CONCENTRATIONS AND SPECIATION OF POLYBROMINATED DIPHENYL ETHERS (PBDE) IN HUMAN AMNIOTIC FLUID

Miller MF[1], Chernyak SM[1], Domino SE[2], Batterman S[1], Loch-Caruso R[1]

[1]Department of Environmental Health Science, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109; [2]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan 48109

Keywords: Polybrominated diphenyl ethers (PBDEs), amniotic fluid, human exposure, pregnancy

Abstract

Polybrominated diphenyl ethers (PBDEs) are persistent organic chemicals used as flame retardants in textiles, plastics, and consumer products. Many of the 209 PBDE congeners have been classified as persistent organic pollutants at the 4th meeting of the Convention of Parties of the Stockholm Convention on Persistent Organic Chemicals. Although PBDE accumulation in humans has been noted since the 1970s, few studies have investigated PBDEs within the gestational compartment and none to date has identified levels in amniotic fluid. This study reports congener-specific PBDE concentrations in second-trimester amniotic fluid from fifteen women in southeast Michigan, USA. BDEs 17, 28, 47, 49, 66, 71, 75, 85, 99, 100, 138, 153, 154, 166, 183, 190, 203, 206, 207, 208 and 209 were measured by GC/MS. The average total PBDE concentration was 3,795 pg/ml amniotic fluid (range 337 – 21,842 pg/ml). High levels of both higher and lower brominated PBDEs were identified. The most abundant congeners were BDE-138 (15% of the total BDE), 207 (15%), 47 (10%), 208 (9.5%), and deca (8.4%). BDEs 47 and 99 were identified in each sample. These results show that high PBDE concentrations in amniotic fluid are common, and they suggest that amniotic fluid may participate in the transfer of PBDEs to the fetus, a previously undocumented exposure route. The relatively high levels of PBDEs found in the human gestational compartment warrant further investigations of exposure pathways and potential impacts to birth outcomes and perinatal health.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of widely used brominated flame retardants that have been incorporated into many consumer electronics, textiles and furniture. These compounds are not chemically bound and thus may leech into the environment. Although production of penta-BDE (tri- to hexa-BDE mixtures) and octa-BDE (hexa- to nona-BDE mixtures) ceased in the United States in 2004, PBDEs remain a human health concern due to production in other regions, import/export of goods containing PBDEs, the wide stock of PBDE-containing materials remaining in use, and PBDE environmental persistence[1]. In addition, deca-BDE (nona- to deca-BDE mixtures) production continues at increasing rates.

Because of their environmental persistence and toxicity, the US EPA has identified PBDEs as a priority human health concern[2]. Recently, the 4th meeting of the Convention of Parties of the Stockholm Convention on Persistent Organic Chemicals listed tetra-, penta-, hexa- and hepta-BDEs as persistent organic compounds, effectively banning their use in over 160 countries[3]. Currently, the human reproductive and developmental health risks associated with PBDE exposure have not been thoroughly addressed and many uncertainties remain. Animal studies show that PBDEs exhibit neurodevelopmental[4,5] hepatic[6,7], immunological[8,9] and thyroid toxicity[7]. Rabbits orally exposed to PBDEs show decreased gestation length[10]. Further research is needed to identify how these animal findings translate to humans and to understand the dose-response relationships for both individual PBDEs and PBDE mixtures.

Human amniotic fluid plays an important role in gestation. Amniotic fluid begins to collect within the amniotic cavity during the third week of gestation and increases in volume through the 35th week of gestation. During the first 20 weeks of gestation amniotic fluid is primarily formed through maternal secretion. The volume of fluid increases throughout the second trimester from approximately 200 ml at 16 weeks gestation to a peak of 1000 ml at 28 weeks gestation[11]. Amniotic fluid surrounds the fetus until birth, providing nourishment from early stages of pregnancy until birth. Throughout gestation, the fetus is continuously swallowing and "inhaling" amniotic fluid. Intake into the gastrointestinal and respiratory tracks along with dermal exposure provide a direct route of transport into the fetus for toxicants that enter the amniotic compartment. Historically amniotic fluid analysis has been used to confirm fetal exposure to nicotine and cotinine, but is not a suitable method for routine assessment of prenatal drug/toxicant exposure due to the invasive nature of sample collection potentially resulting in harm to the fetus[12].

