15	ND	ND–56	42%
2,4,6-TBP	2	91	13 ± 14	ND	ND–46	37%	12 ± 11	ND	ND–28	36%
2,4-DBP	2	106	5.2 ± 5.4	ND	ND–17	44%	2.0 ± 1.8	ND	ND–5.1	16%
∑BRPs	–		17 ± 27	3.8	ND–113	–	12 ± 16	3.7	ND–56	–

LOD: limit of detection; RR: recovery rate; SD: standard deviation; ND: not detected.

concentration of a congener was less than the LOD, a value equal to half the LOD of the analytical method was attributed for statistical analysis, while it was set at zero for sum, mean and median calculations. Normality was confirmed by the Kolmogorov–Smirnov test. Two independent *t*-tests, Wilcoxon rank sum test, one-way ANOVA and Kruskal–Wallis test were used to compare the MeO-/OH-BDE and BRP contaminations among gender and different age groups.

3. Results and discussion

3.1. Concentrations of MeO-/OH-BDEs and BRPs

Concentrations of ∑MeO-BDEs, ∑OH-BDEs, ∑BRPs and ∑PBDEs in blood plasma collected from people in Hong Kong are presented (Table 1). Concentrations of ∑MeO-BDEs ranged from 3.8×10^2 to 52×10^3 pg g^{-1} lipid (median 4.5×10^3 pg g^{-1} lipid) and were comparable to concentrations of ∑PBDEs reported previously for blood plasma collected from people in Hong Kong (5.6×10^2 to 92×10^3 pg g^{-1}, median 5.4×10^3 pg g^{-1}) (Wang et al., under review). It has also been known that concentrations of PBDEs and MeO-BDEs in fish muscle were similar (Wang et al., 2011a). Concentrations of ∑OH-BDEs and ∑BRPs ranged from 5.3 to 4.9×10^2 pg g^{-1} lipid (median 81 pg g^{-1} lipid) and from ND (not detected) to 1.1×10^2 pg g^{-1} lipid (median 3.7 pg g^{-1} lipid), respectively. Concentrations of OH-BDEs and BRPs were 10 to 1000-fold less than those of MeO-BDEs and PBDEs. This result is reasonable because OH-BDEs have polar hydroxyl functional groups and thus are relatively more polar and have greater potential to be excreted than do PBDEs. Therefore, concentrations of OH-BDEs would be less than those of PBDEs in human plasma. Results of previous studies indicated that concentrations of persistent lipophilic compounds such as PBDEs tend to increase proportionally with age and body fat content (Covaci et al., 2002). However, in this study, there were no significant (one-way ANOVA: $p > 0.05$) correlations between concentrations of ∑MeO-BDEs, ∑OH-BDEs, ∑BRPs and ∑PBDEs with lipid content, age or body weight (data not shown). Furthermore, no significant (*t*-test: $p > 0.05$) differences in concentrations of ∑MeO-BDEs, ∑OH-BDEs or ∑BRPs were observed between males and females (data not shown).

There were limited published data concerning the occurrence of these MeO-/OH-BDEs and BPRs in human tissues, particularly for human blood plasma. Concentrations of ∑OH-BDEs observed in blood plasma of residents of Hong Kong were less than their respective concentrations in sera collected from residents of the United States, where total concentration of HO-BDEs and BRPs ranged from 2.0×10^3 to 9.0×10^5 pg g^{-1} lipid, with a mean of 79×10^3 pg g^{-1} lipid, respectively (Qiu et al., 2009). Concentrations of OH-BDEs and BRPs in blood plasma of people of Hong Kong were also less than those in blood serum collected from the general population of India (25 pg g^{-1}, wet weight (w.w.), about 2.0×10^2 pg g^{-1}, lipid) (Eguchi et al., 2010) and Nicaragua (∑OH-BDEs ranged from 0.11 to 1.2×10^2 pmol g^{-1} lipid, about equal to 57 to 6.2×10^4 pg g^{-1} lipid, at an e-waste reprocessing plant, compared with 3.1 to 5.6 pmol g^{-1} lipid, about equal to 1.6×10^3 to 2.9×10^3 pg g^{-1} lipid, in the reference population) (Athanasiadou et al., 2008). The only previous report on quantification of MeO-BDEs in human tissue, reported concentrations of 10 to 25×10^3 pg g^{-1} lipid in breast milk collected from women in Spain (Lacorte and Ikonomou, 2009) were similar to concentrations observed in blood plasma in the present study.

