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Organobromine compound profiling in human adipose: Assessment of sources of bromophenol

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ABSTRACT

Bromophenols (BRPs) have been widely detected in human tissues, however, relative proportions from natural products and/or anthropogenic flame retardants are not clear. 21 polybrominated diphenyl ethers (PBDEs), 15 MeO/OH-PBDEs, and 10 BRPs were simultaneously quantified in adipose collected from people from New York City, USA. An *in vitro* assay utilizing human liver microsomes was performed for detected predominant organobromine. High concentrations of 2,4,6-triBRP and PBDEs were observed, and extremely low concentrations of naturally occurring MeO/OH-PBDEs were detected. Similar biotransformatioin rates of BRPs and MeO/OH-PBDEs indicated that the relative high concentration of 2,4,6-triBRP in humans was not of natural origin. Significant correlation observed between concentrations of 2,4,6-triBRP and BDE-209 suggested that the two chemicals may share a common source. Both 2,4,6-triBRP and BDE-209 were detected in commercial ABS resins, suggesting that plastic products made from ABS resins could be potential sources of co-exposure of the two compounds for humans.

1. Introduction

Due to persistence, bioaccumulation and toxicity, organobromine compounds are of great concern as contaminants in the environment. As an important group of synthetic organobromine compounds, brominated flame retardants (BFRs) including polybrominated diphenyl ethers (PBDEs) are released into the environment during production, use and disposal of products containing the flame retardants (Alaee et al., 2003). Apart from the synthetic organobromine compounds, a variety of naturally occurring brominated compounds have been reported to be produced by marine organisms including algae, marine sponges and bacteria (Malmvärn et al., 2005; Whitfield et al., 1999; Gribble, 2003). The synthetic and natural organobromine compounds can accumulate through the food webs, and are found in human tissues and undergo maternal transfers, posing potential health risks to human offspring (Wan et al., 2010; Zhang et al., 2012).

Bromophenols (BRPs) are a group of brominated compounds, which could originate from both synthetic and natural sources. The chemicals have been reported to elicit a variety of effects on rats including salivation, suppressed body weight gain, increased liver weights, and elevated total protein and alkaline phosphatase activity (ALP) in blood (Tanaka et al., 1999). Besides, 2,4,6-triBRP induced differentiation of human SH-SY5Y neuroblastoma cells (Rios et al., 2003) and inhibition of thyroid hormone activity in human (Craig et al., 2011; Craig and Heather, 2013). 2,4,6-triBRP has also been identified as a natural precursor for toxic brominated dioxins via dimerization catalyzed by bromoperoxidase *in vivo* (Arnoldsson et al., 2012). BRPs have been found to be widely detected in human tissues, with concentrations up to 100 ng/g lipid weight (lw) in serum or adipose samples in Europe, which were 10 to 100- fold higher than those of other brominated flame retardants such as PBDEs (Thomsen et al., 2001; Smeds and Saukko, 2003). BRPs are mainly used as reactive flame retardants with a worldwide production of 9500 in 2001 tonnes (IUCLID, 2003). BRPs have been reported to be related to PBDEs, and some BRPs potentially

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originate from degradation of PBDEs via biotransformation or UV irradiation (Bendig and Vetter, 2013). Besides anthropogenic sources, BRPs have also been linked with natural sources, since these compounds were firstly identified as key natural flavor components of marine fish (Whitfield et al., 1998) and secondary metabolites produced by marine algae and sponges, similar to methoxylated (MeO-) and hydroxylated (OH-) PBDEs (Hassenklover et al., 2006; Sheikh and Djerassi, 1975; Goerke and Weber, 1991). However, limited knowledge was available on exposure source of humans to BRPs, which has important implications for risk assessment and how exposures can be controlled.

Available information about the synthetic and natural sources of predominant organobromines (e.g. PBDEs, MeO-/OH-PBDEs) could help clarify the origin of BRPs by simultaneous quantification of these compounds in human. Occurrences of PBDEs could provide information about exposure to BFRs used as additives in high-impact polystyrene housings for electronics and textiles for furniture, and investigation of naturally occurring MeO-/OH-PBDEs could help determine relative contributions from natural sources since BRPs have similar anthropogenic and natural sources with PBDEs and MeO-/OH-PBDEs, respectively (Malmvärn et al., 2005; Whitfield et al., 1999; Weil, 1993). But the profile of the organobromine compounds in human tissues remains unknown.

Biotransformation of these organobromines in humans is a vital process for the determination of exposure and accumulation profiles of chemicals. For example, human liver microsomes mediated metabolism of three PBDE congeners showed that biotransformation rates of BDE-153 were slower than those of BDE-47 and BDE-99, providing an explanation for the high accumulation of this compound in humans (Lupton et al., 2009). Whereas several studies have examined biotransformation of PBDEs and their MeO-/OH- structural analogues in laboratory animals (Lupton et al., 2009; Erratico et al., 2010, 2011; Benedict et al., 2007; Browne et al., 2009; Letcher et al., 2009; Wan et al., 2009), little information is available on biotransformation of organobromines in humans. Thus, comprehensive determination of biotransformation rate of predominant organobromines in human microsomes together with the exposure sources could clarify sources and accumulation in humans. However, the direct comparison of biotransformation rates of various chemicals tested in different batches was difficult to achieve due to variations within incubations (Fay et al., 2014).

In the present study, forty-six organobromine compounds including 21 PBDEs, 7 OH-PBDEs, 8 MeO-PBDEs and 10 BRPs were simultaneously quantified in adipose of people from New York City, USA. Occurrences and profiles of both natural and anthropogenic organobromines were compared to evaluate sources to humans. An *in vitro* assay utilizing microsomes from human liver was conducted to compare rates of biotransformation of individual organobromines with benzo[a]pyrene (B[a]P) as a "benchmark" compound to normalize for variations among various biological materials. With all the results obtained in the present study, the exposure sources of human to BRPs in human adipose were proposed.

