Hydroxylated Polybrominated Diphenyl Ethers and Bisphenol A in Pregnant Women and Their Matching Fetuses: Placental Transfer and Potential Risks

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · JULY 2010

Impact Factor: 5.33 · DOI: 10.1021/es1002764 · Source: PubMed

12 AUTHORS, INCLUDING:

Kyungho Choi
Seoul National University
133 PUBLICATIONS **2,204** CITATIONS
SEE PROFILE

Paul D Jones
University of Saskatchewan
186 PUBLICATIONS **6,455** CITATIONS
SEE PROFILE

Jong Seong Khim
Seoul National University
104 PUBLICATIONS **2,530** CITATIONS
SEE PROFILE

Jeongim Park
Soonchunhyang University
37 PUBLICATIONS **869** CITATIONS
SEE PROFILE

Environ. Sci. Technol. **2010,** *44,* 5233–5239

Hydroxylated Polybrominated Diphenyl Ethers and Bisphenol A in Pregnant Women and Their Matching Fetuses: Placental Transfer and Potential Risks

YI WAN,[†] KYUNGHO CHOI,[*,‡] SUNMI KIM,[‡] KYUNGHEE JI,[‡] HONG CHANG,[†] STEVE WISEMAN,[†] PAUL D. JONES,[§] JONG SEONG KHIM,[∥] SEOKHWAN PARK,[⊥] JEONGIM PARK,[#] MICHAEL H. W. LAM,[▽] AND JOHN P. GIESY[†,▽,○]

Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada, School of Public Health, Seoul National University, Seoul 151-742, Korea, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5C8B3, Canada, Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, Korea, School of Applied Sciences, Seowon University, Cheongju, Chungbuk 361-742, Korea, College of Natural Sciences, Soonchunhyang University, Asan, Chungnam 336-745, Korea, State Key Laboratory in Marine Pollution and Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong, SAR China, and Department of Zoology and Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan

Received January 26, 2010. Revised manuscript received May 10, 2010. Accepted May 11, 2010.

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) are suspected endocrine disruptors, which can pass through the mammalian placenta and accumulate in the human maternal-fetal-placental unit. However, little is known about mechanisms of placental transfer and the associated risk(s). Ten OH-PBDE congeners, bisphenol A (BPA), total 17β-estradiol (E2), and total thyroxine (T4) were quantified in blood serum from 26 pregnant women and 28 matching fetuses, including three pairs of twins from South Korea. Only 6-OH-BDE-47, a naturally occurring OH-PBDE, was detected at relatively great concentrations (maternal serum: 17.5 $\pm$ 26.3 pg/g ww, fetal cord blood serum: 30.2 $\pm$ 27.1 pg/g ww), which suggests that exposure was related to diets among Korean women. Concentrations of 6-OH-BDE-47 in maternal and cord serum were positively correlated, with concentrations being significantly greater in cord blood serum. The placental transfer ratio between fetal and maternal blood serum for 6-OH-BDE-47 (F/M ratio: 1.4 $\pm$ 1.1) was different than the observed placental transfer ratio of BPA and previously reported values for hydroxylated polychlorinated biphenyls (OH-PCBs). This result is possibly due to large affinities to T4 transport proteins. Lesser concentrations of E2 and T4 were detected in cord blood serum (E2: 4.7 $\pm$ 2.2 ng/mL, T4: 8.5 $\pm$ 1.7 μg/dL) compared to maternal blood serum (E2: 8.0 $\pm$ 3.0 ng/mL, T4: 9.7 $\pm$ 1.8 μg/dL). A major effect of OH-PBDE exposure might be a decrease in serum T4 concentrations. Potential risks associated with disruption of T4 transport to the developing fetus such as negative consequences for fetal neurological development should be considered in further studies.

Introduction

Some chemicals, released into the environment through production and usage, are known to mimic or antagonize hormonal activities at trace concentrations (*1*). Exposure to these chemicals during critical periods of development, including the prenatal period, could potentially influence growth, reproduction, and development of wildlife and humans, causing morphologic and functional alterations (*2*). Since the developing fetus might be more sensitive to the effects of chemicals than adults (*3*), it is important to understand the extent and effect of prenatal exposure to potentially toxic chemicals.

Previous studies have reported that placental transfer of more lipophilic chemicals with log octanol−water partitioning coefficients (log Kow) greater than 5 might be limited or slow (*4, 5*). Chemicals with log Kow values of −0.9 to 5 could relatively easily cross the placental membrane, and the transfer rate might depend on physical-chemical properties that affect diffusion through cell membranes. Consequently, organic compounds, such as phenolic compounds and metabolites of lipophilic chemicals, with both lipophilic and hydrophilic properties could have larger placental transfer rates (*4, 5*). Bisphenol A (BPA), a weakly estrogenic phenolic compound, passes through the mammalian placenta and accumulates in the maternal-fetal-placental unit (*2, 3, 5, 6*). Information on concentrations, placental transfer, and toxicity of BPA has been presented previously (*2, 3, 5*−*7*). This information is valuable when trying to understand the behavior of structurally similar phenolic compounds.

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) are a relatively new group of phenolic compounds that have attracted particular interest due to their biological effects including disruption of thyroid hormone homeostasis, disruption of sex hormone steroidogenesis, and neurotoxic effects (*8*−*15*). Unlike BPA, limited information is available regarding the exposure characteristics of OH-PBDEs among exposed individuals, most notably pregnant women and their fetuses. OH-PBDEs were first reported in humans (pooled blood serum from children) at a municipal waste disposal site in Managua, Nicaragua (*16*). Recently, greater concentrations of OH-PBDEs have been reported in fetal blood ($n = 16$) compared to maternal blood ($n = 4$) of women in the USA, but the limited sample size and the fact that only a single paired maternal and fetal blood sample was studied limited the utility of that study (*17*). However, a study of 6 pairs of matched maternal-fetal samples in Japan found that concentrations of OH-PBDEs were greater in maternal blood than fetal blood (*18*).