3.2. Congener profiles

The predominant congeners of MeO-/OH-BDEs, BRPs and PBDEs in blood plasma collected from residents of Hong Kong are given (Table 1). Concentrations of non-predominant OH-BDEs in blood plasma are given in Table S2. All individual MeO-BDEs, OH-BDEs and BRPs were detected in the blood plasma except 6-MeO-BDE-137, with the least frequency of detection being 6.9% for 6′-OH-BDE-99. Three PBDE congeners (BDE-47, BDE-99 and BDE-28) made up 5.1 to 90% (median 66%) of the ∑$PBDE_{22}$. The proportions of ∑$PBDE_{22}$ contributed by the three dominant PBDE congeners were: BDE-47 (26%) > BDE-99 (18%) > BDE-28 (16%). That BDE-47 was the predominant congener detected in human plasma was similar to the trend observed for BDE-47 in human hair collected from Hong Kong (Kang et al., 2011). The accumulation of less brominated PBDE congeners might be due to their longer half-lives compared with the more brominated PBDEs. The estimated half-lives of BDE-47, which have been estimated to be on average 3.0 years with a range of 1.9 to 4.2 years, and BDE-99 had an estimated half-life of 5.4 years with a range of 3.5 to 7.2 years. These half-lives are longer than that of BDE-209, which was estimated to be approximately 15 days with a range of 11to 18 days (Geyer et al., 2004; Thuresson et al., 2006). Furthermore, half-life was inversely proportional to degree of bromination in the order of deca-BDE, octa-BDE, and hexa-BDE (Sjodin et al., 2004).

Among the 7 detected MeO-BDEs congeners, the most abundant MeO-BDE congener was 3-MeO-BDE-47, with concentrations that ranged from ND to 47×10^3 pg g^{-1} lipid, median 2.2×10^3 pg g^{-1} lipid, and accounted for 8.6 to 46% of total MeO-BDEs (median 26%), followed by 2′-MeO-BDE-28 (median 7.0×10^2 pg g^{-1} lipid) and 2′-MeO-BDE-68 (median 2.0×10^2 pg g^{-1} lipid). The 2′-MeO-BDE-28 congener was that most frequently detected in breast milk collected from women in Spain (Lacorte and Ikonomou, 2009). The results of previous studies have suggested that 3-MeO-BDE-47 was not detected in the blood of marine fish or that the concentration was significantly less than that of 2′-MeO-BDE-68 and 6-MeO-BDE-47 (Covaci et al., 2008; Marsh et al., 2004). However, relatively great concentrations of 3-MeO-BDE-47 were detected in not only the human blood plasma of the present study but also muscle of fishes (mean 2.1×10^2 pg g^{-1}, w.w.) (Wang et al., 2011a), and muscle and liver of frogs (about 4×10^3 pg g^{-1} lipid) (Wu et al., 2009) collected from the Pearl River Delta (PRD). This result is suggestive of local sources of 3-MeO-BDE-47 in South China, but not conclusive.

Of the 15 OH-BDEs congeners analyzed, 6-OH-BDE-47 was detected most frequently (89% in female and 74% in male) and at the greatest concentrations (ranged from ND to

3.0×10^2 pg g^{-1} lipid, median 31 pg g^{-1} lipid), followed by 5′-OH-BDE-99 (ND to 1.0×10^2 pg g^{-1} lipid, median 6.9 pg g^{-1} lipid) and 3-OH-BDE-100 (ranged from ND to 78 pg g^{-1} lipid). The observation that 6-OH-BDE-47 was the predominant congener is similar to the results in tissues of wildlife such as Chinese sturgeon (Zhang et al., 2010), bottlenose dolphin (Houde et al., 2009), and fish (Valters et al., 2005). The concentrations of 6-OH-BDE-47 in the present study (median 30 pg g^{-1}, lipid) were less than those (median 23 pg ml^{-1}, w.w., approximately 200 pg ml^{-1}, lipid) in blood serum in second trimester pregnant women from Northern and Central California (Zota et al., 2011). In the present study, the congeners 4-HO-BDE-42 and 3-HO-BDE-47, thought to be biotransformation products of BDE-47 in mice (Qiu et al., 2007), were detected in 18% (ND — 1.4×10^2 pg g^{-1} lipid) and 11% (ND — 4.1 pg g^{-1} lipid) blood plasma samples, respectively. Results of previous studies revealed greater concentrations of 5′-HO-BDE 99 and 6′-HO-BDE-99 in blood of humans or mice that had been exposed to a commercial mixture of PBDEs (Qiu et al., 2007; Qiu et al., 2009). The present study revealed that 5′-HO-BDE-99 was the second most abundant congener, while 6′-HO-BDE-99 was detected at the least frequency among the 15 investigated OH-BDEs congeners.

