



Published in final edited form as:

Anal Bioanal Chem. 2016 April ; 408(10): 2449–2459. doi:10.1007/s00216-016-9340-3.

Development of an analytical method to quantify PBDEs, OH-BDEs, HBCDs, 2,4,6-TBP, EH-TBB, and BEH-TEBP in human serum

Craig M. Butt¹, Marie Lynn Miranda^{2,3}, and Heather M. Stapleton¹

Heather M. Stapleton: heather.stapleton@duke.edu

¹Nicholas School of the Environment, Levine Science Research Center, Duke University, Room A220, Box 90328, Durham, NC 27708, USA

²Department of Statistics, Rice University, 6100 Main St. MS-138, Duncan Hall, Box 1892, Houston, TX 77005-1827, USA

³Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Abstract

Polybrominated diphenyl ethers (PBDEs) flame retardants (FRs) were phased-out in the mid-2000s (penta- and octaBDE) and 2013 (decaBDE); however, their hydroxylated metabolites (OH-BDEs) are still commonly detected in human serum. Today, novel FRs such as Firemaster[®] 550, a mixture that contains two brominated compounds, EH-TBB and BEH-TEBP are used as replacements for PBDEs in some applications, and there is a need to develop a comprehensive analytical method to assess exposure to both legacy PBDEs and novel FRs. This study developed a solid-phase extraction (SPE)-based method to analyze PBDEs, OH-BDEs, 2,4,6-tribromophenol (TBP), hexabromocyclododecane isomers (HBCDs), EH-TBB, and BEH-TEBP in human serum. Briefly, serum proteins were first denatured with formic acid, and then the target analytes were isolated using a SPE column. Finally, the extract was cleaned and fractionated using a silica SPE column. Method performance was assessed by spiking fetal bovine serum with 1–2 ng of the target analytes, and method accuracy was quantified by comparison to a serum Standard Reference Material (SRM). The developed method showed good recovery and accuracy for all target analytes with the exception of the very low and very high molecular weight PBDE congeners. Using this method, 43 serum samples collected from the Healthy Pregnancy, Healthy Baby Study (HPHB) cohort in Durham, NC, USA were analyzed for FRs. A novel finding was the ubiquitous detection of 2,4,6-TBP, at levels greater than the individual PBDE congeners. Furthermore, 2,4,6-TBP was positively correlated with PBDEs, suggesting that they may have a similar source of exposure, or that 2,4,6-TBP may result from metabolism of PBDEs in vivo.

Electronic supplementary material: The online version of this article (doi:10.1007/s00216-016-9340-3) contains supplementary material, which is available to authorized users.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest with this research.

Keywords

Flame retardants; Serum analysis; Method development; Mass spectrometry

Introduction

Chemical flame retardants (FRs) are used in foams, polymers, and textiles to prevent combustion and suppress fire propagation. Due to strict fire safety regulations, FRs are broadly found in commercial products such as furniture, electronics, and construction material. Prior to the mid-2000s, one of the most common classes of FRs was the polybrominated diphenyl ethers (PBDEs). PBDEs were sold primarily as either penta-, octa-, or decaBDE commercial mixtures. Due to concerns regarding their persistence and human health effects, pentaBDE and octaBDE mixtures were banned in both Europe and the USA during the mid-2000s. Further, pentaBDE and octaBDE mixtures are now listed as persistent organic pollutants under Annex A of the Stockholm Convention [1]. In addition, the decaBDE mixture was phased-out by the end of 2013 [2]. Despite these phase-outs, PBDEs are still commonly detected in household furniture [3] and in house dust samples [4, 5]. PBDEs and their hydroxylated metabolites are also still widely detected in human serum [6–8]. For example, in the most recent NHANES data (2007–2008) BDE 28, 47, 99, 100, and 153 were detected in 100 % of the pooled samples.

In response to PBDE regulatory bans, the chemical flame retardant market has shifted to alternative, and sometimes novel, compound classes. Among these novel FRs is Firemaster 550[®] (FM550), which is a mixture of two brominated compounds [2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (BEH-TEBP)] and a suite of isopropylated aryl phosphates and triphenyl phosphate. Research from our laboratory, as well as other research groups, has shown that FM550 components are widely detected in foams from baby products [9] and sofas [3], as well as house dust [10–12]. Toxicological effects are relatively unknown, but *in vitro* studies have shown that EH-TBB and BEH-TEBP are potential endocrine disruptors [13]. Further, a recent rat study showed that perinatal exposure to FM550 resulted in obesity, anxiety, and early puberty [14].

Such market shifting of compounds, which can be expected to continue, suggests the need for comprehensive analytical methods to measure legacy PBDEs and their hydroxylated metabolites, as well as novel FRs in human tissues (i.e., serum). Most techniques for the analysis of PBDEs and OH-BDEs in human serum are based on those originally described by Hovander et al. [15]. This latter method involves protein denaturation with a strong acid, liquid-liquid extraction, separation of the OH-BDEs from the PBDEs by an alkaline wash, derivatization of the OH-BDEs by diazomethane (or another agent), silica or acid silica gel clean-up, and instrumental analysis by gas chromatography-mass spectrometry (GC-MS) [8, 16–19]. In recent years, liquid chromatography tandem mass spectrometry (LC-MS/MS) has been used for OH-BDE and bromophenol analysis [20–24], but with limited application to human serum [25].