The structures of BPA and OH-PBDEs consist of two benzene rings and phenolic groups (Figure S1 in the

* Corresponding author phone: +82-2-880-2738; fax: +82-2-745-9104; e-mail: kyungho@snu.ac.kr.
[†] Toxicology Centre, University of Saskatchewan.
[‡] Seoul National University.
[§] School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan.
[∥] Korea University.
[⊥] Seowon University.
[#] Soonchunhyang University.
[▽] City University of Hong Kong.
[○] Michigan State University.

10.1021/es1002764
Published on Web 05/28/2010

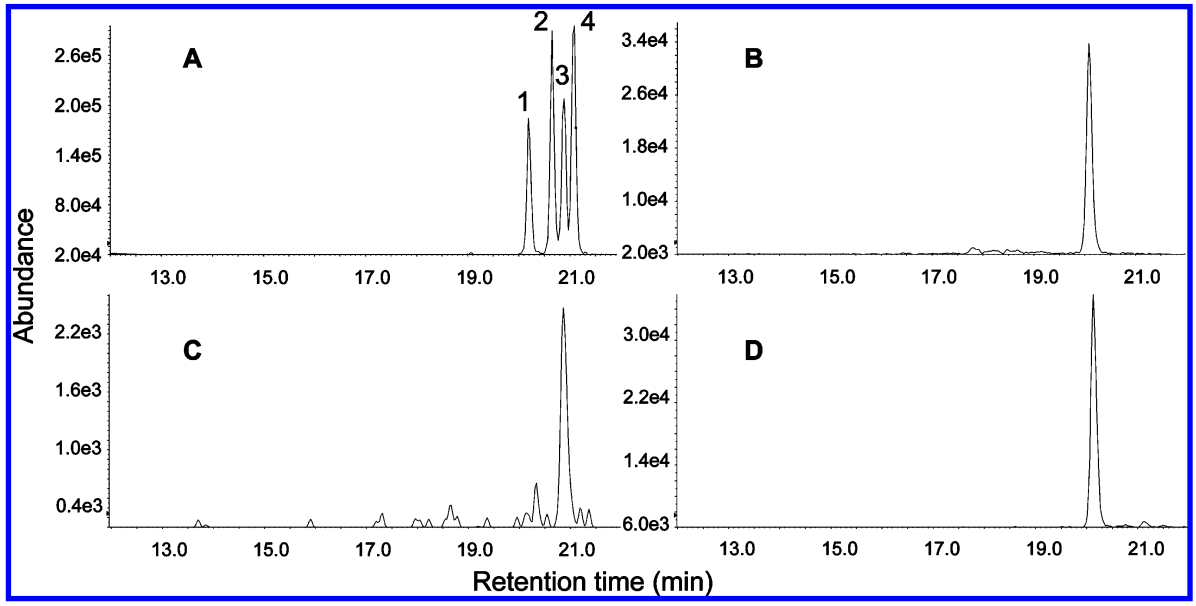

FIGURE 1. LC-MS/MS MRM chromatographic profiles of OH-Tetra-BDEs and BPA detected in human serum. A: OH-Tetra-BDEs standard solution (10 ng/mL), 3-OH-BDE-47 (1), 5-OH-BDE-47 (2), 6-OH-BDE-47 (3), 4′-OH-BDE-49 (4); B: BPA standard solution, 2 ng/mL; C: 6-OH-BDE-47 detected in human serum; D: BPA detected in human serum.

Supporting Information). Like BPA, OH-PBDEs also bind to the estrogen receptor (ER), which suggests that these two compounds have some similar biological activities (*12, 13*). Since BPA and OH-PBDEs are structurally similar, it was postulated that current information on the placental transfer of BPA might be valuable when trying to explain placental transfer of OH-PBDEs. Given the sensitivity of the developing fetus and the potential effects of OH-PBDE (*9, 12, 15*), it is important to understand placental transfer of OH-PBDEs in order to assess potential effect on fetal development. To this end, ten OH-PBDE congeners and BPA were analyzed in blood serum ('serum' hereafter) from 26 pregnant women and 28 matching fetuses from South Korea. To explore possible transfer mechanisms, placental transfer of OH-PBDEs was investigated and contrasted with that of BPA. Finally, the potential risks of prenatal exposure to OH-PBDE to the fetus were assessed by analyzing 17β-estradiol (E2) and total thyroxine (T4). Both E2 and T4 are important in fetal growth and development and are known to be affected by OH-PBDEs (*9, 12, 15*).

Materials and Methods

Study Population. Pregnant women ($n = 26$) were recruited at three hospitals located in Seoul, Cheongju, and Gumi, South Korea between August 2008 and March 2009. With the exception of two subjects whose blood was collected during weeks 20−25 of pregnancy, all blood was drawn during the third trimester of pregnancy (Table S1 in the Supporting Information). In addition, cord blood ($n = 28$, including 3 twin pairs with one fetal cord blood sample missing) was drawn at delivery from the umbilical cord vein of the matching fetuses. Serum was separated on-site using serum-separating tube and stored in polypropylene cryovials at −70 °C until analysis. Participating women completed a questionnaire, which included queries regarding current or previous pregnancy history, medical history, and demographic parameters. Characteristics of infants (e.g., gender) were also gathered. The Institutional Review Board of the School of Public Health, Seoul National University approved the study, and informed consent was obtained from all participating women.