The frequency of detection of BRPs was 40, 36 and 22% for 2, 4, 5-TBP, 2, 4, 6-TBP, and 2, 4-DBP, respectively. These three BRPs had been indentified in blood of mice after exposure to DE-71 (Qiu et al., 2007). To our knowledge, this is the first time that 2, 4-DBP and 2, 4, 6-TBP have been reported to occur in humans from Asia. The frequencies of detection of 2, 4-DBP and 2, 4, 6-TBP were less than those detected in serum collected from humans in Canada (57% and 87% for 2, 4-DBP and 2, 4, 6-TBP, respectively) (Dallaire et al., 2009). Furthermore, concentrations of 2, 4, 6-TBP (ND to 46 pg g^{-1} lipid, median: ND) in blood plasma of people in Hong Kong were also less than those observed in blood of people in Norway (80 to 81×10^3 pg g^{-1} lipid) (Thomsen et al., 2002), a population of Inuit (ND to 280×10^3 pg g^{-1} lipid) (Dallaire et al., 2009) and blood of residents of the United States (median 8.0×10^2 pg g^{-1} lipid) (Qiu et al., 2009).

3.3. Potential source apportionments

For the MeO-BDEs, OH-BDEs and BRPs, there was very limited information concerning their sources and distribution in biota. Correlations among lipid contents and concentrations of dominant congeners of PBDEs, MeO-BDEs, OH-BDEs and BRPs were calculated (Table 2). No significant correlation was observed between lipid contents and concentrations of organo-brominated compounds including PBDEs, MeO-BDEs, OH-BDEs and BRPs, a result which suggests that lipids might not be the sole factor influencing their concentrations in blood plasma of humans. This result is similar to that of previous studies, in which concentrations of MeO-BDEs and OH-BDEs were not significantly correlated with fat content in fish muscle (Wang et al., 2011a) or Chinese sturgeon (*Acipenser sinensis*) (Zhang et al., 2010). This might be due to MeO-/OH-BDEs being inter-converted and their hydrophilicity. Therefore, the fat might not be the most important factor in explaining their distribution among individuals and tissues.

Concentrations of $\sum$PBDEs were not significantly ($p>0.05$) correlated with concentrations of $\sum$MeO-BDEs, $\sum$OH-BDEs or $\sum$BRPs in blood plasma in the present study. The results of this study were consistent with those of previous studies in which there were no significant correlations between concentrations of PBDEs and MeO-BDEs in tissues of marine animals collected from different global locations (Stapleton et al., 2006; Verreault et al., 2005). Furthermore, concentrations of $\sum$OH-BDEs were not correlated with concentrations of $\sum$PBDEs in beluga whale (Kelly et al., 2008) or in livers of tuna, five albatross species, or polar bear from remote marine locations (Wan et al., 2009). Taken together, the results of the study upon which we report here were consistent with the hypothesis that synthetic PBDEs used in flame retardants, are not precursors of MeO-BDEs or OH-BDEs in blood plasma of humans in Hong Kong (Wiseman et al., 2011).

Concentrations of 6-OH-BDE-47 were significantly ($p<0.05$) correlated with those of 6-MeO-BDE-47 ($r=0.36$) and 2′-MeO-BDE-68 ($r=0.64$). These correlations were consistent with those observed in previous studies, in which concentrations of 6-MeO-BDE-47 were correlated with 6-OH-BDE-47 in albatrosses and polar bear from remote marine locations in Canada (Wan et al., 2009) and in anadromous Chinese sturgeon from the Yangtze River (Zhang et al., 2010). Also, no correlation was observed between concentrations of BDE-47 and 6-OH-BDE-47 or 6-MeO-BDE-47 in the Chinese sturgeon. The results of the present study indicated that accumulation of 6-OH-BDE-47 might be from conversion of 6-MeO-BDE-47 and not from phase I transformation of BDE-47. It was further confirmed in hepatic microsomes from Japanese medaka in which the conversion ratios of MeO-BDEs to OH-BDEs (in vitro: 10%; in vivo: 6%) were approximately 1000-fold greater than those between PBDEs and OH-BDEs reported elsewhere (Wan et al., 2010b). This study also revealed an inter-conversion of OH-BDEs and MeO-BDEs in exposed organisms (Wan et al., 2010b). However, in the present study, there was no statistically significant correlation between $\sum$OH-BDEs and $\sum$MeO-BDEs in blood plasma of humans, which suggests that the relationships, including biotransformation between total concentrations of OH-BDEs and MeO-BDEs in humans, need further investigation. Although there were significant ($p<0.05$) positive correlations between PBDE congeners and the metabolized compounds such as BDE-47 and 2′-MeO-BDE-68, BDE-28 and 3-OH-BDE-7, these metabolic relationships have never been reported and need further study.