Liquid-liquid extraction methods require large volumes of organic solvents and typically use diazomethane as the derivatizing agent, which is a potential hazard. Alternatively, solid-

phase extraction (SPE) techniques have been developed to assist in the isolation of halogenated organic compounds (e.g., PCBs, PBDEs) and their hydroxylated metabolites, as well as halogenated phenols (e.g., brominated phenols, triclosan) [7, 26–30], in environmental matrices. However, these methods rely on derivatization for hydroxylated- and phenol-compound instrumental analysis by GC-MS. To our knowledge, no published method exists that can recover and quantify PBDEs, OH-BDEs, tribromophenols (TBPs), EH-TBB, BEH-TEBP, and HBCDs in serum samples.

The present study developed a novel SPE extraction technique for the analysis of PBDEs, OH-BDEs, HBCDs, TBPs, EH-TBB, and BEH-TEBP in human serum. Specifically, our method uses dichloromethane (DCM): ethyl acetate to initially elute the analytes from the SPE cartridge, producing a cleaner extract. Additional purification of the extract and fractionation is performed using non-acidified silica, allowing for conservation of acid-labile compounds such as BEH-TEBP. Finally, our method is capable of analyzing TBPs, EH-TBB, and BEH-TEBP. Representative structures are shown in Fig. 1. PBDEs, TBPs, EH-TBB, and BEH-TEBP were analyzed by GC-MS, and OH-BDEs and HBCDs were analyzed by LC-MS/MS. The developed method was then applied to 43 serum samples collected in 2010–2011 from a pregnancy cohort in Durham County, NC, USA, to evaluate the detection frequency and distribution of these analytes.

Materials and methods

Chemicals

All solvents used were HPLC-grade or better (EMD Millipore Corporation, Billerica, MA). The PBDE calibration standard was prepared from 27 PBDE individual congeners (AccuStandard, New Haven, CT). Standard solutions of α -, β -, and γ -hexabromocyclodecane (HBCD, 99 %), ^{13}C - α -, β -, and γ -HBCD, ^{13}C -BDE 209 (2, 2', 3, 3', 4, 4', 5, 5', 6, 6'-BDE, 99 %), ^{13}C -OH BDE 100 (3-OH 2,2',4,4',6-BDE, 99 %), ^{13}C -6-OH BDE 47 (6-OH 2,2',4,4'-BDE, 99 %), 2,4,5-TBP and 2, 4,6-TBP were purchased from Wellington Laboratories (Guelph, ON). 3-OH BDE 47 (3-OH 2,2',4,4'-BDE, 97 %), 6-OH BDE 47 (100 %), 4'-OH BDE 49 (4'-OH 2,2',4,5'-BDE, 97.8 %), 5'-OH BDE 99 (5'-OH 2,2',4,4',5-BDE, 99.0 %), 6'-OH BDE 99 (6'-OH 2,2',4,4',5-BDE, 99.3 %) and F-BDE 69 (4'-fluoro-2,3',4,6-tetrabromodiphenyl ether) were purchased from AccuStandard. ^{13}C -CDE 141 (2,2'-3,4, 5,5'-hexachlorinated diphenyl ether, 99 %) was purchased from Cambridge Isotope Laboratories (Andover, MA). Formic acid (98 %) was purchased from Sigma-Aldrich.

Sample collection

Serum samples were collected as part of the Healthy Pregnancy, Healthy Baby Study (HPHB) cohort ($n = 43$) in Durham County, NC, USA. These samples all came from one clinic site: Lincoln Community Health Center, a federally qualified health center serving primarily low-income populations. A group of samples from the HPHB cohort were previously analyzed to investigate the association between PBDEs, OH-BDEs, and thyroid hormones [25]. However, the samples analyzed in the present study were from a separate group of individuals within the HPHB cohort. Serum samples were collected between

September 2008 and June 2010 during a routine clinic visit in either the second (24–28 weeks of pregnancy) or third trimester (35–36 weeks). Full details regarding participant recruitment and sample collection have been described previously [25]. In brief, all participants were residents of Durham County in central NC, USA. In our cohort of 43 samples, their average age was 24.3 years (range, 18–36 years), and 55 % reported completing some college. Further, our cohort was predominately non-Hispanic black (60 %), followed by non-Hispanic white (17.5 %), and Hispanic (15 %) (demographic data was not available for 1 individual). Blood was collected in a clinical laboratory, allowed to sit on ice and clot, and then serum was isolated by centrifugation (5 min, 3500 rpm). Serum samples were kept frozen (–20 °C) until analysis. Sample collection and handling was performed according to the human subject research protocol approved by the Duke University Institutional Review Board.