Since the 1970s, many researchers have reported PBDE concentrations and accumulation in human tissues (reviewed by Frederiksen 2009)[13]. Although the human biomonitoring data primarily focuses on breast milk and sera, several studies have begun to address the partitioning among and between gestational compartments through paired sampling of maternal blood, fetal blood and placenta[14-18]. Recently, we identified PBDE accumulation in human gestational membranes[19]. To date, however, the potential for PBDEs to accumulate in human amniotic fluid has not been addressed.

The aim of this study was to measure PBDE profiles, including congener-specific and total PBDE concentrations, in amniotic fluid collected from women in southeast Michigan, USA. This information will help to evaluate the significance of PBDE transport into amniotic fluid and from the amniotic fluid into other gestational matrices, including the developing fetus.

Materials and Methods

Sample collection. In February and March 2009, excess clinical amniotic fluid samples, typically 15 ml, were collected at the University of Michigan Women's Hospital in Ann Arbor, Michigan, USA. Deidentified samples containing excess amniotic fluid from 15 women undergoing clinically-indicated amniocentesis for identification of fetal genetic aberrations during their second trimester were analyzed for PBDE profiles. An average volume of 15 ml of amniotic fluid per sample was received in the cytogenetics laboratory and centrifuged at 280 g for 10 minutes to remove the cellular component. The excess supernatant was transferred into tubes without patient identification and stored at -80 °C. The resulting supernatant was used for all analyses.

The 15 women represent an anonymous sample where personal identifiable data were not collected, in compliance with the University of Michigan Institutional Review Board requirements. The investigators had no direct interaction with the human subjects. All amniotic fluid specimens used in this study would have been otherwise discarded.

Lipid analysis. Amniotic fluid supernatants were evaluated independently for lipid content. For each amniotic fluid sample, 1 ml of amniotic fluid was denatured with 1 ml HCl followed by 6 ml isopropyl alcohol. Samples were then extracted using 5 ml methyl tert-butyl ether and hexane (1:1). Sample fractions were separated by centrifugation and the organic fraction collected. The extraction process was repeated three times and organic fractions were combined in a previously weighed beaker. The organic fraction was volatilized under a flowing nitrogen stream until dry, and then baked at 100°C for 12 h to remove all water. Beakers were reweighed and lipid weight per ml amniotic fluid was calculated.

PBDE analysis. Amniotic fluid supernatants were evaluated for 21 PBDE congeners. For each amniotic fluid sample, 7 ml of amniotic fluid was denatured with 2 ml HCl, followed by 12 ml isopropyl alcohol. Samples were then extracted using 10 ml methyl tert-butyl ether and hexane (1:1). Sample fractions were separated by centrifugation and the organic fraction collected. The extraction process was repeated three times and organic fractions were combined and volatilized under a flowing nitrogen stream until nearly dry. Samples

were resuspended in 5 ml hexane and cleaned with 3 ml sulfuric acid. The organic fraction was removed and neutralized using sodium carbonate.

