DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS IN HUMAN MATERNAL SERUM AND CORD BLOOD SAMPLES USING ACCELERATED SOLVENT EXTRACTION AND GC/EI-MS/MS

Cariton Kubwabo[1], Sandra Gregorovich[2], Rocio Monroy[2], Katherine Morrison[3], Stephanie Atkinson[3], Brian Stewart[1], Koon Teo[4] and Warren G. Foster[2]

[1]Research and Radiation Directorate, Health Canada, Ottawa, Ontario, Canada
[2]Department of Obstetrics & Gynecology, McMaster University, Hamilton, Ontario, Canada
[3]Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada
[4]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

Abstract

Accelerated solvent extraction (ASE) was used for the extraction of polybrominated diphenyl ethers (PBDEs) from biological matrices. The ASE technique proved to be efficient, simple, and rapid. Very low detection limits were achieved through the combination of ASE and GC/EI-MS/MS for the analysis of PBDEs in maternal serum (n = 101) and umbilical cord blood (n =104) samples. Ten PBDE congeners were analyzed in this study: the tri-(BDE 17, 28), tetra-(BDE 47, 66), penta-(BDE 99, 100), hexa-(BDE 153, 154), hepta-(BDE 183), and deca-(BDE-209). The mean recovery ranged from 87% to 127% for the PBDEs under investigation, with the exception of the deca-(BDE-209). Data for the latter were not reported in this study because of the high background interference observed in processing the method blank samples. PBDE-47 was found to be the major congener in both maternal serum and umbilical cord blood, with a mean concentration of 44.3, 44.7 and 76.7ng/g lipid for maternal serum at mid pregnancy, maternal serum at delivery, and in umbilical cord blood, respectively.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that have been used extensively for several decades in a wide variety of manufactured and consumer products including computers, electronics and electrical equipment, televisions, textiles, carpet, foam furniture, insulating foams, and other building materials[1,2]. PBDEs are ubiquitous and have been found worldwide in biological and environmental matrices[3]; in addition, PBDEs have been shown to be potentially toxic, persistent, and bioaccumulative[4]. Although PBDEs have been detected in cord blood and placenta in a few studies, confirming that PBDEs can cross the placenta into fetal circulation[5-9], developmental exposure in expectant Canadian mothers is unknown. In the present study, an improved method was developed to measure PBDE congeners in maternal serum at mid-pregnancy and delivery, and in umbilical cord blood in healthy pregnant women from Hamilton, Ontario, Canada.

Materials and methods

Sample collection

Study subjects were first contacted when they were presented for an obstetrical ultrasound for standard prenatal care. Women consenting to participate in the study and meeting the inclusion criteria had maternal and umbilical cord blood samples collected. Briefly, after consent was

obtained, study subjects were seen at their second trimester visit (24-28 weeks) to complete demographic and obstetrical history questionnaires. Blood was collected from 101 women at the time of their glucose tolerance test (second trimester of pregnancy) and then again at delivery. Blood was collected in two 10 mL red-topped vacutainers, allowed to clot at 4°C, then centrifuged at 1,500 rpm for 20 minutes. The serum was decanted into pre-cleaned Supelco glass vials for chemical analysis (Sigma-Aldrich Canada, Oakville, ON). At delivery umbilical cord blood (UCB) was collected from an umbilical vein immediately following delivery of the placenta. In the case of twins, one cord was marked with a clamp for twin A, the other for twin B was left unclamped. The twins were subsequently identified as twin A and twin B for all future analyses. Within one hour of collection, UCB samples were transported to the laboratory where they were allowed to clot for a minimum of 4 hours at 4°C and were then centrifuged at 1,500 rpm for 20 minutes. Serum was decanted into pre-cleaned Supelco glass vials for chemical analysis (Sigma-Aldrich). The study protocol was approved by the McMaster University Research Ethics Board. In addition, the determination of PBDEs in maternal serum and UCB collected in this study was also approved by Health Canada's Research Ethics Board.

Standards and Reagents

The ^{13}C-labelled internal standard (MBDE-MXC) and the native (BDE-MXC) solutions were purchased from Wellington Laboratories (Ontario, Canada). Hexane (residue analysis grade) was purchased from EMD Chemicals (Gibbstown, NJ). Nonane was obtained from Fluka (Sigma-Aldrich, Switzerland). Cellulose disks were obtained from Dionex Corporation (Sunnyvale, CA). Hydromatrix was purchased from Varian (Varian, U.S.). Anhydrous granular sodium sulfate was purchased from BDH (BDH, Canada) and prior to use was ground into a powder and baked at 700°C for 6 hours. Alumina 80-200 mesh was purchased from Fisher (Fisher, U.S.) and was baked at 400°C for 4 hours and then de-activated with 5% of water prior to use.