2. Materials and methods

2.1. Chemicals

Two OH-PBDEs (3-OH-BDE-47 and 5-OH-BDE-47) were purchased from AccuStandard (New Haven, CT, USA), the remaining five OH-PBDEs (6-OH-BDE-47, 4-OH-BDE-49, 2-OH-BDE-68, 6-OH-BDE-90, and 2-OH-BDE-123), seven MeO-PBDEs (2′-MeO-BDE-68, 6-MeO-BDE-47, 5-MeO-BDE-47, 4′-MeO-BDE-49, 5′-MeO-BDE-100, 4′-MeO-BDE-103, 5′-MeO-BDE-99, and 4′-MeO-BDE-101) and surrogate standards (2′–OH–6′-Cl-BDE-7, 6′-MeO-BDE-17) were synthesized in the Department of Biology and Chemistry, City University of Hong Kong. Purities of all metabolites were greater than 98% (He et al., 2008). Eighteen PBDEs (BDE-7, BDE-11, BDE-25, BDE-28, BDE-47, BDE-66, BDE-71, BDE-77, BDE-85, BDE-99, BDE-100, BDE-118, BDE-119, BDE-138, BDE-153, BDE-154, BDE-183 and BDE-209), and ten bromophenols (BRPs) (2,4,6-triBRP, 2,3,6-triBRP, 2,3,4-triBRP, 2,3,5-triBRP, 2,4,5-triBRP, and 3,4,5-triBRP, 2,3,4,5-tetraBRP, 2,3,4,6-tetraBRP, 2,3,5,6-tetraBRP and 2,3,4,5,6-pentaBRP) were obtained from Wellington Laboratories Inc. (Guelph, ON, Canada). Dichloromethane (DCM), n-hexane, methyl *tert*-butyl ether (MTBE), cyclohexane, pyridine, water, acetone, acetonitrile and methanol were pesticide residue grade obtained from OmniSolv (EM Science, Lawrence, KS, USA). Silica gel (60–100 mesh size), formic acid, hydrochloric acid (37%, A.C.S. reagent), 2-propanol and methyl chloroformate (MCF) were obtained from Sigma–Aldrich (USA). Human liver microsomes (HLM), which were pooled (n = 20) from mixed genders, were obtained from BD Biosciences (USA), and stored at −80 °C prior to *in vitro* studies. NADPH regenerating system was purchased from Promega (Madison, WI, USA). All other biochemical reagents were obtained from Sigma–Aldrich and were reagent grade or better unless stated otherwise. Commercial decabrominated diphenyl ethers (BDE-209) was obtained from Wenhua Chemical (China) and stored at −4 °C before analysis. Three types of epoxy resin adhesives were purchased from Ausbond Co. LTD (China). And three brands of plastic sheets made of acrylonitrile butadiene styrene copolymers (ABS) resin, Chimei (Taiwan, China), Feijin (Japan) and Shengmei (China) were purchased from various vendors.

2.2. Sample collection

Samples (n = 47) of adipose were collected simultaneously with those samples in the previous study in 2003–2004 from an office based liposuction clinic in New York City (Boris et al., 2005). Approval for the study and all specific procedures was obtained from the Institutional Review Board (IRB) of the New York State Department of Health. Details of the collection of these samples have been published previously (Boris et al., 2005). The only known demographic factors were age, gender, and date of collection without personal identifiers. The samples were stored at −20 °C until analysis.

2.3. In vitro microsomal incubations

Individual brominated compounds detected in adipose were incubated, *in vitro,* with HLM to assess the biotransformation rate and possible products in humans. Incubations of individual PBDEs and their structural analogues (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209, 6-OH-BDE-47, 6-MeO-BDE-47, 246-triBRP) with HLM were conducted as described previously with small modifications (Erratico et al., 2010). Briefly, 100 μL HLM (2 mg/mL protein) in phosphate buffer (pH 7.4; 3 mM $MgCl_2$) was dosed with 1 μL standards, including brominated compounds and benzo[a]pyrene both at 0.5 μM. After pre-incubation in a shaking incubator at 37 °C for 5 min, reactions were initiated by addition of a NADPH-generating system (NADP 6.5 mM, glucose 6-phosphate 16.5 Mm, $MgCl_2$ 16.5 Mm, and glucose 6-phosphate dehydrogenase 2 U/mL). Reactions were stopped by addition of 200 μL of ice-cold acetone after 0, 1, 3, 5, 9, 12 and 24 h. Incubations with deactivated microsomes and standards were used as negative controls to assess potential background interference and the possibility of non-enzymatic changes. After the incubation, all the samples were analyzed immediately for identification of metabolites and determination of biotransformation rates.

2.4. Sample preparation

Approximately 1 g of freeze-dried adipose was spiked with ^{13}C-BDE-209, 6′-MeO-BDE-17 and 2′-OH-6′-Cl-BDE-7, as surrogate standards, which had been determined to be absent from the samples. Samples were extracted by accelerated solvent extraction (Dionex ASE-200, Sunnyvale, CA) with two 10 min cycles. The first cycle was performed with n-hexane/dichloromethane (DCM) (1:1) at a temperature of 100°C and pressure of 1500 psi, followed by a second cycle with n-hexane/methyl tert-butyl ether (MTBE) (1:1) at 60 °C and pressure of 1000 psi. Solvents from two extractions were combined and rotary evaporated to near dryness at the temperature of 35 °C. Lipid content in each sample was determined gravimetrically by rotary evaporation of the extract to a constant weight.