Correlation coefficients of some statistical pairs such as 3-OH-BDE-100 and 6-OH-BDE-47, 6-OH-BDE-85 and 5′-OH-BDE-99, 2,4-DBP and 6-OH-BDE-85 are equal to +1.0, while other pairs such as 3-OH-BDE-100 and 5′-OH-BDE-99, 3-OH-BDE-7 and 3-OH-BDE-100, 2,4,6-TBP and 3-OH-BDE-7, 2,4,6-TBP and 2,4-DBP are equal to −1.0, which represent very strong relationships. The perfect positive correlations among different OH-BDEs and BRPs congeners are consistent with these congeners being inter-converted (Fig. 1) via mechanisms reported previously (Wan et al., 2010b). The negative correlations suggested that one compound in the pair might have originated from the other. Significant correlations between OH-BDEs and BRPs that were observed in the present study were consistent with results of a previous study where concentrations of BRPs were correlated with OH-BDEs in mouse plasma after exposure to a commercial mixture of penta bromodiphenyl ethers (Qiu et al., 2007).

The results of a previous study revealed that the marine environment is a natural source of BRPs, where macroalgae synthesize 2, 4-DBP and 2, 4, 6-TBP as major secondary metabolites (Flodin and Whitfield, 1999). Meanwhile, the 2,4,6-TBP is widely used as a flame retardant with a worldwide production of 9500 t in 2001 (IUCLID, 2003). Sources of exposure and pathways of transformation that could result in the occurrence of 2,4-DBP and 2,4,6-TBP in human plasma are not clear. Results of a recent study revealed that consumption of fruits and vegetables has been suggested to be a good predictor of concentrations of 2, 4, 6-TBP in blood serum of humans (Dallaire et al., 2009). Although there is no published data on concentrations of BRPs in fruits and vegetables collected from either Hong Kong or the surrounding areas, our previous study revealed the concentrations of 2, 4, 6-TBP in fish from markets in Hong Kong ranged from ND to 13 pg g^{-1}, w.w. (Wang et al., 2011a). Therefore, accumulation of 2, 4, 6-TBP in blood of residents of Hong Kong might result from both dietary intake and biotransformation of OH-BDEs. The results of a previous study suggested that 2, 4-DBP is a major metabolite of brominated DE-71 formulation in mice through cleavage of the diphenyl ether bond of either PBDE 47 or PBDE 99 (Qiu et al., 2007). However, concentrations of the three BRPs detected in blood plasma of people from Hong Kong were not correlated with PBDE congeners, which are consistent with the results of the study of blood plasma of Inuit adults in Nunavik, Canada (Dallaire et al., 2009). These results might suggest that BRPs in blood of humans are not biotransformation products of PBDEs.

3.4. The accumulation of MeO-/OH- BDEs and BRPs via fish consumption

A review of the recent scientific literature indicated that there were notable coincidences of the comparatively large contribution of fish and seafood, and dairy products (Domingo, 2012). Fish has been found to be the predominant dietary source of PBDEs in some countries such as Finland and Belgium (Kiviranta et al., 2004; Voorspoels et al., 2007), even though consumption rates of fish in those countries were less than those of Hong Kong. Fish has been reported to be the predominant pathways for exposure of the general population of Hong Kong to other organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) (Wang et al., 2010) and organochlorine pesticides (OCPs) (Wang et al., 2011b). Furthermore, our previous study revealed a significant ($r=0.89$, $p<0.001$) positive correlation between concentrations of individual PBDE congeners in market fish and blood plasma of humans from Hong Kong, which is consistent with previous studies that consumption of fish is an important pathway of exposure to PBDEs (Wang et al., under review).

