Serum PBDE Levels Measured In Pregnant Women: Associations with Thyroid Hormones

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Introduction

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants that have been extensively used in consumer products to reduce their flammability and meet regulatory flammability standards. The chemical structures of PBDEs are very similar to polychlorinated biphenyls, and thus they share similar properties, including the capacity to bioaccumulate and biomagnify in food webs and elicit endocrine disrupting and neurodevelopmental effects in laboratory-exposed organisms. While two of the three PBDE commercial formulations have been phased out or banned in different regions of the world, PBDEs continue to be detected in human tissues. Levels in the U.S. and Canada are significantly higher than any other region of the world examined, suggesting higher exposures in North America. This higher exposure is believed to result from exposure to flame retardants present in indoor dust that may be inadvertently ingested or inhaled.

Given the concerns about endocrine disruption and neurodevelopmental effects from PBDEs, it is important to evaluate prenatal exposure to this persistent class of contaminants. Very few studies have measured body burdens of PBDEs in pregnant women, particularly in the U.S. This study was undertaken to investigate the levels of PBDEs in serum collected from pregnant women late in their third trimester and to determine if there were any significant associations with thyroid hormone levels. Here we present PBDE data from the first 79 participants enrolled in our study.

Materials and Methods

Participant Recruitment The Duke University Institutional Review Board approved a research protocol for recruiting pregnant women into this study. Participants in this study were recruited within an ongoing parent study assessing the effect of social, environmental, and host factors on pregnancy outcomes (the Healthy Pregnancy, Healthy Baby Study). A subset of enrolled patients, (greater than 34 weeks pregnant) were approached during their pre-natal appointments at the Durham County Health Department's Prenatal Clinic which is held at the Lincoln Community Health Center in Durham, NC and asked if they wished to participate. Consenting individuals filled out a short questionnaire about lifestyle characteristics, and two tubes of blood were collected during their visit. Blood samples were collected in 4 mL and 10 mL tubes. Both tubes were centrifuged at 3500 RMP for 5 minutes to isolate the serum. The 10 mL tubes (approximately 4-5 grams of serum) were then stored at -20°C until analysis. The 4 mL tubes were sent to the Duke Clinical Laboratory at Duke Hospital for analysis of Thyroid Stimulating Hormone (TSH), free and total thyroxine (T4), and free and total triiodothyronine (T3) using standard radioimmunoassays. Results presented here are from samples collected between September 2008 and June 2009.

Chemicals. All solvents used for the analysis were HPLC-grade or better. A fluorinated BDE standard, 4'-2,3',4,6-tetrabromodipheyl ether (FBDE-69) (Chiron Inc., Trondheim, Norway) and ^{13}C labeled BDE-209 (Wellington Laboratories, Guelph, Ontario) were used as internal standards. The recovery of FBDE-69 was assessed by addition of ^{13}C labeled 2,2',3,4,5,5'-hexachlorinated diphenyl ether (Cambridge Isotope Laboratories, Andover, MA). PBDE calibration solutions were prepared from neat standards purchased from Accustandard Inc. (New Haven, CT).

Sample Extraction Serum samples analyzed at Duke University were extracted using a previously published method (*1*). Serum samples were transferred to 50 mL centrifuge tubes and spiked with the two internal standards, FBDE-69 (25 ng) and ^{13}C BDE 209 (15 ng). The serum was extracted and cleaned and then reduced in volume to 0.5 mL, transferred to a GC auto-sampler vial and spiked with 25 ng of the recovery standard, ^{13}C CDE-141.

Sample Analysis Extracts were analyzed for PBDEs using a gas chromatograph (GC, Agilent 5890) coupled to a mass spectrometer (Agilent 5975) operated in electron capture negative ionization (GC/ECNI-MS) mode. The method information is reported in Stapleton et al (*2*).

*Quality Control***.** Both bovine serum and deionized water were used as a laboratory blanks. SRM 1958 (NIST, Gaithersburg, MD) was used for quality control and to test performance of the extraction method. Minor laboratory contamination with BDE-47, BDE-99, and BDE-209 was observed and all samples were blank subtracted using the average blank measurement for these three congeners. Method detection limits (MDL) were calculated at 3 times the standard deviation of the blanks divided by the sample size for BDE-47, BDE-99, and BDE-209. The MDLs for the remaining congeners were determined by calculating the mass equivalent to three times the noise and dividing by the sample size. Recovery of the internal standard, FBDE-69 averaged 84 ± 14%, and recovery of the BDE congeners in SRM 1958 ranged from 67 to 128%.

Results and Discussion

PBDEs were detected above MDLs in all samples analyzed. The primary congeners measured in these samples includes BDEs 28, 47, 49, 66, 99, 100, 153, 154, 85/155, and 183. Total PBDE concentrations ranged from 1.1 to 736 ng/g lipid and the geometric mean value was 34.1 ng/g lipid. Only congeners 28, 47, 99, 100, 153, and 154 were detected in more than 50% of the samples (Table 1). The congeners that contributed the greatest amount to the total PBDE concentrations were BDE 47>BDE 153>BDE 100~BDE 99. The geometric mean value for BDE 47 was 14.9 ng/g lipid and in the same range as the geometric mean value of 19.6 ng/g lipid determined for females in the 2003/2004 National Health and Nutrition Examination Survey (NHANES) (Sjodin, et al 2008). Our samples presented here were collected in 2008-2009, 4-6 years after the collection of the samples in the NHANES project. The lower geometric mean value measured in our study may suggest that PBDE serum levels are decreasing following the phase out and voluntary withdraw of the commercial flame retardant mixture know as PentaBDE in the U.S in 2004. The primary BDE congeners detected here (i.e. BDE 47, 99, 100 and 153) were present in PentaBDE and are the likely source of exposure for most individuals.