The bulk of high molecular weight compounds in the extracts were removed by gel permeation chromatography (GPC Lab Tech PrepElite-GV, USA; Column: Bio-beads S-X3) using cyclohexane/ethyl acetate (1:1) as the mobile phase. Most of the matrix was eluted in the first fraction at 1-10 min following injection, and the 10-34 min fraction was collected based on elution profile of target compounds. The second fraction was rotary evaporated and then dried by a gentle stream of nitrogen, and derivatization with 480 μL solvent (acetonitrile/methanol/water/pyridine 5:2:2:1; v/v/v/v) and 50 μL of MCF was performed. The reaction mixture was shaken at room temperature for 1 h before the addition of 1 mL water. The aqueous solution was extracted three times with 2 mL of n-hexane, and extracts were concentrated to a volume approximately 1 mL before subjecting to acidified silica gel chromatography. To prepare acidified silica, 50 g silica gel was heated at 120 °C overnight, and then mixed with 27 ml concentrated sulfuric acid. This column was eluted with 15 mL n-hexane and followed by 10 mL DCM. The eluant was concentrated to 50 μL in n-hexane, and analyzed by gas chromatography-electron capture negative ionization mass spectrometry (GC-ENCI-MS) (Shimadzu QP 2010 plus, Japan). By using this method, phenolic compounds were derivatized to methyl formate BDEs and methyl formate BRPs.

For microsomes, incubation mixtures were mixed with 1 mL of water and a surrogate mixture of 6′-MeO-BDE-17, 2′-OH-6′-Cl-BDE-7 and ^{13}C-BDE-209, and extracted three times with 1 mL hexane. The extract was then passed through a Pasteur pipe filled with sodium sulfate to remove moisture and then eluted by 2 mL hexane and 2 mL DCM. After derivatization with MCF as described above, the elute was concentrated to 50 μL in n-hexane for GC-ENCI-MS analysis.

Approximately 0.5 g commercial products of BDE-209, ABS plastic sheets and epoxy resin adhesives were cut into pieces with scissors and combined with a mixture of 6′-MeO-BDE-17, 2′-OH-6′-Cl-BDE-7 and ^{13}C-BDE-209 as surrogates before ultrasonic extraction with 2 mL n-hexane/DCM (1:1) followed by 2 mL n-hexane/MTBE (1:1) and each extraction was performed twice. Commercial products of BDE-209, ABS plastic sheets and epoxy resin adhesives were completely dissolved by the extraction solvents. All the extracts were combined and dried under a gentle stream of nitrogen before derivatization. The derivatives were concentrated to approximately 1 mL and purified by acidified silica gel chromatography. Elute were concentrated to 200 μL in n-hexane before instrumental analysis.

2.5. Preparation of stock and working standards

To prepare calibration standards, five different concentrations of target compounds (0.5, 1, 5, 50, and 100 ng/mL for PBDEs, MeO-PBDEs and BRPs; 2.5, 5, 50, 100, and 500 ng/mL for OH-PBDEs) and a working surrogate standard (50 ng/mL) solution were added to five amber glass tubes. After derivatization with MCF, the mixture was extracted with n-hexane three times and concentrated to 100 μL in n-hexane.

2.6. Instrumental analysis

Target compounds including tri–hepta- PBDEs, MeO-PBDEs, methyl formate BDEs and methyl formate BRPs were identified and quantified by use of gas chromatography with electron capture negative ionization mass spectrometry (GC-ENCI-MS). Bromine (79, 81 m/z) was monitored using SIM mode for target analytes. The ion source was kept at a temperature of 260 °C and the injection port was at 280 °C in splitless mode. A 30 m chromatographic column (DB-5MS, Agilent Technologies) was used for the gas chromatographic separation. The carrier gas was helium at a flow rate of 2 mL/min. The temperature program was from 110 °C (2 min) to 245 °C at a rate of 3 °C/min, then to 320 °C at a rate of 15 °C/min, and the oven temperature was held at 320 °C for 5 min.

For quantification of octa and deca- PBDEs, chromatographic separation was achieved by use of a VF-5MS capillary column (15 cm × 0.25 mm × 0.1 μm film thickness; J&W Scientific, USA). Molecular fragments (486.5 m/z for octa and deca- PBDEs; 494.6 m/z for ^{13}C -BDE-209) were monitored using SIM mode for detection of target analytes. A splitless injector was used, and the injector was held at 290 °C. The temperature program was from 120 °C (2 min) to 310 °C (5 min) at a rate of 25 °C/min. The transfer line temperature and the ion source temperature were maintained at 280 °C and 260 °C, respectively. High pressure injection was applied with the pressure of 300 psi held for 1 min. The carrier gas was helium at a constant flow rate of 5 mL/min. Data acquisition was conducted in selected ion monitoring mode.

2.7. Quality assurance/quality control

Concentrations of tri–hepta-BDEs and MeO-PBDEs in sample extracts were quantified relative to 6′-MeO-BDE-17, phenolic compounds (derived as methyl formate BDEs and methyl formate BRPs) were quantified relative to 2′—OH—6′-Cl-BDE-7, and octa–deca-BDEs were quantified relative to ^{13}C-BDE-209. The procedure described above was validated by analyzing spiked oil (matrix spike samples) with spiking concentrations at least three times the basal concentrations. Mean (±SD) recoveries of PBDEs, MeO-PBDEs, OH-PBDEs and BRPs were 97—118%, 108—117%, 60—95%, and 67—104%, respectively (Table S1). Mean recoveries of 6′-MeO-BDE-17, 2′—OH—6′-Cl-BDE-7 and ^{13}C-BDE-209 in samples were 92 ± 30%, 82 ± 19% and 98 ± 20%, respectively. To avoid sample contamination, glassware was rinsed with acetone followed by n-hexane. A laboratory blank was incorporated in the analytical procedures for every batch of 10 samples. Method detection limits (MDLs) were 0.002—0.09 ng/g wet weight (ww) for tri–hepa- PBDEs, 4.0 ng/g ww for deca-PBDE, 0.007—0.02 ng/g ww for MeO-PBDEs, 0.006—0.04 ng/g wm for OH-PBDEs and 0.004—0.3 ng/g ww for BRPs in adipose samples (Table S1).

3. Results and discussion

3.1. Occurrences in human adipose

MCF was used in the derivatization of target brominated organic compounds and showed high reactivity towards phenolic hydroxyl groups of organobromines at room temperature. Derivatization with MCF can also help discriminate MeO-PBDEs from OH-PBDEs, as OH-PBDEs are derivatized to methyl formate BDEs (Hovander et al., 2000). Acceptable resolution of chromatographic peaks for more than forty organobromines including PBDEs, MeO-PBDEs, methyl formate BDEs and methyl formate BRPs was achieved by

Table 1
Concentrations of predominant brominated compounds detected in adipose of people from New York, NY United States of America (USA). (ng/g, lw; mean ± SD).