Sample extraction and analysis

Serum was thawed and gravimetrically transferred (~3.0–4.0 g) to a glass test tube. All glassware was baked-out overnight (450 °C) and rinsed with DCM and hexane prior to use. Internal standards were spiked (2.5 ng of F-BDE 69 and ¹³C-BDE 209; 1 ng of ¹³C-6-OH BDE 47 and ¹³C- α -, β -, γ -HCBd) prior to the serum addition. Formic acid (2 ml) and water (6 ml) were added to denature the serum proteins and the mixture was sonicated for 20 min. The analytes were isolated on a Waters Oasis HLB column (500 mg, 6 ml). The column was conditioned with 5 ml each of dichloromethane (DCM), methanol (MeOH), and water; the sample was loaded, and the column washed with 5 ml water. The analytes were eluted with 10 ml of DCM:ethyl acetate, with the eluent blown to near dryness under a gentle nitrogen stream and reconstituted in 1 ml of hexane. The analytes were cleaned and fractionated using a silica column (1 g, Waters, Sep-Pak) that was initially conditioned with 10 ml of hexane. The first fraction was eluted with 10 ml of hexane, reduced in volume to ~100 μ l, and analyzed by GC-MS for a suite of 27 PBDEs and 2,4,5- and 2,4,6-TBP. Prior to analysis, 5 ng of ¹³C-CDE 141 was spiked to assess F-BDE 69 recovery. The second fraction was eluted with 10 ml of DCM, blown to dryness, reconstituted in ~100 μ l MeOH and analyzed by LC-MS/MS for the HBCDs and OH-PBDEs. After LC-MS/MS analysis, the MeOH fractions were solvent switched to hexane, 5 ng of ¹³C-TBB added, and analyzed for EH-TBB and BEH-TEBP by GC-MS.

Instrumental analysis was performed using methods previously developed by our laboratory [25, 31]. The GC-MS was operated in electron capture negative ionization mode. The LC-MS/MS conditions were altered slightly and are briefly described. Analytes were separated using a C18 column (Agilent Zorbax Eclipse XDB-C18, 4.6 \times 50 mm, 1.8 μ m particle size) under gradient conditions and mobile phases were water and acetonitrile (ACN), both modified with 5 mM acetic acid. Initial conditions were 40:60 water:ACN, held for 1 min, increased to 10:90 over 0.5 min, increased to 5:95 over 4.0 min, held for 0.5 min, returned to initial conditions over 0.25 min, and held for 4.25 min. The column temperature was 40 °C, the injection volume was 20 μ l and flow rate was 400 μ l/ml. The internal standards were ¹³C- α HCBd for the HBCDs and ¹³C-6-OH BDE 47 for the OH-BDEs. The multiple reaction monitoring transitions (MRMs) observed for the OH-tetraBDEs were m/z 500.7 > 418.8, m/z 500.7 > 265.9 and m/z 500.7 > 78.9. MRMs monitored for the OH-pentaBDEs

were m/z 580.7 > 498.8, m/z 580.7 > 343.8, and m/z 580.7 > 78.9. We found that the position of the hydroxyl group gave diagnostic MRMs, as has been shown by other researchers [24]. For example, the m/z 500.7 > 418.8 and m/z 580.7 > 498.8 (formation of [M-Br]⁻) was unique to meta-OH BDEs. Also, the m/z 500.7 > 265.9 and m/z 580.7 > 343.7 (formation of the bromobenzoquinone) was indicative of para-OH BDEs. Finally, the ortho-OH BDEs showed only the m/z 500.7 > 78.9 and m/z 580.7 > 78.9 transitions (formation of Br⁻). In addition to retention time, these diagnostic MRMs were used to positively identify the OH-BDEs. The following OH-BDEs were quantified: 4'-OH BDE 49, 3-OH BDE 47, 6-OH BDE 47, 6'-OH BDE 99, and 5'-OH BDE 99. MRM chromatograms for a 0.1 ng standard (10 pg on-column) are shown in the Electronic Supplementary Material (ESM) (Fig. S1).

Lipid determination

Serum triglycerides (TG) and total cholesterol (CHOL) were determined using enzymatic techniques (Duke University Health System Clinical Laboratories, Durham, NC). The serum lipid content (TL) was estimated using the previously reported correlation equation, $TL = 1.33 \times TG + 1.12 \times CHOL + 1.48$ (g/l) [32].

Quality control

Fetal bovine serum (PAA Laboratories, Etobicoke, ON) and deionized water were both used as laboratory blanks. Method detection limits (MDLs) were calculated as three times the standard deviation of the laboratory blanks. Analytes levels were similar in both the fetal bovine serum and the deionized water and therefore both matrices were used for the MDL calculations. If analytes were not detected in the blanks, then the MDL was quantified as three times the standard deviation of the baseline noise. The only analytes detected in the laboratory blanks were BDE 47 (mean = 890 pg/g lw), BDE 99 (380 pg/g lw), BDE 100 (585 pg/g lw), and 6-OH BDE 47 (440 pg/g lw). For PBDEs, the accuracy of the developed method was tested by analyzing SRM 1958 [Fortified Human Serum; NIST (National Institute of Standards and Technology), Gaithersburg, MD].

Data analysis

Statistical analysis was performed using JMP[®] Pro (v. 11.0.0, 2013, SAS Institute Inc., Cary, NC). Values below MDL were assigned a value of half the detection limit for statistical analysis only. Summary statistics were only calculated for those variables with detection frequencies >50 %. Analyte data were assessed for normality using the Shapiro-Wilkes test and log-transformed as necessary. Correlations among analytes, and between serum lipid level and analyte concentrations, were examined using the Spearman rank-order correlation. Statistical significance was set at $\alpha < 0.05$.