Previous reports of PBDE levels and congener patterns in human tissues have frequently observed significant contributions of BDE 47 and BDE 99, with lesser contributions from the higher brominated congeners like BDE 153(*3-4*). However, recent reports have now begun to identify a shift in BDE congener patterns with increasing detection of samples with greater proportions of BDE 153 to the total composition (*5-6*). Several hypotheses have been offered to explain this shift, including, metabolic debromination of BDE 209 to form BDE 153, shifts in the commercial mixtures that are being used, and longer half-lives of BDE 153 relative to BDE 99 and BDE 47. Due to the fact that PentaBDE and OctaBDE were phased out from use or banned in 2004, this second hypothesis is unlikely. BDE 153 is comprised of a 2,2',4,4',5,5'-hexabromo-substitution pattern, and is very similar in structure to the recalcitrant PCB 153 which has a very long half-life in human tissues. In this data set, approximately 20% of the samples had higher levels of BDE 153 relative to BDE 47, which is in contrast to previous studies which consistently observed higher proportions of BDE 47 relative to BDE 153. This may suggest that chronic exposure to PentaBDE may be subsiding and that this shift in patterns is due to increased metabolism of the lower molecular weight congeners relative to BDE 153.

Associations between PBDE levels and thyroid hormone measurements were examined for any significant correlations. The only significant association was between the contribution of BDE 153 to total PBDE levels and free thyroxine levels ($r = -0.26$, $p=0.02$). No association between BDE 153 and free T4 was observed. Because BDE 153 has a longer half-life than most of the other common BDE congeners found in tissues, a higher contribution of BDE 153 to the total PBDE burden in some individuals may reflect a longer time period between the participants exposure to PBDEs and the time in which they participated in the study. It is possible that exposure to the more recalcitrant BDE congeners, and/or increased metabolism of the lower weight congeners contribute to this affect on thyroid hormone levels. Previous studies have demonstrated that the PBDE metabolites are a more competitive substrate for thyroid hormone transporters found in serum relative to the parent PBDE chemicals (*7*). In addition, laboratory studies have also observed a depression of free thyroxine upon exposure to PBDEs (*8-10*).

In addition to PBDEs, several alternate flame retardants are currently being measured in these serum samples. These alternate flame retardants include hexabromocyclododecane (HBCD), dechlorane plus (DP) and the two brominated components of Firemaster 550 referred to as 2-ethylhexyl-tetrabromobenzoate (TBB) and bis(2-ethylhexyl)-tetrabromophthalate (TBPH) (*11*). Samples are being pooled and re-analyzed for these compounds and will be presented at the conference. This will be the first report of DP, TBB and TBPH in serum samples collected from the U.S.

Table 1. PBDE concentrations (ng/g lipid) measured in serum from pregnant women (n=79).

Congener	Detection Frequency (%)	MDL	Minimum	Maximum	Geometric Mean	95th Percentile
BDE 28	**52**	**1.9**	**<MDL**	**7.8**	**1.6**	**6.3**
BDE 47	**91**	**3.4**	**<MDL**	**297**	**14.9**	**95.4**
BDE 99	**60**	**3.3**	**<MDL**	**249**	**5.5**	**40.2**
BDE 100	**95**	**0.74**	**<MDL**	**107**	**4.4**	**20.8**
BDE 85,155	**18**	**0.74**	**<MDL**	**10.5**	**NA**	**NA**
BDE 153	**96**	**0.74**	**<MDL**	**67.6**	**5.7**	**36.7**

BDE 154	**57**	**0.74**	**<MDL**	**52.9**	**1.1**	**7.1**
BDE 183	**3**	**0.74**	**<MDL**	**5.2**	**NA**	**NA**
ΣPBDEs			**1.1**	**736**	**34.1**	**163.4**

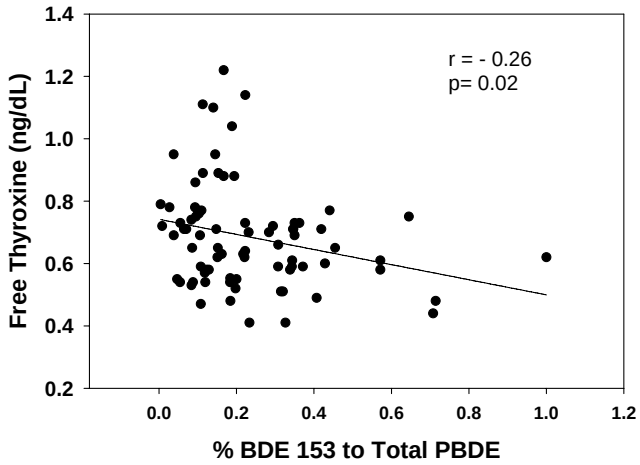

Figure 1. Correlation between the contribution of BDE 153 to total PBDE burden with the levels of free thyroxine in serum.

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