Group (n)	Sample	Age (year)			Gender	
	All (47)	≤30 (20)	30–40 (18)	>40(9)	Female (28)	Male (19)
BDE-28	0.31 ± 0.57	0.32 ± 0.52	0.23 ± 0.28	0.47 ± 1.00	0.39 ± 0.69	0.20 ± 0.27
BDE-47	6.00 ± 10.8	5.61 ± 9.06	6.33 ± 12.1	6.20 ± 13.1	6.16 ± 10.4	5.76 ± 11.8
BDE-100	2.22 ± 5.29	1.92 ± 4.37	2.78 ± 6.77	1.79 ± 4.12	2.00 ± 4.30	2.56 ± 6.60
BDE-119	0.03 ± 0.04	0.03 ± 0.03	0.04 ± 0.04	0.03 ± 0.04	0.03 ± 0.03	0.03 ± 0.04
BDE-99	2.27 ± 5.63	2.01 ± 4.33	2.60 ± 7.30	2.17 ± 4.89	2.17 ± 4.48	2.42 ± 7.12
BDE-85	0.33 ± 0.90	0.25 ± 0.49	0.47 ± 1.35	0.23 ± 0.45	0.26 ± 0.48	0.44 ± 1.31
BDE-154	0.96 ± 1.43	0.49 ± 0.56	1.36 ± 1.96	1.21 ± 1.40	0.88 ± 1.41	1.09 ± 1.49
BDE-153	5.78 ± 12.5	2.82 ± 4.42	10.1 ± 19.0	3.69 ± 4.45	4.17 ± 5.92	8.15 ± 18.4
BDE-183	0.07 ± 0.06	0.07 ± 0.05	0.09 ± 0.09	0.05 ± 0.04	0.07 ± 0.05	0.08 ± 0.08
tri-hepta BDEs	18.2 ± 34.8	13.8 ± 22.4	24.3 ± 47.7	16.04 ± 27.9	16.4 ± 24.4	21.0 ± 46.7
BDE-206	0.69 ± 3.19	1.26 ± 4.88	0.17 ± 0.14	0.44 ± 0.68	0.98 ± 4.12	0.26 ± 0.49
BDE-207	1.20 ± 4.53	2.00 ± 6.93	0.50 ± 0.27	0.80 ± 0.93	1.62 ± 5.84	0.57 ± 0.67
BDE-208	0.26 ± 1.25	0.48 ± 1.91	0.05 ± 0.06	0.20 ± 0.36	0.37 ± 1.61	0.10 ± 0.26
BDE-209	5.27 ± 5.06	4.99 ± 5.12	5.00 ± 5.06	6.41 ± 5.35	5.95 ± 6.10	4.27 ± 2.81
octa-deca BDEs	7.41 ± 10.2	8.74 ± 14.3	5.72 ± 5.49	7.85 ± 5.55	8.92 ± 12.77	5.20 ± 3.27
∑PBDEs	25.7 ± 35.3	22.5 ± 24.7	30.0 ± 47.8	23.9 ± 28.1	25.28 ± 26.6	26.2 ± 46.1
2′-MeO-BDE-68	0.03 ± 0.03	0.03 ± 0.03	0.02 ± 0.02	0.03 ± 0.03	0.03 ± 0.02	0.03 ± 0.03
6-MeO-BDE-47	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.04	0.02 ± 0.03	0.02 ± 0.01
∑MeO-PBDEs	0.07 ± 0.05	0.07 ± 0.04	0.07 ± 0.04	0.09 ± 0.06	0.08 ± 0.05	0.06 ± 0.04
6-OH-BDE-47	0.04 ± 0.09	0.03 ± 0.04	0.06 ± 0.12	0.06 ± 0.11	0.04 ± 0.07	0.05 ± 0.11
6-OH-BDE-90	0.43 ± 0.76	0.17 ± 0.18	0.52 ± 0.64	0.80 ± 1.43	0.36 ± 0.54	0.52 ± 1.01
∑OH-PBDEs	0.54 ± 0.88	0.23 ± 0.22	0.65 ± 0.79	1.00 ± 1.59	0.47 ± 0.68	0.64 ± 1.13
2,4,6-triBRP	5.05 ± 9.74	3.13 ± 6.12	5.25 ± 12.9	8.91 ± 8.87	6.23 ± 11.6	3.31 ± 6.04

the 30 m DB-5MS chromatographic column (Fig. S1). Linear calibration curves for PBDEs and MeO-PBDEs were not affected by derivatization of OH-PBDEs and BRPs, and coefficients of determination for target analytes were higher than 0.98. Due to the high sensitivities of NCI sources for brominated compounds, the IDLs (instrument detection limits) for methyl formate BDEs (0.2–1.4 ng/mL) and methyl formate BRPs (0.1–1.0 ng/mL) were comparable to those determined by LC–MS/MS (0.6–1.1 ng/mL), but lower than those by GC-HRMS (0.5–1 ng/mL) (Hua et al., 2005; Lacorte et al., 2010). Among the target brominated compounds, thirteen PBDE congeners (BDE-28, 47, 85, 99, 100, 119, 153, 154, 183, 206, 207, 208, 209), four MeO-PBDE congeners (6-MeO-BDE-47, 2′-MeO-BDE-68, 5-MeO-BDE-47, 4′-MeO-BDE-49), three OH-PBDE congeners (6-OH-BDE-47, 6-OH-BDE-90, 2-OH-BDE-123) and one BRP congener (2,4,6-triBRP) were detecctd in human adipose samples. Concentrations of ∑PBDEs, ∑OH-PBDEs, ∑MeO-PBDEs and ∑BRPs in adipose were in the range of 3.0–202 ng/g lipid weight (lw) (median: 11 ng/g lw, mean: 26 ng/g lw), 0.03–5.2 ng/g lm (median: 0.24 ng/g lw, mean: 0.54 ng/g lw), 0.03–0.19 ng/g lw (median: 0.06 ng/g lw, mean: 0.07 ng/g lw) and 0.23–54 ng/g lw (median: 0.28 ng/g lw, mean: 5.1 ng/g lw), respectively (Table 1).