Extraction and Cleanup of OH-PBDEs, BPA, and E2. Phenolic compounds, including eight OH-PBDEs (2′-OH-6′-Cl-BDE-7, 6′-OH-BDE-17, 6-OH-BDE-47, 4′-OH-BDE-49, 2′-OH-6′-Cl-BDE-68, 6-OH-BDE-90, 2-OH-BDE-123, and 6-OH-BDE-137), E2, and BPA, were separated, identified, and quantified using a previously developed method (*19*). Chemical information was provided in the Supporting Information. Briefly, approximately 0.5 mL of each serum sample was transferred into amber tubes and spiked with d_4-βE2, d_{16}-BPA, and 6′-OH-BDE-17. Samples were extracted two times with 5 mL of hexane/methyl *tert*-butyl ether (MTBE) (1:1; v/v) after the addition of 2 mL of pure water, 50 μL of hydrochloric acid (HCl, 37%), and 3 mL of 2-propanol. Extracts were washed with 4 mL of pure water four times and dried under a gentle stream of nitrogen. The target compounds in the extracts were then derivatized with dansyl chloride. The dried residues were dissolved in 200 μL of aqueous sodium bicarbonate (100 mmol/L, pH adjusted to 10.5 with NaOH). Dansyl chloride (200 μL of 1 mg dansyl/mL in acetone) was added and mixed by gentle vortexing for 1 min and incubated at 60 °C for 5 min. After cooling, 1 mL of water and 3 × 3 mL of hexane were added. The hexane layers were collected and combined before being subjected to silica gel column (4 g of silica gel and 4 g of sodium sulfate) fractionation. After application of the hexane extract, the column was rinsed with 15 mL of hexane/dichloromethane (DCM) (1:1, v/v) and then eluted with 20 mL of DCM and 30 mL of DCM/acetone (9:1; v/v). The 20 mL DCM fraction contained OH-PBDEs, and the DCM/acetone eluate contained estrogens and BPA. The two fractions were evaporated to dryness and reconstituted with 50 μL of acetonitrile:water (60:40) before liquid chromatography-tandem mass spectrometry (LC-MS/MS). Details of the total T4, LC-MS/MS analysis and quantification and quality assurance are provided in the Supporting Information.

Results

Among the ten OH-PBDE congeners investigated in maternal serum ($n = 26$) and their matching umbilical cord serum ($n = 28$), only 6-OH-BDE-47 was detected (Figure 1). Detection frequencies were 42% and 80% in maternal and umbilical cord serum samples, respectively (Figure S2 in the Supporting Information). Concentrations of 6-OH-BDE-47 ranged from <4 pg/g ww to 117 pg/g ww (mean = 17.5 ± 26.3 pg/g ww, median ≤4 pg/g ww) in maternal serum and from <4 pg/g ww to 127 pg/g ww (mean = 30.2 ± 27.1 pg/g ww, median = 26 pg/g ww) in umbilical cord serum (Table 1).

TABLE 1. Concentrations of 6-OH-BDE-47 (pg/g ww) in Fetal and Maternal Blood Sera of People from Different Geographical Regions

		6-OH-BDE-47						
	lipid (%)	*n*	*n* > LOD	mean ± SD	range	median	region	ref
fetal	-	25[a]	20	30.2 ± 27.1	<4−127	26	2008−2009, Korea	this study
	-	16	16	44.6[b]	-	4.5[b]	2003−2004, USA	17
	-	6	4	1.4 ± 2.0	<0.6−5.2	0.6	2005−2006, Japan	18
	-	9	0	-	-	-	2001−2002, Netherlands	28
maternal	-	26	11	17.5 ± 26.3	<4−117	<4	2008−2009, Korea	this study
	-	4	4	1.4[b]	-	0.9[b]	2003−2004, USA	17
	-	6	4	8.5 ± 12	<1−27	2.1	2005−2006, Japan	18
	-	90	0	-	-	-	2001−2002, Netherlands	28
	0.5 ± 0.1	4[c]	4[c]	7.4 ± 4.1	4.1−12.9	6.3	2002, Nicaragua	16
children	0.4 ± 0.1	10[c]	10[c]	8.9 ± 8.7	1.7−25.7	6.8	2002, Nicaragua	16

[a] Twin data were averaged. [b] Lipid content (0.45%) was from *16*, and 6-OH-BDE-47 was not the predominant congener in this study. [c] Number of pooled samples is shown.

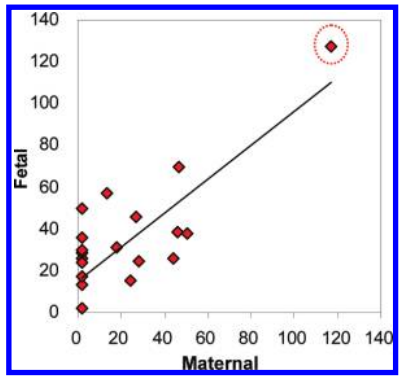

FIGURE 2. Association between maternal and fetal concentrations of 6-OH-BDE-47 (pg/g ww), $Y = 15.02 + 0.81 \times$ ($r = 0.625$, $p = 0.001$). The predictive relationship is $Y = 17.94 + 0.55 \times$ ($r = 0.567$, $p = 0.005$), when the circled outlier was removed from the analysis.

Concentrations of 6-OH-BDE-47 were significantly greater in umbilical cord serum than in maternal serum ($n = 25$, Wilcoxon test, $p = 0.002$, $Z = -3.061$). Concentrations of 6-OH-BDE-47 in umbilical cord serum were significantly correlated with concentrations in maternal serum (Figure 2, $r = 0.625$, $p = 0.001$). This significant correlation remained ($r = 0.567$, $p = 0.005$) even after an outlier was removed from the analysis. The mean concentration of 6-OH-BDE-47 was 35.5 pg/g ww in males (SD 31.7 pg/g, $n = 13$) and 24.5 pg/g ww in females (SD 20.8 pg/g, $n = 12$). No statistical difference between concentrations of 6-OH-BDE-47 in fetal serum of males and females was observed.

Because of similarities in their structures, possible mechanisms of OH-PBDE placental transfer were investigated by comparing ratios of 6-OH-BDE-47 in maternal and fetal serum with those of BPA. BPA was detected in only 27% and 8% of maternal and umbilical cord sera, respectively (Figure S2 in the Supporting Information). Concentrations of BPA in maternal serum ranged from <0.6 to 5.4 ng/mL (0.7 ± 1.0 ng/mL), while those in fetal serum ranged from <0.6 to 0.7 ng/mL (Table 2).