Instrumental analyses used GC/MS (Agilent 6890, Palo Alto, CA, USA), negative chemical ionization mode, a DB-5 column (30m, 0.25 mm i.d., 0.25 μm film thickness, J&W Sci, Folsom, CA, USA) and a 2 μl splitless injection. The carrier gas was helium (0.7 ml/min, inlet pressure 5.43 psi, average velocity 37 cm/s), and methane was the reagent gas. The injector was set at 280 ^{o}C. The oven temperature started at 80 ^{o}C, held for 2 min, ramped at 10 ^{o}C/min to 300 ^{o}C, and held for 40 min. Calibration standards included BDEs 17, 28, 75, 49, 71, 47, 66, 100, 99, 85, 154, 153, 138, 166, 183, 190, 203, 208, 207, 206 and 209 (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). Standards were run for a wide range of concentrations (100 to 5000 ng/ml) that encompassed the range expected in field samples. The MS was operated in selected ion monitoring mode, and quality matching routines included at least two ions. Structural verification used additional ions and confirmed spectra by requiring proper ratios of ions for each analyte. Complete details of the instrumental analysis have been published previously[20].

Quality assurance. In parallel with the analyses, field, lab and method blanks were processed and shown to be clear of contamination. Linearity and drift checks were analyzed with each sample batch. Repeat analysis of a standard injected every fifth sample varied by less than 10% and linearity plots produced r^2 values less than 0.999. Surrogate spike recoveries ranged from 82-106%.

Data analysis. Measurements falling below the method detection limit (MDL) were assigned a value of one-half the MDL. Descriptive statistics, including the mean, the standard error of the mean, median, range, and the percentage of observations above the MDL, were calculated for each congener. For each sample, total PBDE (∑PBDE) concentrations were calculated as the sum of the 21 congeners, and a grand mean was calculated as the average of the sample ∑PBDEs. Concentrations are expressed as volumetric and lipid-based fractions. Congener-specific abundances were calculated for each sample as the congener concentration divided by the total PBDE loading and expressed in percent. Sample statistics utilized the mean, median and sum of these abundances. The relationship of lipid content to BDE levels was evaluated using the Spearman rank order correlation.

Results and Discussion

Lipid content. The lipid content averaged 0.94 ± 0.03% and ranged from 0.74 to 1.25%. The lipid content reported here is lower than previous reports which show values as high as 22.5 ± 1.2%[21]. This is the result of using amniotic fluid supernatant in this study. Cellular debris, which is lipid rich, was removed by centrifugation prior to lipid and PBDE measurements.

PBDE concentrations. Concentrations varied widely among the samples, but BDE-47 and 99 were found in all samples tested. On a volumetric basis, the ∑PBDE concentration in amniotic fluid averaged 3,795 ± 1,529 pg/ml (median = 1,253 pg/ml; n=15; Table 1), and individual samples ranged from 337 to 21,842 pg/ml. On a lipid basis, this is equivalent to a ∑PBDE concentration of 404 ± 163 ng/g lipid (median = 133 ng/g).

The correlation between lipid content and ∑PBDE concentration was low and not statistically significant (p=0.86). Similarly, no correlation was found between lipid content and any of the individual congeners (p=0.15-0.99). These results are not unexpected given the small amount of variation in lipid content. For example, the highest ∑PBDE concentration (21,842 pg/ml = 2,324 ng/g lipid) was found in a specimen with a lipid content of 1.01%, just slightly above the study average. Significantly, this ∑PBDE level is comparable to the highest U. S. reports of PBDE levels in breast milk[22] (1,900 ng/g lipid), although it is below that reported in adipose tissue[23] (9,600 ng/g lipid). Interestingly, tri- to octa-congeners accounted for 82% of the ∑PBDEs in this sample, well above the 60% average for all samples.

Many factors can affect an individual's PBDE body burden and the concentrations of PBDEs found in amniotic fluid. These include variation in an individual's PBDE exposure before and during pregnancy, the

number of previous children for each woman, the timing of amniocentesis within the second trimester, and the woman's age. Due to the nature of sample collection, these factors were not controlled in this study.