However, there is no published information on the role of dietary intake in accumulation of MeO-BDEs, OH-BDEs or BRPs. The results of a previous study revealed that estimated daily intakes of MeO-BDEs, OH-BDEs and BRPs by residents of Hong Kong ranged from 5.0×10^2 to 4.3×10^3, 22 to 4.3×10^2, and 0 to 2.1×10^2 pg kg^{-1} body weight day^{-1}, respectively (Wang et al., 2011a). To evaluate accumulation of MeO-BDEs and OH-BDEs via consumption of fish, regression analyses were performed between concentrations of individual MeO-/OH-BDEs congeners in fish (data from our previous study (Wang et al., 2011a)) and blood plasma of people in Hong Kong. There were no statistically significant ($p>0.05$) correlations between concentrations of individual MeO-BDEs, OH-BDEs or BRPs congeners in fish muscle and human blood plasma collected from people in Hong Kong. For the sum concentrations of $\sum$PBDEs, $\sum$MeO-BDEs, $\sum$OH-BDEs and $\sum$BRPs, the association between accumulation of these compounds in blood and dietary intake of foodstuffs, particularly fish, was marginally significant ($p=0.082$, $df=3$). These results suggest that the diet and particularly seafood might be a source of these compounds in human plasma. Furthermore, considering the estimated half-lives of "parent" PBDE compounds (BDE-28, BDE-47 or BDE-99) for the dominant MeO-BDE congeners (3-MeO-BDE-47 and 2′-MeO-BDE-28) and OH-BDE congeners (6-OH-BDE-47 and 5′-OH-BDE-99) are very long (several years) (Geyer et al., 2004), it is reasonable to assume that these dominant MeO-BDE and OH-BDE congeners are directly accumulated from fish in the diet. This conclusion is consistent with the result that some congeners such as 3-MeO-BDE-47 and 2′-MeO-BDE-28 were found to be the dominant congeners in fish products in our previous study (Wang et al., 2011a) and in blood plasma of humans during the present study. However, the relative contributions of digestion and inter-conversion in of fish in humans are in need of further study.

Considering that other food items such as meat and fruits also contain organo-brominated compounds (Dallaire et al., 2009), future studies of daily intake should also include these items. Furthermore, there can be biotransformation and inter-conversions between organo-brominated compounds once they are accumulated. Therefore, there might be multiple sources of organo-brominated compounds to which humans are exposed. Responses of *in vitro* competitive bioassays have shown that OH-BDEs bind to transthyretin (TTR), a principal thyroid hormone transport protein in mammals, with greater affinity than thyroxine (T4) (Legler and Brouwer, 2003). Therefore, potential effects of those contaminants on lipids and concentrations of thyroid hormone in blood serum of residents of Hong Kong will be assessed in forthcoming reports.

4. Conclusions

The general population of Hong Kong is exposed to organo-brominated compounds including MeO-BDEs, OH-BDEs and BRPs. In

Table 2
Pairwise correlations between concentrations of lipid and concentrations of PBDEs, MeO-BDEs, OH-BDEs and BRPs in blood plasma of humans in Hong Kong.