Concentrations of both E2 and T4 were measured in serum and associations with 6-OH-BDE-47 determined. Concentrations of E2 in maternal serum (8.0 ± 3.0 ng/mL) were significantly greater than those in cord serum (4.7 ± 2.2 ng/mL, Wilcoxon test, $p < 0.001$, $Z = -3.542$) (Table 2), which is comparable to previous investigations (*20, 21*). T4 concentrations were also significantly greater in maternal serum (9.7 ± 1.8 μg/dL) compared to cord serum (8.5 ± 1.7 μg/dL, Wilcoxon test, $p < 0.05$, $Z = -2.344$). There was no statistically significant difference between hormone concentrations in female and male fetal serum.

No significant relationships between concentrations of 6-OH-BDE-47 and E2 ($r = 0.013$, $p = 0.838$) were observed in fetal serum (Figure 3A). Negative relationships without statistical significance were found between concentrations of 6-OH-BDE-47 and T4 ($r = -0.217$ to -0.238, $p = 0.308$ to 0.275) in fetal serum (Figure 3B). After correcting for the covariates age and BMI of the mother, which were shown to influence T4 concentrations in the study population, the multivariate model also resulted in no statistically significant correlations between concentrations of 6-OH-BDE-47 and hormones (T4, E2) in cord serum ($\beta = -0.03$ and $p = 0.398$ for T4; $\beta = -0.03$ and $p = 0.702$ for E2, multiple regression).

Discussion

Concentrations of OH-PBDEs. The fact that only 6-OH-BDE-47 of the ten OH-PBDE congeners investigated was detected in either maternal or fetal serum (Figure 1) is consistent with the results of most other studies. 6-OH-BDE-47 was also the only congener observed in maternal blood, cord blood, and samples of umbilical cord tissues collected in Japan (*18*). Similarly, 6-OH-BDE-47 was the predominant congener identified in serum collected from reference sites in Nicaragua (*16*). Although it has been suggested that this congener could originate from metabolism of PBDEs (*16*), a recent publication indicated that metabolism of the naturally occurring methoxylated PBDEs (e.g., 6-MeO-BDE-47) could be a primary source of OH-PBDEs (*22*). Recent investigations have reported that the average concentrations of MeO-PBDEs (in invertebrates, 12.5−417 pg/g ww; in fish, 11.5−15237 pg/g ww) were greater than those of PBDEs (in invertebrates, 23.5−103 pg/g ww; in fish, 33−2452 pg/g ww) in five invertebrates and nine fishes collected from Bohai Sea, the innermost bay of the Yellow Sea bordered with the Korean peninsula (*23*). Relatively greater concentrations of MeO-PBDEs (92.3−140 ng/g lipid weight (lw)) compared to PBDEs (98.3−116.5 ng/g lw) were also reported for Japanese common squid from Korean offshore waters (*24*). 6-MeO-BDE-47 is the predominant congener in marine organisms and related food products (*22*−*26*) and has been identified as a natural product in marine organisms (*26*). Consequently, consumption of marine products could represent an important source of 6-OH-BDE-47 exposure among pregnant women in the present study. Indeed, marine products are an important part of the diet of South Koreans. Although specific data pertaining to consumption of marine fish is unavailable, a combined daily intake of marine and freshwater fish of 70.5 g has been reported among Korean women (*27*). This dietary practice could contribute to the relatively great concentrations of 6-OH-BDE-47 in Korean maternal and fetal serum observed in this study.

TABLE 2. Concentrations of BPA and E2 (ng/mL) in Fetal and Maternal Blood Sera of People from Different Geographical Regions

		BPA			E2			
	n	*n* > LOD	mean ± SD	range	mean ± SD	range	region	ref
fetal	25[a]	2	<0.6	<0.6−0.7	4.7 ± 2.2	1.6−10.8	2008−2009, Korea	this study
	300	120	1.13 ± 1.43	<0.63−8.86	-	-	Korea	6
	37	37	2.9 ± 2.5	0.2−9.2	-	-	2000−2001, Germany	2
	32	32	2.2 ± 1.8	-	-	-	Japan	29
	-	-	-	-	12.4 ± 12.2	0.4−81.1	1994−1998, USA	20
	-	-	-	-	5.9 ± 5.2	0.6−41.3	2000−2001, Japan	21
maternal	26	7	0.7 ± 0.1	<0.6−5.4	8.0 ± 3.0	4.2−15.8	2008−2009, Korea	this study
	300	252	9.04 ± 14.03	<0.63−66.48	-	-	Korea	6
	37	37	4.4 ± 3.9	0.3−18.9	-	-	2000−2001, Germany	2
	37	37	1.4 ± 0.9	-	-	-	Japan	29
	-	-	-	-	24.1 ± 10.7	0.8−75.1	1994−1998, USA	20
	-	-	-	-	21.6 ± 7.0	7.4−38.0	2000−2001, Japan	21

[a] Twin data were averaged.

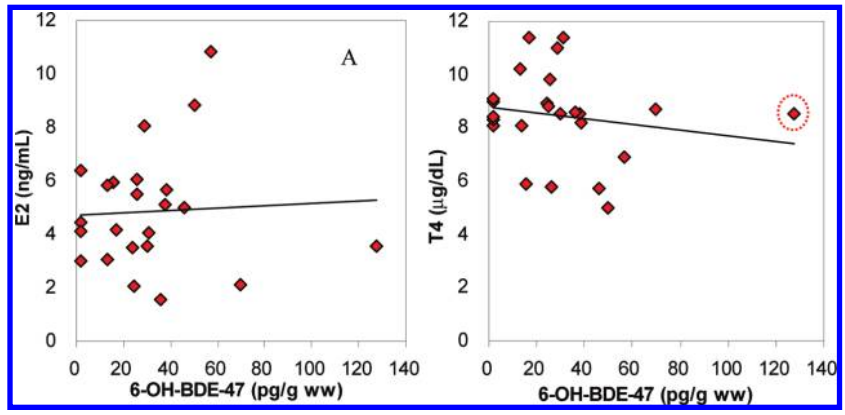

FIGURE 3. Correlations between concentrations 6-OH-BDE-47 in umbilical cord blood serum and concentrations of A) E2 and B) T4. A: $Y = 4.64 + 0.004 \times$ ($n = 25$, $r = 0.013$, $p = 0.838$); B: $Y = 8.77 - 0.01 \times$ ($n = 24$, $r = -0.217$, $p = 0.308$) and $Y = 9.09 - 0.02X$ ($n = 23$, $r = -0.238$, $p = 0.275$), when the circled outlier was removed from the analysis.