Sample Extraction

Samples were extracted using a Dionex ASE 200 Automated Solvent Extractor (ASE) system (Dionex Corporation, Sunnyvale, CA). 11 mL ASE cells were packed with two pre-cleaned cellulose disk followed by a layer of 5% deactivated alumina. Pre-cleaned cellulose disk was placed on top of alumina layer and 3 g of anhydrous sodium sulphate was added. Fourth pre-cleaned cellulose disk was inserted, followed by the addition of 1.1 g of Hydromatrix. 1.0 mL of sample and 50 µL of 100ppb internal standard solution were pipetted directly onto the Hydromatrix. Finally, the last pre-cleaned cellulose disk was put on top and the cell was sealed. The cell was then placed in the ASE with the serum sample at the top such that the extract could pass through the sodium sulfate and alumina layers to the collection vial. Extraction was carried out with hexane at 60°C and 1500 psi, using two extraction cycles per sample, 10 min each. The extracts were dried under nitrogen at 40°C and dissolved in 100 µL nonane.

GC/MS/MS

PBDE congeners were separated using an Agilent 6890 GC (Agilent Technologies, Palo Alto, CA) equipped with Agilent 7683 series autosampler and Agilent 7683B series split/splitless injector set at 260°C. The column used was 15m DB1-HT, 0.25mm I.D. with 0.1µm film thickness (Agilent Technologies, Palo Alto, CA). GC oven temperature program used was as

follows: heat from 140°C to 220°C at 10°C/min, heat to 320°C at 20°C/min and hold for 3 min. Flow rate was 1.0 mL/min. PBDE concentrations were determined using the Quattro Micro triple quadrupole mass spectrometer (Waters, Beverly, MA) in MRM operating mode. Source temperature was set at 180°C and GC interface temperature was at 270°C. MS /MS was operated in EI positive mode at 70 eV.

Method Performance

The method detection limit (MDL) was determined according to the EPA Regulation 40 CFR part 136 (Appendix B) method. Seven replicates of horse serum spiked at 0.025 ng/mL with a mixture of target analytes were processed through the entire extraction procedure and analyzed. Standard deviation associated with the analysis multiplied by the Student's t value of 3.143 appropriate for a 99% confidence level provides the method detection limit. The limit of quantification (LOQ) has been calculated as 10 times the standard deviation associated with 7 replicate analyses of an individual compound. Tables 1 and 2 summarize the method performance data.

Lipid Content Determination

Total cholesterol and triglycerides were measured by standard clinical chemistry enzymatic methods. The total lipid concentration of each sample was calculated based on the concentrations of total cholesterol and triglycerides by the following formula: Total lipid concentration = 0.9 + 1.3 x (Cholesterol + Triglycerides)[13].

Results and Discussion

The study population was composed of pregnant women presenting for their first obstetrical ultrasound assessment at McMaster University Medical Centre who enrolled in the "Family Study" and provided complete maternal and UCB samples. Maternal blood samples (n = 101) collected at 24-28 weeks of gestation and at the delivery as well as 105 serum samples from UCB (four twin pregnancies) collected at delivery were analyzed for PBDE residue levels. PBDE-28, -47 and -99 were present at concentrations above the LOQ in 100 % of serum samples from expectant mothers at mid-pregnancy and delivery. All of the UCB samples had quantifiable levels of these congeners. PBDE-66 and -183 were detected at levels above the MDL in 61-65 % and 71-78 % of the samples analyzed, respectively. The arithmetic mean concentrations and the range of 9 PBDE congeners detected in this study are given in Table 3. Of the congeners measured in maternal and umbilical cord blood, PBDE-47 was present at higher concentration, with a mean of 76.7 ± 11.9 ng/g lipid in umbilical cord blood, compared to maternal serum at mid pregnancy and delivery with a mean of 44.3 ± 9.9 and 44.7 ± 9.3 ng/g lipid, respectively.

Table 1. Limits of detection and linear calibration range

Analyte	Detection Limits		Linearity range (ng/mL)	R^2
	MDL (ng/mL)	LOQ (ng/mL)		
PBDE-17	0.03	0.10	0.1 – 25	0.998
PBDE-28	0.03	0.10	0.1 – 25	0.998
PBDE-47	0.06	0.20	0.1 – 25	0.997
PBDE-66	0.03	0.11	0.1 – 25	0.997
PBDE-99	0.05	0.16	0.1 – 25	0.999
PBDE-100	0.04	0.14	0.1 – 25	0.999
PBDE-153	0.04	0.14	0.1 – 25	0.998
PBDE-154	0.03	0.10	0.1 – 25	0.998
PBDE-183	0.05	0.17	0.1 – 25	0.998

Table 2. Percent recovery and precision of individual PBDEs from spiked horse serum