Results and discussion

General method development

Our SPE extraction methods were adapted from previous techniques using Oasis HLB SPE columns for the extraction of PBDEs, HBCDs, and OH-BDEs from serum [27–29, 33]. A flow chart of the developed method is shown in Fig. 2. Due to the range of analyte polarity,

selection of an appropriate elution solvent was critical to elute the analytes only and avoid matrix co-elution. DCM was not sufficiently polar to extract the OH-BDEs from the HLB column, but was sufficient for PBDEs, HBCDs, EH-TBB, and BEH-TEBP (data not shown). Whereas other studies have used 1:1 DCM:MeOH as the elution solvent, we found that this solvent mixture eluted the serum matrix from the column. In particular, the matrix co-elution caused severe signal suppression and poor peak shape for the OH-BDEs and HBCDs during the LC-MS/MS analysis. Instead, we used 1:1 DCM:ethyl acetate to elute the target analytes. This elution mixture was sufficient to elute the polar analytes, while eluting a minimal amount of matrix.

Additional extract clean-up and analyte fractionation was performed using a silica gel SPE column. Previous studies have used sulfuric acid silica gel columns to clean the serum matrix, but in our experiments the use of acidic silica resulted in the near complete loss of BEH-TEBP (~5 % recovery, $n = 3$). Further, aggressive cleaning techniques were not required since very little matrix was eluted by the DCM:ethyl acetate. The first fraction (hexane) only contained the PBDEs; additional fractions with 1:1 DCM:hexane and DCM did not elute additional PBDEs. The OH-BDEs, HBCDs, EH-TBB, and BEH-TEBP were eluted in 1:1 DCM:hexane. Additional tests demonstrated equal extraction efficiency using DCM as the eluant, and thus DCM was selected due to the higher polarity.

Spike and recovery

Method performance was assessed by spiking 1–2 ng of all analytes (PBDEs, 2,4,6-TBP, α -HBCD, and OH-BDEs) into 3 ml of fetal bovine serum (Table 1). Overall, the method showed excellent recovery for the tri- to hexa-BDEs, with a mean recovery of 81 % (range, 61–106 %). The octa- and nona-BDE congeners showed lower recovery (mean, 55 %, range, 40–61 %), demonstrating that the method was not as efficient at capturing the higher brominated BDEs which is consistent with previous studies [34]. Further, BDE 209 was not recovered in the method. The 2,4,6-TBP, α -HBCD, EH-TBB, BEH-TEBP, and OH-BDEs generally showed >90 % recovery, but the tetra-OH BDE recovery was slightly lower, ranging from 59–75 %.

Standard reference material

Method accuracy for PBDEs was assessed by extracting NIST Standard Reference Material (SRM) 1958 (Fortified Human Serum). Most of the PBDEs showed good accuracy: ranging from 67 % (standard error = 2.9 %) for BDE 99 to 99 % (5.2 %) for BDE 154, with the exception of BDE 153 (mean recovery = 46 %, SE = 2.4 %) (Table 2). However, the method showed poor accuracy for the lower brominated (i.e., BDE-17) and higher brominated (i.e. BDE-183) congeners. Overall, these results suggest that the method developed was satisfactory for most tri- through hepta-BDE congeners.

Method application to maternal serum

Associations between serum lipid and flame retardant levels—Hydrophobic organic compounds tend to partition into the lipid component of tissues. As a result, normalizing the serum chemical concentrations by the total serum lipid level [35] has been suggested, and this approach is typically used in the literature [6, 36]. To our knowledge,

though, very few studies actually report lipid content-organic contaminant correlations [37]. In our cohort, the lipid content was not correlated with the predominate BDE congeners or 2,4,6-TBP (BDE-47: $p = 0.60$; BDE-99: $p = 0.64$; BDE-100: $p = 0.43$; BDE-153: $p = 0.75$; BDE-154: $p = 0.69$; sum-BDE: $p = 0.58$; 2,4,6-TBP: $p = 0.27$). To be consistent with the literature, we are here reporting values in ng/g lipid weight (ng/g lw). However, the non-lipid-normalized values are presented in the supporting information.

PBDEs—Seven of the 27 PBDE congeners were detected above the MDL in the 43 maternal serum samples, and at least 1 PBDE was detected in every sample (Table 3). The congeners frequently detected included: BDE 28/33, 47, 99, 100, 153, 154, and 183. Total PBDEs ranged from 0.71–252 ng/g lipid in the serum samples. Total PBDEs, BDE-47, BDE-100, and BDE-99 were log-normally distributed, but BDE-154 and BDE-153 were neither normally nor log-normally distributed.

BDE-47 was the most abundant congener, contributing approximately 50 % of the total BDE burden in the serum samples. BDE-47 was detected in 98 % of samples with a geometric mean of 13.4 ng/g lipid (95 % confidence interval, 10.0–18.1). The second most abundant congeners were BDE-153 and BDE-99, with geometric mean levels of 3.8 ng/g lipid (95 % CI, 2.8–5.2) and 3.6 ng/g lipid (95 % CI, 2.6–5.1), respectively. These PBDE levels and patterns were similar to those previously reported for this cohort, with the exception of BDE-153, which was lower in the current study [25]. The lower BDE-153 levels are presumably due to the low method accuracy as reflected in the SRM 1958 sample analysis. In addition, the PBDE congener patterns in the current study are similar to those from other regions of North America [6, 7].