	Lipid%	BDE-47	BDE-28	BDE-99	∑PBDE	3-MeO-BDE-47	2′-MeO-BDE-28	2′-MeO-BDE-68	6-MeO-BDE-47	∑MeO-PBDE	6-OH-BDE-47	5′-OH-BDE-99	3-OH-BDE-100	3-OH-BDE-7	6-OH-BDE-85	∑OH-PBDE	2,4-DBP	2,4,5-TBP	2,4,6-TBP	∑BPRs
Lipid%	1																			
BDE-47	**−0.46****	1																		
BDE-28	−0.18	0.08	1																	
BDE-99	**−0.53****	**0.90****	0.06	1																
∑PBDEs	−0.39	**0.75****	**0.50****	**0.75****	1															
3-MeO-BDE-47	−0.14	0.02	0.21	0.05	0.11	1														
2′-MeO-BDE-28	−0.16	−0.10	0.17	0.08	−0.04	0.09	1													
2′-MeO-BDE-68	−0.20	**0.44****	−0.11	**0.30***	0.23	0.05	−0.12	1												
6-MeO-BDE-47	−0.22	−0.07	0.07	0.07	−0.03	0.13	**0.47****	0.04	1											
∑MeO-BDEs	−0.17	0.04	0.21	0.10	0.12	**0.98****	0.22	0.08	0.22	1										
6-OH-BDE-47	**−0.34***	0.00	−0.08	0.24	−0.04	−0.01	**0.64****	0.01	**0.36***	0.03	1									
5′-OH-BDE-99	−0.05	0.27	−0.16	0.10	−0.17	0.16	0.13	0.35	0.17	0.19	0.14	1								
3-OH-BDE-100	−0.49	0.19	−0.29	0.77	0.42	0.74	0.69	−0.41	**0.90***	0.73	**1.00***	**−1.00****	1							
3-OH-BDE-7	−0.11	0.12	**0.53***	0.20	0.04	0.39	0.27	0.38	0.30	0.42	0.40	−0.29	**−1.00****	1						
6-OH-BDE-85	0.20	−0.24	−0.05	−0.32	−0.24	−0.29	−0.16	−0.24	0.18	−0.28	0.15	**1.00****	[a]	[a]	1					
∑OH-BDEs	−0.26	−0.00	−0.13	0.24	−0.12	0.14	**0.36****	0.06	**0.46****	0.18	**0.92****	**0.49****	**0.90***	0.23	0.70*	1				
2,4-DBP	−0.32	0.34	0.04	−0.01	0.05	0.08	−0.32	−0.28	−0.37	−0.01	0.00	−0.42	0.09	−0.26	**1.00****	−0.17	1			
2,4,5-TBP	−0.14	0.33	−0.23	0.08	0.24	0.21	−0.10	0.09	−0.06	0.21	0.34	0.47	0.05	−0.24	[a]	0.05	−0.66	1		
2,4,6-TBP	−0.06	0.33	0.24	0.11	0.27	0.14	0.34	0.34	0.32	0.18	0.41	**0.56***	[a]	**−1.00****	[a]	**0.55***	**−1.00****	**0.74****	1	
∑BPRs	0.19	−0.10	0.05	−0.19	−0.09	0.16	−0.04	−0.04	0.09	0.16	−0.14	0.12	0.23	0.22	0.75*	0.01	0.06	**0.92****	**0.80****	1

Bold means a statistical significance.
[a] Cannot be computed because at least one of the variables is constant.
** $p<0.01$ (2-tailed).
* $p<0.05$ (2-tailed).

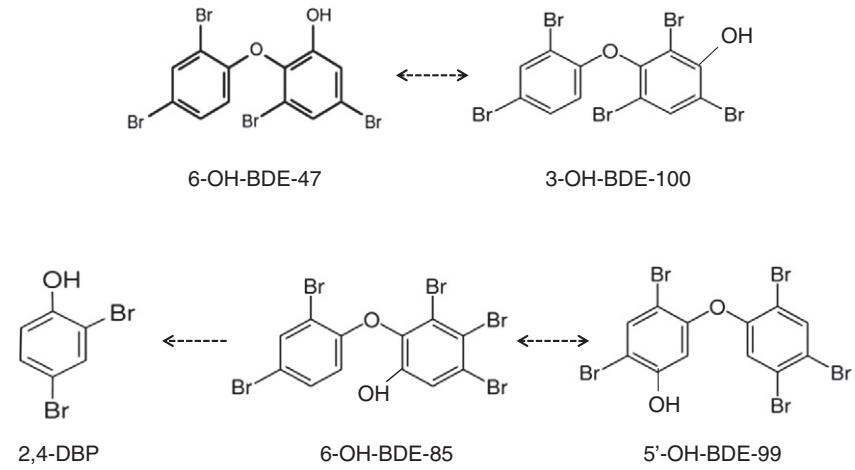

Fig. 1. Proposed scheme for biotransformation of OH-BDEs and BRPs in humans (Wan et al., 2010b).

human blood plasma, concentrations of OH-BDEs and BRPs were less than those of MeO-PBDEs. The dominant congeners of MeO-BDEs, OH-BDEs and BRPs were 3-MeO-BDE-47, 6-OH-BDE-47 and 2, 4, 5-TBP, respectively. Concentrations of these organo-brominated compounds in human blood serum were not correlated with age, fat and did not vary between genders. The origin of MeO-BDEs, OH-BDEs and BRPs was from natural products and inter-conversion of naturally occurring compounds rather than from synthetic PBDEs used as flame retardants. Potential effects of those contaminants on lipids and concentrations of thyroid hormone in blood serum of residents of Hong Kong will be assessed in forthcoming reports.

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Appendix A. Supplementary data

The detail procedures of sample extraction, clean up and instrumental quantification are attached. Two tables showing detailed quantification parameters of target compounds and concentrations of non-predominant OH-BDEs in blood plasma of humans from Hong Kong are attached. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envint.2012.06.004.

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