TABLE 1: PBDE concentration in human amniotic fluid. (N=15)

		Amniotic Fluid Concentration (pg/ml fluid)			
PBDE	**MDL**	**Mean (SEM)**	**Median**	**Range**	**N>MDL (%)**
17	0.01	108 (91)	11	<MDL-1237	80
28	0.02	206 (206)	<MDL	< MDL -1374	27
47	0.05	380 (295)	49	9.11-4491	100
49	0.02	50 (43)	<MDL	< MDL -655	40
66	0.02	11 (6)	4.92	< MDL -90	53
71	0.01	31 (29)	<MDL	< MDL -434	33
75	0.02	12 (8)	<MDL	< MDL -119	40
85	0.01	75 (25)	24	< MDL -308	73
99	0.06	188 (95)	31	4-1124	100
100	0.05	187 (127)	37	< MDL -1916	93
138	0.01	565 (312)	37	< MDL -4456	67
153	0.03	143 (77)	18	< MDL -1119	67
154	0.02	192 (140)	36	< MDL -2134	53
166	0.01	20 (18)	<MDL	< MDL -273	20
183	0.05	28 (17)	<MDL	< MDL -263	40
190	0.05	36 (25)	<MDL	< MDL -371	27
203	0.05	177 (61)	97	< MDL -839	60
206	0.22	149 (69)	79	< MDL -957	60
207	0.22	559 (346)	71	< MDL -5213	67
208	0.22	362 (181)	189	< MDL -2598	67
209	0.65	319 (102)	133	< MDL -1286	80
∑PBDE (pg/g tissue)		3795 (1529)	1253	337-21842	
∑ Tri-octaBDEs		2407 (1183)	788	81-17977	
∑PBDE (ng/g lipid)		404 (163)	133	36-2324	
∑ Tri-octaBDEs		256 (126)	84	9-1912	

Congener abundances. The median congener abundances, interquartile range and max/min values are displayed in Figure 1. We identified a wide range of congeners in this study, and all 21 target congeners were measured above MDLs in at least three of the fifteen samples (20%). As noted earlier, BDE-47 and 99 were identified in all samples; additionally, BDE-100, 209, and 17 were identified in 93%, 80% and 80% of samples, respectively. Based on median abundances, the dominant congeners were BDE-206, 209, 203, 208 and 207, representing 23, 16, 12, 10 and 9%, respectively, of the ∑PBDEs.

The contribution of PBDE homologues are shown in Figure 2. The PBDE profile of the amniotic fluid shows a shift toward higher brominated congeners as compared to profiles reported for breast milk and serum[13]. This may be due to altered partitioning in amniotic fluid or a shift in exposure. Although the lower brominated congeners (tri-octaBDEs) are no longer produced in the United States, the homologue profile shows that they still constitute the bulk (60%) of the ∑PBDE loading. Mobilization of body burdens, exposure from the import/export of goods containing PBDEs, the wide stock of PBDE-containing materials still in-use, and

environmental persistence may explain the legacy of these congeners we see accumulating in nascent amniotic fluid.

FIGURE 1: Congener profile for PBDEs in human amniotic fluid.
(median, interquartile range, max/min; N=15)