PBDE levels from the current North Carolina cohort were lower than those reported in the 2003–2004 National Health and Nutrition Examination Survey (NHANES) from the USA [36]. The NHANES 2003–2004 dataset is the most recent national representative dataset for which samples were analyzed on the individual level. For example, the 2003–2004 NHANES 20–39 year age group levels were 1.6-fold and 1.4-fold lower in the more recent North Carolina cohort for BDE-47 and BDE-99, respectively. The NHANES cohort includes individuals from diverse race/ethnicity groups, whereas our cohort was predominantly African-American. However, PBDE levels in the NHANES non-Hispanic Black, female sub-group were higher than those of the overall pooled mean for the full NHANES sample, suggesting that racial/ethnic differences were not responsible for the higher NHANES levels. The North Carolina cohort was collected at a significantly later time period (2008–2010), and PBDE serum levels have generally shown decreasing trends in the USA since the early 2000s [7, 38], which may explain some of the variability between the NHANES and NC samples. In addition, differences in the geographic scope of study areas and the representation of low-income individuals between the cohorts may be responsible for the observed differences. Similarly, PBDE serum levels from the 2007–2009 Canadian Health Measures Survey (20–39 year group) were 1.6-fold higher for both BDE-47 and BDE-99, respectively [6]. Again, these differences may be driven by geographic, temporal, or socioeconomic differences.

OH-BDEs and TBPs—Five OH-BDE congeners were monitored in the present study, but only 5'-OH BDE 99 and 4'-OH BDE 49 were detected in >50 % of the samples. Geometric mean levels were 0.062 ng/g lw and 0.029 ng/g lw for 5'-OH BDE 99 and 4'-OH BDE 49, respectively. These levels were 1–2 orders of magnitude lower than the PBDEs; this finding is consistent with previous studies in human serum [8, 19, 25, 38]. The 4'-OH BDE 49 levels in our cohort (geometric mean = 29 pg/g lw) were somewhat lower than those reported from a different subset of the study cohort (geometric mean = 110 pg/g lw) [25]. It is difficult to directly compare the OH-BDE levels in our study to those from other regions since most report levels on ng (or pg) per ml basis. However, comparing wet weight concentrations (ESM Table S1) shows that the 4'-OH BDE 49 and 5'-OH BDE 99 levels in our cohort were ~10- to 60-fold lower than from other regions in the USA [8, 38, 39]. The higher concentrations in the previously published studies [38, 39] may be due to the fact that the cohorts were from California, which is known to have higher PBDE exposure due to California Technical Bulletin 117.

2,4,6-TBP was detected in 100 % of samples, but 2,4, 5-TBP was not detected in any sample. Interestingly, the levels of 2,4,6-TBP were greater than individual PBDE congeners. For example, the 2,4,6-TBP geometric mean was 19.2 ng/g lw and the BDE-47 geometric mean was 13.4 ng/ g lw. Although studies are limited, 2,4,6-TBP has also been reported in serum from Japan [40], Hong Kong [19, 41], Norway [42], Pakistan [27], northern Quebec [43], and the USA [16]. However, reported 2,4,6-TBP concentrations vary greatly among studies. For example, Qui et al. [16] reported mean 2,4,6-TBP levels of 0.8 ng/g lw in maternal serum from Indiana in 2003–2004, ~25-times lower than our study. 2,4, 6-TBP exposure is not well understood, but can originate from multiple sources. 2,4,6-TBP is used as a flame retardant and is an intermediate in the production of other flame retardants chemicals [44]. For example, 2,4,6-TBP was recently detected in a novel brominated triazine-based flame retardant—2,4, 6-tris(2,4,6-tribromophenoxy-1,3,5-triazine (TTBP-TAZ), a compound used in various electronics plastics [45]. As such, 2,4,6-TBP has been detected near e-waste sites [46], as well as in indoor air [47]. In addition, 2,4,6-TBP is formed naturally in marine algae [48] and sponges [49] and can bioaccumulate in higher trophic level marine mammals [49]. Finally, 2,4, 6-TBP could also be formed through the metabolism of certain PBDEs, in a mechanism similar to the formation of 2,4, 5-TBP from BDE-99 [50, 51].

HBCDs—HBCDs were infrequently detected (7 % of individuals), with only the α -HBCD isomer observed (range, <0.02–3.0 ng/g lw). Dominance of the α -HBCD isomer in serum is consistent with previous studies with the exception of a recent study from South Korea that showed the γ -HBCD as the dominant isomer. HBCDs are widely detected in house dust, at levels comparable to PBDEs [5], suggesting that the low serum levels may be due to poor uptake or rapid metabolism. Overall, there are few literature reports of HBCDs in human serum, but levels are generally within the range observed in the present study [6, 52–54]. In contrast to our findings, a recent Canadian study observed 100 % detection of α -HBCD and 23 and 35 % detection of β - and γ -HBCD, respectively (geometric mean Σ HBCD = 0.85 ng/g lipid) [6].