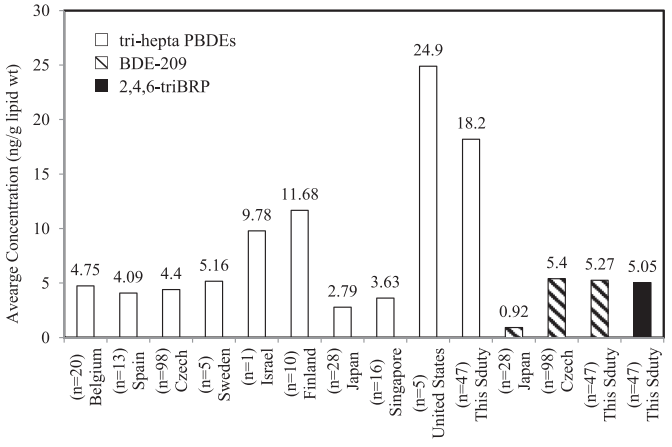

Fig. 1. Concentrations of predominant brominated organic compounds in human adipose analyzed in the present study compared with those reported in other countries including Belgium (Covaci et al., 2002), Spain (Meneses et al., 1999), Czech (Pulkrabova et al., 2009), Sweden (Meironyte et al., 2001), Israel (de Boer et al., 1998), Finland (Strandman et al., 1999), Japan (Kunisue et al., 2007), Singapore (Li et al., 2005), United States (Boris et al., 2005) (ng/g, lw).

Among all the brominated organic compounds analyzed, tri–hepta-BDEs were the predominant congeners detected in all samples. BDE-47 was the prevalent congener, accounting for 23.4% of the total PBDE concentrations in human adipose tissue, followed by BDE-153 (22.5%), BDE-99 (8.8%) and BDE-100 (8.7%) (Table 1). Concentrations of ∑tri–hepta-BDEs in adipose tissue from New York ranged from 0.47 to 199, ng/g lw (median: 6.02 ng/g lw; mean: 18.2 ng/g lw (Table 1). These concentrations were higher than those reported in adipose from people in Europe and Asia (4.09–11.68 and 2.79–3.63 ng/g lw, respectively) (Covaci et al., 2002; Meneses et al., 1999; Pulkrabova et al., 2009; Meironyte et al., 2001; de Boer et al., 1998; Strandman et al., 1999; Kunisue et al., 2007; Li et al., 2005), but comparative to those reported previously for people in the United States (24.9 ng/g lw) (Fig. 1) (Boris et al., 2005). The relatively high concentrations of PBDEs in the U.S. population were possibly due to the predominant (>90% of global production) usage of the penta-BDE mixture in North America (Bromine Science and Environmental Forum, 2003).

Besides the low brominated PBDE congeners, octa–deca-BDEs were detected at comparable concentrations (7.41 ng/g lw) with BDE-47, accounting for over 20% of the total concentrations of PBDEs, although some European countries or states in the USA have recently banned the use of deca-BDE (USEPA, 2010; Clean Production Action, 2006). Concentrations of BDE-209 in adipose tissue from New York were comparable to those in the Czech Republic (5.4 ng/g, lw) (Pulkrabova et al., 2009), but higher than concentrations in adipose from people in Japan (0.92 ng/g lw) (Kunisue et al., 2007). Statistically significant correlations were observed among individual tri–hepta-BDEs (BDE-47, BDE-99, BDE-100, BDE-153, BDE-183), and among octa-BDE, nona-BDE and deca-BDE congeners (Fig. S2). However, no significant correlation was found between tri–hepta-BDEs and octa–deca-BDEs, possibly suggesting the differences in sources of human exposure to low and high brominated BDEs (e.g. commercial penta-BDE mixtures and

deca-BDE mixtures).

Concentrations of MeO-/OH-PBDEs and BRPs in human adipose provide a complete overview of PBDEs and their structure-analogues in human tissues. To our knowledge, this is the first report on concentrations of MeO-/OH-PBDEs in human adipose. Concentrations of MeO-PBDEs and OH-PBDEs in adipose were very low, and only four MeO-PBDEs and three OH-PBDEs were detected (Table 1). Of all the MeO-/OH-PBDEs, 2′-MeO-BDE-68, 6-MeO-BDE-47, and 6-OH-BDE-47 were detected frequently, which is consistent with results of previous studies of occurrences of MeO-/OH-PBDEs in human blood (Wan et al., 2010; Athanasiadou et al., 2008; Zota et al., 2011; Chen et al., 2013). Relatively high detection frequency of 6-OH-BDE-90 was also observed, possibly suggesting the preferential accumulation of highly brominated OH-PBDEs in adipose tissue. Of the BRPs, 2,4,6-triBRP was detected at significantly high concentrations (mean: 5.05 ng/g lw, median: 0.28 ng/g lw), in comparison with MeO-/OH-PBDEs in adipose samples. (Table 1). Concentrations of 2,4,6-triBRP were similar to those of BDE-209, and in some samples even higher than those of BDE-47 and BDE-153. The predominance of 2,4,6-triBRP in human tissues has been reported previously, and similarly high concentrations of this compound were detected in serum of people in Norway (0.077–81 ng/g lm) and adipose from people in Finland (2.16–53.8 ng/g lm) (Thomsen et al., 2001; Smeds and Saukko, 2003). 2,4,6-triBRP is a naturally occurring compound in marine organisms, but also widely used as a flame retardant with a worldwide production in 2001 of 9500 tonnes (IUCLID, 2003). However, limited knowledge was available for the sources of human exposure to triBRPs, which has important implications for risk assessment and how exposures can be controlled.