Congener	Recovery ±RSD (%)			Precision (%RSD)	
	Spiked conc.: 0.25 ppb (n = 5)	Spiked conc.: 0.5 ppb (n = 5)	Spiked conc.: 2.5 ppb (n = 5)	Intra-day (n = 10)	Inter-day (n = 30)
PBDE-17	84.2 ± 6.7	82.6 ± 5.7	99.2 ± 4.6	7.4	8.2
PBDE-28	82.0 ± 6.2	80.1 ± 6.1	97.1 ± 4.3	5.8	6.9
PBDE-47	104.9 ± 11.6	102.8 ± 6.5	113.4 ± 2.6	10.5	19.3
PBDE-66	115.6 ± 11.5	123.1 ± 9.0	133.1 ± 4.4	6.6	10.6
PBDE-99	96.2 ± 6.9	94.1 ± 3.3	105.7 ± 3.8	11.6	10.3
PBDE-100	102.0 ± 9.7	102.6 ± 9.1	114.4 ± 3.3	7.0	9.1
PBDE-153	107.6 ± 6.3	100.6 ± 4.0	115.8 ± 0.9	8.5	10.2
PBDE-154	108.3 ± 6.0	111.7 ± 3.6	121.3 ± 5.3	8.1	9.8
PBDE-183	87.4 ± 11.2	89.6 ± 7.2	106.4 ± 5.3	8.5	12.4

Table 3. Mean (± SEM) concentration of PBDE congeners in maternal and umbilical cord serum.

PBDE-Congener	Maternal serum at mid pregnancy (N =101)		Maternal serum at delivery (N = 101)		Umbilical cord blood serum (N = 104)	
	Mean±SEM	Range	Mean±SEM	Range	Mean±SEM	Range
PBDE-17	1.56±0.13	0.11 – 5.8	0.84±0.06	0 – 2.48	2.88±1.21	0 – 9.87
PBDE- 28	2.71±0.19	0.18 – 12.3	2.61±0.17	0.4 – 13.2	6.71±0.43	0.5 – 26.8
PBDE-47	44.3±9.86	9.34 – 799.3	44.7±9.31	3.76 – 653.6	76.7±11.9	13.7 – 997.1
PBDE-66	2.95±0.15	0.48 – 7.17	1.18±0.11	0 – 3.92	4.01±0.38	0 – 13.4
PBDE-99	15.0±3.04	2.85 – 276.7	14.9±2.57	0 – 230.1	25.2±2.67	4.13 – 236.3
PBDE-100	7.90±2.12	0.52 – 172.4	8.00±2.30	0 – 200.2	10.6±2.43	0 – 206.4
PBDE- 153	8.33±2.03	0.19 – 169.9	7.81±1.66	0 – 138.7	7.77±1.51	0 – 147.5
PBDE-154	2.26±0.22	0.007 – 14.4	1.75±0.21	0 – 13.3	3.20±0.29	0 – 14.2
PBDE-183	3.65±0.25	0.019 – 9.16	1.91±0.21	0 – 9.46	4.73±0.49	0 – 24.6

Results of the present study demonstrate that, in a cohort of high socio-economic status Canadian women from Hamilton, Ontario, all had exposure to PBDE-28, -47 and -99 and these chemicals crossed the placenta and were quantifiable in UBC blood. Of note, the concentrations of the PBDEs were slightly higher in UCB samples compared to maternal blood, suggesting that the placenta and the fetus may have higher exposure. The congener with the highest measured exposure in both expectant mothers and UBC blood was PBDE-47. Although exceptions have been reported describing PBDE-209 as the most common congener[12], most of the studies describe PBDE-47 as the dominant congener in serum samples, a finding that is consistent with the PBDE profile documented in our study. On the other hand, no differences have been depicted in terms of concentrations of maternal and cord serum PBDEs for most of the European studies. Figure 1 presents congener profiles of PBDE in maternal serum and in umbilical cord blood, where the mean level of each congener was determined as a percentage of the total of the 9 PBDEs detected in the samples. The pattern in Figure 1 is very similar to that observed in other studies in the US[8, 13], probably due to similar exposure sources in North America.