EH-TBB and BEH-TEBP—EH-TBB and BEH-TEBP were not detected in any serum sample, but these analytes had relatively high MDLs (56,000 pg/g lw for EH-TBB and 24,000 pg/g lw for BEH-TEBP). To our knowledge, only one other study has investigated EH-TBB and BEH-TEBP in human serum [55]. The previously reported cohort comprised nursing women from Quebec, Canada and collected samples in 2008–2009 ($n = 102$). The detection frequencies for the Quebec cohort were 57 % for EH-TBB (geometric mean concentration = 1300 pg/g lw) and 17 % for BEH-TEBP (geometric mean not reported) [55]. Although exposure to EH-TBB via house dust appears to be ubiquitous [3, 5], EH-TBB is rapidly metabolized to TBBA [56] which may explain the non-detect serum levels in our cohort.

Associations between PBDEs, OH-BDEs, and 2,4,6-TBP—Considering the lipid normalized concentrations, all PBDEs and Σ PBDE were highly correlated with Spearman rank correlation coefficients ranging from 0.40–0.95 ($p < 0.05$ for all relationships) (Fig. 3 and Table 4). Since the lipid levels were not associated with the PBDE and 2,4,6-TBP levels in our cohort, Spearman coefficients were also calculated using non-lipid normalized concentrations and negligible differences were observed. These trends suggest a common exposure source such as house dust [57]. Further, 4'-OH BDE 49 and 5'-OH BDE 99 were correlated with most BDEs. A notable exception was that 5'-OH BDE 99 was not correlated with BDE 99 ($p = 0.14$). This was unexpected considering that 5'-OH BDE 99 is metabolite of BDE 99 [50]. As well, 2,4,6-TBP and the PBDE/ Σ PBDE congeners were correlated with the exception of BDE-47. These relationships were not as strong as among the individual PBDE congeners. This may indicate multiple exposure sources or that some of the PBDEs are metabolizing to 2,4,6-TBP. To our knowledge, this is the first report describing the relationships between 2,4,6-TBP and PBDE congeners in blood. However, a recent study from a Hong Kong cohort showed a positive relationship between Σ 2,4,6-TBP-glucuronide and sulfate conjugates in urine and Σ 2,4,6-tribromo-BDEs in human serum [41].

Conclusions

A comprehensive analytical method was developed to measure legacy PBDEs and their hydroxylated metabolites, as well as HBCDs, EH-TBB, and BEH-TEBP in human serum. The nearly ubiquitous detection of PBDEs in serum indicates that, despite their phase-out in the mid-2000s and 2013, PBDEs continue to be present in human tissues. Future monitoring is essential. In addition, two metabolites of PBDEs—5'-OH BDE 99 and 4'-OH BDE 47—were broadly detected. 2,4,6-TBP was ubiquitously detected at levels that were generally greater than individual PBDE congeners. The detection of OH-BDEs and 2,4,6-TBP is important because these compounds have been shown to inhibit thyroid hormone regulating enzymes, with potencies greater than the PBDEs [58, 59]. There are very few reports of 2,4,6-TBP in human serum; future monitoring should be expanded to include this compound. Finally, as newer and alternative flame retardants are introduced into the marketplace, existing analytical techniques will need to be modified to expand monitoring programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank all of our participants in this study. We also would like to thank Dr. Pamela Maxson for managing the recruitment effort and to Claire Osgood for data management support. This work was supported by a grant from the National Institute of Environmental Health Sciences, R01 ES020430, and a grant from the USEPA (RD-83329301).

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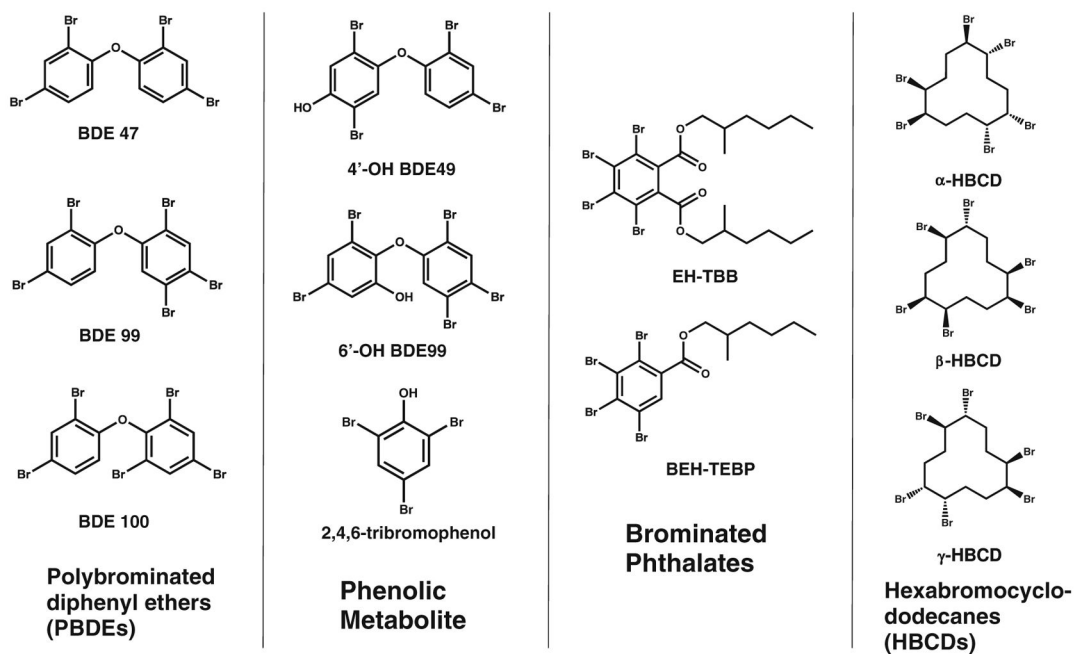