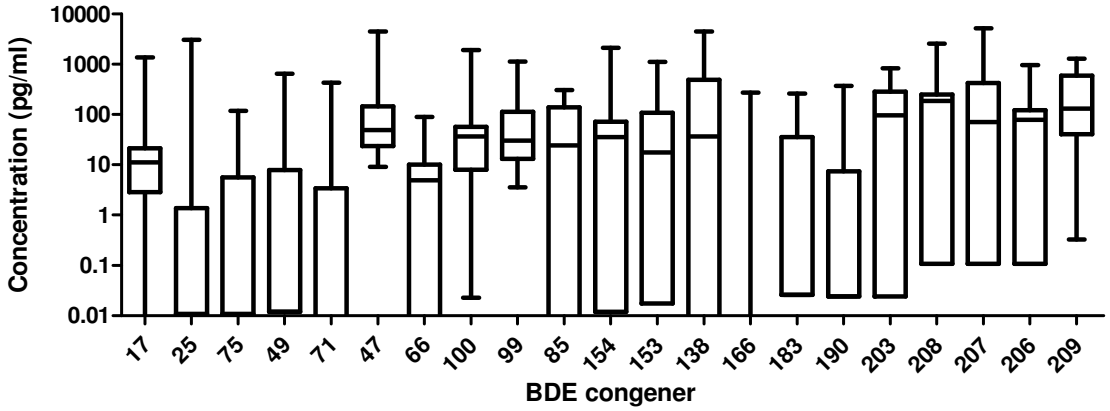
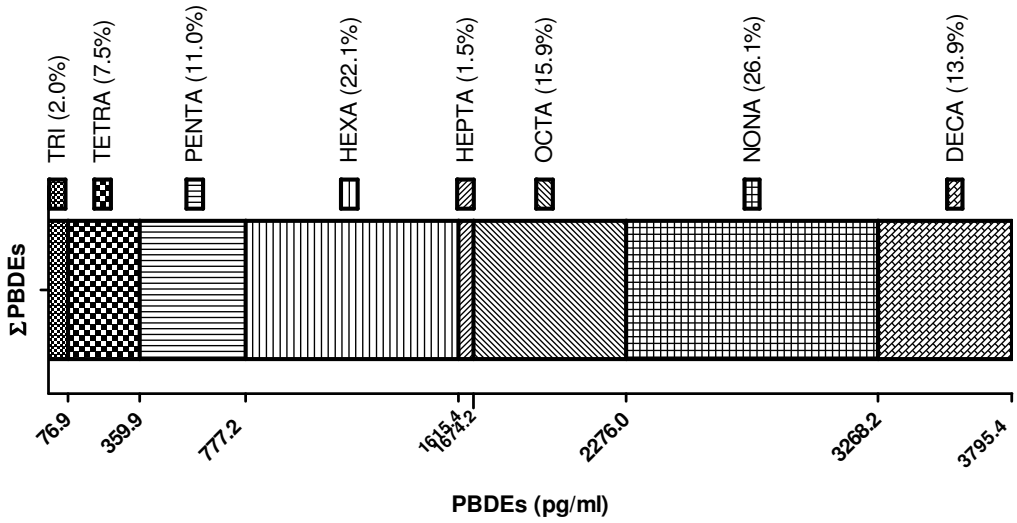


FIGURE 2: Profile of PBDE homologues in human amniotic fluid. Based on median abundances.



The contribution of PBDE homologues are shown in Figure 2. The PBDE profile of the amniotic fluid shows a shift toward higher brominated congeners as compared to profiles reported for breast milk and serum[13]. This may be due to altered partitioning in amniotic fluid or a shift in exposure. Although the lower brominated congeners (tri-octaBDEs) are no longer produced in the United States, the homologue profile shows that they still constitute the bulk (60%) of the ∑PBDE loading. Mobilization of body burdens, exposure from the import/export of goods containing PBDEs, the wide stock of PBDE-containing materials still in-use, and environmental persistence may explain the legacy of these congeners we see accumulating in nascent amniotic fluid.

The PBDE concentrations reported here add significantly to the biomonitoring of PBDEs within human populations. This study is the first to report concentrations and congener abundances for PBDEs within human amniotic fluid. Because it surrounds the fetus during gestation, amniotic fluid is possibly highly correlated to the exposure to the fetus. While our sample size is modest, the measured concentrations appear significant, and suggest the need for further investigation into the partitioning of PBDEs between amniotic fluid, the developing fetus, and the greater reproductive environment. Our results also suggest the need for further investigation of an exposure pathway not considered previously: the amniotic fluid-to-fetus route.