3.2. Assessment of sources of bromophenol

Profiles of target organobromine compounds with available source information together with biotransformation rates of the compounds in human could help determine the exposure sources of chemicals accumulated from the environment. Previous studies have found that *meta-/para*-substituted OH-PBDE congeners could

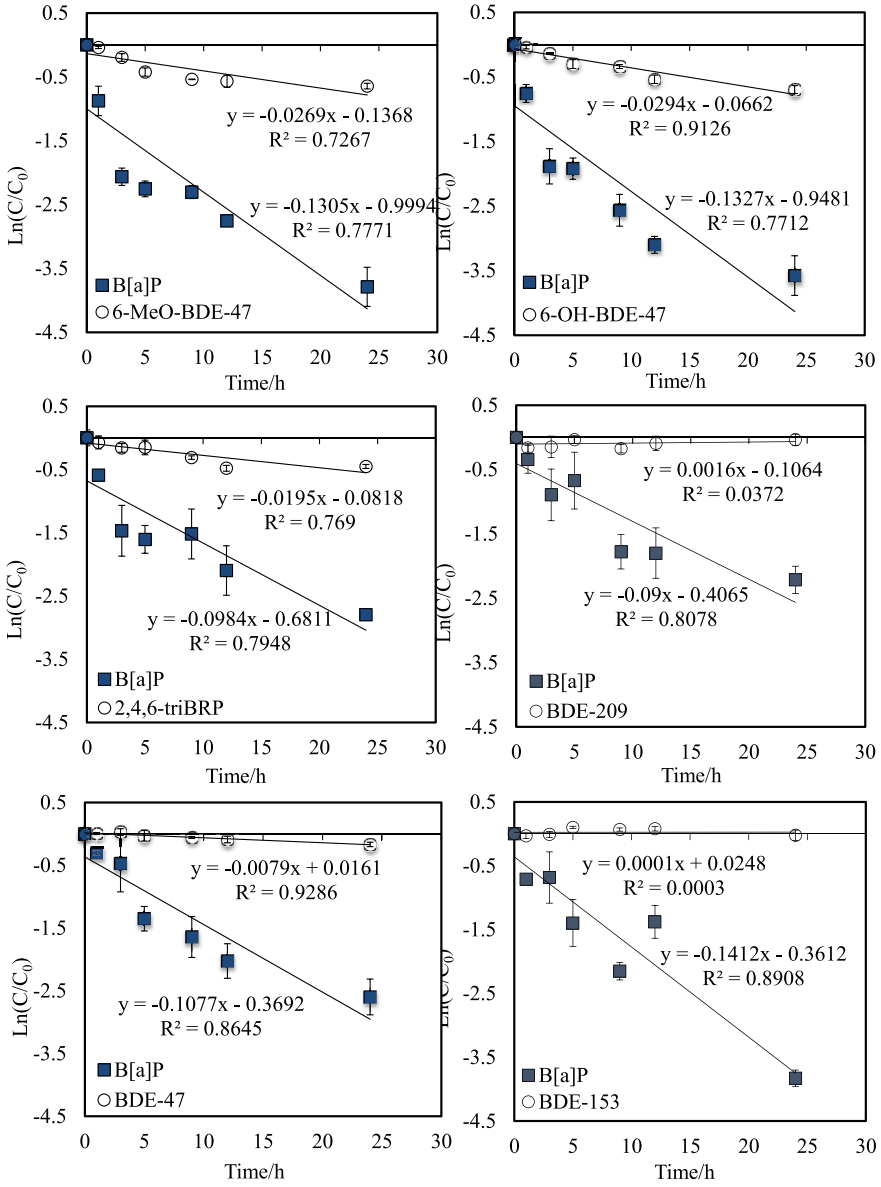

Fig. 2. Substrate depletion curves from *in vitro* incubation of BDE-47, BDE-153, 2,4,6-triBRP, BDE-209, 6-MeO-BDE-47, and 6-OH-BDE-47. Error bars are standard deviations from triplicate incubations.

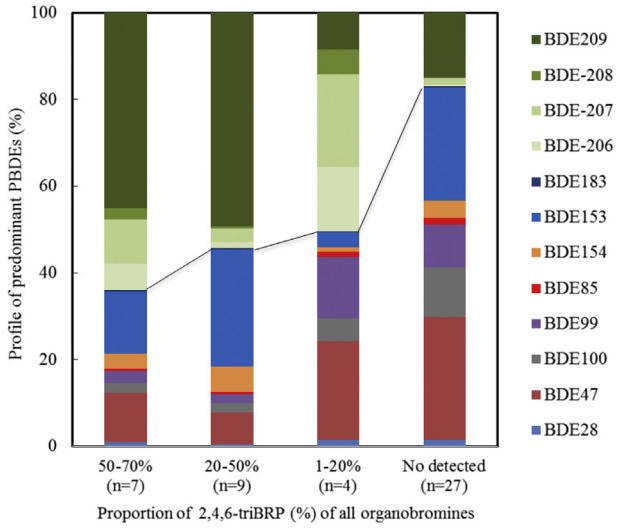

Fig. 3. Relative proportions (%) of PBDEs along with the proportion of 2,4,6-triBRP in human adipose of people from New York, NY, USA.