Other *para*- or *meta*-substituted OH-PBDE congeners could originate from exposure to synthetic brominated flame-retardants (e.g., PBDEs (*16, 17*)) or by metabolism of MeO-PBDEs, such as penta-MeO-PBDEs (*22*). These congeners have been reported to be in serum collected from people residing near waste disposal sites in Nicaragua (*16*), and profiles with a predominance of 5-OH-BDE-47 (*meta* substituted OH-PBDE) were observed in maternal blood from the USA (*17*). However, these congeners were not detected in our study, which is consistent with OH-PBDEs in Korean pregnant women originating primarily from natural sources.

The mean concentration of 6-OH-BDE-47 in fetal serum in the present study ($n = 25$) was similar to that reported in the USA ($n = 16$ (*17*)) but greater than reported concentrations in Japan ($n = 6$ (*18*)) and The Netherlands ($n = 9$ (*28*)) (Table 1). The mean concentration of 6-OH-BDE-47 in maternal serum in this study was greater than the concentrations reported in maternal serum collected in the USA (*17*), Nicaragua (*16*), Japan (*18*), and The Netherlands (*28*) (Table 1). However, 6-OH-BDE-47 was not the predominant congener identified in maternal blood collected in the USA, and the mean concentration of ΣOH-PBDEs observed in that study (32.7 pg/g ww based on a mean lipid content of 0.45% (*16, 17*)) was greater than that observed in this study. The results suggest that pregnant South Korean women are exposed to relatively great concentrations of OH-PBDEs compared with people in other geographical regions.

Placental Transfer. The average placental transfer ratio between fetal and maternal serum (F/M ratio) for 6-OH-BDE-47 was 1.4 ± 1.1 in the 10 mother-fetal pairs with detectable 6-OH-BDE-47. The observation that concentrations of 6-OH-BDE-47 in maternal serum were significantly less than those in umbilical cord serum is consistent with results reported for the USA (*17*) but somewhat divergent from the results reported for Japan (*18*). Sixteen fetal and 4 maternal blood samples with only one matching mother-fetus pair were analyzed in the USA study. The Japanese study included six paired maternal and fetal blood samples, but only 4 paired samples contained 6-OH-BDE-47, and concentrations exhibit a large amount of variation (*18*). A significant positive correlation between concentrations of 6-OH-BDE-47 in maternal and cord sera was found in the current study (Figure 2). This is consistent with placental transfer being the primary pathway of 6-OH-BDE-47 accumulation in fetal serum. The M/F ratio calculated in the present study is based on an assumption that variation in gestational age at when blood was collected (week 36 ± 5.1) did not influence the concentration of 6-OH-BDE-47 to any significant extent. No significant differences between concentrations of 6-OH-BDE-47 in female and male fetal serum suggested that placental transfer was independent of gender.

The placental transfer rate of BPA was less than that of 6-OH-BDE-47. BPA was detected in both greater concentration and frequency in maternal serum compared to cord serum. Similar patterns of placental transfer have been reported for BPA in several studies of mammals (*2, 3, 6, 29*). With the exception of one study in Japan (*29*) concentrations of BPA in maternal blood have been greater than those in cord blood (*2, 6*) (Table 2). Additionally, an *in vivo* exposure of monkeys to BPA showed greater concentrations of BPA in maternal serum compared to umbilical cord and fetal serum (*3*).

Placental transfer of chemicals is known to be affected by their physicochemical characteristics that govern diffusion

through cell membranes. Generally chemicals with both lipophilic and hydrophilic properties (log Kow = −0.9 to 5) move readily across the placental membrane to the developing fetus, compared to more lipophilic chemicals (log Kow = >5 *4, 5*). The log Kow of 6-OH-BDE-47 is 6.36−6.50 (calculated by Crippen's and Viswanadhan's fragmentation methods, ChemOffice Ultra 11, CambridgeSoft, Cambridge, MA, USA), while the log Kow of BPA is 3.32 (*5*). Therefore, it was hypothesized that placental transfer of BPA would be greater than that of 6-OH-BDE-47. However, the observation that concentrations of 6-OH-BDE-47 in cord sera were greater than concentrations of BPA indicates that lipophilicity is not the only factor driving the differential placental transfer of these two compounds.

Although the actual mechanism(s) responsible for the observed difference in placental transfer of 6-OH-BDE-47 and BPA are unknown, there is evidence that a T4 transport plasma protein, transthyretin (TTR), might be involved. It is known that the potential for placental transfer depends on binding with plasma proteins (*30*). TTR is one of the three plasma proteins in humans responsible for transport of T4, and approximately 15% of circulating T4 is bound to TTR (*31*). TTR is able to cross the placental membrane (*32*) and blood-brain barrier (*33*) thereby facilitating delivery of T4 to the developing fetus. The results of previous studies have suggested that binding of hydroxylated polychlorinated biphenyls (OH-PCBs) to TTR is possibly responsible for accumulation of these phenolic xenobiotics in the developing fetus, which results in lesser concentrations of T4 in fetal blood (*18, 34, 35*). The observation of particular relevance to this study is that 6-OH-BDE-47, as a potent competitive-binding TTR agonist, has a greater affinity for TTR than T4 (*11, 15, 36*). In contrast to 6-OH-BDE-47, BPA does not displace TTR-bound T4 (*11, 36*). Consequently, binding to TTR might be a more important determinant of placental transfer of these phenolic compounds than lipophilicity. In addition, recent studies have shown that placental cells synthesize TTR (*37*), and this TTR is secreted and binds T4 resulting in increased internalization of the TTR-T4 complex (*38*). Although this might represent a critical mechanism whereby maternal T4 is transferred to fetal circulation, placental TTR might also be responsible for the accumulation of 6-OH-BDE-47, but not BPA, by the developing fetus.