Fig. 1.
Representative chemical structures for target analytes

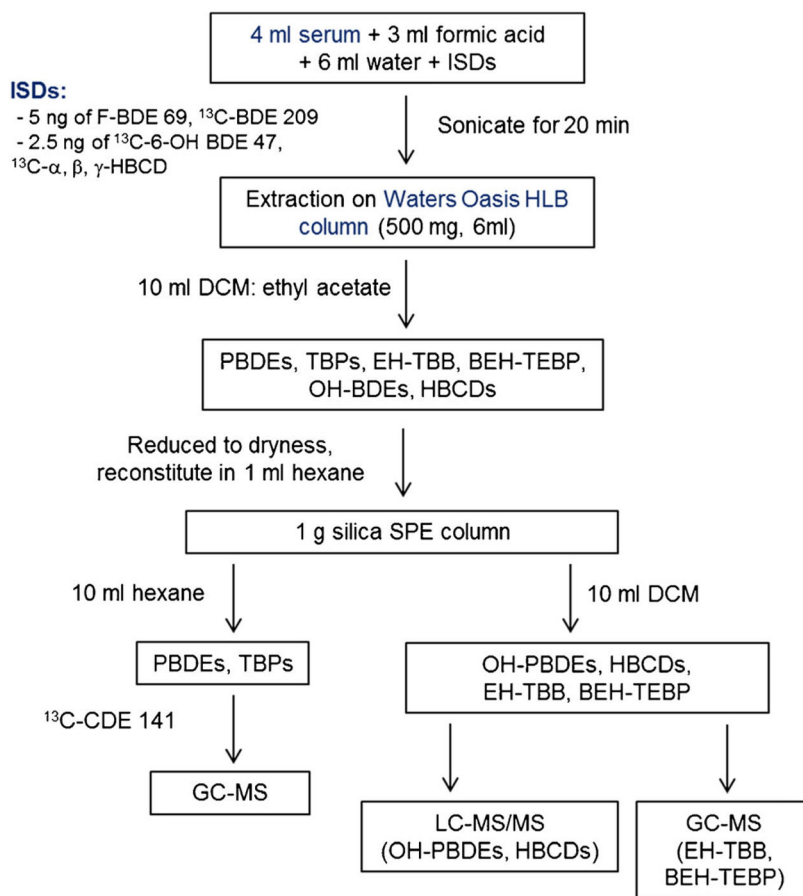


Fig. 2.
Flow chart of extraction and analysis methods

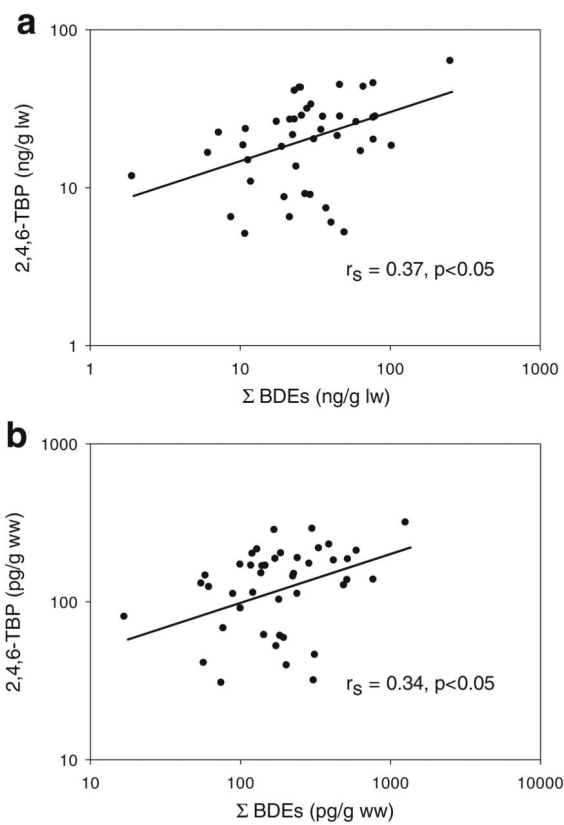


Fig. 3. Scatter plot 2,4,6-TBP versus ΣBDEs in serum from the HPHB cohort in North Carolina using **a** lipid normalized and **b** non-lipid normalized concentrations