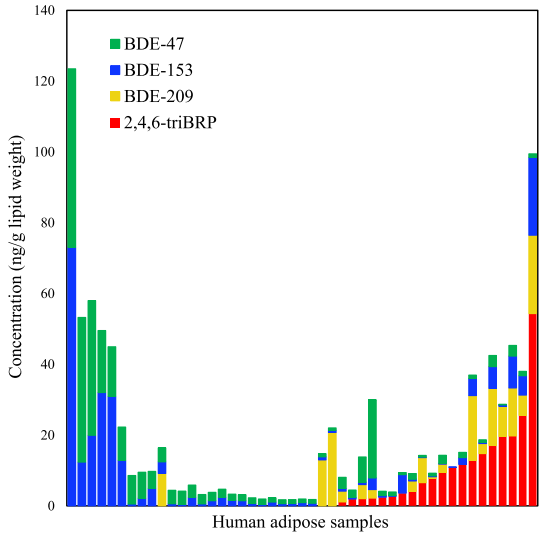

Fig. 4. Concentrations of BDE-47, BDE-153, BDE209, and 2,4,6-triBRP in adipose of people from New York, NY, USA (Zero was set for non-detectable levels in the figure).

originate from exposure to synthetic PBDEs, while *ortho*-substituted OH-PBDE congeners were mainly natural compounds (Wiseman et al., 2011). In the present study, only the *ortho*-substituted OH-PBDE was detected, and no significant correlation were found between PBDEs and their corresponding MeO-/OH-structural analogues, which was consistent with the relationship among PBDEs, MeO-PBDEs and OH-PBDEs in serum from residents living near a coastal area in India (Eguchi et al., 2012). The results indicated that OH-PBDEs in humans in New York were mainly originated from natural sources. It has been reported that OH-PBDEs had similar natural sources with triBRPs in the marine environment (e.g. marine algae) (Hassenklover et al., 2006; Sheikh and Djerassi, 1975; Goerke and Weber, 1991). Concentrations of OH-PBDEs (11–316 pg/g ww) were reported to be comparable to those of BRPs (3.7–126 pg/g ww) in marine fish and dietary intakes of OH-PBDEs and BRPs for human were reported to be 23–352 pg/kg day and 1.6–208 pg/kg day on the basis of the 50th and 95th centile concentrations, respectively (Wang et al., 2011), suggesting the similar dietary exposure of these natural compounds. Since biotransformation and assimilation are two important toxicokinetic processes to determine the bioaccumulation of chemicals in mammalian species (Weisbrod et al., 2009), an *in vitro* incubation was firstly performed to determine biotransformation rates of 2,4,6-triBRP, 6-OH-BDE-47 and 6-MeO-BDE-47. B[a]P was added as a benchmark compound in the incubation mixture to normalize the variation in transformation rates that occurred in different batches of analyses. Similar biotransformation rates were observed for 2,4,6-triBRP, 6-OH-BDE-47 and 6-MeO-BDE-47, of which the $K/K_{B[a]P}$ values were 0.20, 0.22 and 0.21, respectively (Fig. 2, Table S2), which suggest that these compounds were all metabolized at a comparative rate approximately 5-fold lower than that of B[a]P. The assimilation of chemicals generally take place via passive diffusion as the primary means by which chemicals cross the surfaces of gut or skins mainly depend on lipophilicity of the chemicals (logKow) (Weisbrod et al., 2009). The logKow of 2,4,6-triBRP (4.24) was reported to be relatively lower than that of 6-OH-BDE-47 (6.59) (Kuramochi et al., 2004; Yu et al., 2008), suggesting that 2,4,6-triBRP was more difficult to be accumulated. Thus, concentration of 2,4,6-triBRP would be lower than that of 6-OH-BDE-47 in human assuming that BRPs were only originated from similar natural sources with OH-PBDEs. However, the relatively high concentrations of BRPs in comparison with extremely low concentrations of naturally occurring MeO-PBDEs and OH-PBDEs in human adipose samples indicated that BRPs was not of natural origin.

BRPs were found to have close relationship with PBDEs in these samples. It is interesting to note that human samples containing 2,4,6-triBRP had a PBDE profile with high contributions of octa-deca-BDEs, and relative contributions of octa–deca-BDEs in PBDEs were directly proportional to proportion of 2,4,6-triBRP (Fig. 3). The detection frequency of 2,4,6-triBRP (20/47) was similar with that of BDE-209 (17/47), and a significant correlation between concentrations of 2,4,6-triBRP and BDE-209 was also observed in detected samples ($p < 0.05$, Fig. S3). Furthermore, the presence of the three predominant PBDE congeners (BDE-47, BDE-153 and BDE-209) and 2,4,6-triBRP in adipose samples demonstrated that samples with detection of 2,4,6-triBRP had high proportion of BDE-209 (Fig. 4). The distribution of the two compounds was different from that of BDE-47 and BDE-153, which was reported to be related to exposures of technical mixtures of penta-and octa-BDE (Fig. 4). To explore the co-exposure sources of 2,4,6-triBRP and BDE-209 in human adipose, the persistence of the compounds were assessed in

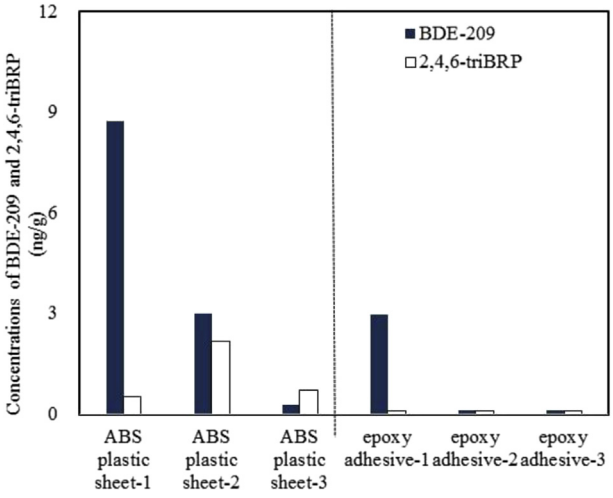

Fig. 5. Profiles of brominated flame retardants (BFRs) detected in ABS plastic sheets and epoxy resin adhesives, PBDEs (except BDE-209), OH-PBDEs and MeO-PBDEs were all lower than limits of detection.

human microsomes and compared with other predominant PBDEs. No significant variation was observed in the concentrations of all PBDE congeners with the incubation time and $K/K_{B[a]P}$ ranged from <0.001 to 0.09, suggesting that PBDEs were resistant to metabolism and their biotransformation rates are 10 times lower than that of B[a]P (Table S2). Among PBDEs, concentrations of BDE-47 and BDE-154 decreased slowly with the incubation time (Fig. 2 and Fig. S4), and the relatively high biotransformation rate of BDE-47 and BDE-153 was consistent with the results of a previous study on human liver microsome-mediated metabolism of BDE-47, BDE-99 and BDE-153 (Lupton et al., 2009). Whereas BDE-209 was biotransformed rapidly when incubated with liver microsomes from fish or rat (McKinney et al., 2011; Wan et al., 2013), this compound was found to be resistant to biotransformation in the presence of microsomes from liver of humans, which is also consistent with those for BDE-209 exposed *in vitro* to human hepatocytes (Stapleton et al., 2009). Based on the above results, the relatively high persistence of BDE-209 and 2,4,6-triBRP in human microsomes and co-occurrences of the two compounds revealed that BDE-209 and 2,4,6-triBRP may share a common exposure source in humans.