In addition to TTR, thyroxine binding globulin (TBG) could also facilitate the placental transfer of 6-OH-BDE-47 in pregnant women. The F/M ratio of 6-OH-BDE-47 obtained in the current study (1.4 ± 1.1) was relatively greater than those reported for OH-PCBs in pregnant women and their infants in The Netherlands (0.6−0.7 (*28*)) and Japan (0.1−0.9 (*18*)). This is an interesting observation since both OH-PCBs and OH-PBDEs are lipophilic, and binding affinities of OH-PCBs were comparable to those of OH-PBDEs with human TTR *in vitro* (*36*). The greater F/M ratio for 6-OH-BDE-47 compared to OH-PCBs could be explained by their different affinity toward TBG. Stronger affinities to TBG were found for OH-PBDEs (especially for 6-OH-BDE-47), while TBG binding was negligible for most OH-PCBs and BPA (*36*). As a major T4 transport protein in human plasma, TBG is responsible for binding with approximately 75% of circulating T4 (*36*). This suggests that TBG could also contribute to placental transfer of 6-OH-BDE-47.

Potential Effects. OH-PBDEs have the potential to elicit a variety of effects on humans including disruption of thyroid hormone homeostasis, altered estradiol synthesis, neurotoxicity, and inhibition of human placental aromatase activity (*8, 9, 11, 12, 15, 36*). Consequently, it is important to determine risks that OH-PBDEs might pose to the developing fetus. The mean concentration of 6-OH-BDE-47 detected in fetal serum was 30.2 ± 27.1 pg/g ww or 0.06 nM, while the maximum detected concentration was 127 pg/g ww or 0.25 nM. It has been reported that OH-PBDEs could compete with thyroid hormones for binding sites on human plasma proteins. Based on *in vitro* studies of human cells, median inhibitory concentrations (IC50s) range from 22.3 to 107.8 nM for TTR and from 100 to 867 nM for TBG, depending on the congener (*36*). Concentrations of OH-PBDEs of 100−1000 nM have been reported to cause estrogenic activities (*12*), concentrations of 1000−5000 nM can cause neurotoxic effects (*9*), and concentrations of 5000−10000 nM can inhibit human placental aromatase activity (*8*). The concentrations of 6-OH-BDE-47 observed in fetal serum were approximately 100-fold less than the IC50 value for T4-TTR binding and E2 agonism obtained from *in vitro* studies (*11, 36*). Associations between concentrations of 6-OH-BDE-47 and E2 or T4 in cord serum were not statistically significant from one another (Figure 3). After being corrected for the covariates age and BMI of the mother, the relationships were still not statistically significant. But the concentration of 6-OH-BDE-47 in fetal serum was closer to the effect concentration for TTR or TBG binding than other potential effects including effects on steroid hormones and placental aromatase activity.

Information on concentrations of OH-PBDEs in humans is limited. However, relatively great concentrations of OH-PBDEs have been reported in marine organisms, such as red algae from the Baltic Sea (estimated concentration: about 200 nM ww), glaucous gulls from Norwegian Arctic (on average 7.0 nM in whole blood and 7.1 nM in liver), and polar bears from east Greenland (average concentration: 5.8 nM in whole blood) (*39−41*). People living in places with great exposure to OH-PBDEs could accumulate significant quantities of these compounds. Although concentrations of OH-PBDEs measured in fetal serum in this study are more than 100-fold less than the IC50 for binding to human TTR (*36*), the effects of OH-PBDEs in more exposed people (especially the developing fetus) deserve some attention.

Normal fetal brain development is dependent upon a regulated control of T4 supplies. Synthesis and secretion of fetal T4 by the hypothalamus-pituitary-thyroid (HPT) axis are not initiated until the end of the first trimester of development (*42*). Consequently, during the first 16 wk of gestation the fetus depends on maternal supply of thyroid hormones. Disruption of this supply could have consequences for fetal neurological development (*43, 44*). Therefore, it is not prudent to ignore a possibility of disruption in T4 balance in later stages of pregnancy especially among fetuses exposed to greater concentrations of OH-PBDEs through the placenta. Along with the fact that many other contaminants have also been observed to affect T4 concentrations, this observation warrants further studies on prenatal exposure to T4 transport disrupting chemicals.

Acknowledgments

The research was supported by a Discovery Grant from the National Science and Engineering Research Council of Canada (Project 326415-07) and a grant from Western Economic Diversification Canada (Project # 6971 and 6807). Prof. Giesy was supported by the Canada Research Chair program and an at large Chair Professorship in the Department of Biology and Chemistry and Research Centre for Coastal Pollution and Conservation, City University of Hong Kong. The authors wish to acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructure. This research was supported, in part, by a grant (Project # 08182KFDA499) from the Korea Food and Drug Administration.

Supporting Information Available

Detailed information on chemical analysis and data analysis and characteristics of participating humans and structure of

study chemicals. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Colborn, T. Environmental estrogen: Health implications for humans and wildlife. *Environ. Health Perspect.* **1995**, *103*, 135–136.

(2) Schönfelder, G.; Wittfoht, W.; Hopp, H.; Talsness, G. E.; Paul, M.; Chahoud, I. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environ. Health Perspect.* **2002**, *110*, 703–707.

(3) Uchida, K.; Suzuki, A.; Kobayashi, Y.; Buchanan, D. L.; Sato, T.; Watanabe, H.; Katsu, Y.; Suzuki, J.; Asaoka, K.; Mori, C.; Arizono, K.; Iguchi, T. Bisphen*ol-A administration durin*g pregn*ancy* results in fetal exposure in mice and monkeys. *J. Health Sci.* **2002**, *48*, 579–582.