Table 1

Analyte recovery (%) in fetal bovine serum matrix spikes

	Mean	Std Error
BDE 30	87	3
BDE 17	97	5
BDE 25	106	4
BDE 28,33	82	3
BDE 75	74	6
BDE 49	n/a	n/a
BDE 71	85	1
BDE 47	71	6
BDE 66	90	6
BDE 100	79	6
BDE 119	81	5
BDE 99	61	5
BDE 116	83	4
BDE 85,155	79	5
BDE 154	75	5
BDE 153	74	5
BDE 138	81	5
BDE 156	77	5
BDE 183	60	4
BDE 191	57	4
BDE 181	59	3
BDE 190	61	3
BDE 203,200	53	9
BDE 205	40	2
EH-TBB	111	4
BEH-TEBP	101	5
2,4,6-TBP	93	5
α -HBCD	87	15
5'-OH BDE 99	94	7
6-OH BDE 99	89	4
3-OH BDE 47	64	8
4'-OH BDE 49	59	3
6-OH BDE 47	75	0.3

$n = 5$ for PBDEs; $n = 3$ for EH-TBB, BEH-TEBP, 2,4,6-TBP, α -HBCD and OH-BDEs

Spike levels: PBDEs, EH-TBB, BEH-TEBP = 1–2 ng; 2,4,6-TBP = 2 ng; α -HBCD and OH-BDEs = 1 ng

For the FBS matrix spikes only, BDE 49 and BDE 71 co-eluted and could be quantified separately

Table 2

Method performance (percent accuracy) for PBDEs in NIST SRM 1958

	SRM 1	SRM 2	SRM 3	Mean (SE)
BDE 17	51	51	60	54 (3.2)
BDE 28/33	80	80	88	82 (2.6)
BDE 47	76	77	85	80 (2.8)
BDE 66	74	79	86	79 (3.5)
BDE 100	71	74	85	77 (4.1)
BDE 99	64	64	73	67 (2.9)
BDE 85/155	69	75	82	75 (3.8)
BDE 154	90	100	107	99 (5.2)
BDE 153	42	45	50	46 (2.4)
BDE 183	20	25	25	23 (1.4)

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Table 3

Serum concentration (pg/g lw)

Variable	MDL (pg/g lw)	Detection frequency (%)	Geometric mean (95 % CI)	Min	Max	Percentile			
						25th	50th	75th	95th
PBDEs									
BDE 28/33	360	49	NA	<360	2600	NA	NA	NA	NA
BDE 47	1100	98	13,400 (1000–18,100)	<1100	122,000	7600	13,700	25,300	48,400
BDE 66	1300	0	NA	<1300	<1300	NA	NA	NA	NA
BDE 99	750	93	3600 (2600–5100)	<750	78,300	2100	3500	7000	31,700
BDE 100	490	93	2500 (1800–3500)	<490	27,800	700	3000	5800	14,300
BDE 153	240	98	3800 (2800–5200)	<240	22,100	2300	4400	6900	15,100
BDE 154	140	81	430 (310–620)	<140	7500	200	600	900	1700
BDE 183	310	5	NA	<310	1800	NA	NA	NA	NA
ΣPBDEs			27,500 (21,000–35,800)	710	252,000	19,000	27,200	46,300	97,800
Phenolic metabolites									
2,4,5-TBP	1200	0	NA	<1200	<1200	NA	NA	NA	NA
2,4,6-TBP	1800	100	19,200 (15,700–23,400)	5100	63,400	11,800	21,500	28,200	45,600
5'-OH BDE 99	2.1	53	62 (34–110)	<2.1	1800	10	30	330	1200
6-OH BDE 99	5.6	0	NA	<5.6	<5.6	NA	NA	NA	NA
3-OH BDE 47	20	5	NA	<20	610	NA	NA	NA	NA
4'-OH BDE 49	1.4	58	29 (19–46)	<1.4	370	10	50	120	220
6-OH BDE 47	340	0	NA	<340	<340	NA	NA	NA	NA
Brominated phthalates									
EH-TBB	56,000	0	NA	<56,000	<56,000	NA	NA	NA	NA
BEH-TEBP	24,000	0	NA	<56,000	<56,000	NA	NA	NA	NA
Hexabromocyclodecanes									
α-HBCD	84	7	NA	<84	3000	NA	NA	NA	NA
β-HBCD	84	0	NA	<84	<84	NA	NA	NA	NA
γ-HBCD	84	0	NA	<84	<84	NA	NA	NA	NA

Table 4
Spearman rank correlation matrix for PBDEs (ng/g lw) with frequency of detection >50 %

Variable	BDE-47	BDE-100	BDE-99	BDE-153	BDE-154	ΣBDEs	2,4,6-TBP	4'-OH BDE49
PBDEs								
BDE-47								
BDE-100	0.94##							
BDE-99	0.82##	0.80##						
BDE-153	0.45**	0.60##	0.48#					
BDE-154	0.66##	0.63##	0.65##	0.40**				
ΣBDEs	0.95##	0.95##	0.89##	0.71##	0.63##			
Phenolic metabolites								
2,4,6-TBP	0.29	0.37**	0.30*	0.43**	0.30*	0.37*		
4'-OH BDE 49	0.47**	0.43**	0.25*	0.19	0.17	0.41**	0.01	
5'-OH BDE 99	0.36*	0.39**	0.23	0.38*	0.11	0.34*	-0.03	0.57#

* $p < 0.05$,** $p < 0.01$,# $p < 0.001$,## $p < 0.0001$