3.3. Potential anthropogenic sources of 2,4,6-triBRP

Considering the relationship between 2,4,6-triBRP and BDE-209 in human tissues, it is hypothesized that BDE-209 might be a precursor of 2,4,6-triBRP, or the two compounds might share a similar anthropogenic exposure source. Results of recent studies have shown that UV-induced hydrodebromination has been identified as a major abiotic transformation pathway for highly brominated PBDEs, and that hydrodebromination of PBDEs was a possible source of bromophenols (2,4-diBP, 2,5-diBP, 2,4,6-triBRP, and 2,4,5-triBRP) in the environment (Bendig and Vetter, 2013). However, ratio of BRPs formed from PBDEs was lower than 5%. In contrast, concentrations of 2,4,6-triBRP found in human tissues were comparable or higher than those of PBDEs. Furthermore, in the present study, BRPs were not detected during incubation of any PBDEs including BDE-209 with human microsomes. These results indicate that BDE-209 was not an important precursor that would produce large amounts of 2,4,6-triBRP in human tissues.

Considering the possibility that BRPs are formed as byproducts during the manufacture of deca-BDE mixtures, which could result in concurrent exposure to 2,4,6-triBRP and BDE-209, BRPs were quantified in the commercial deca-BDE mixtures. However, BRPs including 2,4,6-triBRP were not detected in commercial deca-BDE mixtures. Thus, it is possible that BDE-209 and 2,4,6-triBRP might be used simultaneously as additives in commercial flame-retarded products. It has been reported that 2,4,6-triBRP was used as an intermediate for production of flame retardants (tetrabromobisphenol A, tribromophenyl allyl ether, and 1,2-bis(2,4,6-tribromophenoxy) ethane) used in brominated epoxy resins and ABS resins (Weil, 1993). These epoxy resins are widely used as adhesive agents for electric materials, and ABS resin is one of the most popular resins in industrial manufacturing. Three brands of ABS plastic sheets and three types of epoxy resin adhesives were analyzed for BFRs. As shown in Fig. 5, BDE-209 and 2,4,6-triBRP were both detected in all ABS plastic sheets, but their concentrations were lower than MDL (2,4,6-triBRP: 0.02 ng/g; BDE-209: 0.5 ng/g) in epoxy resin adhesives (Fig. 5). Concentrations of 2,4,6-triBRP and BDE-209 were 0.5–2.2 and 1.4–9.8 ng/g dry mass in three brands of ABS plastic sheets. The possible reason for the low concentrations of 2,4,6-triBRP and BDE-209 in ABS plastic sheets could be that raw materials of ABS resins were used for analysis, and 2,4,6-triBRP was not used directly as a flame retardant, but rather as an intermediate for production of flame retardants used in ABS resins (Weil, 1993). It has been reported that BDE-209 was transferred to dust via physical processes, such as abrasion or weathering of polymers bound to particles of the original polymer matrix (Webster et al., 2009; Ma et al., 2014), and 2,4,6-triBRP was reported to be an important products from decomposition of tetrabromobisphenol A at high temperature (Marongiu et al., 2007). Thus 2,4,6-triBRP would be expected to be released from electronics and plastic products made from ABS resins and a potential source of exposure for humans.

3.4. Potential effects

To our knowledge, no previous studies have reported potential toxicological effects of brominated phenols in humans. However, brominated phenols have the potential to elicit a variety of effects in rodents including salivation, body weight gain suppression, increased absolute and relative liver weights, and elevated total protein, albumin, albumin/globulin ratios and ALP in blood (Tanaka et al., 1999). 2,4,6-triBRP reduced cell growth and increased acetylcholinesterase activity in cultured SH-SY5Y human neuroblastoma cells and the differentiated cells were more sensitive to 2,4,6-triBRP than native cells (Rios et al., 2003). Besides, 2,4,6-triBRP induced inhibition of thyroid hormone activity in human (Craig et al., 2011; Craig and Heather, 2013). Results of recent studies have shown that 2,4,6-triBRP was a natural precursor for polybrominated dibenzo-p-dioxins (PBDDs) in biota from the Baltic Sea (Arnoldsson et al., 2012). Based on the high concentration of 2,4,6-triBRP (up to 54.32 ng/g) in human tissues, and potential adverse effects on humans, further studies are needed on the toxicity and risk assessment of brominated phenols in humans.

4. Conclusions

In the present study, 46 organobromine compounds were simultaneously quantified in adipose collected from people from New York City, USA, and biotranformation of the predominant compounds were assessed in *in vitro* assay utilizing human liver microsomes. High concentrations of 2,4,6-triBRP and PBDEs were observed, whereas extremely low concentrations of naturally occurring MeO/OH-PBDEs were detected. Similar biotransformatioin rates of OH-PBDEs, MeO-PBDEs and BRPs with identical natural resources suggested that the relative high concentration of 2,4,6-triBRP in humans was not of natural origin. Significant correlation between concentrations of 2,4,6-triBRP and BDE-209 suggested that the two chemicals may share a common exposure source in humans. PBDEs including BDE-209 were found to be resistant to biotransformation in humans and not a primary precursor of 2,4,6-triBRP. Both 2,4,6-triBRP and BDE-209 were detected in commercial ABS resins, which indicates that plastic products made from ABS resins could be potential sources of co-exposure for humans.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.04.015.

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