(4) Frederiksen, M.; Vorkamp, K.; Thomsen, M.; Knudsen, L. E. Human internal and external exposure to PBDEs - A review of levels and sources. *Int. J Hyg. Environ. Health* **2009**, *212*, 109–134.

(5) Takahashi, O.; Oishi, S. Disposition of orally administered *2,2-bis*(4-hydroxyphenyl)propane (Bisphenol A) in pregnant rats and the placental transfer to fetus. *Environ. Health Perspect.* **2000**, *108*, 931–935.

(6) Lee, Y. J.; Ryu, H. Y.; Kim, H. Y.; Min, C. S.; Lee, J. H.; Kim, E.; Nam, B. H.; Park, J. H.; Jung, J. Y.; Jang, D. D.; Park, E. Y.; Lee, K. H.; Ma, J. Y.; Won, H. S.; Im, M. W.; Leem, J. H.; Hong, Y. C.; Yoon, H. S. Maternal and fetal exposure to bisphenol A in Korea. *Reprod. Toxicol.* **2008**, *25*, 413–419.

(7) Shin, B. S.; Yoo, S. D.; Cho, C. Y.; Jung, J. H.; Lee, B. M.; Kim, J. H.; Lee, K. C. Maternal-fetal disposition of bisphenol A in pregnant Sprague-dawley rats. *J. Toxicol. Environ. Health A* **2002**, *65*, 395–406.

(8) Canton, R. F.; Scholten, D. E. A.; Marsh, G.; de Jong, P. C.; van den Berg, M. Inhibition of human placental aromatase activity by hydroxylated polybrominated diphenyl ethers (OH-PBDEs). *Toxicol. Appl. Pharmacol.* **2008**, *227*, 68–75.

(9) Dingemans, M. M. L.; de Groot, A.; van Kleef, R. G. D. M.; Berman, Å.; van den Berg, M.; Vijverberg, H. P. M.; Westerink, R. H. S. Hydroxylation increase the neurotoxic potential of BDE-47 to affect exocytosis and calcium homeostasis in PC12 cells. *Environ. Health Perspect.* **2008**, *116*, 637–643.

(10) Hamers, T.; Kamstra, J. H.; Sonneveld, E.; Murk, A. J.; Visser, T. J.; Van Velzen, M. J. M.; Brouwer, A.; Bergman, A. Biotransformation of brominated flame retardants into potentially endocrine-disrupting metabolites, with special attention to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). *Mol. Nutr. Food Res.* **2008**, *52*, 284–298.

(11) Meerts, I. A. T. M.; van Zanden, J. J.; Luijks, E. A. C.; van Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman, A.; Brouwer, A. Potential competitive interactions of some brominated flame retardants and related compounds with human transthyretin *in vitro*. *Toxicol. Sci.* **2000**, *56*, 95–104.

(12) Meerts, I. A. T. M.; Letcher, R. J.; Hoving, S.; Marsh, G.; Bergman, Å.; Lemmen, J. G.; van der Burg, B.; Brouwer, A. *In vitro* estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environ. Health Perspect.* **2001**, *109*, 399–407.

(13) Mercado-Feliciano, M.; Bigsby, R. M. Hydroxylated metabolites of the polybrominated diphenyl ether mixture DE-71 are weak estrogen receptor-alpha ligands. *Environ. Health Perspect.* **2008**, *116*, 1315–1321.

(14) Song, R.; He, Y.; Murphy, M. B.; Yeung, L. W.; Yu, R. M.; Lam, M. H.; Lam, P. K.; Hecker, M.; Giesy, J. P.; Wu, R. S.; Zhang, W.; Sheng, G.; Fu, J. Effects of fifteen PBDE metabolites, DE71, DE79 and TBBPA on steroidogenesis in the H295R cell line. *Chemosphere* **2008**, *71*, 1888–1894.

(15) Ucán-Marín, F.; Arukwe, A.; Mortensen, A.; Gabrielsen, G. W.; Fox, G. A.; Letcher, R. J. Recombinant transthyretin purification and competitive binding with organohalogen compounds in two gull species (*Larus argentatus* and *Larus hyperboreus*). *Toxicol. Sci.* **2009**, *107*, 440–450.

(16) Athanasiadou, M.; Cuadra, S. N.; Marsh, G.; Bergman, A.; Jakobsson, K. Polybrominated diphenyl ethers (PBDEs) and bioaccumulative hydroxylated PBDE metabolites in young humans from Managua, Nicaragua. *Environ. Health Perspect.* **2008**, *116*, 400–408.

(17) Qiu, X. H.; Bigsby, R. M.; Hites, R. A. Hydroxylated metabolites of polybrominated diphenyl ethers in human blood samples from the United States. *Environ. Health Perspect.* **2009**, *117*, 93–98.

(18) Kawashiro, Y.; Fukata, H.; Omori-Inoue, M.; Kubonoya, K.; Jotaki, T.; Takigami, H.; Sakai, S. I.; Mori, C. Perinatal exposure to brominated flame retardants and polychlorinated biphenyls in Japan. *Endocrin. J.* **2008**, *55*, 1071–1084.

(19) Chang, H.; Wan, Y.; Naile, J.; Zhang, X. W.; Wiseman, S.; Hecker, M.; Lam, M. H. W.; Giesy, J. P.; Jones, P. D. Simultaneous quantification of multiple classes of phenolic compounds in blood plasma by liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr., A* **2010**, *1217*, 506–513.

(20) Troisi, R.; Potischman, N.; Roberts, J. M.; Harger, G.; Markovic, N.; Cole, B.; Lykins, D.; Siiteri, P.; Hoover, R. N. Correlation of serum hormone concentrations in maternal and umbilical cord samples. *Cancer Epidemiol. Biomarkers Prevent.* **2003**, *12*, 452–456.

(21) Nagata, C.; Iwasa, S.; Shiraki, M.; Sahashi, Y.; Simizu, H. Association of maternal fat and alcohol intake with maternal and umbilical hormone levels and birth weight. *Cancer Sci.* **2007**, *98*, 869–873.