Acknowledgements

We gratefully acknowledge support of this work by grants to R.L.-C. from the NIH/NIEHS (ES014860) and University of Michigan, Michigan Institute for Clinical and Health Research. Additional support for M.F.M. was provided by a NIEHS Institutional Training Grant (T32 ES07062), NIH Institutional Training Grant (T32 HD007048), and the Department of Environmental Health Sciences of the University of Michigan School of Public Health. We thank Dr. Diane Roulston in the Department of Pathology at the University of Michigan for assistance collecting amniotic fluid samples.

References

1. Birnbaum L.S. and Staskal D.F. *Environ Health Perspect* 2004; 112(1): 9.
2. U.S. Environmental Protection Agency *Polybrominated diphenyl ethers (PBDES) project plan* 2006.
3. Stockholm Convention News Release 2009/04.
4. Branchi I., Capone F., Alleva E. and Costa L.G. *Neurotoxicology* 2003; 24(3): 449.
5. Viberg H., Johansson N., Fredriksson A., Eriksson J., Marsh G. and Eriksson P. *Toxicol Sci* 2006; 92(1): 211.
6. Zhou T., Ross D.G., DeVito M.J. and Crofton K.M. *Toxicol Sci* 2001; 61(1): 76.
7. Zhou T., Taylor M.M., DeVito M.J. and Crofton K.M. *Toxicol Sci* 2002; 66(1): 105.
8. Fowles J.R., Fairbrother A., Baecher-Steppan L. and Kerkvliet N.I. *Toxicology* 1994; 86(1-2): 49.
9. Thuvander A. and Darnerud P.O. *Toxicological and Environmental Chemistry* 1999; 79: 229.
10. Breslin W.J., Kirk H.D. and Zimmer M.A. *Fundam Appl Toxicol* 1989; 12(1): 151.
11. Queenan J.T. *Contemp Obstet Gynecol* 1991; 36: 60.
12. Lozano J., García-Algar O., Vall O., de la Torre R., Scaravelli G and Pichini S. *Ther Drug Monit* 2007; 29(6):711.
13. Frederiksen M., Vorkamp K., Thomsen M. and Knudsen L.E. *Int J Hyg Environ Health* 2009; 212: 109.
14. Mazdai A., Dodder N.G., Abernathy M.P., Hites R.A. and Bigsby R.M. *Environ Health Perspect* 2003; 111(9): 1249.
15. Bi X., Qu W., Sheng G., Zhang W., Mai B., Chen D., Yu L. and Fu J. *Environ Pollut* 2006; 144(3): 1024.
16. Gomara B., Herrero L., Ramos J.J., Mateo J.R., Fernandez M.A., Garcia J.F. and Gonzalez M.J. *Environ Sci Technol* 2007; 41(20): 6961.
17. Herbstman J.B., Sjodin A., Apelberg B.J., Witter F.R., Patterson D.G., Halden R.U., Jones R.S., Park A., Zhang Y., Heidler J., Needham L.L. and Goldman L.R. *Environ Health Perspect* 2007; 115(12): 1794.
18. Main K.M., Kiviranta H., Virtanen H.E., Sundqvist E., Tuomisto J.T., Tuomisto J., Vartiainen T., Skakkebaek N.E. and Toppari J. *Environ Health Perspect* 2007; 115(10): 1519.
19. Miller M.F., Chernyak S.M., Batterman S. and Loch-Caruso R. *Environ Sci Technol* 2009.
20. Batterman S., Chen T-Z., Chernyak S. and Godwin C. *J Envioron Monit* 2009; 11: 858.
21. Das S.K., Foster H.W., Adhikary P.K., Mody B.B. and Bhattacharyya D.K. *Obstet Gynecol* 1975; 45(4): 425.
22. Johnson-Restrepo B., Addink R., Wong C, Arcaro K. and Kannan K. *J Envioron Monit* 2007; 9: 1205.
23. Johnson-Restrepo B., Kannan K., Rapaport D.P. and Rodan B.D. *Environ Sci Technol* 2005; 39: 5177.