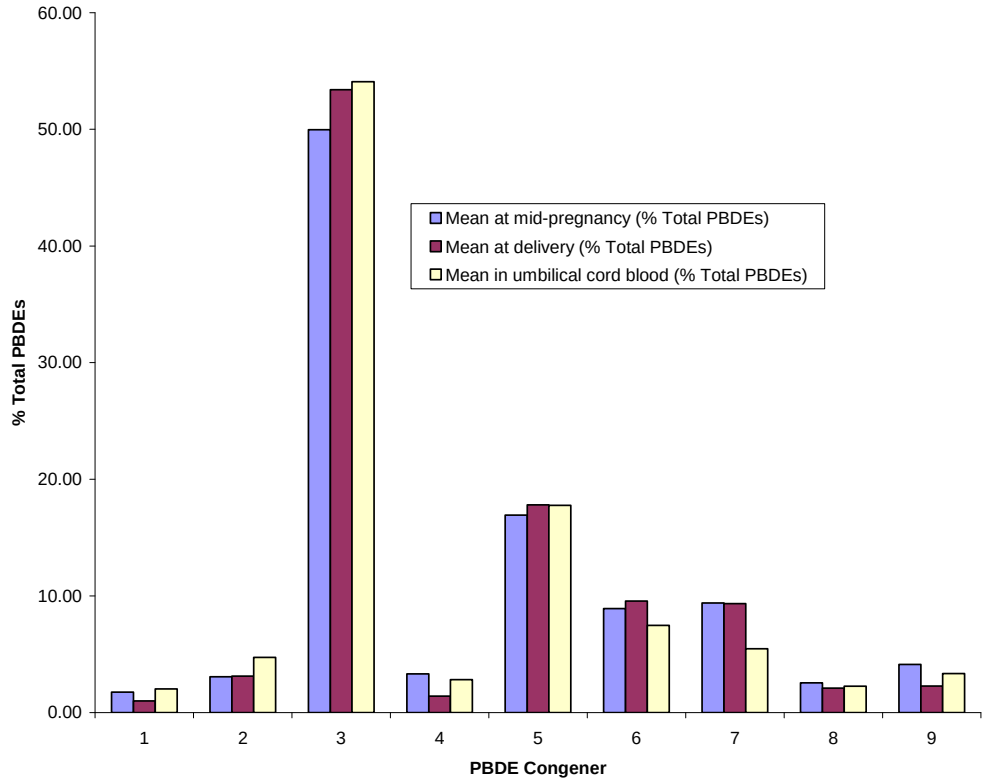

Figure 1. Congener profiles of PBDEs in maternal serum (at mid-pregnancy and term) and umbilical cord blood.

In this study, the deca-BDE (PBDE-209) was not analyzed due to very high background concentration levels. However, accelerated ASE is a simple, sensitive, reproducible, and rapid sample preparation technique for the determination of PBDEs in human serum; and DB1-HT column proved to be the most suitable for the separation of PBDEs under investigation.

Acknowledgments
We are grateful for the help of our research nurses Julie Gross, Liz Helden, Sue Steele and Mary Louise Beecroft for managing patient recruitment and sample collection for this study. Funding for the project was provided by the Canadian Institutes of Health Research (CIHR) and the American Chemistry Council (ACC). Salary support (WGF) was provided by the CIHR/Ontario Women=s Health Council. Authors thank Lisa Mattioli for running the samples.

References

1. Birnbaum L. S.; Staskal D. F. *Environ. Health Perspec*. 2004; 112: 9.
2. Alaee M.; Arias P.; Sjodin A.; Bergman A. *Environ. Int*. 2003; 29: 683.
3. de Wit, C. A. *Chemosphere* 2002; 46: 583.
4. Domingo J. L. *J. Chromatogr. A* 2004; 1054: 321.

5. Guvenius D.M., Aronsson A., Ekman-Ordeberg G, Bergman A., Norén K. *Environ. Health Perspect*. 2003; 111: 1235.
6. Mazdai, A.a , Dodder, N.G.b , Abernathy, M.P.a , Hites, R.A.b , Bigsby, R.M. *Environ. Health Perspec.* 2003; 111: 1249.
7. Carrizo D., Grimalt J.O., Ribas-Fito N., Sunyer J., Torrent M. *Environ. Sci. Technol*. 2007; 41: 4907.
8. Gómara B., Herrero L., Ramos J.J., Mateo J.R., Fernández M.A., García J.F., González M.J. *Environ. Sci. Technol*. 2007; 41: 6961.
9. Herbstman J.B., Sjödin 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., Goldman L.R. *Environ. Health Perspect.* 2007; 115: 1794.
10. Meijer, L., Weiss, J., Van Velzen, M., Brouwer, A., Bergman, A., Saure, P. *Environ. Sci. Technol*. 2008; 42: 3428.
11. Kawashiro Y., Fukata H., Omori-Inoue M., Kubonoya K., Jotaki T., Takigami H., Sakai S.-I., Mori C. *Endocrine Journal* 2008; 55: 1071.
12. Antignac, J., Cariou, R., Zalko, D., Berrebi, D., Cravedi, J., Maume, D., Marchand, P., Monteau, F., Riu, A., Andre, F., Le Bizec, B. *Environ. Pollut*. 2009; 57: 164.
13. Rylander, L., Nilsson-Ehle, P., Hagmar, L. *Chemosphere* 2006; 62: 333.