(22) Wan, Y.; Wiseman, S.; Chang, H.; Zhang, X. W.; Jones, P. D.; Hecker, M.; Kannan, K.; Tanabe, S.; Hu, J. Y.; Lam, M. H. W.; Giesy, J. P. Origin of hydroxylated brominated diphenyl ethers: natural compounds or man-made flame retardants. *Environ. Sci. Technol.* **2009**, *43*, 7536–7542.

(23) Zhang, K.; Wan, Y.; An, L. H.; Hu, J. Y. Trophodynamics of PBDEs and MeO-PBDEs in a marine foodweb. *Environ. Toxicol. Chem.* In press.

(24) Kim, G. B., Stapleton, H. M. PBDEs, Methoxylated PBDEs and HBCDs in Japanese common squid (*Todarodes pacificus*) from Korean offshore waters. *Mar. Pollut. Bull.* In press doi:10.1016/j.marpolbul.2010.03.025.

(25) Covaci, A.; Voorspoels, S.; Vetter, W.; Gelbin, A.; Jorens, P. G.; Blust, R.; Neels, H. Anthropogenic and naturally occurring organobrominated compounds in fish oil dietary supplements. *Environ. Sci. Technol.* **2007**, *41*, 5237–5244.

(26) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science* **2005**, *307*, 917–920.

(27) Jang, J. Y.; Jo, S. N.; Kim, S. J.; Cheong, H. K. Korea Exposure Factors Handbook. Korea Ministry of Environment: Seoul, 2007.

(28) Meijer, L.; Weiss, J.; van Velzen, M.; Brouwer, A.; Bergman, A.; Sauer, P. J. J. Serum concentrations of neutral and phenolic organohalogens in pregnant women and some of their infants in the Netherlands. *Environ. Sci. Technol.* **2008**, *42*, 3428–3433.

(29) Ikezuki, Y.; Tsutsumi, O.; Takai, Y.; Kamei, Y.; Taketani, Y. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum. Reprod.* **2002**, *17*, 2839–2841.

(30) Little, B. B.; VanBeveren, T. T. Placental transfer of selected substances of abuse. *Semin. Perinatol.* **1996**, *I*, 147–153.

(31) Schussler, G. C. The thyroxine-binding proteins. *Thyroid* **2000**, *10*, 372.

(32) Brouwer, A.; Morse, D. C.; Lans, M. C.; Schuur, A. G.; Murk, A. J.; Klasson-Wehler, E.; Bergman, A.; Visser, T. J. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol. Ind. Health* **1998**, *14*, 59–84.

(33) Schreiber, G.; Southwell, B. R.; Richardson, S. J. Hormone delivery systems to the brain—transthyretin. *Exp. Clin. Endocrin.* **1995**, *103*, 75–80.

(34) Meerts, I. A. T. M.; Assink, Y.; Cenijn, P. H.; van den Berg, J. H. J.; Weijers, B. M.; Bergman, A.; Koeman, J. H.; Brouwer, A. Placental transfer of a hydroxylated polychlorinated biphenyl and effects on fetal and maternal thyroid hormone homeostasis in the rat. *Toxicol. Sci.* **2002**, *68*, 361–371.

(35) Purkey, H. E.; Palaninathan, S. K.; Kent, K. C.; Smith, C.; Safe, S. H.; Sacchettini, J. C.; Kelly, J. W. Hydroxylated polychlorinated biphenyls selectively bind transthyretin in blood and inhibit amyloidogenesis: rationalizing rodent PCB toxicity. *Chem. Biol.* **2004**, *11*, 1719–1728.

(36) Marchesini, G. R.; Meimaridou, A.; Haasnoot, W.; Meulenberg, E.; Albertus, F.; Mizuguchi, M.; Takeuchi, M.; Irth, H.; Murk, A. J. Biosensor discovery of thyroxine transport disrupting chemicals. *Toxicol. Appl. Pharmacol.* **2008**, *232*, 150–160.

(37) Mckinnon, B.; Li, H.; Richard, K.; Mortimer, R. Synthesis of the thyroid hormone binding proteins transthyretin and albumin by human trophoblast. *J Clin. Endocrinol. Metab.* **2005**, *90*, 6714–6720.

(38) Landers, K. A.; McKinnon, B. D.; Li, H.; Subramaniam, N.; Mortimer, R. H.; Richard, K. Carrier-mediated thyroid hormone transport into placenta by placental transthyretin. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 2610–2616.

(39) Verreault, J.; Shahmiri, S.; Gabrielsen, G. W.; Letcher, R. J. Organohalogen and metabolically-derived contaminants and associations with whole body constituents in Norwegian Arctic glaucous gulls. *Environ. Int.* **2007**, *33*, 823–830.
(40) Gebbink, W. A.; Sonne, C.; Dietz, R.; Kirkegaard, M.; Riget, F. F.; Born, E. W.; Muir, D. C. G. Letcher. Tissue-specific congener composition of organohalogen and metabolic contaminants in East Greenland polar bears (*Ursus maritimus*). *Environ. Pollut.* **2008**, *152*, 621–629.
(41) Malmvarn, A.; Marsh, G.; Kautsky, L.; Athanasiadou, M.; Bergman, A.; Asplund, L. Hydroxylated and Methoxylated brominated diphenyl ethers in the red algae *Ceramium tenuicorne* and blue mussels from the Baltic Sea. *Environ. Sci. Technol.* **2005**, *39*, 2990–2997.
(42) Calvo, R. M.; Jauniaux, E.; Gulbis, B.; Asunción, M.; Gervy, C.; Contempré, B.; Morreale de Escobar, G. Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 1768–1777.
(43) Morreale de Escobar, G.; Obregón, M. J.; Escobar del Rey, F. Is neuropsychological development related to maternal hypothyroidism, or to maternal hypothyroxinemia. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 3975–3987.
(44) Morreale de Escobar, G.; Obregon, M. J.; Escobar del Rey, F. Role of thyroid hormone during early brain development. *Eur. J. Endocrinol.* **2004**, *151*, 25–37.

ES1002764